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Physiology and Pharmacology of Corticotropin-releasing Factor*

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I. Introduction and Historical Perspectives

CRF‡ is the major physiological regulator of the secretion of ACTH, β-endorphin, and other POMC-derived peptides from the anterior pituitary gland. Simply stated, CRF is the predominant chemical messenger by which the CNS controls the activity of the pituitary-adrenal axis and is, therefore, ultimately responsible for orchestrating the endocrine response to stress. This has been hypothesized to be the case since the 1950s and has been decisively demonstrated to be so for nearly a decade. In the past 5 years, overwhelming evidence has accumulated that is concordant with the hypothesis that CRF also acts as a neurotransmitter within the CNS. Taken together, the extant CRF literature strongly suggests that CRF integrates not only the endocrine but also the autonomic, immunological, and behavioral responses of mammalian organisms to stress. Moreover, inappropriate CRF neuronal activity may manifest itself in a number of psychiatric illnesses including affective dis-

‡Abbreviations: CRF, corticotropin-releasing factor; ACTH, adrenocorticotropic hormone; POMC, pro-opiomelanocortin; CNS, central nervous system; LHRH, luteinizing hormone-releasing hormone HPA, hypothalamic-pituitary-adrenal; LH, luteinizing hormone; oCRF, ovine CRF; mRNA, messenger ribonucleic acid; PVN, paraventricular nucleus; BNST, bed nucleus of the stria terminalis; cAMP, cyclic adenosine monophosphate; i.c.v., intracerebroventricular(ly); AVP, arginine vasopressin; GABA, γ-aminobutyric acid; 5-HT, 5-hydroxytryptamine; i.v., intravenous(ly); FSL, Flinders sensitive line; SHR, spontaneously hypertensive rat; ECT, electroconvulsive therapy; NK, natural killer; IL, interleukin; MAP, mean arterial pressure; EEG, electroencephalographic; CSF, cerebrospinal fluid.

orders, anxiety disorders, anorexia nervosa, and Alzheimer's disease.

Although such an all-encompassing role for a single neurotransmitter substance may initially seem somewhat surprising, the burgeoning database supports what is quite simple and elegant teleologically. The amino acid sequence of CRF has been highly conserved throughout the evolutionary process. CRF has been identified in mammals and birds, with homologs of CRF demonstrated in amphibians and fish. Therefore, it appears that CRF-like compounds have been utilized by organisms for millions of years in which they apparently function in an adaptive role to mediate stress responses. Although these compounds may have originally functioned simply to mobilize sources of energy to help flee predators or other threatening conditions, as animals developed evolutionarily, CRF appears to have taken on a more complex role in integrating the organism's responses (endocrine, behavioral, autonomic, and immunological) to stress. Recent evidence suggests that a similar evolutionary sequence of events may have occurred for LHRH (gonadotropin-releasing hormone). Thus, not only does this decapeptide control the secretion of sex steroids by functioning as the major physiological regulator of pituitary gonadotropin release but it also appears to directly influence behavioral aspects of reproductive behavior by acting on CNS neurons.

In the present review, it is our intention to summarize the well-established data concerning CRF (i.e., physical properties, localization, neuroendocrine function), as well as to scrutinize the experimental data suggesting a behavioral, autonomic, and immunological function for CRF in the organism's response to stress. Moreover, is dysregulation of CRF neuronal activity responsible for some aspects of mental illness and, if this is the case, what are the clinical implications for future treatment strategies? We have used pertinent references gathered up until the early part of 1991.

Early morphological investigation of the anterior pituitary showed that it was completely free of direct neuronal innervation. In the early 1930s, Popa and Fielding (1933) described the existence of a hypothalamohypophysial portal vessel system. However, controversy arose concerning the direction of blood flow, and their findings were subsequently disregarded as unimportant. At this same time, the notion of humoral control of the anterior pituitary gland by the brain was first hypothesized. In a number of animal and human studies which utilized laboratory animals or soldiers who had been war casualties, lesions of the hypothalamus resulted in profound decreases in pituitary function as evidenced by adrenal and gonadal atrophy. It was not until the late 1940s that Harris (1948) and colleagues in England confirmed the neurohumoral control of anterior pituitary hormone secretion. In this "chemotransmitter-portal vessel hypothesis," chemical messengers are transported via the portal vessels from the hypothalamus to the anterior pituitary. However, although the humoral control of anterior pituitary function was confirmed in the late 1940s and early 1950s, nearly 20 years elapsed before the chemical identity of the first hypothalamic releasing factor, thyrotropin-releasing hormone, was achieved by Schally's group (Bøler et al., 1969) and Guilleman's group (Burgus et al., 1969).

Because Hans Selye (1936) observed that the "general adaptation syndrome" following exposure to stress activated the pituitary-adrenocortical axis, elucidation of the chemical identity of CRF was clearly of paramount importance to any comprehensive understanding of the neural mechanisms that mediate the HPA response to stress. The clinical importance of the HPA axis, and the availability of bioassays to measure ACTH, led researchers in the early 1950s to focus on CRF prior to attempting characterization of other putative hypothalamic releasing factors. However, elucidation of the structure of CRF proved difficult for several reasons. First, the ease with which almost any novel stimulus (mild stressor) activates the HPA axis in experimental animals has confounded many studies. Second, because many neurotransmitters in tissue extracts other than authentic CRF possess CRF-like activity and can enhance ACTH secretion, extreme caution was necessary before any endogenous substance could be deemed the physiological CRF. This was a particular problem with regard to the bioassay used to identify CRF activity in which ACTH release was measured from hemipituitaries in vitro. Finally, the radioimmunoassay for ACTH also proved to be particularly problematic, because of poor sensitivity and specificity.

The systematic search for CRF in hypothalamic tissue began with the work of Saffran and Schally (1955) and Guillemin and Rosenberg (1955). Thereafter, Schally and Guillemin working together at McGill University used extracts of neurohypophysial tissue and gel filtration chromatography to identify CRF-like activity in three separate fractions which they labeled α_1, α_2 (similar to α-melanocyte-stimulating hormone) and β (similar to vasopressin) (Schally et al., 1960, 1962). They also discovered that extracts of porcine hypothalamus contained ACTH and α- and β-melanocyte-stimulating hormone (Guillemin et al., 1962). Although remarkable in that this finding anticipated by 16 years the discovery of POMC-derived peptides in the brain (Krieger and Liotta, 1979), this finding further increased the difficulty of interpreting the assay data, and serious work on the isolation of CRF was brought to a halt (Fink, 1981).

The more than 25-year delay in the isolation and characterization of CRF after unequivocal evidence for its existence can be attributed, as noted above, to several factors. The bioassays were problematic because of their lack of specificity, although their sensitivity was often remarkable. Interpretation of even the best in vitro as-

says could be confounded by the fact that other substances can directly stimulate ACTH release, albeit less potently than authentic CRF, and/or potentiate the effects of CRF itself. Whole hypothalamic extracts also contain ACTH. Furthermore, because the sizes of CRF (41 amino acids) and ACTH (39 amino acids) are similar, the two peptides are generally not easily separable by liquid chromatography. The in vitro bioassay systems were also vulnerable to nonspecific secretagogues found in tissue extracts, such as myelin basic protein, histones, K^+, and the components of a variety of buffers and solvents (Vale et al., 1983a).

In 1981, Wylie Vale and colleagues at the Salk Institute (Vale et al., 1981; Spiess et al., 1981; Rivier et al., 1982c) isolated and characterized a 41-amino acid peptide from extracts of ovine hypothalamus with the following primary structure: H-Ser-Gln-Glu-Pro-Pro-Ile-Ser-Leu-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Glu-Val-Leu-Glu-Met-Thr-Lys-Ala-Asp-Gln-Leu-Ala-Gln-Gln-Ala-His-Ser-Asn-Arg-Lys-Leu-Leu-Asp-Ile-Ala-NH_2. Starting material for this purification was a side fraction of nearly 500,000 fragments of ovine hypothalamus initially processed during the characterization of LHRH. A comprehensive review of the isolation and characterization of CRF was written by Vale et al. (1983a).

The structure of oCRF is homologous with several known peptides including sauvagine and urotensin I (Pallai et al., 1983). Sauvagine was isolated from the skin of the South American frog *Phylomedusa sauvagei*. More than 50% of the residues in sauvagine are identical with those in oCRF; the majority of the remaining residues are conservative substitutions. Both sauvagine and oCRF are closely related to a third peptide, urotensin I, isolated from the urohypophysis of two species of fish, *Catostomus cyprimus* and *Catostomus catostomus*. CRF also shares some homology with calmodulin and angiotensinogen. The tetrapeptide Phe-His-Leu-Leu is common to both angiotensinogen and oCRF and is the site in angiotensinogen of renin and converting enzyme cleavage. This may reflect a distant ancestral relationship between angiotensinogen and oCRF, each of which can modulate adrenocortical function (Vale et al., 1983a).

Rat and human CRF have an identical structure and differ from oCRF in only seven of the 41 residues (Spiess et al., 1983). Although detailed determination of the active portion of the CRF molecule has been studied by a number of investigators, other than the work of Jean Rivier and colleagues (1984) at the Salk Institute, little has been published. However, utilizing ACTH secretion and adenylate cyclase activity in vitro, Aguilera et al. (1983) determined that bioactivity resides within the COOH-terminal 27 amino acid residues. However, the weak CRF partial agonist, α-helical CRF_{9-41}, which is used as an antagonist, has much weaker affinity for the CRF receptor than does the native CRF molecule containing the first eight residues. Therefore, many regions of the CRF molecule are necessary for full function.

Numa and colleagues were the first to clone the DNA sequences complementary to the human and ovine mRNA encoding the CRF precursor (Furutani et al., 1983; Shibahara et al., 1983). Comparison of the amino acid sequence of oCRF precursor with that of the ACTH-β-lipotropin precursor and the AVP-neurophysin II precursor suggests that these precursor proteins may be evolutionarily related as alluded to earlier. The structures of porcine, caprine, and bovine CRF have also been isolated and sequenced (Ling et al., 1984; Patthy et al., 1986, Gouth et al., 1987). The structure of porcine CRF shows greater homology to rat and human CRF than it does to caprine and bovine CRF; the latter two more closely resemble oCRF.

II. Distribution of Corticotropin-releasing Factor-containing Neurons and Receptors

A. Corticotropin-releasing Factor Peptide and Messenger RNA Localization

1. Localization of corticotropin-releasing factor in the central nervous system. Following the isolation, characterization, and synthesis of CRF, a number of groups generated polyclonal antibodies against CRF for use in immunohistochemical and radioimmunoassay mapping studies of CRF-containing neurons. Cell bodies and fibers that stain positively for CRF are located heterogeneously throughout the CNS. The most widely recognized and intensively studied population of CRF neurons is located in the parvocellular region of the PVN of the hypothalamus. Their major projection is to the median eminence, the site of the primary plexus of the hypothalamohypophysial portal system (Bloom et al., 1982; Kawata et al., 1982, 1983; Pelletier et al., 1982; Antoni et al., 1983; Liposits et al., 1983a,b; Liposits and Paull, 1985; Schipper et al., 1984; Daikoku et al., 1985; Piekut and Joseph, 1985; Rho and Swanson, 1987), although some fibers project to other hypothalamic nuclei and extrahypothalmic brain areas. CRF-immunoreactive cell bodies are also present in a number of other hypothalamic nuclei as well, although to a lesser extent than in the PVN. These CRF-positive cell bodies have been observed in the supraoptic, suprachiasmatic, preoptic, premammillary, periventricular, arcuate, and magnocellular paraventricular nuclei (Kawata et al., 1982; Antoni et al., 1983; Daikoku et al., 1984, 1985; Piekut and Joseph, 1985). Although some of these other hypothalamic nuclei also project to the median eminence, the majority of their projection fields are unknown.

During the past decade, numerous investigators have convincingly demonstrated that several chemical transmitters may be colocalized within the same neuron. This has also been shown to be true for CRF. Thus, immunohistochemical double-staining methods have revealed that a subset of CRF perikarya in the PVN also contain

vasopressin. Moreover, adrenalectomy induces the expression of vasopressin in the majority of CRF cells in the PVN (Roth et al., 1982; Sawchenko et al., 1984; Whitnall et al., 1985, 1987; Piekut and Joseph, 1986; Alonso et al., 1986). The increase in the number of CRF neurons also containing vasopressin following adrenalectomy is clearly beneficial because vasopressin not only stimulates ACTH secretion but also potentiates the actions of CRF on anterior pituitary corticotrophs. Oxytocin has also been found to coexist with CRF in a number of cells in both the parvocellular and magnocellular regions of the PVN (Sawchenko et al., 1984; Papadopoulos et al., 1985; Pretel and Piekut, 1990a). A number of CRF cells in the PVN also stain positively for enkephalin (Hökfelt et al., 1983; Hisano et al., 1986; Ceccatelli et al., 1989a; Sakanaka et al., 1989) or express enkephalin mRNA (Pretel and Piekut, 1990b). There have been singular reports of colocalization of dynorphin (Roth et al., 1983), neurotensin (Ceccatelli et al., 1989a), and peptide histidine isoleucine amide (Hökfelt et al., 1983; Berkenbosch et al., 1986) with CRF in the PVN. The physiological function of colocalization of neuropeptides remains obscure, but it is plausible that one peptide may modulate the function of the other at the anterior pituitary. Alternatively, several different anterior pituitary hormones could be released following depolarization of neurons containing multiple peptides.

As noted earlier, CRF neurons have a widespread, but selective, distribution throughout the CNS. A number of investigators have examined the CNS distribution of CRF utilizing either immunohistochemical (Merchenthaler et al., 1982; Cummings et al., 1983; Joseph and Knigge, 1983; Swanson et al., 1983; Merchenthaler, 1984; Sakanaka et al., 1987a) or radioimmunoassay techniques (Fischman and Moldow, 1982; Côté et al., 1983; Palkovits et al., 1985; Skofitsch and Jacobowitz, 1985; Kilts et al., 1987). Of these studies, those of Swanson et al. (1983), Merchenthaler (1984) and Sakanaka et al. (1987a) provide an excellent overview with a large number of detailed schematic diagrams of CRF-immunopositive perikarya and fibers (fig. 1). CRF neurons are localized throughout the cortex, limbic system, and brainstem nuclei associated with autonomic functioning. Briefly, the highest density of CRF neurons are found in the amygdala, BNST, lateral hypothalamus (distinct from the PVN), central gray area, dorsal tegmentum, locus ceruleus, parabrachial nucleus, dorsal vagal complex, and inferior olive. It is of interest to note that these areas are interconnected via the median forebrain bundle and its caudal extension in the reticular formation or dorsally through a periventricular system in the thalamus and central gray area. Although CRF fibers are found coursing throughout the median forebrain bundle, the direction of fibers in these systems is unclear. Therefore, there is an unfortunate paucity of data regarding the actual projection fields of the various groups of CRF perikarya.

Recently, a small number of CRF pathways outside of the hypothalamus have been traced. CRF cell bodies are widely distributed throughout the neocortex, but relatively more CRF neurons are observed in the prefrontal, cingulate, and insular cortical areas (Swanson et al., 1983). These CRF neurons appear to be predominantly localized to layers II and III of the cortex where they appear to represent cortical interneurons. However, it should be noted that recent data suggest that CRF is actually present in a diverse group of neurons and processes in the neocortex dispersed throughout various cortical lamenae (Lewis et al., 1989; Lewis and Lund, 1990).

Gray and colleagues have provided detailed descriptions of the morphology of CRF neurons in the central nucleus of the amygdala (Cassell and Gray, 1989) and have traced their pathways directly into the parvocellular

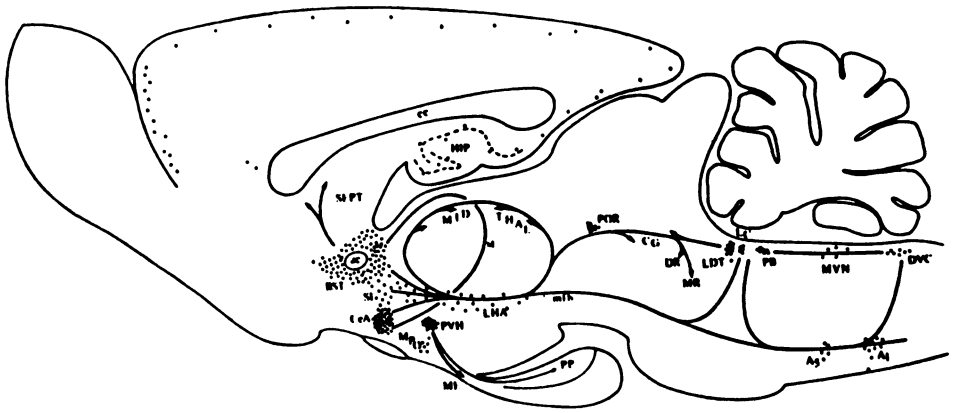

FIG. 1. Major CRF-stained cell groups (dots) and fiber systems in the rat brain. CC, corpus callosum; HIP, hippocampus; SEPT, septal region; AC, anterior commissure; BST, bed nucleus of the stria terminalis; SI substantia innominata; CcA, central nucleus of the amygdala; MPO, medial preoptic area; PVH, PVN of hypothalamus; ME, median eminence; PP, posterior pituitary; LHA, lateral hypothalamic area; mfb, medial forebrain bundle; MID THAL, midline thalamic nuclei; ST, stria terminalis; POR, perioculomotor nucleus; CG, central gray; DR, dorsal raphe; MR, median raphe; LDT, laterodorsal tegmental nucleus; LC, locus ceruleus; PB, parabrachial nucleus; MVN, medial vestibular nucleus; DVC, dorsal vagal complex; A_5, A_1, noradrenergic cell groups. Reprinted with permission from Swanson et al. (1983).

region of the PVN (Gray et al., 1989). Additionally, they (Moga and Gray, 1985) have traced a pathway from the central nucleus of the amygdala to the parabrachial nuclei in the brainstem which play a role in controlling cardiovascular and respiratory responses. Sakanaka and Lederis (1986) have also reported a similar pathway from the amygdala to the parabrachial nuclei. They found that this descending amygdalofugal projection appears to pass through the BNST, the lateral hypothalamus, and the reticular formation to these brainstem nuclei. Moreover, along this pathway, a number of CRF neurons in the amygdala terminated in the BNST and ventromedial hypothalamus rather than in the brainstem. These findings are similar to those of Moga et al. (1990) who reported that large numbers of CRF-staining cells are found in the lateral hypothalamus which project to the parabrachial nuclei. The observed staining was not in cell bodies but, rather, primarily in fibers of passage. In addition to this lack of direct projections from the lateral hypothalamus, <1% of CRF perikarya in the PVN project to the parabrachial nuclei.

The largest concentration of CRF cell bodies outside the hypothalamus is found in the BNST. Anatomically, the BNST is the major pathway for amygdaloid inputs into the hypothalamus as well as being a region containing a number of reciprocal connections with brainstem nuclei involved in autonomic functioning. As in the amygdala, CRF neurons of the BNST project directly to the parabrachial nuclei (Moga et al., 1989) and to the dorsal vagal complex, both of which can regulate autonomic functioning (Gray and Magnuson, 1987). Some CRF neurons in the central nucleus of the amygdala and BNST also contain neurotensin immunoreactivity (Ju and Han, 1989; Shimada et al., 1989).

In addition to the above mentioned descending CRF projections, ascending projections from these nuclei have been reported. Within the dorsal vagal complex, the nucleus of the solitary tract contains CRF cell bodies that ascend to the parabrachial nuclei (Herbert and Saper, 1990). Neuroanatomically, the nucleus of the solitary tract provides a major projection to the parabrachial nuclei. This ascending projection is believed to be the main source of somatosensory visceral information to the forebrain. Moreover, the nucleus of the solitary tract is a major relay for descending pathways from the parabrachial nuclei and forebrain implicated in autonomic regulation. Lind and Swanson (1984) reported a pathway originating from CRF cell bodies in the parabrachial nucleus and terminating in the medial preoptic nucleus of the hypothalamus. CRF was found alone as well as colocalized in a subpopulation of cholinergic cell bodies in the lateral dorsal tegmentum near the locus ceruleus and parabrachial nuclei (Crawley et al., 1985). A number of these cholinergic/CRF neurons projected directly to the medial frontal cortex. Vincent and Satoh (1984) reported CRF projections from the lateral dorsal tegmentum to the sacral spinal cord.

It is important to note that not all CRF neurons in the hypothalamus project to the median eminence. Cells in the PVN and anterior hypothalamus have been shown to terminate in the lateral septum (Sakanaka et al., 1988). Some of those in the lateral hypothalamus project to the inferior colliculus where they may play a role in modulating auditory processing (Sakanaka et al., 1987b).

An olivocerebellar CRF pathway has been reported in the cat (Cummings et al., 1988; Kitahama et al., 1988), opossum (Cummings et al., 1989, Cummings and King, 1990), and primate brain (Cha and Foote, 1988; Foote and Cha, 1988). These CRF neurons originating in the inferior olive project throughout the cerebellum within the flocculus and paraflocculus.

CRF-immunoreactive fibers have been found to terminate in various layers of the spinal cord. Some of these fibers originate in the Edinger-Westphal nucleus (Chung et al., 1987), sympathetic (Krukoff, 1986) and sensory ganglia (Skofitsch et al., 1985), and the spinal cord itself (Merchenthaler et al., 1983). Although the function of CRF neurons in the spinal cord is unclear, it is plausible that they may play a role in modulating sensory input and/or autonomic outflow.

This heterogeneous distribution of CRF discussed above is concordant with a role for the peptide as a neurotransmitter in the CNS. To some extent, functional roles for chemical messengers in the CNS can be inferred from neuroanatomical localization. Thus, consistent with a role for CRF as a hypothalamic releasing factor regulating pituitary-adrenocortical activity is the presence of CRF in high concentrations in the PVN and median eminence, two of the so-called hypophysiotropic areas of the hypothalamus. Relatively high concentrations of CRF have been observed following immunohistochemistry and radioimmunoassay studies in subcortical limbic and brainstem structures (e.g., amygdala, BNST, raphe nuclei, locus ceruleus), brain regions traditionally associated with control of arousal and affect. Furthermore, considerable variance in the concentrations of CRF is observed among component nuclei of the amygdala and hypothalamus (Kilts et al., 1987), suggesting that the functional role of CRF would most likely vary among these individual nuclei. High concentrations and dense staining are also noted in many of the brain regions containing the major perikarya for the catecholamine and indoleamine transmitters. Thus, CRF is strategically positioned to influence the activity of the major monoamine-containing neuronal systems in the CNS.

2. Localization of corticotropin-releasing factor in endocrine, gastrointestinal, immune, and other peripheral tissues. Although generally thought of as a brain peptide regulating pituitary-adrenal function, CRF immunoreactivity has been observed in a number of peripheral tis-

sues, many of which are apparently unrelated to HPA axis activity. CRF-like immunoreactivity is present in the pituitary stalk and neurointermediate lobe of the posterior pituitary gland in rats (Saavedra et al., 1984; Jeandel et al., 1987) and humans (Ohtani et al., 1987). This CRF-like immunoreactivity is thought to be of brain origin. The physiological role of CRF in these areas is unclear at present. However, it has been suggested that CRF in the neurointermediate lobe might act presynaptically on autoreceptors located on CRF terminals in the median eminence and/or act locally in a paracrine fashion to release ACTH from anterior pituitary corticotrophs. Both of these hypotheses must be considered highly speculative at this time.

CRF is present in the adrenal medulla of rats (Hashimoto et al., 1984), cows (Edwards and Jones, 1988; Minamino et al., 1988), humans (Suda et al., 1984a), and dogs (Bruhn et al., 1987) where it appears to be in close proximity to small blood vessels. In contrast to other investigators, Rundle et al. (1988) utilized immunohistochemical methods to demonstrate the presence of CRF in sheep adrenal cortex where it appears in small nerve fibers associated with blood vessels. They suggested that this CRF may be present in postganglionic autonomic nerve fibers. Although the physiological role of adrenal CRF is not known, both direct stimulation of the splanchnic nerve (Edwards and Jones, 1988) and hemorrhagic stress (Bruhn et al., 1987) increase the concentration of CRF in adrenal venous plasma. Such a plasma concentration of CRF is too low to increase pituitary-adrenal activity, and, therefore, it may play a paracrine role in the adrenal.

In a preliminary study, we (Ritchie et al., 1986) detected CRF immunoreactivity in lymphocytes. This immunoreactivity was dilutable, suggesting that it is the authentic peptide. This finding has recently been confirmed by Stephanou et al. (1990) who detected both immunoreactive CRF and CRF mRNA in lymphocytes.

Yoon and colleagues (1988) identified CRF immunoreactivity in the rat testis by both radioimmunoassay and immunocytochemistry. This immunoreactivity was observed in Leydig cells, advanced germ cells, and even epididymal spermatozoa. These investigators found that hypophysectomy significantly reduced the concentrations of CRF observed in the testis. This suggests that the CRF-containing cells are under the influence of pituitary hormones, perhaps gonadotropin.

CRF has also been found in the pancreas, stomach, and small intestine in a number of different mammals. For example, CRF is found in a large number of cells within the endocrine pancreas (Petrusz et al., 1983, 1984), where they are in close topographical association with glucagon-secreting cells. In addition, Petrusz et al. (1984) and Kruseman et al. (1984) identified CRF-containing cells in the gastric epithelium. Similar to the localization of CRF in the adrenal, CRF immunostaining in nerve fibers in the rat duodenum was reported by Wolter (1984, 1985) to be closely associated with myenteric and submucosal blood vessels. These fibers may arise from autonomic neurons in the myenteric and submucosal plexus.

CRF has been measured in blood plasma obtained from the peripheral circulation of rats, horses, and humans, albeit at much lower concentrations than that found in the plasma of portal vessels supplying the anterior pituitary (Suda et al., 1985a; Sumitomo et al., 1987; Alexander et al., 1991; Hohtari et al., 1988). Because plasma CRF concentrations in humans exhibited an apparent diurnal rhythm paralleling plasma ACTH and cortisol concentrations, plasma CRF was proposed to be of hypothalamic origin (Watabe et al., 1987). However, recent evidence from the rat suggests that this is not the case; Plotsky et al. (1990) reported that neither bilateral destruction of CRF perikarya in the PVN nor stalk transection alters peripheral plasma CRF concentrations. Moreover, the increase in plasma CRF concentrations following nitroprusside-induced hypotension was shown to be neither of hypothalamic nor adrenal origin. Thus, the source(s) and function of plasma CRF is uncertain at present, and any measurement of its concentration as a marker of HPA activity should be viewed with skepticism. However, this is not the case during pregnancy. Plasma CRF in pregnant women, the source of which is the placenta, undergoes a sharp increase during the third trimester of pregnancy. Although plasma CRF concentrations were either undetectable early in pregnancy (Sasaki et al., 1984; Goland et al., 1986; Laatikainen et al., 1987) or low (Campbell et al., 1987), concentrations increased 6- to 40-fold late in the third trimester or at parturition. Plasma CRF concentrations do not increase during the stress of labor nor are plasma ACTH or cortisol concentrations increased at any time during the course of pregnancy (Goland et al., 1986; Laatikainen et al., 1987; Campbell et al., 1987). In contrast to the suggestion of Goland et al. (1986), the lack of any correlation between plasma CRF concentrations during pregnancy and pituitary-adrenocortical hormone concentrations strongly suggests that maternal CRF of placental origin does not modulate maternal or fetal HPA axis activity during gestation. This is likely due to the presence of a specific circulating CRF-binding protein which was first semipurified from maternal plasma during pregnancy (Suda et al., 1988a, 1989; Linton et al., 1990) and has now been purified and sequenced by Potter et al. (1991). This CRF-binding protein is present in both plasma and brain and may well play an important physiological role in regulating CRF availability.

Considerable variability in the concentrations of plasma CRF has been reported by different investigators. This is almost certainly due to differences in extraction and radioimmunoassay procedures. As a result, interin-

vestigator comparisons of experimental data are difficult, if not impossible, to interpret at present.

3. Localization of corticotropin-releasing factor messenger RNA. In 1983, Numa and colleagues succeeded in cloning the gene encoding the CRF prohormone in ovine (Furutani et al., 1983), human (Shibahara et al., 1983), and rat genomic libraries (Jingami et al., 1985b; Thompson et al., 1987). Southern blot analysis has since tracked the locus of the human CRF gene to the long arm of chromosome 8 (Arbiser et al., 1988). Thompson et al. (1987) found that the rat and human CRF gene were quite similar in sequence homology and basic organization. Both genes contain two exons separated by an intervening intron. The first exon encodes most of the 5′-untranslated region of the mRNA, and the second exon contains all the prohormone-coding sequences and some 3′-untranslated regions of the mRNA (fig. 2). The 5′-flanking DNA sequences are likely to contain the DNA sequence elements responsible for glucocorticoid regulation, tissue-specific expression, and second messenger regulation of CRF gene expression. In fact, a cAMP-responsive element from the CRF gene was isolated following transfection into rat pheochromocytoma (PC-12) cells in which the cAMP-responsive element allows the cells to express the gene encoding chloramphenicol acetyltransferase (Seasholtz et al., 1988). The cAMP-responsive element was localized to a 59-base pair region located between 238 and 180 base pairs 5′ to the CRF mRNA cap site.

Localization of CRF mRNA by in situ hybridization or Northern blot analysis coincides quite well with immunohistochemical localization of CRF, although high concentrations of peptide do not necessarily infer proportional amounts of mRNA and vice versa. This is a result of mRNA being localized in cell bodies, whereas CRF, or its prohormone, is localized in terminal fields, axons, and cell bodies. However, there is increasing evidence for the presence of mRNA in axonal processes (Jirikowski et al., 1990). Although the antisera used in immunocytochemical and radioimmunoassay studies is raised against CRF, it is unclear while reviewing the literature as to whether these antisera recognize any form of the CRF prohormone or just the final processed peptide. Nonetheless, CRF mRNA is certainly found in those regions previously found to contain CRF-immunoreactive perikarya.

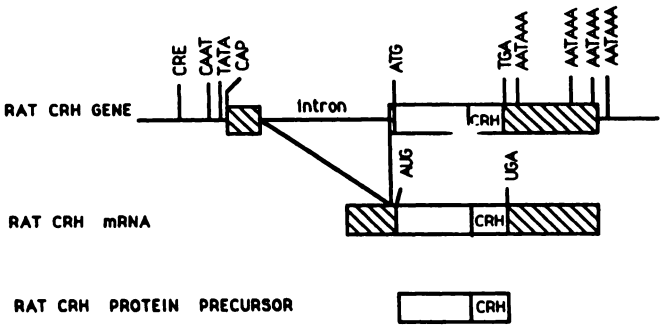

FIG. 2. Structural organization of the rat corticotropin-releasing hormone gene, mRNA, and protein precursor. Top, schematic representation of the rat corticotropin-releasing hormone (CRH) gene. The exons are shown as blocks; the intron, 5′-flanking and 3′-flanking sequences are shown as lines. The 5′-untranslated and 3′-untranslated regions of the exons are shaded. The cAMP-responsive element (CRE), CAAT and TATA sequences, cap site, translation initiation ATG, and translation termination TGA are indicated. Four polyadenylation addition signals (AATAAA) are indicated. All hypothalamic CRH complementary DNA clones isolated to date appear to use the second or third polyadenylation addition signals. The location of the CRH peptide is indicated by CRH. The structure of the rat CRH mRNA (1400 nucleotides) and rat CRH protein precursor (187 amino acids) are also diagrammed. This figure was kindly provided by A. F. Seasholtz.

In situ hybridization studies have identified CRF mRNA in a number of brain areas including the parvocellular region of the PVN (Young et al., 1986a; Lightman and Young, 1987), magnocellular regions of the PVN and supraoptic nucleus of the hypothalamus (Lightman and Young, 1987), inferior olive (Young et al., 1986b; Palkovits et al., 1987; Barmack and Young, 1990), and olfactory bulb (Imaki et al., 1989). Similarly, CRF mRNA has been found in the PVN (Jingami et al., 1985b; Thompson et al., 1987; Beyer et al., 1988; Suda et al., 1988b) and cerebral cortex (Thompson et al., 1987; Suda et al., 1988b) by Northern blot analysis. Beyer et al. (1988) reported the presence of CRF mRNA in extracts of tissue from the amygdala, BNST, and supraoptic nucleus. In fact, Thompson et al. (1987) reported the existence of CRF mRNA in every major brain region with the exception of the cerebellum. When expressed as a percentage of total polyadenylated mRNA, the concentration of CRF mRNA can be represented as: brainstem >> cerebral cortex = hypothalamus > midbrain > striatum > hippocampus. This should not be totally unexpected because immunohistochemical and radioimmunoassay studies have previously demonstrated a widespread distribution of CRF neurons throughout the CNS.

As with CRF itself, CRF mRNA has been observed in a number of peripheral tissues including the human placenta (Grino et al., 1987; Frimm et al., 1988). In concert with an increase in placental CRF peptide concentrations, CRF mRNA concentrations increased more than 20-fold in the 5-week period preceding parturition. Lightman and coworkers (Stephanou et al., 1990) observed CRF mRNA in T and B lymphocytes and neutrophils. Thompson et al. (1987) reported the existence of CRF mRNA in the testes and, in contrast to Jingami et al. (1985b), in whole pituitary and adrenal glands. CRF mRNA was not found in samples taken from kidney, duodenum, thymus, or liver. The reason for this discrepancy between these two groups is unclear but may be the result of methodological differences or the possibility of transient CRF mRNA expression.

A number of investigators have scrutinized CRF mRNA in the PVN, and these data will be discussed in

the section concerning CRF regulation of endocrine function (section III).

B. Corticotropin-releasing Factor Receptors

1. Biochemical characterization of the corticotropin-releasing factor receptor. Initial attempts to biochemically characterize the ligand-binding subunit of the CRF receptor revealed that in a number of species there were distinct differences between cortical and anterior pituitary CRF receptors (Grigoriadis and De Souza, 1988; Grigoriadis et al., 1989b). The ligand-binding portion of the CRF receptor in cortex consisted of a protein with an apparent molecular weight of 58,000, whereas the anterior pituitary receptor binding subunit resided on a protein with an apparent molecular weight of 75,000. This difference has since been found to be the result of differential glycosylation of cortical and pituitary receptors; deglycosylation generated virtually identical peptide fragments which indicates that the ligand-binding portion of the CRF receptor in both tissues resides on a polypeptide of approximately 40,000 to 45,000 molecular weight (Grigoriadis and De Souza, 1989a). Binding of various CRF ligands to the receptor is increased by divalent cations and decreased by guanyl nucleotides (Perrin et al., 1986; De Souza, 1987). This is consistent with CRF receptors as members of the G protein-coupled family of receptors.

2. Localization of corticotropin-releasing factor receptors in pituitary and brain. A number of biochemical and autoradiographic studies have described CRF receptors throughout the pituitary and CNS. CRF receptors are found in greatest density in the anterior pituitary but are also found in the neurointermediate lobe as well, albeit in much lower numbers (Wynn et al., 1983, 1984; De Souza et al., 1984b, 1985a; De Souza, 1987; Millan et al., 1987; Grigoriadis and De Souza, 1989b).

Many investigators have studied the distribution of CRF receptors in the CNS. De Souza (1987) described in detail the binding characteristics of CRF in membrane homogenates from the anterior pituitary and 11 other brain regions. This binding is saturable, reversible, and, on Scatchard analysis, reveals a high-affinity component (K_d) of 0.1 to 0.2 nM and a low-affinity-binding site (K_d) of 20 nM (fig. 3). In brain tissues, highest concentrations of CRF receptors are found in the olfactory bulb, followed by cerebellum, followed by cortical and limbic regions. This was initially somewhat surprising because the olfactory bulb and cerebellum appear to possess relatively few CRF-containing fibers, although a olivocerebellar CRF pathway does exist. However, as will be discussed later, many of these receptors may not be functional or possess limited second messenger-generating capability.

More precise anatomical localization of CRF receptors can be found in the detailed autoradiographic studies of De Souza et al. (1984a, 1985b) and Wynn et al. (1984). Highest densities were again found in the cerebellum with somewhat lower densities in the pons-medulla and cortex. Although high concentrations of CRF receptors were visualized in these regions, substantial heterogeneity was exhibited among the component nuclei of the brainstem as well as the different laminae and areas of cortex. Lower, although substantial, binding was found throughout the majority of brain regions studied including the spinal cord.

Two novel methods of identifying CRF receptors have recently been reported. One involves the use of anti-idiotypic antibodies (Piekut and Knigge, 1989) and the other uses a fluorescent analog of CRF (Schwartz et al., 1986).

3. Localization of corticotropin-releasing factor receptors in other endocrine, gastrointestinal, and immune system tissues. Although CRF immunoreactivity has been observed in a number of peripheral tissues (vide supra), CRF receptors have been identified in only a few peripheral tissues. CRF receptors are present in the adrenal medulla (Dave et al., 1985; Udelsman et al., 1986b) and sympathetic ganglia (Udelsman et al., 1986b). Adrenal CRF receptors are functionally coupled to adenylate cyclase and have been postulated to modulate the release of catecholamines. Alternatively, we believe they may reside on the walls of local blood vessels within the adrenal where they may alter local blood flow patterns. In fact, Dashwood et al. (1987) obtained autoradiographic evidence of CRF receptors present on rabbit aortic endothelium where they were hypothesized to play a role in regulating vascular tone. Dave et al. (1985) also reported the existence of small numbers of CRF receptors in the prostate, spleen, liver, kidneys, and testis. De Souza and colleagues (Webster and De Souza, 1988; Webster et al., 1989, 1990) also identified CRF receptors in the spleen. These receptors appear to be restricted to a population of resident splenic macrophages and are also coupled to adenylate cyclase. Neither our group nor De Souza's group (E. B. De Souza, personal communication) have found any evidence of CRF receptor binding on lymphocytes or erythrocytes as was previously suggested by Smith et al. (1986) and Dave and Eskay (1986), respectively.

4. Signal transduction via second messenger systems. Following binding, the CRF receptor is positively coupled to adenylate cyclase. The resultant increases in cellular cAMP represent the second messenger associated with CRF receptor activation in the CNS (Labrie et al., 1982a,b, 1983; Giguere et al., 1982; Wynn et al., 1984; Litvin et al., 1984; Hoffman et al., 1985; Sobel, 1985; Millan et al., 1987). When pituitary cell cultures, pituitary membrane homogenates, or AtT-20 mouse pituitary tumor cells are studied, increases in cAMP are observed within minutes following the addition of CRF to these preparations. Giguere et al. (1982) observed 4-fold increases in cAMP 2 minutes following addition of CRF and 8-fold increases between 10 and 180 minutes. Aguilera et al. (1983) observed 4- to 6-fold increases in cAMP

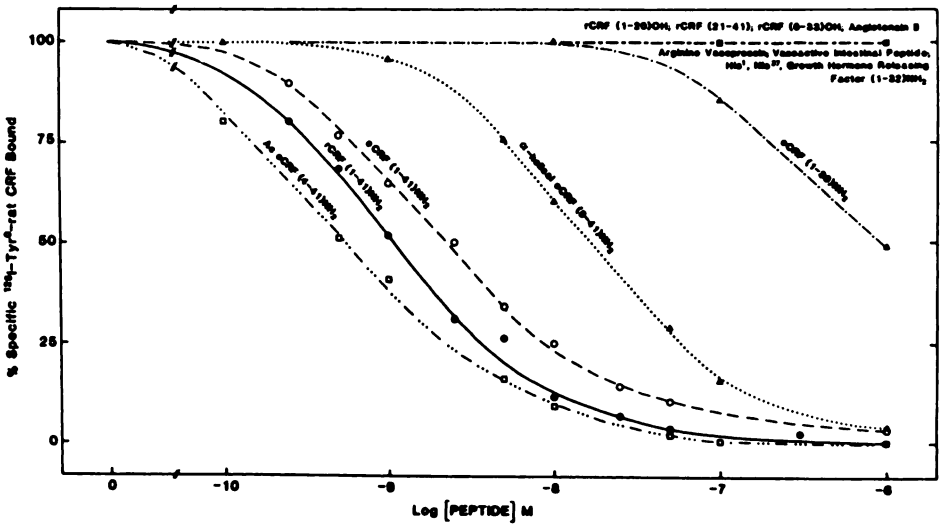

FIG. 3. Characterization of the pharmacological specificty of ^{125}I-Tyr^0 rat/human CRF binding in rat olfactory bulb membranes. Crude mitochondrial/synaptosomal membranes were incubated for 120 minutes at room temperature in the presence of 0.1 nM ^{125}I-Tyr^0 rat/human CRF and varying concentrations of CRF-related and unrelated peptides. Nonspecific binding was determined in the presence of 1 μM rat CRF and was subtracted from total binding. The data shown are from representative experiments. Each point, mean of a triplicate determination; SEM <10%. Shown are acetyl (Ac) oCRF (4–41)NH_2; rCRF (1–41)NH_2; oCRF (1–41)NH_2; α-helical oCRF (9–41)NH_2; oCRF (1–39)NH_2, and one of several noncompeting rCRF fragments or unrelated peptides. Reprinted with permission from De Souza (1987).

levels 3 minutes following CRF stimulation with maximal 10- to 15-fold increases occurring by 30 minutes. Litvin et al. (1984) reported that a 2-fold increase in cAMP following the addition of 30 nM CRF resulted in maximal ACTH secretion in vitro; however, ACTH release was not substantially increased, although cAMP levels were eventually increased up to 20-fold using the phosphodiesterase inhibitor 3-methylisobutylxanthine. These observed increases in cAMP were produced with CRF concentrations near the reported K_d for receptor binding [i.e., 0.28 to 1.3 nM (Aguilera et al., 1983) and 3.3 nM (Millan et al., 1987)]. These increases in cAMP apparently are essential for CRF-induced ACTH release because the cAMP-dependent protein kinase inhibitor blocks both the ACTH secretory response and the POMC gene expression produced by CRF (Reisine et al., 1985).

De Souza and colleagues conducted a detailed study of CRF-mediated cAMP production in a variety of brain regions (Battaglia et al., 1987). The rank order of potency for CRF analogs and fragments in stimulating adenylate cyclase activity was directly correlated to their binding affinities for CRF receptors. However, the regional distribution of receptor density (vide supra) did not correspond with regional CRF-stimulated adenylate cyclase activity (frontoparietal cortex > olfactory bulb > cerebellum > midbrain > hippocampus > striatum > hypothalamus > spinal cord). The authors suggest that this disparity may derive from some populations of CRF receptors being coupled to other second messenger systems (vide infra). Alternatively, certain populations of CRF receptors may not be functionally coupled to any second messenger system and/or may represent "spare" receptors. CRF receptors in peripheral tissues, including the spleen (Webster et al., 1989), rat adrenal membranes, and bovine chromaffin cells (Dave et al., 1985), are also positively coupled to adenylate cyclase.

As suggested above, other second messenger systems may be involved in CRF receptor-mediated signal transduction. For example, in a pilot study our group (C. D. Kilts and C. B. Nemeroff, personal communication) observed CRF-induced increases in phosphoinositide hydrolysis in rat hypothalamic brain slices. Cronin et al. (1986) obtained evidence that protein kinase C can potentiate cAMP production subsequent to CRF receptor activation. It should be noted that in this case this would not be a direct result of CRF receptor occupancy but a synergistic effect of other neurotransmitters on that cell that activate protein kinase C.

Using electrophysiological methods, Aldenhoff (1986) demonstrated that the calcium channel blocker, verapamil, blocked the excitatory effects of CRF on hippocampal neuronal activity. This raises the possibility that CRF alters membrane calcium fluxes with possible resultant alterations in potassium ion conductance and membrane potentials. In addition, there has been one report describing a role for calcium-mediated second messenger systems in modulating the actions of CRF. It is known that binding of calcium to calmodulin leads to activation of a calmodulin-dependent kinase that may be important in the stimulus-secretion coupling in various cells. Murakami et al. (1985) found that the calmodulin inhibitor (W-7) inhibits CRF-stimulated ACTH release in vitro without effecting CRF-stimulated cAMP accumulation. Although these results suggest that CRF exerts its effects on ACTH release through both a cAMP system and a calcium-calmodulin system, this hypothesis awaits further study and confirmation.

Following coupling to its receptor, CRF increases the methylation of phosphatidylethanolamine to phosphatidylcholine (Hook et al., 1982). Although this reaction has not been well studied, it has been suggested that phospholipid methylation may be a possible membrane transduction mechanism for receptor-mediated events. In addition, this same group (Heisler et al., 1983) reported that CRF also stimulates methylation of free carboxyl groups on glutamyl and/or aspartyl residues of various protein substrates by the enzyme protein carboxymethylase. This latter action of CRF appears to be important in exocytosis secretion mechanisms in general and in CRF-mediated ACTH release in particular.

Little is known regarding termination of the action of CRF following its synaptic release. Although there is evidence that CRF is degraded by one or more peptidases, there is evidence that the ligand-receptor complex is internalized following receptor activation and metabolized internally. In a preliminary report, Ritchie et al. (1990) observed that, following incubation of rat/human CRF with brain tissue extracts, high-pressure liquid chromatography fractionation showed diminution of the parent CRF peak as well as the presence of two other peaks not previously observed. The latter peaks may represent CRF metabolites resulting from the action of peptidases. Leroux and Pelletier (1984), using ^{125}I-CRF electron microscopic autoradiography, found that, within 15 minutes of administration to intact animals, silver grains were observed primarily over lysosomes and the Golgi apparatus of anterior pituitary corticotrophs. In addition, by 30 minutes, no labeling could be detected. This suggested that, following binding to plasma membrane receptors, CRF is rapidly internalized. Similar findings were reported by Childs et al. (1986) who used a biotinylated CRF analog. Internalization was observed as early as 1 to 3 minutes following exposure to pituitary cell cultures. Although further work is needed, these studies suggest that internalization of CRF-receptor complexes, cleavage of the CRF molecule in the synaptic cleft, and binding of CRF by its binding protein represent three complimentary methods for termination of the action of CRF. All three of these mechanisms represent possible means for long-term modulation of CRF neurotransmission.

III. Corticotropin-releasing Factor Regulation of Neuroendocrine Function

A. Regulation of the Pituitary-Adrenal Axis

1. Corticotropin-releasing factor as the major regulator of pro-opiomelanocortin-derived anterior pituitary hormone secretion. In this section we briefly review the literature supporting a seminal role for CRF in neuroendocrine function. As noted earlier, Vale et al. (1981) elucidated the structure of CRF approximately a decade ago. CRF was found to stimulate the release of ACTH and β-endorphin both in vivo (Rivier et al., 1982a; Donald et al., 1983) and in vitro (Vale et al., 1983b). These actions of CRF are antagonized by the CRF antagonist, α-helical CRF_{9-41} (Rivier et al., 1984c), or by immunoneutralization with polyclonal (Rivier et al., 1982b) or monoclonal (van Oers et al., 1989) anti-CRF antibodies. In addition, CRF administered i.c.v. also stimulates activation of the HPA axis (Rock et al., 1984; Ono et al., 1985a). Final proof that CRF is the major physiological regulator of the increased HPA activity that occurs in response to stress comes from data showing almost complete blockade of pituitary-adrenal responses to a variety of stressors following administration of CRF antisera (Rivier and Vale, 1983a; Linton et al., 1985; Nakane et al., 1985; Ono et al., 1985b) and from studies in which CRF was measured in hypophysial portal blood (Plotsky and Vale, 1984).

2. Ontogeny of the hypophysiotropic corticotropin-releasing factor system. CRF immunoreactivity in the PVN of the rat fetus can be observed beginning at approximately gestation day 18 or 19 and gradually increases in density during development before finally attaining adult levels (Bugnon et al., 1982; Chatelain et al., 1988; Rundle and Funder, 1988). Similarly, Grino et al. (1989b) detected CRF mRNA in the PVN on day 17 of gestation. Concentrations of CRF mRNA increased gradually through birth, then decreased during the perinatal period, before finally increasing to adult levels thereafter. Emanuel et al. (1989) first detected CRF mRNA on gestation day 20 and measured both the peptide and mRNA from this prenatal period until postnatal day 15. CRF concentrations increased throughout the study period, but CRF mRNA concentrations did not change.

Although exposure to stress does not elicit marked increases in plasma ACTH concentrations until 14 days of age (vide infra), Walker et al. (1986) showed that exogenous CRF can directly stimulate ACTH release throughout postnatal days 3 to 21. Moreover, urethane-induced stress can result in a small increase in ACTH secretion that can be blocked by CRF immunoneutralization as early as day 3 postnatally. The limited capability of the rat to mount a robust ACTH and corticosterone response to stress during the first week of life has been termed the stress-nonresponsive period. Whereas one current hypothesis to explain the stress-nonresponsive period is an increased glucocorticoid negative feedback on POMC and CRF peptide synthesis during this time, Grino et al. (1989a) showed that, in contrast to the adult, adrenalectomy does not alter CRF gene expression in the PVN of 7 day old rats. This suggests that CRF gene expression, rather than being particularly sensitive to glucocorticoid negative feedback, may in fact be unresponsive to feedback of any sort, such as the ability to respond to the need for increased CRF and glucocorticoid production under any circumstance. It would be of interest to determine the activity of the glucocorticoid-re-

sponsive elements in the promoter region of the CRF gene during this stress-nonresponsive period.

CRF receptors in pituitary and cerebral cortex are detectable by prenatal day 17 (Insel et al., 1988). Interestingly, receptor number in whole brain increases to three times their adult concentration by postnatal day 8 and then decreases to adult concentrations by day 21. In addition to changes in the density of CRF receptors, there are alterations in the distribution of CRF receptors that occur during development. For example, CRF receptors are found in very high density in the striatum prenatally, but postnatally and in the adult, CRF receptor binding is much more dense in the cerebral cortex with minimal binding in the striatum.

3. Circadian rhythmicity. It is well established that the HPA axis exhibits a circadian rhythm in humans, rats, and other mammals. This rhythm is generally thought to be controlled predominantly by the differential release of CRF from nerve terminals in the median eminence into the portal vessels supplying the anterior pituitary corticotrophs. However, other neuroregulators such as vasopressin, oxytocin, and epinephrine also are known to possess ACTH-releasing activity, and immunoneutralization of CRF does not completely abolish circadian rhythms of plasma ACTH (Carnes et al., 1989, 1990).

We (Owens et al., 1990a) reported that CRF concentrations in the median eminence and hypothalamus (minus median eminence) increase with the normal circadian increase in plasma corticosterone concentrations. These increases in CRF concentrations may reflect increased synthesis, storage, and release (i.e., turnover) of CRF necessitated by the greater secretion of ACTH and corticosterone that occurs in rodents in the late afternoon. Although it is likely that CRF concentrations measured in the median eminence reflect stored CRF in vesicles, the majority of CRF measured in the hypothalamus minus median eminence probably represents peptide found in cell bodies in the PVN and their axons. In contrast to our findings, Moldow and Fischman (1984) reported that the lowest hypothalamic concentrations of CRF occur at the time of peak plasma corticosterone concentrations. More recently, and in agreement with our findings, Yokoe et al. (1988) reported that increased hypothalamic and plasma concentrations of CRF vary in parallel with alterations in the pituitary-adrenal axis and circulating glucocorticoids. Further evidence supporting our findings comes from the work of Watts and Swanson (1989) who found that the content of CRF precursor mRNA in the PVN begins to decline sometime between midday and the beginning of the dark phase. This decline in mRNA synthesis occurs at or near the time of maximal peptide accumulation in the neurons.

4. Actions of corticotropin-releasing factor on pituitary corticotrophs. As mentioned previously, following interaction of CRF with its receptor on the corticotrophs, the formation of cAMP is markedly increased, which leads to a cascade of little understood events that ultimately results in the secretion of POMC-derived peptides into the peripheral circulation. However, during chronic or excessive exposure to CRF, the corticotrophs undergo a number of changes.

It has consistently been shown that CRF receptor occupancy or cAMP analogs increase the concentration of POMC mRNA both in vivo and in vitro (Affolter and Reisine, 1985; Gagner and Drouin, 1985, 1987; Loeffler et al., 1985; Dave et al., 1987; Knight et al., 1987). In addition to these increases in POMC peptide synthesis, CRF also appears to possess trophic actions on the pituitary as well. For example, Westlund et al. (1985) reported that following a 48-hour subcutaneous infusion of CRF in rats, corticotroph cell area and ACTH-immunoreactive staining was increased. Similarly, McNicol et al. (1988) reported that both adrenalectomy or daily intraperitoneal injections of CRF increased corticotroph cell volume. Gertz et al. (1987) demonstrated that CRF infusion (10 μg/day $\times$ 52 days) resulted in continuously elevated corticosterone concentrations, increased adrenal weight, increased numbers of ACTH-immunostaining cells, and increased diameter of peptide-forming and storage granules, but no increase in corticotroph cell area. It is unclear from a review of the literature whether the increased number of ACTH-staining cells is the result of hyperplasia or expression of the POMC gene in cells previously quiescent.

However, in contrast to the above cited actions of CRF on corticotrophs, which bolster the production of ACTH and related peptides, is the desensitization that occurs in response to continuous or excessive exposure to CRF. In vivo studies clearly show that continuous exposure to CRF substantially reduces ACTH secretion when compared to initial responsiveness; however, CRF still stimulates ACTH secretion above baseline (Rivier and Vale, 1983b, 1985a; Evans et al., 1985).

The tolerance that develops to CRF exposure appears to be primarily at the level of the CRF receptor and its coupling to adenylate cyclase in a manner similar to that seen with other G protein receptors. De Souza et al. (1985a) initially reported that adrenalectomy markedly decreased CRF receptor density ($\approx$70%) in the anterior pituitary, but no changes were observed in the neurointermediate lobe. This CRF receptor down-regulation persisted for as many as 9 weeks postsurgery. In a more detailed study, Wynn et al. (1985) observed significant (29%) down-regulation of anterior pituitary CRF receptors 24 hours following adrenalectomy. Receptor number progressively declined by another 20% by day 4. Comparable decreases in CRF-stimulated adenylate cyclase activity and sensitivity were observed in these adrenalectomized animals. In addition, these changes, like many others induced by adrenalectomy, were reversed by dexamethasone supplementation. The same group performed a similar study evaluating the effects of chronic

CRF infusion rather than adrenalectomy (Wynn et al., 1988). A 46% decrease in CRF receptor binding was observed following 48 hours of CRF infusion (100 ng/minute). Again, the changes in CRF receptor number were accompanied by comparable decreases in CRF-stimulated adenylate cyclase activity. The findings comparing chronic CRF infusion to adrenalectomy suggest that additional factors (vasopressin?) may be involved in the modulation of CRF receptor kinetics following adrenalectomy. In vitro studies by Reisine's group (Reisine and Hoffman, 1983; Hoffman et al., 1985) have shown that the ability of CRF to stimulate cAMP accumulation and the ability of cAMP analogs to stimulate ACTH secretion are decreased following exposure to CRF for short periods of time. Moreover, the HPA stimulatory actions of CRF recovered rapidly within several hours following removal of chronic CRF treatment.

5. Potentiation of the action of corticotropin-releasing factor on the corticotroph. It is well established that a number of endogenous substances in addition to CRF also possess ACTH-releasing properties. These include neurohypophyseal peptides and catecholamines. The most widely studied of these hormones is AVP. A number of in vivo (Rivier and Vale, 1983c; Rivier et al., 1984b; Fischman and Moldow, 1984) and in vitro (Culler et al., 1983; Murakami et al., 1984) studies have shown that AVP weakly stimulates ACTH release alone but markedly potentiates the actions of CRF on ACTH release (Schoenenberg et al., 1987). It appears that these actions of AVP are not mediated through alterations of CRF binding (Holmes et al., 1984); rather, AVP interacts with its receptor subtype, V_1 (Rivier et al., 1984b), to potentiate CRF-stimulated cAMP accumulation (Giguere and Labrie, 1982; Hoffman et al., 1985; Bilezikjian et al., 1987). Interestingly, although AVP does potentiate CRF-stimulated cAMP accumulation, it appears that AVP acts on functionally distinct corticotrophs that do not contain CRF receptors (Schwartz and Vale, 1988; Jia et al., 1991). In fact, Plotsky et al. (1985) reported that during hypoglycemic stress CRF plays predominantly a permissive role, whereas AVP represents the dynamic mediator of ACTH secretion. Also of interest is the report by Levin et al. (1989) who replicated the findings of AVP potentiation of ACTH release but observed that, whereas CRF increased POMC gene transcription and peptide synthesis, AVP, if anything, decreased POMC gene expression.

There are a number of reports that suggest that in the sheep, in contrast to the rat and most other mammals, AVP is a more potent stimulator of ACTH secretion than is CRF (Familari et al., 1989). This appears to result from the fact that the concentration of AVP receptors in the sheep anterior pituitary is twice that of the rat, whereas CRF receptor density is only 10% of those in the rat (Shen et al., 1990).

In addition to AVP, oxytocin also potentiates CRF-stimulated ACTH release both in vitro (Gibbs et al., 1984; Schwartz and Vale, 1988) and in vivo (Gibbs, 1985). Another hormone that exerts synergistic effects with CRF on ACTH secretion is angiotensin II, which also stimulates ACTH release alone as well as potentiating CRF-stimulated cAMP accumulation and ACTH secretion. Another peptide that potentiates CRF-stimulated ACTH release in vitro is the intestinal peptide PHI-27, which is present in the median eminence (Tilders et al., 1984).

B. Regulation of the Corticotropin-releasing Factor Neuron

1. Neurotransmitter regulation of corticotropin-releasing factor release. A decade prior to elucidation of the sequence of CRF, investigators were already studying the neurotransmitter regulation of CRF release in vitro using bioassays for ACTH and adrenal glucocorticoids as a measure of "CRF" activity. Even with the availability of sensitive and specific radioimmunoassays for CRF, considerable controversy exists concerning the role of various neurotransmitters in regulating the secretion of hypothalamic CRF. We have previously discussed these findings in detail (Owens and Nemeroff, 1990) and will, therefore, only briefly review these findings.

Using a specific immunocytochemical marker for catecholaminergic neurons, Liposits et al. (1986b) demonstrated tyrosine hydroxylase-immunoreactive nerve terminals innervating CRF-containing perikarya in the PVN. This same group, using a specific marker for epinephrine-containing neurons, also found that phenylethanolamine-N-methyl transferase immunoreactive nerve terminals arising from the C_1 (ventral lateral medulla) and C_2 (dorsal vagal complex) cell groups establish direct synaptic contact with PVN CRF-containing perikarya (Liposits et al., 1986a; Cunningham et al., 1990). Recently, evidence for direct serotonergic (Liposits and Paull, 1987; Soghomonian et al., 1988), dopaminergic (Liposits and Paull, 1989), and GABAergic (Meister et al., 1988) innervation of the CRF perikarya of the PVN has also been provided. In addition, it appears that CRF neurons may possess recurrent collaterals that innervate CRF neurons, presumably as a form of feedback (Silverman et al., 1989). These findings provide direct anatomical evidence for modulation of hypothalamic CRF secretion by a number of neurotransmitter systems.

Because of the tortuous pathway taken by the CRF neuron from the PVN to the median eminence, in vitro hypothalamic explants are by necessity large (2 to 3 mm^3). However, the vast majority of literature from other fields that utilize in vitro incubations (i.e., cerebral ischemia studies, hippocampal slice physiology) repeatedly has established the fact that tissue explants of this size quickly become hypoxic. Of the large number of groups who have attempted to study the in vitro release of CRF using nearly identical incubation setups, only a select few have been able to succeed. These investigators are

responsible for nearly all of the published work to date. Until further proof is independently provided, it would be prudent to regard many of the reports as preliminary as almost all neurotransmitter systems studied appear to directly alter CRF secretion.

As might be expected, negative feedback effects of glucocorticoids, ACTH, and CRF itself on CRF release have been demonstrated in these in vitro experiments. Both Suda et al. (1985b) and Calogero et al. (1988b) reported a dose-dependent inhibition of CRF secretion by glucocorticoids, suggesting a direct long-loop negative feedback of adrenal steroids on the hypothalamus. Suda's group reported that the effects of dexamethasone on the hypothalamic explant were exerted above the level of the median eminence. A rebound increase in the basal secretion of CRF was seen after removal of dexamethasone, suggesting that short-term incubation with the steroid could decrease release without altering CRF synthesis. These findings are concordant with several studies demonstrating the presence of glucocorticoid receptors on PVN CRF neurons (Liposits et al., 1987; Sawchenko, 1987b). Furthermore, both Suda et al. (1986) and Calogero et al. (1988b) reported a short-loop negative feedback role for ACTH on CRF release. The exact anatomical site(s) where ACTH acts within the hypothalamus (i.e., PVN or median eminence) or where the ACTH originates has not yet been determined. Calogero et al. (1988b) further reported on a possible ultrashort-loop negative feedback of CRF directly on itself. Evidence of local CRF neuronal circuits in the PVN also supports this possibility (vide supra).

The majority of in vitro and in vivo studies demonstrate both stimulatory cholinergic and serotonergic components to hypothalamic CRF release. Although there certainly appears to be a stimulatory cholinergic component, it remains to be clarified whether it is predominantly muscarinic or nicotinic or a combination of the two receptor subclasses (Suda et al., 1987b; Calogero et al., 1988a, 1989c; Tsagarakis et al., 1988).

In vitro studies clearly suggest a robust stimulatory role for serotonin on CRF release (Nakagami et al., 1986; Calogero et al., 1989a). Calogero et al. (1989b) reported that the effects of 5-HT were completely blocked by ritanserin, suggesting that the action of serotonin is mediated by the 5-HT_2 receptor subtype; this was later confirmed through stimulation of CRF release by the relatively specific 5-HT_2 agonist (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane. They also report a dose-dependent stimulation of CRF release by the 5-HT_{1A} agonist 8-hydroxydipropylaminotetralin and by the 5-HT_{1B} agonist *m*-chlorophenylpiperazine, albeit at lower maximal responses than that produced by (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane.

We (Owens et al., 1991a) reported significant increases in plasma ACTH and corticosterone concentrations at doses of the 5-HT_2 and 5-HT_{1C} agonist (±)-1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane as low as 100 μg/kg. However, tolerance to the stimulatory effects of (±)-1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane on HPA axis activity were evident by 7 days of treatment as evidenced by down-regulation of anterior pituitary CRF receptor binding and cortical and hypothalamic 5-HT_2 receptor binding (Owens et al., 1991b). These results are in agreement with those of Nash et al. (1989) who reported that acute (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane administration increased plasma corticosterone concentrations. Similarly, Bagdy et al. (1989) reported that (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane dose dependently increased plasma ACTH and corticosterone concentrations in the rat.

In agreement with the in vitro work on 5-HT_{1A} receptor stimulation of CRF release, our group (Owens et al., 1990b) and others (Koenig et al., 1987; Aulakh et al., 1988; Lorens and van de Kar, 1987) reported that the 5-HT_{1A} agonists, 8-hydroxydipropylaminotetralin and ipsapirone, stimulate HPA axis activity in intact rats. Moreover, Haleem et al. (1989) reported that this is probably a direct serotonergic effect on CRF neurons because 8-hydroxydipropylaminotetralin (500 to 1500 ng) microinjected into the PVN increases plasma corticosterone concentrations.

The effects of norepinephrine and the opioid peptides on CRF release in vitro are less clear. Suda et al. (1987c) reported that norepinephrine has a potent inhibitory effect mediated by α_1- and β-receptors. In contrast, Tsagarakis et al. (1988) and Joanny et al. (1989) report a stimulatory effect of norepinephrine on CRF release mediated through β-receptors. In agreement with the two latter reports, Widmaier et al. (1989) reported noradrenergic stimulation of CRF release via β-receptors in rat hypothalamic cell cultures obtained from 1-week-old rats. The elegant work of Plotsky (1987), using portal vessel cannulation for sampling CRF release in vivo, also supports a stimulatory role for norepinephrine. Norepinephrine produces a bell-shaped dose-response curve, with low doses stimulating CRF release via α_1-receptors and higher doses inhibiting CRF release via β-receptors. An excellent review of the catecholaminergic regulation of HPA axis activity was recently provided by Plotsky et al. (1989).

Buckingham (1986, 1987) reported that various opioid peptides directly stimulate CRF release in vitro. The only exception is the bell-shaped dose-response curve generated by β-endorphin. Concentrations of β-endorphin >100 nM were found to inhibit basal CRF release, whereas concentrations <100 nM retain their stimulatory activity. In contrast, Yajima et al. (1986) reported that a variety of opioid peptides, including β-endorphin, inhibited CRF release at all concentrations tested. Tsagarakis et al. (1989b) reported that morphine, without affecting basal CRF release, potently inhibited CRF release stimulated by 5-HT, acetylcholine, and norepinephrine. In

agreement with the findings of studies using the μ-agonist morphine, Nikolarakis et al. (1987) utilizing CRF immmunoneutralization techniques reported that endogenous opioids tonically inhibit CRF release but that μ-agonists release ACTH via non CRF-dependent mechanisms and that κ-agonists stimulate CRF release directly.

Of the in vitro studies not previously discussed in our recent review (Owens and Nemeroff, 1990), both hypoglycemia (Widmaier et al., 1988) and neuropeptide Y (Tsagarakis et al., 1989c) stimulate, whereas GABAergic/benzodiazepine (Kalogeras et al., 1990) mechanisms inhibit, CRF release. In addition, cocaine has been reported to stimulate CRF release through mechanisms unrelated to its actions on monoamine-containing neurons but, rather, through its local anesthetic properties which are not adequately understood (Calogero et al., 1989b).

As we have implied previously, the in vivo techniques in which viability of the tissue is not a problem, such as the portal vessel cannulations used by Plotsky and colleagues, are superior to the hypothalamic explant incubations. However, this technique can suffer from the fact that the secretagogue under study, if administered systemically, must be able to cross the blood-brain barrier and those that do will certainly be acting at other brain areas in addition to the PVN, thus confounding any attempt to study transmitter regulation at the level of the hypothalamic CRF perikarya themselves. It should be remembered that this problem is common to all studies in which systemic drug administration is used, including our own.

2. Feedback and stress-induced effects on the corticotropin-releasing factor neuron. Feedback regulation of the HPA axis, in general, and CRF neurons, in particular, by glucocorticoids is complex and a detailed description is beyond the scope of this review. Nevertheless, we will briefly review recent studies focusing on alterations in paraventricular CRF neuronal function. Those seeking more detailed, although already somewhat out of date, information would do well to start with the review by Antoni (1986).

There is undeniably convincing evidence that circulating glucocorticoids exhibit part of their negative feedback effects directly at the level of the CRF perikarya of the PVN. Strong evidence of such effects comes from recent immunocytochemical studies revealing the presence of glucocorticoid receptors in CRF-containing neurons of the PVN (Liposits et al., 1987; Sawchenko, 1987b; Uht et al., 1988). In addition to observing glucocorticoid receptor immunoreactivity in the PVN, Cintra et al. (1987) also reported glucocorticoid receptor immunoreactivity in CRF cell bodies in the BNST and in central and medial amygdaloid nuclei. Although it is not known what effect glucocorticoids exert on these extrahypothalamic CRF neurons, it does not necessarily involve a reduction in CRF gene expression because the role of glucocorticoid regulatory elements near the CRF gene may differ among brain regions. In fact, glucocorticoid administration has been reported to increase (Swanson and Simmons, 1989) or have no effect (Beyer et al., 1988) on CRF mRNA concentrations in the central nucleus of the amygdala.

Other evidence for glucocorticoid regulation of CRF neurons comes from the technically difficult studies of Plotsky and colleagues who directly measured the release of CRF from the median eminence into the portal vessels supplying the anterior pituitary. Plotsky and Vale (1984) initially reported that hemorrhage stress increased portal vessel concentrations of CRF from an initial level of 430 ± 34 (approximately 0.1 nM) to 839 ± 170 pg/ml. These increases were blocked by dexamethasone (100 μg/kg). The calculated basal secretory rate from the median eminence was approximately 1.6 pg/minute. Hypotensive stress-induced increases in CRF release were suppressed by plasma corticosterone concentrations between 80 and 120 ng/ml (Plotsky et al., 1986). However, basal CRF release was only decreased by corticosterone concentrations >400 ng/ml. Conversely, the effects of a lack of glucocorticoid feedback, produced by pharmacological adrenalectomy with metyrapone and aminoglutethimide, initially produced decreases in CRF release during the first 24 hours but increases in CRF secretion (2.2-fold) by 72 hours (Plotsky and Sawchenko, 1987).

A number of investigators have directly examined the effects of stress on CRF concentrations in the hypothalamus as well. Although measurement of peptide concentrations alone is insufficient to determine whether changes in release, storage, or synthesis are responsible, differences between treatment groups clearly represent alterations in the activity of the neurons. Chappell et al. (1986) reported that acute (3-hour cold immobilization) or chronic (a series of different stressors for 14 days) stress resulted in a 50% decrease in median eminence CRF concentrations. These decreases are thought to represent the release of CRF from terminal stores in the acute situation and continued release in the chronic paradigm where new synthesis cannot keep pace with the demands for more secretion. Whether this is actually the case is unclear. Murakami et al. (1989) reported a rapid increase in the content of median eminence CRF 2.5 minutes after ether stress. This transient increase disappeared by 5 minutes. The increase was thought to be too rapid to be explained by new synthesis but more likely represented rapid changes in the processing or packaging of CRF in granules. ACTH concentrations had already risen by this time point; therefore, CRF was thought to have been secreted during this time. Moldow et al. (1987) reported that during restraint stress a significant decrease in hypothalamic CRF concentrations occurred 15 minutes after the initiation of stress. This was followed by increases at 60 minutes that could be blocked by cycloheximide pretreatment, indicating that new synthesis was the likely cause of the increased CRF

concentrations observed at this time point. Finally, in agreement with the mRNA data (vide infra), Haas and George (1988) reported that 24 hours after a single 5-minute foot shock, significant increases in median eminence CRF content were observed. Inhibition of protein synthesis with anisomycin completely abolished the increase in CRF and resulted in decreased hypothalamic concentrations; this was undoubtedly due to unreplenished stores being released during the previous 24 hours.

In addition to feedback at the level of the CRF perikarya, glucocorticoids can act at both the anterior pituitary and at higher CNS areas such as the hippocampus. At the level of the anterior pituitary corticotroph, preincubation of pituitary cells in vitro with 10 nM dexamethasone for 18 hours did not alter CRF-stimulated cAMP accumulation but did markedly reduce ACTH release (Giguere et al., 1982). This suggests that glucocorticoids exerted their effect at a point distal to cAMP generation by CRF. In contrast, Sobel (1985) and Bilezikjian and Vale (1983) reported that glucocorticoids significantly attenuated CRF-stimulated cAMP generation, suggesting that glucocorticoids exert at least part of their negative feedback effects prior to cAMP generation. There are several methodological differences between these reports that may account for the discrepancies. For example, an 18-hour preincubation with glucocorticoid was used by Giguere et al. (1982), whereas only a 60-minute preincubation was used by the other investigators. It has been suggested that local production of prostaglandin E_2, which in turn alters intracellular calcium concentrations, may represent a portion of the negative feedback actions of glucocorticoids at the corticotroph (Vlaskovska et al., 1984; Sobel, 1987).

The most recent data have considerably strengthened the hypothesis that the hippocampus plays a significant role in glucocorticoid feedback. Sapolsky et al. (1989) showed that fornix transection, which disrupts hippocampal input to the hypothalamus, renders the normally glucocorticoid-sensitive increased release of CRF during stress resistant to glucocorticoid negative feedback. Additional studies by this group (Sapolsky et al., 1990) suggested that a major regulator of basal CRF concentrations in the portal vessel system is predominantly related to the occupancy of hippocampal type II glucocorticoid receptors, often in combination with hippocampal type I or hypothalamic receptors, whereas secretion of CRF induced by hypotensive stress is a function of both hippocampal type I and II receptor occupancy. In addition to these studies, hippocampectomy or destruction of the dorsal hippocampus results in a 4-fold increase in PVN CRF mRNA production and increases in plasma β-endorphin and corticosterone concentrations (Herman et al., 1989b). Similar findings were observed following hypothalamic deafferentation that removed much of the hippocampal input into the hypothalamus (Herman et al., 1990). Moreover, because the hypothalamus itself is intact, it was shown that local actions of glucocorticoids directly on CRF perikarya are insufficient to maintain normal CRF mRNA expression.

In addition to the previously described feedback actions on CRF release into portal vessels, glucocorticoids, or lack thereof, alter CRF gene expression and peptide content of the median eminence. Our group (Owens et al., 1990a) and others (Suda et al., 1984b; Yokoe et al., 1988; Jessop et al., 1990) have found that glucocorticoid administration decreases CRF immunoreactivity in the hypothalamus. Conversely, Sawchenko (1987a) observed that the lack of glucocorticoid feedback available following adrenalectomy results in increased CRF immunostaining in the PVN; this effect is abolished by glucocorticoid replacement.

A number of investigators have directly studied CRF gene expression by measuring CRF mRNA either by Northern blot gel analysis or by in situ hybridization histochemistry. Following adrenalectomy, c-*fos* immunoreactivity in CRF-containing cells of the PVN (Jacobson et al., 1990) and CRF mRNA concentrations increase anywhere from 90% to 275% (Jingami et al., 1985a; Young et al., 1986a; Beyer et al., 1988). These increases are also abolished by glucocorticoid replacement. In fact, dexamethasone implants into the PVN (Kovács and Mezey, 1987) have been reported to cause a total inhibition of hybridizable CRF mRNA above background. Similarly, Swanson and Simmons (1989) observed that CRF mRNA hybridization remains normal at plasma corticosterone concentrations <50 ng/ml, declines sharply at steroid concentrations between approximately 60 and 130 ng/ml, and is barely detectable at higher concentrations of corticosteroids.

As expected, various physical and behavioral stressors that activate the HPA axis also alter CRF gene expression. Intraperitoneal hypertonic saline, naloxone-precipitated opiate withdrawal, swimming, or restraint stress increased CRF mRNA expression within 4 hours and remained elevated for 24 hours (Lightman and Young, 1988; Harbuz and Lightman, 1989a,b). Further studies by Suda et al. (1988b) revealed that hypoglycemic stress increased CRF mRNA concentrations in the PVN, but not in the cortex, to 130% of control values by 30 minutes, and these increases reached a peak of 186% by 2 hours (fig. 4). These changes followed decreases in median eminence CRF concentrations at earlier time points (30 to 60 minutes). Lightman and Young (1989a) reported that dexamethasone administration in the fast or intermediate feedback time domains, 5 minutes and 2 hours, respectively, did not alter the CRF mRNA response to hypertonic saline stress. However, dexamethasone administered during a 2-day period reduced both basal and stress-induced CRF mRNA concentrations. Finally, Lightman and Young (1989b) also reported that, although there are a number of hypothalamic changes that are known to occur during lactation, lactation was

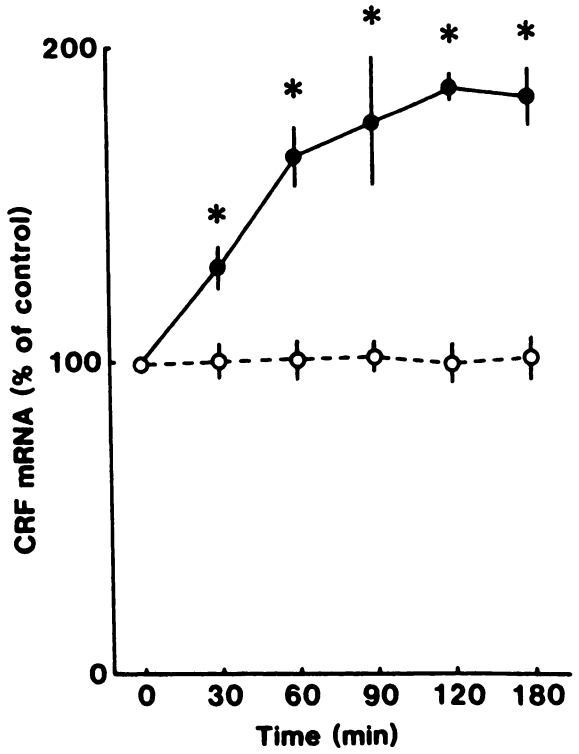

FIG. 4. Effect of insulin-induced hypoglycemia on CRF mRNA levels in the hypothalamus without the median eminence. Relative changes in CRF mRNA compared with control values are shown above. There were four pools of animals with four rats per pool. ●, insulin; ○, saline. *$P < 0.05$. Values are mean ± SE. Reprinted with permission from Suda et al. (1988b).

found to abolish stress-induced, but not adrenalectomy-induced, CRF mRNA responses. These findings suggest that normal hypothalamic stress responses are altered during lactation but return to normal within 2 days following removal of pups from their mother. Although this is presumably an adaptive response, the neurotransmitter alterations responsible for this remain obscure.

Some of the most interesting current studies involve examination of the regulatory elements preceding the CRF gene proper. Emanuel et al. (1990) showed that both forskolin and phorbol esters stimulate CRF gene expression in dispersed rat fetal hypothalamic cultures. A more detailed analysis was performed by Holsboer and coworkers (Van et al., 1990) who transfected the human CRF gene promoter region containing a 760-base pair segment into AtT-20 cells and linked it to the bacterial chloramphenicol acetyltransferase gene. Expression was enhanced by 8-bromo-cAMP but not by phorbol esters. They also observed that the core sequence for a cAMP-responsive element was 5′-TGACGTCA-3′ −221 base pairs from the putative CRF mRNA cap site. In addition, treatment with 500 nM dexamethasone reduced activity approximately 2-fold in cAMP-stimulated cells, suggesting that a portion of the glucocorticoid regulatory element(s) resides in this region. Similar findings with dexamethasone were observed by Adler et al. (1988) following introduction of an 8-kilobase DNA fragment containing the entire human CRF gene including approximately 6 kilobases of the 5′-flanking sequence and 0.8 kilobases of the 3′-sequence into AtT-20 cells.

3. Feedback- and stress-induced effects on anterior pituitary corticotropin-releasing factor receptors. The stimulation of ACTH release is dependent upon three major factors involving CRF neurotransmission. Actually, this can be said for most forms of neurochemical transmission. These include (*a*) neurotransmitter synthesis, which can be assessed by measurement of CRF mRNA expression, (*b*) CRF secretion, as determined by measurement of portal vessel concentrations of CRF, and (*c*) neurotransmitter receptor functioning, as determined by measurement of receptor affinity and density or second messenger generating capabilities, which constitutes a portion of the final aspect of neurotransmission. Regarding receptor function, removal of the negative feedback action of glucocorticoids by adrenalectomy results in CRF hypersecretion and a reduction (down-regulation) in anterior pituitary CRF receptor concentrations, effects that can be prevented by glucocorticoid supplementation (Wynn et al., 1983, 1984; Aguilera et al., 1986; Holmes et al., 1987).

In addition to these changes, chronic administration of corticosterone (0.5 to 150 mg/day) for 1 to 4 days also causes a dose-dependent decrease in anterior pituitary CRF receptor number (Hauger et al., 1987). This may be the result of decreased synthesis of new CRF receptors in light of excessive glucocorticoid tone or may represent another means by which circulating glucocorticoids inhibit further ACTH secretion. It will be interesting to identify the presence of a glucocorticoid responsive regulatory element near the CRF receptor gene after it has been sequenced. In agreement with these findings, Childs and Unabia (1990), while trying to identify corticotrophs by cytochemical binding with biotinylated analogs of CRF, found that glucocorticoids decreased the ability of cells to bind CRF within 60 minutes of exposure to the steroids, findings that could be mediated by a reduction in CRF receptor numbers at the cell surface. However, in contrast to these observations suggesting glucocorticoid-induced reduction in CRF function at the level of the pituitary corticotroph, Ceda and Hoffman (1986) observed that glucocorticoids are necessary to prevent development of CRF desensitization in vitro. This suggests that a mechanism exists by which, even in the face of high circulating concentrations of glucocorticoids, the development of substantial CRF desensitization is prevented in vivo. This is, in fact, the case because no specific desensitization to exogenous CRF is seen in chronically stressed animals (Young and Akil, 1985; Rivier and Vale, 1987), although CRF receptor concentrations in the anterior pituitary are decreased (Hauger et al., 1988).

C. Involvement of Corticotropin-releasing Factor Neurons in Other Endocrine Functions

1. Effects on growth hormone secretion. One of the most well-documented endocrine responses to stress in

the rat is the inhibition of growth hormone secretion. Recent evidence suggests that this response is controlled by CRF neurons. Rivier and Vale (1984a) and McCann and colleagues (Ono et al., 1984) initially reported that i.c.v. administration of CRF dose dependently decreases growth hormone secretion. This appears to be the result of CRF-induced stimulation of somatostatin release from the median eminence; this has been demonstrated in vitro (Peterfreund and Vale, 1983; Aguila and McCann, 1985) and in vivo (Mitsugi et al., 1990). Corroborating studies by Rivier and Vale (1985b) showed that both i.c.v. CRF-induced and stress-induced decreases in plasma growth hormone concentrations are blocked by the CRF antagonist, α-helical CRF_{9-41}, or immunoneutralization of somatostatin. It should be noted that, in contrast to rodents, stress increases growth hormone secretion in primates. Therefore, the data derived from rodent experiments may not be relevant to nonhuman primates and humans. Unrelated to its actions on growth hormone secretion, but of interest nevertheless, CRF has also been reported to stimulate the release of dynorphin and β-endorphin from hypothalami in vitro (Nikolarakis et al., 1986).

Interestingly, somatostatinergic neurons can alter stress-induced ACTH secretion from the anterior pituitary by one of two apparent mechanisms. Somatostatin-28 and $desAA^{1,2,4,5,12,13}$[d-Trp^{8}]somatostatin, but not somatostatin-14, given i.c.v. prevent stress-induced ACTH secretion by inhibition of CRF release (Brown et al., 1984). Additionally, CRF-stimulated adenylate cyclase activity and ACTH secretion from AtT-20 cells can be dose dependently inhibited up to 50% by somatostatin. Higher doses can further inhibit ACTH secretion apparently through a non-cAMP-dependent protein kinase mechanism (Litvin et al., 1986).

2. Effects on reproductive hormone function. It is well established that stress inhibits reproductive functioning. Although reproduction is clearly of paramount importance to the survival of an organism, during times of life-threatening stress, energy is best expended solely for survival. It is, therefore, not surprising that activation of CRF neurons may result in inhibited sexual functioning.

Rivier and Vale (1984b) and Ono et al. (1984) reported that i.c.v. CRF administration produced dose-dependent decreases in plasma LH, but not follicle-stimulating hormone, concentrations in rats. Rivier and Vale (1984b) showed that this effect was powerful enough to inhibit ovulation and to disrupt pregnancy. In addition to these findings, Rivier et al. (1986) reported that i.c.v. administration of the CRF antagonist blocked stress-induced decreases in plasma LH concentrations. In vitro studies utilizing hypothalamic slices have shown that the CRF antagonist increases gonadotropin-releasing hormone (LHRH) release (Nikolarakis et al., 1988). Moreover, immunocytochemical studies have shown direct evidence for synaptic contact between CRF terminals and LHRH-containing neurons in the rat hypothalamus (Maclusky et al., 1988). These studies suggest that CRF neurons can act centrally to directly inhibit LHRH release from the median eminence.

CRF, administered peripherally, also decreases plasma LH concentrations. The mechanism(s) by which this occurs is unclear. CRF decreased plasma LH concentrations in male and female rats and plasma testosterone concentrations and seminal vesicle weights in male rats but did not alter plasma follicle-stimulating hormone concentrations (Rivier and Vale, 1985a). Moreover, these actions of CRF could be mimicked by ACTH administration and were abolished by adrenalectomy. These observations strongly suggested that the peripheral actions of CRF on LH secretion were mediated by pituitary-adrenal activation. A different set of findings have been observed in primates. CRF decreases both plasma LH and follicle-stimulating hormone in rhesus monkeys when given i.v., and this effect is independent of glucocorticoid secretion (Olster and Ferin, 1987; Gindoff and Ferin, 1987; Xiao et al., 1989). In fact, Gindoff et al. (1989) reported that dexamethasone actually blocks the effects of CRF on gonadotropin secretion. Ferin and colleagues proposed that the mechanism involves CRF-stimulated release of endogenous opioids which then inhibit LHRH release centrally. It is unclear how, or even whether, opioid peptides from the anterior pituitary are responsible for this. Finally, i.v. CRF decreases electrical activity in an area of the mediobasal hypothalamus thought to contain the LHRH pulse generator (Williams et al., 1990). These actions of CRF were unrelated to glucocorticoid levels but were partially blocked by naloxone, lending some credence to the above opioid hypothesis.

Further study of the function of CRF neurons in the regulation of the hypothalamic-pituitary-gonadal axis may provide potentially useful information regarding the pathophysiology and treatment of fertility problems.

D. Responses of Corticotropin-releasing Factor Neurons to Miscellaneous Experimental Manipulations

1. Lesion studies. Several lesion studies have been performed that were not conceived primarily as indirect methods of tracing anatomical pathways but, rather, as an experimental manipulation. These have primarily focused on lesions of the PVN. Bruhn et al. (1984) observed a 90% decrease in median eminence CRF content 4 to 6 days following bilateral lesions of the PVN. This was associated with a 75% reduction in the ACTH response to stress. In addition, hyperresponsiveness to exogenous CRF was observed, probably as a result of CRF receptor up-regulation. Similarly, another group reported that 6 weeks following surgery <5% of the CRF in the median eminence observed in sham-operated rats was seen in lesioned rats (Dohanics et al., 1986; Makara et al., 1986). Although these rats had normal basal plasma ACTH levels, probably maintained by other ACTH secretagogues, they exhibited either no (Makara

et al., 1986) or a markedly reduced (Dohanics et al., 1986) ACTH response to surgical or ether stress, respectively.

Beaulieu et al. (1989) determined the effects of destruction of the central nucleus of the amygdala, a region containing large numbers of CRF-staining cell bodies, on CRF immunoreactivity in the median eminence. The authors found a >50% decrease in CRF immunostaining in the median eminence 2 weeks after bilateral lesions of the central amygdala. The interpretation of this finding is not easy. A direct pathway from the central nucleus to the median eminence may exist, although there is no other evidence for this. More likely, the pathways from the central amygdala to the PVN could alter the neuronal activity of PVN CRF neurons that project to the median eminence. Alternatively, the existence of projections from the central amygdala to the brainstem and back to the hypothalamus could help explain these observations. Unfortunately, measures of HPA axis activity were not undertaken in this study to help determine the physiological role of this decrease in CRF staining.

2. Role in animal models of genetic disorders. A number of animal models for various diseases have been shown to be associated with alterations in HPA activity. With this in mind, several studies were conducted to examine the role of CRF neurons in these models. The FSL of rats was developed by selective breeding for muscarinic cholinergic receptor supersensitivity. These rats have been proposed as a genetic model of depression because they share many similarities with depressed patients. Because these rats and depressed humans have exaggerated HPA responses to cholinergic agonists, and because many depressed patients are hypercortisolemic, our group investigated CRF neuronal activity in FSL rats (Owens et al., 1991c). In nonstressed FSL animals, we found decreased basal plasma ACTH concentrations and increased anterior pituitary CRF receptor concentrations with no differences in median eminence CRF concentrations compared to control rats of the Flinders resistant line. The Flinders resistant line rats have generally been shown to resemble normal Sprague-Dawley rats in previous studies. Thus, under basal conditions, this strain of rat appears to possess diminished HPA activity and is, therefore, dissimilar to what is observed in many depressed individuals.

Genetically obese (*fa/fa*) Zucker rats are characterized by increased parasympathetic and decreased sympathetic tone. As a result, they are hypometabolic and gain weight more efficiently than their heterozygote controls. Hypercortisolemia or enhanced adrenal responsiveness is thought to contribute to the etiology of this disorder because adrenalectomy can reverse most facets of the syndrome. These animals are hypercortisolemic and exhibit a blunted ACTH response to exogenous CRF (Cunningham et al., 1986). Although there are few data to date, the increased HPA activity appears to be of central origin and involves excessive CRF secretion (Guillaume-Gentil et al., 1990). It is not known whether alterations in hypothalamic CRF neuronal activity is a primary or secondary factor in the etiology of this syndrome.

The last syndrome that has been investigated is the SHR strain. SHRs appear to possess abnormal HPA activity that contributes to the development of hypertension. Like depressed patients, SHRs exhibit blunted ACTH responses to exogenous CRF and are somewhat hypercortisolemic at all times (Hashimoto et al., 1989). However, these rats have lower concentrations of CRF in the median eminence compared with normotensive rats. It is believed that excessive glucocorticoid tone due to enhanced adrenocortical function, rather than CRF hypersecretion, is responsible for the hypercortisolemia and may be essential to the development of hypertension. Recent evidence suggests that adrenalectomy delays the onset of hypertension in SHRs by several weeks but does not prevent it. Neonatal sympathectomy with 6-hydroxydopamine, on the other hand, does prevent the development of the hypertension.

3. Miscellaneous pharmacological treatments. A variety of compounds have been tested for their actions on HPA axis activity with particular emphasis on their actions on CRF neurons. The majority of these studies are similar to those described in the section entitled, "Neurotransmitter Regulation of Corticotropin-releasing Factor Neurons," but because they are poorly selective for any particular neurotransmitter system, they are discussed separately.

Reserpine causes a transient decrease in median eminence CRF concentrations that precedes the increase in plasma ACTH concentrations following a single acute injection (Bugnon et al., 1983; Suda et al., 1987a). Three days of reserpine administration produced similar results in the median eminence and posterior pituitary (Tizabi et al., 1985). It has been suggested from these data that the initial release of monoamines, particularly norepinephrine, prior to the depletion of these substances by reserpine may stimulate CRF release. These findings support other studies specifically focusing on noradrenergic stimulation of hypothalamic CRF release. Moreover, lesions that decrease the noradrenergic innervation of the hypothalamus result in decreases in CRF-immunostaining 14 to 17 days afterward. Without catecholaminergic input, CRF neurons apparently do not produce, and presumably secrete, CRF at normal rates (Sawchenko, 1988).

Although some consider ethanol a nonspecific stressor capable of disrupting membrane fluidity, it is also believed to act largely via a GABAergic mechanism. Because it is unclear which, if either, mechanism is correct, we have included it here rather than in the previous section. Ethanol increases CRF release from hypothalamic tissue in vitro (Redei et al., 1988). In addition, immunoneutralization of CRF abolishes the increase in plasma ACTH observed following acute administration

of ethanol (Rivier et al., 1984a). Animals exposed continuously to ethanol vapors for 14 days exhibited decreased anterior pituitary CRF receptors and CRF-stimulated cyclase activity (Dave et al., 1986). This was associated with decreases in plasma β-endorphin concentrations.

In unrelated studies, central administration of the putative neurotransmitter neuropeptide Y increased plasma ACTH and median eminence CRF concentrations 45 minutes postinjection (Haas and George, 1987). Finally, because alterations in CRF neurons have been proposed to play a role in the pathophysiology of major depression, the effects of electroconvulsive shock on CRF neurons have been examined in the rat (Herman et al., 1989a). Following seven daily ECT treatments, CRF mRNA was significantly increased in the PVN, whereas CRF concentrations were decreased. The finding of increased synthesis with decreased tissue concentrations suggests an increased activity of the HPA axis following electroconvulsive treatment. This has not been observed in depressed patients in whom measures of HPA activity generally decrease toward more "normal" values.

IV. Corticotropin-releasing Factor Regulation of Immune Function

A. Evidence for Direct Communication between Immune Tissues and Corticotropin-releasing Factor-secreting or Corticotropin-releasing Factor-receptive Neuronal and Endocrine Tissues

1. Effects of corticotropin-releasing factor on the immune system. Substantial evidence gained during the past several years has demonstrated that the CNS interacts with, and can modulate the activity of, various elements of the immune system. This interaction between these two major communication systems is likely involved in regulating host defense. Much of the interest involving CRF and the immune system resulted from the long-standing observations of stress-induced decreases in immune function in animals and humans. Then, Smith et al. (1986) reported that CRF stimulated the release of ACTH and β-endorphin from leukocytes. This effect was reportedly blocked by the synthetic glucocorticoid dexamethasone and suggested that, like pituitary corticotrophs, the POMC gene may be similarly expressed in leukocytes. However, as noted earlier, CRF receptors have not been found on lymphocytes. Although CRF receptors have yet to be identified on lymphocytes, our group (Ritchie et al., 1986) and others (Stephanou et al., 1990) have found CRF immunoreactivity and CRF mRNA in lymphocytes. CRF may be released from lymphocytes to exert local paracrine actions on other immune system cells or on inflammatory responses (vide infra).

Irwin and colleagues have repeatedly shown that the central, but not peripheral, administration of CRF decreases NK cell cytotoxicity and that this is blocked by central administration of the CRF antagonist, α-helical CRF_{9-41} (Irwin et al., 1987). This action can be observed 10 minutes after i.c.v. injection and persists for <60 minutes following injection, although plasma corticosterone concentrations remained elevated for some time (Irwin et al., 1989). This suggests that these actions are not mediated by glucocorticoids. Moreover, these reductions in NK activity are also observed following footshock stress and can be blocked by central administration of CRF antiserum (Irwin et al., 1990). Finally, this effect of CRF on NK activity is blocked by the ganglionic blocking agent, chlorisondamine (Irwin et al., 1988). Overall, these exciting findings suggest that, under certain stressful conditions, specific CRF neurons, likely extrahypothalamic, activate autonomic outflow to the spleen resulting in reductions in the activity of NK cells. Irwin et al. (1988, 1990) suggested that this may possibly be mediated by sympathetic norepinephrine release in the spleen.

Although central CRF systems can decrease NK activity and possibly lead to a temporary immunosuppressive effect, the inability to mount a proper CRF response may also lead to potential immune-related problems. Sternberg et al. (1989a,b) have evidence that the arthritis-susceptible Lewis strain of rats lack the ability to generate a proper HPA axis response to a given stimulus. This defect appears to be at the level of CRF gene expression in the PVN. Their findings suggest that some diseases characterized by inappropriate or inadequate immune/inflammatory regulation (e.g., cancer, autoimmune diseases) may additionally be the result of CNS defects and not immune system defects alone.

2. Actions of cytokines on hypothalamic corticotropin-releasing factor neurons. There is now considerable evidence that elements of the immune system, during times of stress (e.g., infectious challenge), can stimulate glucocorticoid secretion through activation of the HPA axis. This is thought to help provide a means by which the body can rapidly activate a stress response to infection as well as modulate immune function. The evidence to date clearly indicates that various lymphokines increase hypothalamic CRF secretion as a means of activating the HPA axis.

The earliest report was that of Woloski et al. (1985) who reported that both IL-1 and hepatocyte-stimulating factor stimulated ACTH release from AtT-20 cells. However, in subsequent reports, lymphokine activation of plasma HPA axis activity was demonstrated to occur by stimulation of hypothalamic CRF secretion and not by direct release of adenohypophysial ACTH. Besedovsky et al. (1986) reported that systemic injection of IL-1, but not tumor necrosis factor, IL-2, or γ-interferon, increased plasma ACTH and glucocorticoid concentrations in mice and rats. Rivier et al. (1989) reported that activation of the HPA axis by endotoxin (lipopolysaccharide) is mediated by activation of IL-1 receptors on hypothalamic CRF cells. A number of different techniques have

been used to determine that activation of CRF neurons is responsible for these actions of lymphokines. Uehara et al. (1987) found that IL-1-induced increases in plasma ACTH could be blocked by CRF immunoneutralization. Sapolsky et al. (1987) found similar results with immunoneutralization of CRF as well as observing direct increases in the concentration of CRF in the hypothalamohypophysial portal vessels after IL-1. Similarly, Barbanel et al. (1990), using a push-pull cannula implanted in the median eminence, found that intrahypothalamic infusion of IL-1β directly stimulates release of CRF. Further evidence has recently come from Suda et al. (1990) who also found increases in plasma ACTH concentrations following injection of IL-1α or IL-1β. Moreover, concomitant decreases in the content of median eminence CRF and increases in CRF mRNA in the PVN were observed. Finally, a preliminary report of 30 cancer patients receiving immunotherapy with IL-2 or IL-2 plus lymphokine-activated killer cells suggests that IL-2 can also profoundly activate the HPA axis (Denicoffet al., 1989). Whether this is through stimulation of CRF secretion has yet to be determined.

Although reservations exist regarding the confidence that can be placed on in vitro incubation studies because of problems of tissue viability, the results obtained also support the hypothesis that CRF neurons mediate lymphokine activation of the HPA axis. Tsagarakis et al. (1989a) reported that both IL-1α and IL-1β stimulated CRF release from isolated hypothalamic blocks in vitro. Similar findings were reported by Bernardini et al. (1990a) who proposed that IL-1's actions were mediated by arachidonic acid metabolites. In fact, they suggested that arachidonic acid metabolites may be responsible for the actions of a number of neurotransmitters on CRF release including serotonin and acetylcholine (Bernardini et al., 1989b). Navarra et al. (1991) reported that both IL-1 and IL-6 stimulated CRF release from hypothalamic explants, but not from median eminences alone, in vitro. They found that these actions were antagonized by blockade of the cyclooxygenase, but not lipooxygenase, pathway. Neither IL-2, tumor necrosis factor, α_2-interferon, nor γ-interferon altered CRF or ACTH release. In contrast to the above results, Bernardini et al. (1990b) reported that tumor necrosis factor does stimulate CRF release both in vivo and in vitro. In this case, CRF release was inhibited by both cyclooxygenase and lipooxygenase inhibitors. Finally, platelet-activating factor has been reported to stimulate CRF release both in vivo (Rougeot et al., 1990) and in vitro (Bernardini et al., 1989a). Rougeot et al. (1990) reported that platelet-activating factor acts directly on the median eminence rather than on perikarya in the PVN.

B. Analgesic and Anti-Inflammatory Properties of Corticotropin-releasing Factor

Although the mechanism(s) responsible has not been determined, Wei and colleagues have convincing data that CRF and related peptides (i.e., sauvagine and urotensin I) possess analgesic and anti-inflammatory properties (Wei and Kiang, 1989). Increased exudation of plasma proteins into the rat paw produced by antidromic stimulation of the saphenous nerve has been termed neurogenic plasma extravasation and is inhibited by a number of opiate analgesics. CRF inhibited neurogenic plasma extravasation in both hypophysectomized and adrenalectomized rats, indicating that the effects are not secondary to release of ACTH, β-endorphin, or glucocorticoids (Wei et al., 1986). These effects were also seen after local intradermal injection of very small doses of CRF into the innervated paw. Similarly, increased vascular permeability in the trachea following antidromic stimulation of the right vagus nerve or exposure to formaldahyde vapors is also attenuated by peripheral CRF administration (Wei and Kiang, 1987).

Thermal injury (Kiang and Wei, 1987; Wei et al., 1988) and exposure to concentrated acids (Tian and Wei, 1989) produce protein extravasation and edema into the rat paw as part of the acute inflammatory response; the effects of both noxious agents are attenuated by CRF. When administered i.v. in microgram doses or intradermally in nanogram doses, CRF is effective when administered up to 4 hours prior to or 20 minutes following exposure to the noxious stimuli. These actions of CRF are completely abolished by administration of the CRF antagonist, α-helical CRF_{9-41}. These investigators suggested that CRF acts directly on endothelial cells lining the local vascular system near the site of injury.

Because CRF stimulates the release of β-endorphin during a stress response, it was hypothesized that CRF administration may possess indirect analgesic properties through an endogenous peripheral opioid system. In fact, Hargreaves et al. (1987) reported that, in humans recovering from molar extraction, exogenous CRF administration resulted in significant analgesia compared to placebo. Moreover, in the rat paw-lick test, CRF produced analgesia comparable in length and intensity to that of morphine. They later found that the analgesic properties are not attenuated by hypophysectomy or adrenalectomy and are present when injected locally (Hargreaves et al., 1989). This is in contrast to their most recent report (Hargreaves et al., 1990) in which the antinociceptive actions of CRF were abolished by hypophysectomy, dexamethasone, naltrexone, naltrexone methyl bromide, and immunoneutralization of β-endorphin, observations that would clearly favor a role for endogenous opioids in mediating these analgesic effects. In contrast to these findings, Ayesta and Nikolarakis (1989) found that the analgesic effects of peripherally administered CRF were not modified by naloxone administration nor in rats previously rendered tolerant to morphine. These findings suggest that opioids do not mediate CRF-induced antinociception. In trying to determine the mechanism of action, Poree et al. (1989)

reported that, when recording from the spinal trigeminal nucleus, CRF dose dependently inhibited the evoked response of these cells following exposure to thermal injury. Again, these responses were unaltered by hypophysectomy or adrenalectomy. These effects did not occur following i.c.v. administration and were abolished by peripheral administration of the CRF antagonist. Interestingly, CRF increased the spontaneous firing of cold-responsive units by 57%, suggesting that CRF selectively inhibits neuronal responses to noxious heat.

In summary, CRF appears to exert anti-inflammatory and analgesic properties that may be partly mediated by the HPA axis and partly independently. The anti-inflammatory properties may result from direct interaction of CRF with endothelial cells lining blood vessels. As described earlier, there was a report of CRF receptors on aortic endothelial tissues. A second possibility involves the direct interaction of CRF with leukocytes infiltrating the area of injury. The analgesic effects appear to be the result of direct alterations of sensory neurons that respond to pain. Alternatively, interactions with immune cells and the reduced local production of various pain-mediating chemicals or increases in endogenous opioid secretion may contribute to the observed actions.

V. Corticotropin-releasing Factor Regulation of Autonomic Function and Other Peripheral Actions

A. Cardiovascular Responses to Central and Peripheral Administration of Corticotropin-releasing Factor

In concert with the HPA axis response, various stressors elicit rapid alterations in autonomic nervous system activity readying the body for the "fight or flight" response and inhibiting vegetative functions. As will be discussed, it appears that CRF neurons in the CNS play an important role in this response. Although we will cite a number of studies relevant to this topic, the recent review by Fisher (1989) is more comprehensive (fig. 5).

Following the availability of synthetic CRF, early studies found that relatively high doses of CRF, administered i.v., produced vasodilation and hypotension. In dogs, a decrease in MAP was associated with a rebound increase in heart rate that followed an increase in mesenteric blood flow (Lenz et al., 1985). Similar findings were reported in rats (Kiang and Wei, 1985), monkeys (Kalin et al., 1983b; Udelsman et al., 1986a), and humans (Hermus et al., 1987). In contrast, Kalin et al. (1983a) did not observe any such effect in sheep when oCRF was administered, although the oCRF did produce a profound endocrine response. This finding is difficult to explain because the hypotensive actions of CRF are hypothesized to be mediated by increased β-endorphin secretion, and this was observed in the sheep. Whether there actually is species selectivity in CRF-induced hypotension or different species-specific responses to oCRF versus rat/human CRF has not been further studied.

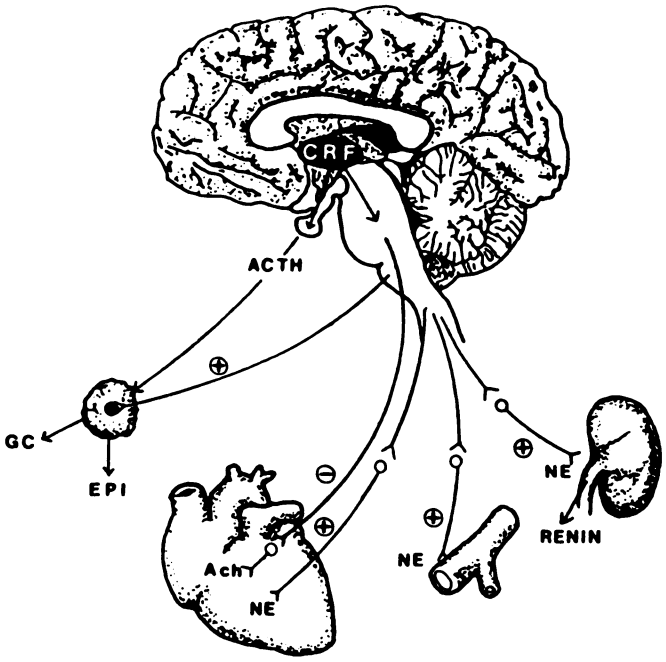

FIG. 5. Some of the autonomic actions of CRF. When released within the hypophysiotropic zone, CRF is transported to the pituitary where it stimulates secretion of ACTH, which in turn elicits glucocorticoid (GC) release from the adrenal cortex. Anatomical, pharmacological, and physiological data support the notion that CRF acts at additional CNS sites to (*a*) stimulate sympathetic tone to the adrenal medulla, resulting in epinephrine (epi) secretion; (*b*) stimulate sympathetic noradrenergic outflow to the heart, kidney, and selected vascular beds; and (*c*) inhibit cardiac parasympathetic (ACh) nervous activity. NE, norepinephrine. Reprinted with permission from Fisher (1989).

Of considerably greater interest is the repeated findings of increased MAP and heart rate following central administration of the peptide, actions that are not related to, and are clearly separate from, activation of the HPA axis. Initial work by Brown and colleagues (Brown and Fisher, 1983; Fisher and Brown, 1984) and subsequently by others (Saunders and Thornhill, 1986) found that i.c.v. administration of CRF resulted in increases in MAP, heart rate, plasma norepinephrine, and epinephrine. These effects, consistent with adaptive responses to threatening situations, are not the result of increased physical activity (Overton and Fisher, 1989a) and can be blocked by chlorisondamine (Brown and Fisher, 1983; Fisher and Brown, 1984; Lenz et al., 1987) or i.c.v administration of the antagonist α-helical CRF_{9-41} (Brown et al., 1986). The response can also be somewhat attenuated by i.c.v. administration of $dynorphin_{1-17}$ and selected dynorphin-related peptides (Overton and Fisher, 1989b). Under normal circumstances, increases in MAP are associated with decreases in heart rate via activation of the baroreceptor reflex. However, under stressful conditions the baroreflex function can be altered such that simultaneous elevations of arterial pressure and heart rate can occur. Central administration of CRF does not alter baroreceptor sensitivity; rather, it increases sympathetic and decreases central parasympathetic outflow

(Fisher, 1988, 1989; Overton et al. 1990). Increases in sympathetic outflow are likely responsible for the elevations in plasma catecholamines and increased MAP, whereas diminished vagal tone probably represents a large component of the tachycardic response and decreased baroreceptor reflex. Attempts to localize the anatomical sites for this action of CRF have not been successful because CRF microinjection into a number of sites have been found to increase sympathetic outflow, as determined by increases in plasma catecholamine concentrations (Brown, 1986). This could either be the result of diffusion away from the injection site or, as suggested by Brown, an anatomical redundancy of regions sensitive to CRF and capable of modulating autonomic function.

Finally, Saitoh et al. (1990) recently found that CRF possessed a positive inotropic effect on guinea pig myocardium in vitro. This was qualitatively different from that produced by cardiac glycosides and was hypothesized to result from an increase in the slow inward Ca^{2+} current. Although CRF receptors or CRF immunoreactivity have not been previously demonstrated in the heart and because CRF in the systemic circulation does not have a definitive physiological role, these actions on the heart would be consistent with a role for CRF in an adaptive circulatory response during the fight or flight syndrome.

B. Metabolic Responses to Corticotropin-releasing Factor Administration

As discussed previously, CNS administration of CRF increases sympathetic outflow. In addition to the increases in circulating plasma catecholamine concentrations associated with increased sympathetic activity, i.c.v. CRF increases physical activity, total oxygen consumption, plasma glucose, and glucagon concentrations (Brown et al., 1982a,b, 1985). As with the above changes in circulatory physiology, these effects are unrelated to HPA axis activation and are abolished both by the ganglionic blocker chlorisondamine (Brown et al., 1982b) and by central administration of the CRF antagonist, α-helical CRF_{9-41}. Moreover, the CRF antagonist also blocks stress-induced increases in plasma epinephrine concentrations by inhibiting sympathetic outflow to the adrenal (Brown et al., 1985). Central administration of α-helical CRF_{9-41} also inhibits the increased oxygen consumption and sympathetic outflow associated with i.c.v. administration of the glucocorticoid antagonist, RU-486 (Hardwick et al., 1989), suggesting that these effects of RU-486 are secondary to CRF hypersecretion. Chronic (7 days) i.c.v. administration of CRF, at doses that do not alter HPA axis activity, has been shown to abolish the excessive weight gain normally observed in genetically obese (*fa/fa*) rats (Rohner-Jeanrenaud et al., 1989). This was not related to changes in food intake and likely represents changes in sympathetic function which appears to be dysfunctional in this strain of rats.

An electrophysiological study recently demonstrated that i.c.v. CRF directly increases sympathetic nervous activity in brown fat tissue, i.e., in cells that have been implicated in thermogenesis and energy mobilization (Egawa et al., 1990). Acute in vitro exposure of pancreatic islets of Langerhans to CRF resulted in increased glucagon, but not insulin (Moltz and Fawcett, 1985a), release over a small range of CRF concentrations (50 to 200 pg/ml; approximately 10 to 40 pM). These investigators also reported that CRF inhibited insulin release from perfused rat pancreas with no effect on glucagon release following i.v. CRF administration (Moltz and Fawcett, 1985b). In contrast, Torres-Aleman et al. (1984) reported that i.v. CRF increased insulin concentrations in the hepatic portal vein of rats without changing plasma glucose or glucagon concentrations. It should be remembered that it is the central actions of CRF that appear to alter autonomic output and not actions at peripheral target organs. Also note that, in the numerous studies in animals and humans, no obvious effect of CRF on plasma insulin or glucagon concentrations has been found.

A brief review by Rothwell (1990) of the effects of CRF on metabolism and energy balance with emphasis on a potential role for CRF neurons in the pathophysiology of obesity and cachexia was recently published. Although any peripheral extrapituitary physiological actions of CRF remain to be determined, the evidence strongly supports a role for central CRF neurons in helping to mobilize energy stores during times of stress. These are actions clearly expected from increased sympathetic nervous activity which CRF neurons may help orchestrate.

C. Gastrointestinal Responses to Corticotropin-releasing Factor Administration

During times of stress, vegetative functions such as digestion are diminished to ensure an adequate blood supply to more vital organs. If one assumes that CRF is involved in modulating the stress response, it is plausible that CRF may alter digestive function by decreasing parasympathetic outflow in a manner similar to that seen in the circulatory system (vide supra). Tache and colleagues reported that i.c.v (Garrick et al., 1988; Stephens et al., 1988) or intrahypothalamic (Gunion and Tache, 1987) administration of CRF decreases gastric acid secretion and gastric motility. These actions are blocked by concurrent administration of an CRF antagonist. Bueno and Fioramonti (1986) reported that i.c.v., but not i.v., CRF administration decreased gastric motility. This was assessed by diminished migrating motor complexes which are the rhythmical smooth muscle contractions originating in the stomach and propagating to the ileum. However, Williams et al. (1987) reported that both i.c.v. and i.v. CRF administration decreased gastric emptying and small intestinal motility and increased colonic motility and fecal excretion. In contrast to these

reports and their own previous work, Tache's group (Pappas et al., 1987) also reported that i.v., but not i.c.v., CRF administration inhibited gastric emptying in dogs. Although these reports appear to be experimentally sound, the inconsistent findings suggest that considerably more work is needed before an actual physiological role for CRF in altering digestion during stress can be definitively established. Nonetheless, decreases in gastrointestinal function caused by centrally acting CRF neurons on parasympathetic activity would be consistent with its proposed role in integrating the autonomic nervous system's response to stressful situations.

D. Local Gonadal Actions of Corticotropin-releasing Factor

CRF is present in the testis where it is synthesized locally. CRF receptors are present on Leydig cells where it appears to act via a pertussis toxin-insensitive G protein to inhibit human chorionic gonadotropin-induced cAMP generation and testosterone synthesis (Ulisse et al., 1989, 1990). Although it is still unclear, it appears that human chorionic gonadotropin stimulates the production and release of CRF from populations of Leydig cells. Following release of CRF, CRF receptors on the same or other Leydig cells inhibit the activity human chorionic gonadotropin has on the production of testosterone. Thus, this type of feedback ultimately appears to locally inhibit Leydig cell function (Fabbri et al., 1990). In addition to these findings, CRF receptors on Leydig cells stimulate the release of locally synthesized β-endorphin from these cells (Eskeland et al., 1989). Although these local paracrine actions of CRF are not fully understood, the findings support other data revealing decreased reproductive functioning produced by CRF at local, neuroendocrine, and behavioral levels, actions all aimed at shifting physiological function away from vegetative needs to those needs necessary during times of stress.

VI. Corticotropin-releasing Factor Regulation of Behavior in Laboratory Animals

A. Behavioral Responses to Central and Peripheral Administration of Corticotropin-releasing Factor

1. Locomotor activation. When administered directly into the brain, CRF produces behaviors similar to those observed following exposure to stress. This is consistent with a proposed role for CRF neurons in mediating endocrine, autonomic, and behavioral responses to various degrees of stress. Following the initial reports of CRF modulation of behavior, a plethora of reports emerged confirming many of the initial findings. These reports will be cited below. However, we will focus on recent reports we view as most significant. For those seeking more detailed information, an extensive review of the behavioral actions of CRF in laboratory animals by Dunn and Berridge (1990) was recently published.

Because of prior findings with other hypothalamic peptides, Sutton et al. (1982) examined the effects of i.c.v. CRF administration in the rat, hypothesizing that CRF possessed an important role in modulating behavior. These investigators found that CRF produced dose-dependent increases in locomotor activity in a familiar environment and behaviors in open-field testing consistent with what can be termed increased "emotionality." These effects were not seen following peripheral administration. Thereafter, a number of investigators confirmed these findings (Veldhuis and De Wied, 1984; Eaves et al., 1985; Sherman and Kalin, 1986, 1987; Ehlers and Chaplin, 1987). These actions can be blocked by central administration of the CRF antagonist, α-helical CRF_{9-41} (Britton et al., 1986c), and are not altered by prior administration of dexamethasone at a dose that blocks pituitary-adrenal activation (Britton et al., 1986a, 1986b). Britton and Indyk (1989) reported that ganglionic blocking drugs can partially attenuate the locomotor effects of i.c.v. CRF, suggesting that some of the observed behaviors following CRF administration are secondary to increased sympathetic activation and the resultant increases in MAP and heart rate. However, it is clear that the locomotor effects of CRF are only partially blocked by these drugs and that other behaviors described in the sections that follow are not the result of increases in peripheral sympathetic activity.

The increases in locomotor activity are not altered by destruction of dopamine nerve terminals (Swerdlow and Koob, 1985) and are blocked only by cataleptic doses of antipsychotic drugs and not at all by the opiate receptor antagonist, naloxone (Koob et al., 1984), suggesting that these effects are independent of both dopamine and opioid systems. In addition, Kalivas et al. (1987) found that ventral tegmentum injections of CRF did produce increases in activity that are not blocked by dopamine receptor antagonists. In an attempt to determine which neurotransmitter systems may be involved, Imaki et al. (1987) suggested that CRF may act via α_2-receptor-mediated noradrenergic activity. Although the pharmacological probes used were clearly neither specific nor sensitive, this observation would be consistent with electrophysiological evidence of CRF activation of noradrenergic cells of the locus ceruleus. Sirinathsinghji and Heavens (1989) implicated GABAergic neurons of the basal ganglia in the locomotor-activating properties of CRF. They reported that, using push-pull cannulas, CRF increased the release of GABA into the caudate nucleus and globus pallidus. It is hypothesized that the resultant inhibitory actions of GABA may play a role in CRF-induced behaviors. Finally, Britton and Indyk (1990) recently observed that CRF and caffeine have similarities in their locomotor activational properties. Thus, both caffeine, which also has anxiogenic actions, and CRF increase activity in nonstressful environments and lower activity in novel, stressful environments. Moreover, caf-

feine can substitute for the anxiogenic effects of novelty in altering the actions of CRF on locomotion. Another behavior that may be related to CRF-induced alterations in locomotor activity is the decrease in sleep observed following central CRF administration (Sherman and Kalin, 1986, 1987). However, EEG changes suggest that decreases in sleep are separate from increased locomotor activation (Ehlers 1986; Ehlers et al., 1986).

2. *Feeding behavior.* The decreased food consumption and associated weight loss that is frequently seen following stress may be mediated by CRF neurons. Following i.c.v. CRF administration, feeding is inhibited in a number of different experimental paradigms. In a novel environment, CRF decreases food intake in food-deprived rats (Britton et al., 1982). Similar findings were also seen in familiar environments (Britton et al., 1982, 1986b). Additionally, Gosnell et al. (1983) observed identical findings in rats with unlimited access to food. In sheep, 50% decreases in food intake could be induced by doses of CRF as low as 60 ng/kg i.c.v. (Ruckebusch and Malbert, 1986). In addition, decreases in food intake were still observed even in rats pretreated with drugs known to increase food consumption. These agents include muscimol, norepinephrine, dynorphin, ethylketocyclazocine, and insulin (Levine et al., 1983; Morley et al., 1985). As with the locomotor actions described above, these anxiogenic actions are not the result of increased HPA axis activity, nor are they the result of CRF-induced illness or lassitude in the rats. As with other CRF-induced behaviors, the decreases in food intake following centrally administered CRF can be blocked by the CRF antagonist (Krahn et al., 1986). Moreover, the CRF antagonist partially reversed the decreases in food intake observed following restraint stress, suggesting that endogenous CRF neurons may play a role in stress-induced anorexia. In an attempt to localize the neuroanatomical site of action of CRF on food intake, Krahn et al. (1988) microinjected CRF into several brain regions. CRF only decreased food intake when microinjected into the PVN but not when injected into the lateral hypothalamus, ventromedial hypothalamus, globus pallidus, or caudate of rats. Interestingly, PVN CRF injections also increased grooming behaviors similarly to that observed following i.c.v. injections. The mechanisms by which these changes in food intake occur are unknown but plausibly could involve monoamine systems within the hypothalamus and/or PVN control of autonomic nervous system activity.

In a recent chronic study in which CRF was continuously infused into the third ventricle for 7 days, CRF reduced body weight and increased sympathetic activity, as measured by brown fat thermogenesis, but did not produce any consistent decreases in food intake (Arase et al., 1988). In a related, but less physiologically relevant experiment, Krahn et al. (1990) administered multiple i.c.v. injections of CRF for 5 consecutive days. They observed that the anorectic effect of CRF decreased substantially over time. Weight gain was slowed only at very high doses. The results of these two studies are difficult to interpret. However, the findings suggest the possibility of tolerance to the anorexic actions of CRF which may have implications for the hypothesized role for CRF in anorexia nervosa (vide infra).

3. *Sexual behavior.* Although it has not been studied in great detail, both male and female sexual behavior is potently inhibited by central administration of CRF in a manner similar to that seen in stressed animals. Sirinathsinghji et al. (1983) initially reported that microinjection of CRF into the arcuate-ventromedial area of the hypothalamus or the mesencephalic central gray area potently suppressed sexual receptivity in female rats. Similar findings were observed in male rats and could be blocked by naloxone infusion or microinfusion of gonadotropin-releasing hormone into the medial preoptic area of the hypothalamus (Sirinathsinghji, 1986, 1987). These findings suggest that CRF may exert its effects through mechanisms that involve activation of opioid pathways which can result in decreased LHRH release. It is not clear whether decreased LHRH release into the portal plexus or into other CNS regions or both is responsible for the decreased sexual behavior. Although speculative, it may be that decreased pituitary LH and follicle-stimulating hormone release may result in a physiological decrease of testicular and ovarian function and that decreased central release of LHRH may diminish sexual desire or pleasure. Nevertheless, CRF neurons may mediate some of the previously described deleterious effects of various stressors on reproductive function.

4. *Animal models of anxiety and depression.* The greatest number of studies examining the actions of i.c.v. CRF deal with the hypothesis that CRF may mediate many of the anxiogenic and fear-related aspects of stress. Data from a wide array of behavioral tests support this hypothesis. These preclinical studies have also been a partial impetus for many clinical studies examining a role for CRF in the pathophysiology of affective and anxiety disorders.

It has been suggested that the effects of CRF examined in a novel environment can provide information about the effects of the peptide in a stressful, aversive environment. CRF, when administered to rats i.c.v., increases the frequency of those behaviors normally expressed in response to a novel environment (Britton et al., 1982). Specifically, CRF increases grooming and freezing behaviors and decreases rearing and the number of approaches to a food pellet. Similar findings have been reported in mice (Berridge and Dunn, 1986, 1987) in which reduced time was spent in contact with novel stimuli and resembled behaviors observed following a period of restraint stress. These effects could be blocked by the CRF antagonist, α-helical CRF_{9-41}, and by naloxone. It should be noted that findings related to the

ability of opiate antagonists to block these actions of CRF have not been replicated nor has any further evidence for an opioidergic role in CRF's actions been reported. In fact, Dunn and Berridge (1987) reported that naloxone failed to alter the increases in noradrenergic metabolism produced by i.c.v. CRF in the prefrontal cortex, hypothalamus, and brainstem of mice.

Another test of anxiogenic activity is the conflict test in which CRF produces a suppression of punished and nonpunished responding. The effects on punished responding are not mediated by pituitary-adrenal activation (Britton et al., 1986a) and are blocked by the CRF antagonist, α-helical CRF_{9-41} (Britton et al., 1986c), and are attenuated by ethanol (Britton and Koob, 1986) and chlordiazepoxide (Britton et al., 1985). These last two results suggest that one potential mechanism for the anxiolytic properties of ethanol and benzodiazepines may be through alterations in brain CRF neurons (Owens et al., 1989, 1991d; Grigoriadis et al., 1989a).

A wide variety of other behavioral tests have also linked CRF to anxiety and other stress-related behaviors including depression. Central administration of CRF potentiates acoustic startle in rats, the effects of which can be blocked by the CRF antagonist (Swerdlow et al., 1989) or the anxiolytic benzodiazepine, chlordiazepoxide (Swerdlow et al., 1986). CRF also decreases social interaction in rats without decreasing locomotion (Dunn and File, 1987). This is considered evidence of anxiogenesis and is reversed by the CRF antagonist. CRF facilitates stress-induced fighting induced by inescapable foot shock (Tazi et al., 1987). Moreover, α-helical CRF_{9-41} blocks this and the fighting induced by higher levels of stress alone. Following exposure to odors associated with fear-induced urination and defecation from a different set of rats, α-helical CRF_{9-41} reduces the level of anxiety and hesitation observed in previously "unstressed" rats (Takahashi et al., 1990). Kalin and colleagues have shown that i.c.v. CRF increases stress-induced freezing behaviors elicited by electric shock (Sherman and Kalin, 1988). These stress-induced freezing behaviors are considered an index of a rat's level of fear. Like many of the behaviors and tests described previously, α-helical CRF_{9-41} blocks the effects of CRF-induced and shock-induced freezing behaviors when given 20, but not 40, minutes prior to foot shock (Kalin et al., 1988; Kalin and Takahashi, 1990). This group has also studied the effects of i.c.v. CRF on primate infants (Kalin et al., 1989). Infant rhesus monkeys emit frequent distress vocalizations ("coos") and alter their activity levels when briefly separated from their mothers. CRF, at doses >10 μg i.c.v., inhibited this behavior without affecting distress vocalizations. Moreover, relatively large doses of CRF administered i.c.v. to adult rhesus monkeys produced symptoms of behavioral despair similar to that induced by long-term separation (Kalin et al., 1983c; Kalin, 1990). The behavioral inhibition is not related to nonspecific sickness or sedation and is likely linked to increased fearfulness. Finally, it has been shown that prior restraint stress enhances locomotor responses to saline injections and the intensity of stereotypic behaviors to amphetamine in rats. These sensitizing effects of prior exposure to stress can be attenuated by i.c.v. administration of the CRF antagonist at the time of the initial stressor (Cole et al., 1990).

We and others have attempted to localize the anatomical site of action for many of these behaviors. Because CRF is known to directly increase noradrenergic cell firing in the locus ceruleus and because of the well-established hypothesis linking noradrenergic neurotransmission with stress, anxiety, and depressive disorders, we examined the behavioral effects of microinfusion of CRF into the locus ceruleus (Butler et al., 1990). Anxiogenic activity was assessed in rats placed in an open field containing a small, darkened compartment that was nonthreatening to the rats. Bilateral infusion of CRF (1 to 100 ng) into the locus ceruleus dose dependently increased the time spent in the darkened compartment and decreased the amount of time spent exploring the outside of the compartment or venturing into the inner squares of the open field, all indices of anxiogenic behavior (fig. 6). In addition, significant increases in the concentration of the norepinephrine metabolite 3,4-dihydroxyphenylglycol was seen in forebrain projection areas. These data suggested that CRF produces its anxiogenic effects, at least in part, by increasing the activity of locus ceruleus noradrenergic neurons. In support of this hypothesis are the findings of Cole and Koob (1988) who observed that the β-adrenergic blocker propranolol blocked the reduction in punished responding produced by i.c.v. CRF administration in the conflict test. In contrast to its effects on CRF, propranolol did not alter the decrease in punished responding produced by the

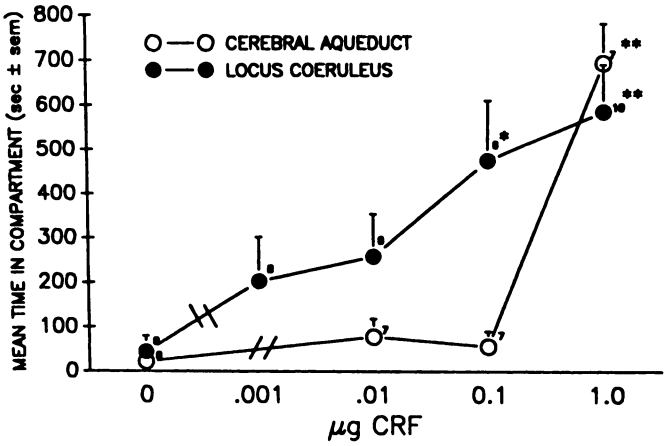

FIG. 6. Time spent withdrawn in a small darkened compartment during a 15-min test period 45 min following infusion of CRF into the cerebral aqueduct or locus ceruleus. The number of animals per group is indicated at each data point. Significantly different from controls using Dunnett's test: $^{*}P < 0.025$, $^{**}P < 0.001$. Reprinted with permission from Butler et al. (1990).

benzodiazepine inverse agonist FG 7142, which is itself anxiogenic. These results are most likely due to postsynaptic β-receptor blockade in forebrain terminal fields of the locus ceruleus and are not thought to be the result of 5-HT antagonism or local anesthetic properties of propranolol. Although central administration of CRF, stress, or administration of the α_2 antagonist, idazoxan, elicited decreases in exploratory behavior, presumably through increased locus ceruleus noradrenergic activity, Berridge and Dunn (1989) suggested that it is noradrenergic activation of CRF release in the CNS, rather than the reverse, that is responsible for the changes observed in stress-induced exploratory behavior. Although it is not inconceivable that certain noradrenergic receptors may regulate the activity of CRF neurons, it does not determine the anatomical site at which CRF produces its effects.

In summary, we believe that the available data support a role for endogenous CRF neurons in increasing locus ceruleus activity as a probable mechanism for some of the observed behaviors and anxiogenic effects produced by stress. As such, this has potentially vital clinical implications in the pathogenesis of a variety of anxiety and depressive disorders. Indeed, many of the effects produced by centrally administered CRF are highly reminiscent of the signs and symptoms of major depression (table 1).

TABLE 1

Similarities between signs and symptoms of major depression (DSM III-R criteria) and the behavioral effects of centrally administered CRF in laboratory animals

DSM III-R major depression	Effects of centrally administered CRF
Depressed mood (irritable mood in children and adolescents) most of day, nearly every day, as indicated either by subjective account or observations by others	Mimics the behavioral despair syndrome observed after maternal separation in rhesus monkey infants
Markedly diminished interest or pleasure in all or almost all activities most of day, nearly every day	Diminishes sexual behavior in male and female rats
Significant weight loss or weight gain when not dieting or decrease or increase in appetite nearly every day	Decreases food consumption in rats
Insomnia or hypersomnia nearly every day	Disrupts normal sleep patterns with concomitant EEG changes
Psychomotor agitation or retardation nearly every day	Increases locomotor activity in a familiar environment and produces "stress-like" alterations in locomotion in a novel environment
Fatigue or loss of energy nearly every day	No data
Feelings of worthlessness or excessive or inappropriate guilt nearly every day	No data
Diminished ability to think or concentrate or indecisiveness nearly every day	No data
Recurrent thoughts of death, recurrent suicidal ideation or a suicide attempt	No data

VII. Electrophysiological Responses to Corticotropin-releasing Factor

A. Single-Unit Recordings

One of the criteria that must be fulfilled for a substance to be considered a neurotransmitter is demonstration of effects of the substance on the electrical activity of neurons. Toward this end, CRF has been used in a number of studies in which the peptide has been shown to alter the electrical activity of various neurons. These electrophysiological studies are generally of three types: (*a*) single-unit recordings from brain slice preparations in vitro, (*b*) single-unit recordings from anesthetized or freely moving rats in vivo, and (*c*) EEG recordings from rats. Although there is a paucity of electrophysiological studies to date, the findings summarized below clearly support a role for CRF as a neurotransmitter in a wide variety of brain areas. For interested readers, in the first comprehensive book published concerning CRF (De Souza and Nemeroff, 1990), three chapters are devoted to electrophysiology.

Several studies of the effects of CRF on the activity of various CNS neurons indicate that the peptide exerts predominantly excitatory actions in the locus ceruleus (fig. 7) (Valentino et al., 1983), cerebral cortex and some regions of the hypothalamus (Eberly et al., 1983), hippocampus (Aldenhoff et al., 1983), and lumbar spinal cord motor neurons (Bell and De Souza, 1989). However, several studies have also indicated predominantly inhibitory actions of CRF in the lateral septum, thalamus, and hypothalamic PVN (Eberly et al., 1983). Many brain regions that contain CRF receptors have not yet had the

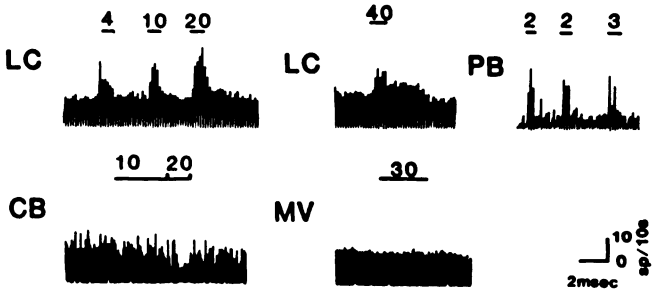

FIG. 7. Effect of pressure application of CRF to different neurons. CRF was directly applied to neurons of the locus ceruleus (LC), cerebellar Purkinje cells (CB), neurons of the mesencephalic nucleus of the trigeminus (MV), or parabrachial cells (PB) for the time period indicated by bars above the records. Numbers above the bars, amount of pressure in pounds per inch that was applied to the CRF-containing micropipette. For CB and MV cells, the pipette used to administer CRF was the same as that used on LC cells in the corresponding records in the top panel. Although CRF was excitatory on both LC cells and this PB neuron, it had no effect on CB or MV cells. Reprinted with permission from Valentino et al. (1983).

effects of CRF determined. At least as far as the hippocampus is concerned, the increased electrical activity appears to result from diminished afterhyperpolarization following bursts of firings (Aldenhoff et al., 1983; Siggins, 1990).

De Souza's group (Sharkey et al., 1989) examined the effects of i.c.v. CRF on glucose utilization, a method used to assess neuronal activity, using [^{14}C]2-deoxyglucose uptake in rat brain. Evidence of increased neuronal activity (evidenced by increased glucose utilization) was observed in the median eminence and lateral hypothalamus consistent with its known hypophysiotropic actions. Moreover, CRF also increased glucose uptake in regions implicated in mediating the stress response, including the locus ceruleus and raphe nucleus. Increases were also observed in several thalamic nuclei, localized areas of the cerebellum, the red nucleus, and inferior olive. Reductions in activity were observed in the prefrontal cortex, nucleus accumbens, and dorsal tegmentum.

The brain region most closely scrutinized electrophysiologically as a target for the actions of CRF is the locus ceruleus. The locus ceruleus contains the noradrenergic perikarya that project >70% of the norepinephrine fibers to the forebrain. As noted previously, CRF-containing nerve terminals have been visualized in both rat and primate locus ceruleus. The origin of these fibers is, however, unknown. Activation of the locus ceruleus and subsequent release of norepinephrine in its projection areas have been implicated in arousal and vigilance responses, as well as in the pathophysiology of anxiety and depression. Briefly, excitation of the locus ceruleus is thought to notify the CNS that incoming sensory information should be attended to. This is a functionally useful adaptive response, particularly in stressful or life-threatening situations (Bloom, 1979; Redmond, 1987). A role for CRF in integrating an organism's response to stress would, therefore, fit nicely with its activational effects in the locus ceruleus.

As alluded to above, i.c.v. CRF increases the spontaneous discharge rate of the locus ceruleus in both anesthetized and unanesthetized rats (Valentino et al., 1983; Valentino and Foote, 1987; Valentino, 1990). Following confirmation of this finding, these investigators studied the action of CRF on evoked discharges from the locus ceruleus. Various sensory and noxious stimuli result in immediate locus ceruleus discharge for approximately 80 to 100 ms after the stimulus. This is followed by a period during which relatively few discharges are observed (postactivational inhibition). I.C.V. CRF, although increasing unstimulated discharge activity as described above, decreases evoked activity and results in more discharges during the postactivational phase (Valentino and Foote, 1988). The overall effect of CRF (1.0 to 3.0 μg) is to decrease the signal to noise ratio between evoked and tonic discharge rates. The activity of locus ceruleus target neurons is generally decreased or unaffected by locus ceruleus activation. This is thought to allow for the actions of other neurotransmitters in the target areas to be increased. Therefore, the signal to noise activity of target neurons is increased when the locus ceruleus fires. One hypothesis suggests that this biases target neurons to a more sensitive deliniation of sensory input. The initial hypothesis that CRF would enhance the response of locus ceruleus neurons to sensory stimuli as an adaptive response to a potentially stressful environment does not fit with the observed data to date. In contrast, CRF, at the doses so far studied, decreases the signal to noise ratio by increasing tonic discharge rates. Perhaps CRF-mediated activation of locus ceruleus activity results in persistent norepinephrine release in target regions and persistent arousal of target neurons to incoming sensory information.

Because central CRF administration increases blood pressure and because activation of the locus ceruleus increases sympathetic outflow, Valentino et al., (1986) sought to determine whether CRF activation of the locus ceruleus was responsible for the previously observed increases in blood pressure. Although CRF did increase locus ceruleus activity, it did not alter blood pressure in anesthetized rats. However, both i.c.v. CRF or the stress of nitroprusside-induced hypotension produced identical effects on locus ceruleus activity (Valentino and Wehby, 1988). Thus, both perturbations increased tonic discharge rates and disrupted locus ceruleus discharge evoked by sensory stimuli such that stimuli were less effective in producing phasic increases in locus ceruleus discharge. The neuronal effects of nitroprusside infusion stress were abolished by prior administration of the CRF antagonist administered i.c.v. but not dexamethasone. Results of this study suggest that the stress produced by nitroprusside-induced hypotension activates locus ceruleus activity via release of CRF from CRF neurons.

B. Electroencephalographic and Convulsive Studies

EEG studies of the effects of CRF were recently comprehensively reviewed by Ehlers (1990). Briefly, i.c.v. CRF results in EEG activation associated with increased behavioral activity and decreased sleep time. In fact, at doses too low to alter locomotor or pituitary-adrenal activity, rats remained awake and vigilant and displayed decreases in slow wave sleep EEG activity compared to saline-injected controls (Ehlers, 1986; Ehlers et al., 1986). In contrast to these findings in rats following i.c.v. CRF administration that are thought to be independent of pituitary-adrenal activation, Holsboer et al. (1988) reported that i.v. CRF administration in humans also decreased slow wave sleep. Whether these changes were the result of large CRF-induced increases in circulating glucocorticoids prior to falling asleep is unclear. In rats, as the CRF dose is increased, paroxysmal EEG activity is observed after a delay of several hours. There is an initial appearance of large spikes in the amygdala which

then spread to the hippocampus and cerebral cortex (Ehlers, 1990); seizure activity then follows. It is after the spread of EEG seizure activity to the cortex that physical signs of seizure activity are observed. Of special interest is the fact that the EEG activity develops in a manner indistinguishable from seizures produced by electrical kindling of the amygdala (Ehlers et al., 1983; Weiss et al., 1986). Weiss et al. (1986) also noted that i.c.v. CRF sensitized the amygdala to electrical kindling, i.e., a reduced number of electrical stimulations were necessary to produce kindling. In contrast, electrically kindled rats were less sensitive to CRF-induced seizures than were saline-treated controls. Moreover, tolerance develops to the seizure-inducing actions of i.c.v. CRF. These results suggest that electrically kindled seizures and CRF-induced seizures, although somewhat similar, are biologically distinct.

Relevant to the reported alterations in polysomnography produced by CRF (vide supra), centrally administered CRF significantly shortens the narcosis induced by pentobarbital (Imaki et al., 1986). These effects are blocked by α-helical CRF_{9-41} and further suggest that CRF may alter normal sleep mechanisms and may even possess intrinsic analeptic properties.

CRF clearly alters the firing rate of various CNS neurons at low doses. Unfortunately, there is a paucity of data regarding the electrophysiological effects of CRF throughout the CNS. The major difficulty we have noticed is the inability, to date, to identify a CRF neuron by its electrical signature. Whereas the effects of various environmental and pharmacological manipulations of individual monoamine-containing neurons are measurable, this is not the case with CRF nor is it with the majority of other putative neuropeptide transmitters.

VIII. Responses of Nonhypophysiotropic Corticotropin-releasing Factor Neurons to Pharmacological and Environmental Perturbation

A. Primary and Secondary Effects of Stress on Nonendocrine Corticotropin-releasing Factor Neurons

1. Effects of stress on corticotropin-releasing factor neurons. Because the data reviewed previously clearly suggest that CRF neurons, apart from, but in concert with, those that regulate pituitary-adrenal axis activity, also mediate the autonomic and behavioral responses of an organism to stress, it is of general scientific interest to study the function and regulation of these nonendocrine CRF neurons. Moreover, because a vast clinical literature indicates that CRF neurons likely are dysfunctional in certain psychiatric illnesses, study of CRF neuronal systems may provide yet untapped and novel treatment modalities.

We (Chappell et al., 1986) previously reported that both acute immobilization stress at 4°C for 3 hours and chronic (14 day) exposure to a series of unpredictable stressors alter the concentration of CRF immunoreactivity in various microdissected brain regions of the rat. Of particular interest is the finding that both acute and chronic stress resulted in a 2-fold increase in the concentrations of CRF in the locus ceruleus, an area known to be electrophysiologically responsive to applied CRF. In addition, chronic stress decreased CRF concentrations in the dorsal vagal complex. The dorsal vagal complex contains various nuclei that are CRF responsive and regulate autonomic function. In a related experiment, Deutch et al. (1987) determined whether mild foot-shock stress altered CRF concentrations in mesotelencephalic dopamine system regions. Because exposure to mild stressors is known to activate mesocortical dopamine neurons, it was plausible to hypothesize that this activation might occur through interactions with CRF neurons. Although stress increased dopamine utilization in the prefrontal cortex and ventral tegmentum, CRF concentrations were unchanged in the dopamine cell body regions of the ventral tegmentum, substantia nigra, and retrorubral field and in the dopamine projection areas of the prefrontal cortex, striatum, and nucleus accumbens. This is in agreement with our earlier findings (Chappell et al., 1986) and suggests that stress-related changes in dopamine and CRF systems are neurochemically distinct.

The protooncogene c-*fos* is expressed in many tissues in response to growth factor stimulation. It appears that induction of the c-*fos* gene may be important in the establishment of long-term functional changes in neurons. Recently, Ceccatelli et al. (1989b) examined the induction of c-*fos* immunoreactivity following exposure of rats to various stressors. As expected, many CRF-containing neurons in the PVN stained positively for c-*fos* immunoreactivity. Of interest to this discussion, c-*fos* immunoreactivity was also induced in cells of the locus ceruleus, the ventrolateral medulla, and the nucleus of the solitary tract in the dorsal vagal complex. Although many of the cells in the locus ceruleus were undoubtedly noradrenergic, a number of cells, such as those in the parabrachial nucleus proximal to the locus ceruleus, are not. Anatomical studies have previously shown that it is possible that those cells in the parabrachial nucleus that contain CRF could easily innervate noradrenergic cells in the adjacent locus ceruleus. Indeed, the increased CRF concentrations observed in the locus ceruleus following stress could possibly emanate from parabrachial CRF neurons, although this has not been determined. Finally, Hauger et al. (1988) studied the effect of a single prolonged immobilization stress (0.25 to 48 hours) on CRF receptor binding. As noted earlier, anterior pituitary CRF receptors were significantly reduced in density (down-regulated); however, CRF receptor density was unaltered in the frontoparietal cortex, olfactory bulb, hippocampus, amygdala, and lateral septum.

2. Secondary effects of stress. Although manipulation

of the HPA axis and its effects on hypothalamic CRF neurons was examined in section III, little mention was made at that time regarding the effects of these manipulations on extrahypothalamic CRF neurons. First, there is a significant circadian variation in the concentration of CRF in a number of extrahypothalamic brain regions (Owens et al., 1990a). These include several cortical, limbic, and brainstem regions. These findings are not surprising because a number of neuronal systems exhibit rhythmical and/or oscillating firing patterns during the course of the day (Llinas, 1989). Moreover, the majority of these diurnal changes are insensitive to circulating glucocorticoids and appear not to be linked to neuroendocrine activity.

Several investigators have examined the effects of exogenous glucocorticoid administration on extrahypothalamic CRF neurons. Beyer et al. (1988) found no effect of high-dose dexamethasone on CRF mRNA in either the central nucleus of the amygdala, BNST, or supraoptic nucleus. Our group (Owens et al., 1990a) recently reported that 7-day corticosterone supplementation to rats with intact adrenal glands was without effect on CRF concentrations in all of the 13 extrahypothalamic brain regions examined, although glucocorticoid supplementation altered the diurnal rhythm of CRF concentrations in several brain regions. Furthermore, Hauger et al. (1987) reported that subchronic (1 to 4 days) administration of corticosterone supplementation did not alter CRF receptor number or affinity in the cortex, hippocampus, amygdala, septum, and olfactory bulb.

In addition to a study of the effects of administered glucocorticoids, the effects of adrenalectomy on extrahypothalamic CRF neurons have also been investigated. It is of paramount importance to note that associated with glucocorticoid deficiency are a number of other effects including hyperactivity of paraventricular CRF neurons. Beyer et al. (1988) reported that adrenalectomy did not alter CRF mRNA in the amygdala, BNST, or supraoptic nucleus as determined by Northern blot analysis, nor did it alter CRF receptor density in any brain region as determined by autoradiography (Wynn et al., 1984). Sawchenko (1987a) reported in one study, but not in another (Swanson et al., 1983), that adrenalectomy increased the number of CRF cell bodies in the perirhinal area of the cortex, the central nucleus of the amygdala, and the BNST. Whether this results from increased synthesis or the expression of CRF in cells previously not expressing CRF is unknown.

In summary, neither adrenalectomy nor increased circulating concentrations of glucocorticoids appears to affect CRF neurons, except for those in the PVN of the hypothalamus.

B. Responses of Extrahypothalamic Corticotropin-releasing Factor Neurons to Pharmacological Manipulation

Because of the data suggesting that extrahypothalamic CRF neurons mediate many of the physiological and behavioral responses of an organism to stress, it is of interest to characterize which neurotransmitter systems interact with these neurons. It should be reiterated at the outset that the paucity of neuroanatomical and physiological information concerning specific extrahypothalamic CRF pathways renders interpretation of drug-induced changes in CRF concentrations difficult. For example, many studies of regional brain CRF concentrations do not distinguish between changes in release, synthesis, storage, or degradation. Nonetheless, any drug-induced changes in CRF concentrations likely denote alterations in the activity of CRF neurons. One would do well to remember that, prior to the advent of advanced neurochemical techniques for measuring the turnover of monoamines, measurement of the concentrations of various neurotransmitters (5-HT, norepinephrine, dopamine, etc.) in discrete brain regions following experimental perturbation were considered important for gauging alterations in the activity of monoamine-containing neurons.

While studying serotonergic regulation of the HPA axis, we observed that chronic (21 days) administration of the 5-HT_{1A} agonists, 8-hydroxydipropylaminotetralin and ipsapirone, increased CRF concentrations in those areas preferentially enriched with 5-HT_{1A} receptors (i.e., hippocampus, entorhinal cortex, and piriform cortex). Another area with somewhat lower 5-HT_{1A} receptor densities, the amygdala, also exhibited increased CRF concentrations. During the course of similar studies of the 5-HT_2 receptor subtype, neither acute nor chronic administration of the potent 5-HT_2 and 5-HT_{1C} agonist, (±)-1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane, altered CRF concentrations in any of 13 brain regions studied, including the median eminence, hypothalamus, prefrontal cortex, frontal/parietal cortex, cingulate cortex, septum, BNST, piriform cortex, amygdala, hippocampus, raphe nuclei, locus ceruleus, and cerebellum (Owens et al., 1990b, 1991a).

Fenfluramine is an amphetamine derivative that is used as a weight-reducing agent in the treatment of obesity. It has been postulated that fenfluramine increases central serotonergic neurotransmission resulting in decreased food intake and altered autonomic outflow which, in turn, increases metabolism (Rowland and Carlton, 1986). As we have noted previously, central administration of CRF produces similar effects on weight and autonomic activity. We (Appel et al., 1991) observed in rats that chronic fenfluramine treatment resulted in dose-dependent decreases in hypothalamic CRF concentrations and reciprocal increases in plasma corticosterone concentrations. These changes in hypothalamic CRF and plasma corticosterone correlated with brain fenfluramine concentrations. In addition to these changes in hypophysiotropic function, fenfluramine treatment significantly increased hippocampal, midbrain, and spinal cord CRF concentrations, whereas levels in the cerebral

cortex, caudate/putamen, thalamus, pons/medulla, and cerebellum were unaffected. Because serotonin is a potent CRF secretagogue, it is hypothesized that the weight-reducing effects of fenfluramine may be mediated, in part, through altered CRF secretion.

While studying regulation of HPA axis function, other groups have also examined the effects of various compounds on CRF concentrations outside the hypothalamus. Haas and George (1987) did not observe any changes in CRF immunoreactivity in the frontal cortex, hippocampus, medulla-pons, midbrain-thalamus, and cerebellum following i.c.v. doses of neuropeptide Y that increased plasma ACTH concentrations (i.e., increased median eminence CRF release). Similarly, Tizabi et al. (1985) did not find any alterations in CRF concentrations in the dorsal or ventral BNST, central nucleus of the amygdala, or a variety of hypothalamic nuclei, excluding the PVN, following the administration of either the monoamine-depleting agent, reserpine, or the tricyclic antidepressant, desipramine, an norepinephrine reuptake inhibitor.

Some of the most interesting pharmacological studies to date pertain to the possibility that clinically efficacious antidepressants and/or anxiolytics may interact with central CRF neurons. Because preclinical and clinical studies suggest that CRF neurons of hypothalamic and extrahypothalamic origin may be involved in the pathophysiology of anxiety and depressive disorders, we examined the actions of a single acute injection of imipramine, alprazolam, or adinazolam on CRF concentrations in 18 rat brain regions (Owens et al., 1989). Imipramine is a prototypical tricyclic antidepressant that, along with its active metabolite, desipramine, inhibits both 5-HT and norepinephrine reuptake. Alprazolam and its dimethylamino analog, adinazolam, are atypical triazolobenzodiazepines (Hester et al., 1971, 1980; Hester and Voigtlander, 1979; Lahti et al., 1983) that possess both anxiolytic properties typical of benzodiazepines and have also been reported to possess clinical antidepressant and antipanic activity unique to these benzodiazepines (Amsterdam et al., 1986; Dunner et al., 1987; Fawcett et al., 1987; Feighner et al., 1983; Rickels et al., 1987). One hour following an acute injection, CRF concentrations were decreased in the locus ceruleus, amygdala, piriform cortex, and cingulate cortex in both alprazolam- and adinazolam-treated rats. Imipramine treatment was without effect on CRF concentrations in all brain regions studied. In a second study, the time course of these effects were studied in which alprazolam decreased CRF concentrations in the locus ceruleus 30 to 180 minutes postinjection (Owens et al., 1991d). The 180-minute time course corresponds very closely with the bioavailability and metabolism of alprazolam. Moreover, CRF concentrations in the locus ceruleus remained decreased during the course of 13 days of continuous administration, which indicates a lack of tolerance to this effect of the drug. In addition, CRF concentrations in the dorsal vagal complex were decreased 24 hours following abrupt alprazolam withdrawal. These changes during drug withdrawal are, not surprisingly, similar to those observed following stress (Chappell et al., 1986).

Of particular interest is the finding of decreased CRF concentrations in the locus ceruleus following acute or chronic alprazolam treatment, which is opposite to that observed following exposure to either acute or chronic stress (fig. 8). As noted previously, CRF has been shown to increase the firing rate of noradrenergic locus ceruleus neurons. Thus, CRF may intrinsically modulate the activity of the major CNS noradrenergic cell body population, one that has long been implicated in the pathophysiology of stress, anxiety, and depression (Bloom, 1979; Klein, 1987; Redmond, 1987). It is unclear at present whether classical benzodiazepines (diazepam, chlordiazepoxide, etc.) alter regional CRF immunoreactivity or whether treatment with anxiolytics or antidepressants abolishes stress-induced changes in CRF neurons.

A complementary study from De Souza's laboratory (Grigoriadis et al., 1989a) examined the effects of chronic tricyclic antidepressant or benzodiazepine treatment on CRF receptor kinetics in a variety of rat brain regions. They reported that, although there were trends toward increased CRF binding in the brain stem, cerebellum, hypothalamus, and frontal cortex following imipramine treatment, the changes were only statistically significant in the brainstem. CRF receptor-binding density was significantly decreased in the frontal cortex and hippocampus following chronic treatment with either alprazolam, adinazolam, or diazepam. The authors accurately point out that the relatively small effects of these drugs on CRF receptor binding do not necessarily imply a lack of effect on CRF neurons but rather may reflect their

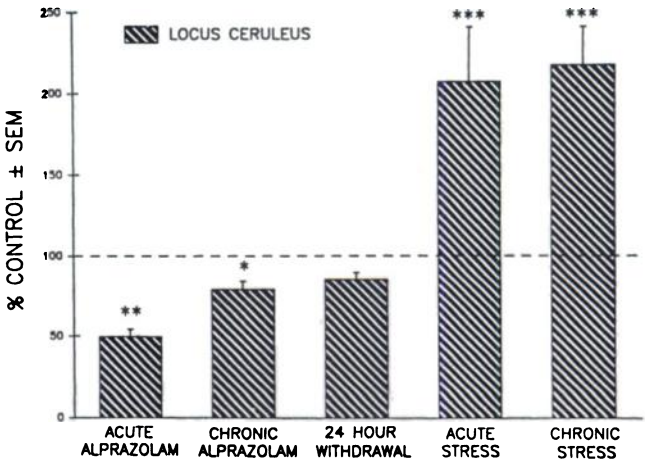

FIG. 8. Effects of alprazolam and stress on CRF concentrations in the locus ceruleus. The anxiolytic/antidepressant triazolobenzodiazepine, alprazolam, produces effects in the locus ceruleus opposite to those of acute or chronic stress but not alprazolam withdrawal. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with controls. Data compiled from Chappell et al. (1986) and Owens et al. (1989, 1991d).

relatively small effects on CRF synthesis and release in the basal state. Because of the inability at present to directly measure CRF release in extrahypothalamic brain regions, experiments aimed at measuring CRF concentrations, CRF receptors, and CRF mRNA during basal states and during exposure to stress or in animal models that mimic anxious or depressive human states are sorely needed.

Based upon their previous electrophysiological experiments, Valentino et al. (1990) examined the effects of acute and chronic antidepressant treatment on noradrenergic neurons of the locus ceruleus. They found that chronic (21 days) treatment with desipramine attenuated locus ceruleus activation following hypotensive stress, an effect thought to require CRF release (Valentino and Wehby, 1988). Locus ceruleus activation by i.c.v. CRF was not altered in desipramine-treated rats. In contrast to the effects of desipramine, chronic administration of the serotonergic reuptake inhibitor, sertraline, did not alter locus ceruleus activity by either stress or i.c.v. CRF. However, the response of the cells to repeated sciatic nerve stimulation was opposite to that produced by CRF. They suggested from these data that certain antidepressants may interfere with the effects of CRF in the locus ceruleus as a possible mechanism of action. This may occur via noradrenergic reuptake inhibitors attenuating stress-induced locus ceruleus activation, an action that requires CRF release, possibly by inhibiting local CRF release. In contrast to desipramine, sertraline and other serotonin reuptake inhibitors may functionally antagonize the actions of CRF by producing opposing effects on the locus ceruleus. Although their conclusions are based on limited data, the findings clearly do not provide any evidence to preclude the hypothesis that the CRF innervation of the locus ceruleus is involved in the therapeutic actions of antidepressants.

Clinical studies (section IX) have implicated both neuronal degeneration of CRF and cholinergic neurons in Alzheimer's disease. To mimic the purported cholinergic deficit observed in Alzheimer's disease, De Souza and Battaglia (1986) examined the effects of chronic muscarinic receptor blockade (by continuous atropine infusion) on CRF receptor kinetics in rat brain. They found that CRF receptors increased in the frontal/parietal cortex, a finding similar to that in Alzheimer's disease reported by De Souza et al. (1986), but receptor number did not change in the olfactory bulb, cerebellum, striatum, or hippocampus. However, the increases in CRF receptors in the cortex do not appear to be functional as assessed by second messenger generation. These increases are thought to represent either spare receptors, alternate second messenger systems (i.e., they are not coupled to adenylate cyclase), or incomplete coupling to G proteins. In a subsequent experiment in which CRF receptors were examined in dissociated fetal rat cortex cell cultures, Kapcala and De Souza (1988) found that, although CRF incubation with the cells decreased the concentration of its own receptor by 36%, atropine did not alter the number of CRF receptors. Again the lack of data regarding CRF anatomical pathways and synaptic interactions with other transmitter systems renders any firm hypothesis regarding CRF involvement in the etiology of Alzheimer's disease, or its interactions with the cholinergic system, tenuous.

C. Miscellaneous Changes in Extrahypothalamic Corticotropin-releasing Factor Neurons

We studied rats from the FSL which have been bred for differences in sensitivity to cholinergic agonists (Owens et al., 1991c). The FSL rats have been proposed as a genetic animal model of depression because, like some depressed patients, they are more sensitive to cholinergic agonists, are less active, exhibit higher rapid eye movement density, and "respond" to classical antidepressants. In addition, these rats exhibit increased concentrations of muscarinic receptors in the striatum and hippocampus. We observed in two studies that FSL rats had decreased concentrations of CRF in the locus ceruleus, prefrontal cortex, and median eminence and decreased CRF receptors in the anterior pituitary.

Hashimoto et al. (1985) measured the regional brain CRF concentration of SHR and reported that CRF immunoreactivity was significantly reduced in midbrain, medulla, cortex, and hypothalamus. It was suggested that the CRF differences may be responsible for the abnormalities in the pituitary-adrenal axis, autonomic responses, and behaviors of SHR, although the large size of the brain regions studied severely limits any conclusions that can be drawn. Again, the lack of anatomical and physiological knowledge surrounding extrahypothalamic CRF neurons hinders the interpretation of these data.

Considerably more useful and increasingly more often utilized is the measurement of CRF mRNA concentrations following various experimental paradigms with the belief that increases or decreases in mRNA levels reflect increases or decreases in the neuronal rate of peptide synthesis due to changes in secretion rate. As mentioned previously, these studies have proven particularly useful in studying PVN CRF neuronal regulation. The only study to date examining extrahypothalamic CRF neurons is that of Barmack and Young (1990) who placed rabbits in a rotating drum which essentially stimulated the animals field of vision in a constant left-to-right or right-to-left manner. Left-to-right stimulation of the left eye increased evoked activity in the contralateral inferior olive neurons in the right caudal cap. The authors found that CRF mRNA levels increased 4- to 7-fold after 48 hours of optokinetic (rotation) stimulation and by more than 10-fold after 144 hours. These increases then declined to control values 30 hours following completion of the experiment. These observations specifically implicate CRF in visual pathways involving the olivocerebellar

system which may predominantly involve fine control of eye movement. Of greater significance may be the fact that this study validates the use mRNA quantitation as a tool to study the effects of various experimental perturbations on changes in CRF neuronal activity.

At the present time, it would appear that the most accurate methods used to study changes in CRF neuronal function require the combined measurement of (*a*) CRF concentrations in discrete brain regions, (*b*) CRF receptor number and affinity and CRF-stimulated second messenger generation in the same brain regions, and (*c*) CRF mRNA concentrations. Note that CRF mRNA measurement is most useful when the anatomical pathway from cell body (location of mRNA) to terminal fields (location of CRF peptide in vesicles and CRF receptors at the synapse) is known. Future techniques being developed include microdialysis to measure CRF release in vivo, electrophysiological identification of CRF neurons, and peptidase inhibitors to alter degradation of CRF following secretion into the synaptic cleft.

IX. Clinical Studies implicating a Role for Corticotropin-releasing Factor Hypersecretion in the Pathophysiology of Psychiatric Illness

A. Major Depression and Anxiety Disorders

1. Corticotropin-releasing factor stimulation test. Preclinical studies clearly support a prominent role for CRF neurons of both hypothalamic and extrahypothalamic origin in orchestrating an organism's response to stress. Moreover, stress has been implicated in precipitating depressive episodes in genetically vulnerable individuals (Anisman and Zacharko, 1982). Therefore, the possibility exists that CRF neuronal dysregulation could contribute to human illness. With this in mind, one of the most reproducible findings in biological psychiatry is the hyperactivity of the HPA axis as evidenced by hypercortisolemia and dexamethasone nonsuppression in patients with endogenous depression. A number of investigators have attempted to study the mechanism(s) that result in this hypercortisolemia. Although there is some evidence for enhanced cortisol responses to ACTH in depression, most evidence points to a primary alteration in the CNS that leads to hyperactivity of the HPA axis, with CRF neuronal hyperactivity the most plausible candidate.

The most widely studied, and perhaps least direct, method to elucidate the pathophysiology of the HPA axis is the measurement of the neuroendocrine response to exogenously administered CRF. Both rat/human CRF and oCRF produce robust ACTH, β-endorphin, β-lipotropin, and cortisol responses following i.v. or subcutaneous administration in normal subjects (Hermus et al., 1984; DeBold et al., 1985; Watson et al., 1986). Although there is a diminished sensitivity of ACTH secretion to negative feedback regulation by glucocorticoids in older men, the ACTH response to exogenous CRF does not decline with age (Pavlov et al., 1986).

Pharmacokinetic studies have shown that, when administered i.v., CRF has an apparent volume of distribution equal to that of plasma volume and a final elimination half-life ranging from 45 to 180 minutes (Schulte et al., 1982, 1984; Tsukada et al., 1984). Schulte et al. (1985) studied pituitary desensitization to prolonged CRF infusion and found that following infusion of CRF (1 μg/kg/hour) for 24 hours, a bolus dose of 1 μg/kg failed to produce any response immediately afterward. Using a less sound experimental design, Désir et al. (1986) still found a vigorous ACTH and cortisol response following repeated CRF injections (0.3 to 0.4 μg/kg every 4 hours for 72 hours). Of interest, both groups still found a persistent diurnal variation in plasma ACTH concentrations in the presence of a continuous CRF infusion. This suggests that the circadian periodicity of ACTH cannot be explained solely by median eminence CRF secretion.

Administering CRF i.v. in what is commonly termed the CRF stimulation test, a number of investigators have observed a blunted ACTH response with a normal total cortisol response in patients with major depression compared with controls (Holsboer et al., 1984a,b; Gold and Chrousos, 1985; Gold et al., 1984, 1986b; Amsterdam et al., 1987; Lesch et al., 1988b; Kathol et al., 1989; Krishnan et al., 1991). Rupprecht et al. (1989) observed blunted ACTH, but normal β-endorphin, responses in depressed individuals. In contrast to these findings, Young et al. (1990) observed blunted β-endorphin/β-lipotropin responses to relatively low doses of CRF in depressed patients. Their blunted responses appear to represent a shortened length of pituitary stimulation from CRF rather than a decreased initial release of these POMC-derived peptides.

In a single, recent study, Amsterdam et al. (1988) found that depressed, patients exhibited a normal ACTH response to CRF following clinical recovery suggesting that the blunted ACTH response, like dexamethasone nonsuppression, may be a "state" marker for depression.

In contrast to all of these findings, Leake et al. (1989) found no evidence for a blunted ACTH response in depressed individuals. Moreover, when ambient cortisol concentrations in depressed and normal individuals were neutralized (i.e., equally suppressed to low concentrations) using the steroid synthesis inhibitor metyrapone, an augmented response to CRF was observed in the depressed population (Lisansky et al., 1989).

The CRF stimulation test has also been used to study other pituitary hormone responses. For example, whereas controls exhibit no growth hormone response to CRF, depressed patients exhibit a significant net increase in growth hormone secretion (Lesch et al., 1988a). In addition, whereas control subjects exhibit a slight increase in plasma δ sleep-inducing peptide concentrations, depressed patients show a marked decrease following CRF administration (Lesch et al., 1988b).

The CRF stimulation test has been applied to patients with other psychiatric diagnoses. Smith et al. (1989) reported that CRF administration (1 μg/kg i.v.) produces a blunted ACTH response in patients with posttraumatic stress disorder, some of whom also fulfilled the criteria for major depression defined in *Diagnostic and Statistical Manual of Mental Disorders*, ed. 3. Blunted ACTH responses are also observed following short-term (12 to 72 hours) abstinence in chronic alcoholics (Heuser et al., 1988). Similar results were observed by Adinoff et al. (1990) at 1 and 3 weeks of abstinence but not at longer periods of time. Roy-Byrne et al. (1986) reported blunted ACTH responses to administered CRF in patients with panic disorder. In contrast to the report of Roy-Byrne et al. (1986), Rapaport et al. (1989) observed no differences in the ACTH response to low-dose CRF (0.03 μg/kg).

Of interest is the work of Sapolsky (1989) who administered the CRF stimulation test to wild baboons living freely in East Africa. He previously showed that, in baboons that live in a stable dominance hierarchy, socially subordinate males are hypercortisolemic relative to dominant animals. These subordinate males that are under long-term stress, both social and physical, show blunted ACTH responses following CRF administration. It is unclear whether the stressors experienced by subordinate animals produce chronic CRF hypersecretion that then results in a blunted ACTH response to CRF (vide infra). Alternatively, the author previously showed that those animals that become subordinates on the social hierarchical scale may possess somewhat dysfunctional or maladaptive sex and stress hormone axes to begin with. Nonetheless, his studies suggest that inappropriate HPA axis activity (CRF neuronal activity?) is associated with poor outcome (i.e., decreased ability to find a mate and continue the gene pool) in a natural habitat. These findings have implications for human disorders such as depression, generalized anxiety disorder, and chronic stress syndromes.

Several hypotheses have been introduced to explain the mechanism of this blunted ACTH response to administered CRF. One hypothesis is that the blunted ACTH responses result primarily from decreased pituitary responsiveness to CRF in the face of long-term hypersecretion of CRF from the median eminence and the resultant down-regulation of pituitary CRF receptors. The data, when scrutinized, support this hypothesis more strongly than an alternative hypothesis suggesting altered sensitivity of the pituitary to glucocorticoid negative feedback, although this may play some role. This has not yet been tested directly by measurement of anterior pituitary CRF receptors or CRF mRNA in the PVN in postmortem tissue from depressed patients. In fact, two recent studies (von Bardeleben and Holsboer, 1989; Krishnan et al., 1991) indicated that following dexamethasone pretreatment depressed patients exhibit greater increases in plasma ACTH and cortisol concentrations than normal persons following CRF administration (i.e., depressed patients escape from dexamethasone suppression). These results further suggest that the blunted ACTH responses to exogenously administered CRF observed in depressed patients are not due to hypercortisolemic negative feedback. Although most studies are concordant, differences exist and are likely the result of patient population (depression severity, misdiagnosis, etc.) and/or ACTH assay methodological differences. In any case, further studies are needed. However, no matter the number of studies, these neuroendocrine studies purported to be a "window of the brain" will always be a secondary measure of CNS activity.

2. Cerebrospinal fluid corticotropin-releasing factor concentrations. To directly test the hypothesis that the synaptic availability of CRF is increased in depression, and possibly other psychiatric illnesses, we and others have measured the concentration of CRF in CSF. Post et al. (1982) showed that, for neuropeptides found in both CSF and plasma, there is a marked CSF-plasma dissociation indicating that neuropeptides are secreted directly into CSF from brain tissue and that CSF neuropeptide concentrations are not derived from the systemic circulation due to the presence of the blood-brain barrier. Thus, plasma CRF concentrations likely represent secretion from hypothalamic CRF neurons terminating in the median eminence and from other peripheral sources, whereas CSF CRF concentrations likely reflect the activity of extrahypothalamic CRF neurons.

Following the first report demonstrating the existence of CRF in human CSF (Suda et al., 1983), a number of basic and clinical studies were conducted. CRF was found to be cleared from CSF in adult rhesus monkeys more rapidly than can be accounted for by bulk flow, suggesting that a transport system exists for the active removal of CRF from CSF; however a mechanism for how this would function has not been described (Oldfield et al., 1985). Further evidence that CSF CRF concentrations are derived from nonhypophysiotropic CRF have been provided from studies in which CSF CRF concentrations were repeatedly measured during the course of the day. Garrick et al. (1987) reported that CSF CRF concentrations were not positively correlated with CSF cortisol concentrations in rhesus monkeys, which directly reflect plasma cortisol concentrations. In fact, CSF CRF exhibits peak concentrations that precede those of CSF cortisol by approximately 14 hours (almost inversely related); it is similarly dysynchronous with plasma ACTH concentrations. Kalin et al. (1987) also reported that CSF CRF concentrations in rhesus monkeys are not entrained with pituitary-adrenal activity. It is evident that CSF CRF is not merely a reflection of median eminence CRF release. Although the source of CSF CRF remains unknown, CRF neurons in cortical, limbic, and brainstem regions are all in close proximity to the ven-

tricular system and may all contribute to the CSF CRF pool.

In a developmental study of pediatric patients, CSF CRF concentrations were highest in the immediate postnatal period (Hedner et al., 1989). CSF CRF concentrations decreased significantly during the first postnatal year compared with the immediate postnatal period and by 1 year of age were similar to that observed in adults.

We showed in a series of studies that CRF concentrations are significantly elevated in the CSF of drug-free patients with major depression (Nemeroff et al., 1984; Arató et al., 1986; Banki et al., 1987; France et al., 1988; Widerlöv et al., 1988; Risch et al., 1991) or following completion of suicide (Arató et al., 1989). In our first study, we measured the CSF concentration of CRF in 10 normal controls, 23 depressed patients, 11 schizophrenics, and 29 demented patients. The CSF concentration of CRF was elevated in the depressed patients compared to all of the other groups; 11 of the 23 depressed patients had CSF CRF concentrations higher than the highest normal controls (Nemeroff et al., 1984). In our second study, we measured the CSF concentration of CRF in 54 depressed patients, 138 neurological controls, 23 schizophrenic patients, and 6 manic patients (fig. 9). The depressed patients exhibited a marked 2-fold elevation in CSF CRF concentrations (Banki et al., 1987). In a third study, we found that patients with major depression had higher CSF CRF levels than patients with chronic pain (France et al., 1988). Our fourth study, conducted in Budapest, also indicated increased CSF CRF concentrations in depressed patients (Arató et al., 1986). Finally, a fifth study was conducted in which we measured CSF CRF concentrations collected postmortem from the intracisternal space in depressed suicide victims and "sudden death" controls. Again, CSF CRF concentrations were elevated in the depressed group (Arató et al., 1989). Although as a total group, Roy et al. (1987) did not find any difference between depressed patients and controls, those patients who were dexamethasone nonsuppressors exhibited higher concentrations of CSF CRF than depressed dexamethasone suppressors.

To determine whether elevated CSF CRF concentrations in depression represent a state or trait marker, we (Nemeroff et al., 1991) measured CSF CRF concentrations in depressed patients before and after a course of ECT. Before ECT, depressed patients exhibited elevated CSF CRF concentrations compared with controls. Twenty-four hours after their final ECT, a significant decrease in CSF CRF concentrations was observed. This finding indicates that CSF CRF concentrations, like hypercortisolemia, represent a state, rather than a trait, marker. Moreover, these findings are not discordant with the hypothesis that CRF neuronal hyperactivity contributes to the signs and symptoms of major depression.

3. Corticotropin-releasing factor receptors in postmortem tissue. Depression is a major determinant of suicide

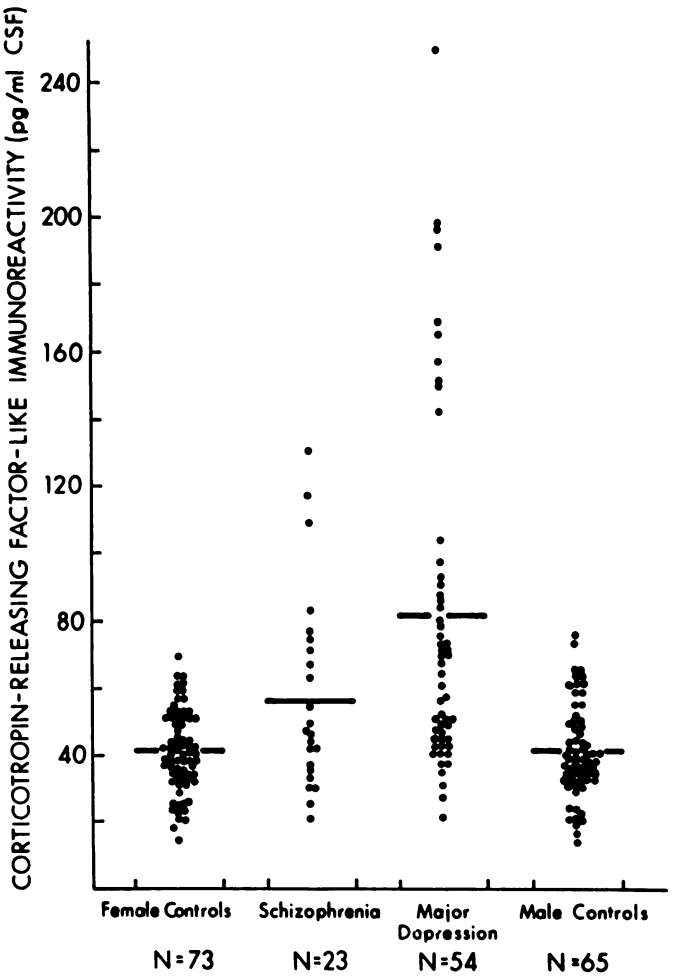

FIG. 9. CSF CRF-like immunoreactivity in patients with schizophrenia [as defined in *Diagnostic and Statistical Manual of Mental Disorders*, ed. 3 (DSM-III)], patients with DSM-III major depression, and control subjects with various peripheral neurological diseases. Patients with DSM-III major depression exhibited a markedly higher (almost 2-fold) CSF CRF concentration than the control subjects (Newman-Keuls test, $P < 0.001$). The mean CSF CRF concentration in the depressed group was also significantly higher than that of the schizophrenic group (Newman-Keuls test, $P < 0.05$). Six (26%) of the 23 schizophrenic patients and 24 (44%) of the 54 depressed patients had higher CSF CRF concentrations than the highest value among the sex-matched control subjects. Reprinted with permission from Banki et al. (1987).

(Van Praag, 1985) and >50% of completed suicides are accomplished by patients with major depression. We therefore hypothesized that, if CRF is chronically hypersecreted in major depression, a reduced (down-regulated) number of CRF receptors may be present in the brain tissue of suicide victims. To test this hypothesis, we measured the number and affinity of CRF receptors in the frontal cortex of 26 suicide victims and 28 control subjects (Nemeroff et al., 1988). The suicide group exhibited a 23% reduction in the number of CRF-binding sites compared with controls (fig. 10). This finding further suggests that CRF is hypersecreted in the CNS of patients with major depression. Clearly, further studies examining CRF receptors and CRF mRNA in other brain regions are of great interest.

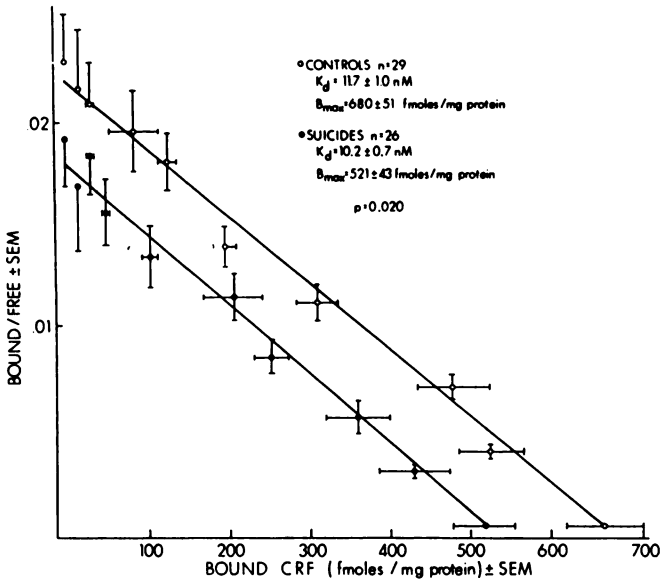

FIG. 10. Composite Scatchard analysis utilizing each individual frontal cortical sample. Points, means (bars, ±SE) for all samples from suicides (●) and controls (○). Reprinted with permission from Nemeroff et al. (1988).

B. Anorexia Nervosa

1. Endocrine and cerebrospinal fluid studies. Many patients with anorexia nervosa share the hypercortisolemia observed in the majority of patients with major depression and also exhibit depressive symptoms. These findings, together with preclinical studies, suggest that CRF hypersecretion may also play a role in the pathophysiology of this disease. For example, there is evidence that fenfluramine-induced anorexia in laboratory animals may be secondary to increased CRF activity (Appel et al., 1991). As observed in major depression, underweight patients with anorexia nervosa exhibit blunted ACTH responses after i.v. CRF administration (Gold et al., 1986a; Hotta et al., 1986). Gold and colleagues (1986a) reported that the patients' responses to CRF normalized 6 months, but not immediately, after correction of weight loss. In contrast, Hotta et al. (1986) reported that ACTH responses to CRF normalized immediately following weight gain.

In CSF studies, both Hotta et al. (1986) and Kaye et al. (1987) found elevated CSF CRF concentrations in these patients. As with the ECT study described in the previous section, the increase in CSF CRF concentrations appears to be a state-dependent marker because Kaye and colleagues reported both normalized pituitary-adrenal function and CSF CRF concentrations after weight recovery. Moreover, these authors reported that CSF CRF concentrations were significantly correlated with depression severity ratings in the weight-corrected patients.

C. Alzheimer's Disease

1. Alterations in regional brain corticotropin-releasing factor concentrations and receptors. Alzheimer's disease is a neurodegenerative disease characterized by a progressively worsening dementia, pathologically by the appearance of neurofibrillary tangles and plaques in particular areas of the CNS, and biochemically by degeneration of cholinergic neurons in the substantia innominata, as well as a number of other neurotransmitter alterations (McDonald and Nemeroff, 1991). Bissette et al. (1985) reported a marked reduction in CRF concentrations in the frontal and temporal cortex (≈50%) as well as in the caudate nucleus (≈70%) from postmortem Alzheimer's disease brain tissue. These findings were confirmed and extended by De Souza et al. (1986) who observed decreased CRF concentrations in the frontal, temporal, and occipital cortex in patients with Alzheimer's disease and reciprocal increases (up-regulation) of CRF receptors (fig. 11). In addition, immunocytochemical studies have demonstrated abnormal CRF-immunoreactive neurons in the amygdala, some of which contained amyloid plaques (Powers et al., 1987).

2. Cerebrospinal fluid studies. Along with the reduction of cortical CRF concentrations, CSF concentrations of CRF have been reported to be decreased in severe end-stage Alzheimer's disease. Decreases in CRF concentrations have been reported without any signs of pituitary-

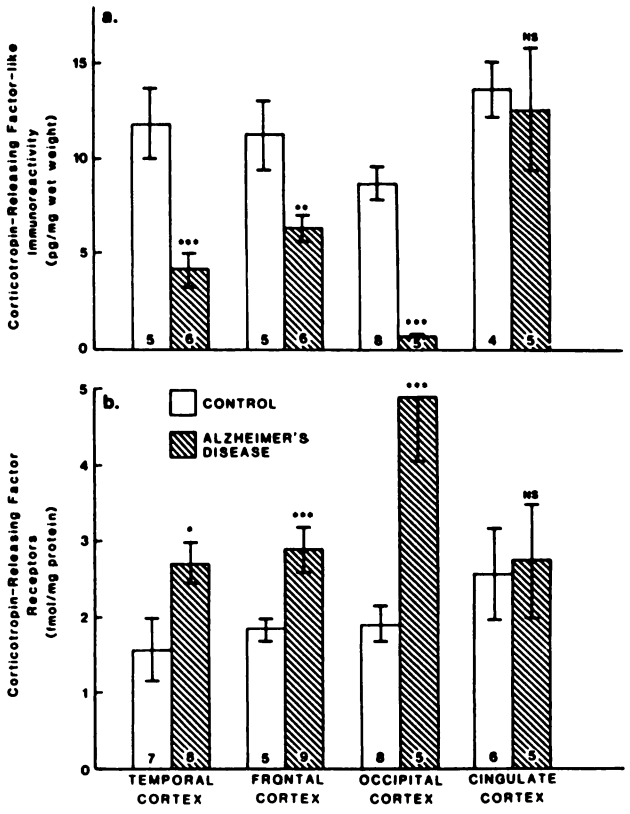

FIG. 11. CRF-like immunoreactivity (a) and CRF receptor binding (b) in discrete regions of the cerebral cortex of patients with Alzheimer's disease and controls. Columns, means (bars, ±SE). The number of patients in each group is given at the bottom of each histogram. Data were analyzed for differences using a Student's *t* test. $^{*}P < 0.05$, $^{**}P < 0.025$, $^{***}P < 0.005$ compared with control group. NS, not significant. Reprinted with permission from De Souza et al. (1986).

adrenal dysfunction (May et al., 1987) or without direct correlation with disease severity or decreased cognitive functioning (Mouradian et al., 1986). In contrast to the findings of Mouradian et al. (1986), Pomara et al. (1989) found a significant correlation between global neuropsychological impairment ratings and lower CSF CRF concentrations without significant overall decreases in CSF CRF concentrations in the Alzheimer's group as a whole, although the patients studied had only mild to moderate dementia.

D. Other Psychiatric and Neurological Illnesses

Reductions in CRF concentrations have been observed in the cerebral cortex from patients with Parkinson's disease and progressive supranuclear palsy (Whitehouse et al., 1987). As in Alzheimer's disease, CRF concentrations are decreased in the caudate/putamen in Huntington's disease (De Souza et al., 1987). However, unlike in Alzheimer's disease, CRF concentrations were unchanged in various cortical regions. Finally, CSF CRF concentrations have been reported to be decreased approximately 50% in patients with amyotrophic lateral sclerosis (Klimek et al., 1986).

X. Conclusions and Future Directions

From the considerable evidence described in this monograph, it is clear that CRF integrates the overall physiological and behavioral responses of an organism to stress. The neuroendocrine response to stress is primarily controlled by CRF neurons whose perikarya originate in the PVN of the hypothalamus, although vasopressinergic neurons likely contribute to some extent. It is not known which CRF neurons are responsible for the behavioral and autonomic responses accompanying stress. However, CRF neurons in the cerebral cortex and limbic system and CRF neurons of the medulla and pons are logical candidates for regulation of many of the behavioral and autonomic responses, respectively.

Because of the vast array of functions over which CRF may exert a modulatory influence, it is plausible, and the clinical evidence quite convincing, that inappropriate regulation of CRF neurons may contribute to human illness. To date, CRF dysregulation appears to occur in a number of psychiatric disorders, including major depression, posttraumatic stress disorder, and panic disorder.

Although the number of papers dealing with the study of various aspects of CRF neurobiology has increased exponentially in the past several years, much has yet to be learned regarding the detailed description of the mechanisms by which CRF alters behavior and autonomic activity. As additional neuroanatomical studies are undertaken, the greater understanding of CRF neurocircuitry gained will undoubtedly result in elucidation of which specific CRF neuronal populations mediate the various actions of CRF. As more is learned about the regulatory elements flanking the CRF gene in discrete brain regions (there is no a priori reason that the CRF gene should be under the same regulation throughout the brain), there will be better understanding of normal CRF gene expression and function. Indeed, manipulation of the regulatory elements may provide an avenue for future treatment strategies aimed at altering CRF gene expression.

Although there is limited evidence at present, it is plausible that some anxiolytic and antidepressant drugs may exert a portion of their efficacy through alterations in CRF neuronal functioning. Although tricyclic antidepressants alter serotonergic and noradrenergic neurotransmission acutely and chronically, and benzodiazepine anxiolytics potentiate GABA-stimulated Cl^- flux, it is still unclear how these actions result in their clinical efficacy. As we have noted previously, future techniques including the use of microdialysis probes to measure CRF release in vivo, the possibility of electrophysiological identification of CRF neurons, and the use of specific peptidases and peptidase inhibitors, in addition to the present techniques that can be used to measure CRF peptide, CRF receptors, and CRF mRNA, will greatly increase our ability to understand CRF neurobiology. Moreover, it is important to determine whether the recently described CRF-binding protein actively controls the synaptic availability of CRF.

Clearly, one of the most exciting areas of research is the possibility of using a CRF antagonist for the treatment of depression and/or anxiety. Although computer-aided drug design of such a large peptide presents problems, the recent cloning of the CRF-binding protein and the eagerly awaited cloning of the CRF receptor will greatly aid in the elucidation of the active portion of the peptide or the active site on the receptor. These discoveries may lead to the rational design of lipophillic drugs which may possess clinical utility. Moreover, these compounds could lead to useful ligands for positron emission tomography or single photon emission computerized tomography neuroimaging studies. Another possible means of producing potentially useful compounds would depend upon the synthesis of peptidase-resistant CRF analogs having the ability to permeate the blood-brain barrier.

Although the scientific design of CRF-active drugs may prove difficult in the near future, the one tried and true method of drug discovery, serendipity, may prove fruitful. Indeed, a CRF receptor-binding assay is now being used in new drug-screening processes at many pharmaceutical companies.

Considering that CRF was first isolated and characterized barely 10 years ago, one marvels at the wealth of knowledge that has been gathered to date. We believe that in the next decade many of the experiments and techniques mentioned above will come to fruition and provide a wealth of detailed information regarding basic CRF physiology. Even more exciting to us are the possibilities that pharmacological agents based upon neu-

ropeptides in general, and CRF in particular, may find usefulness in the therapy of human mental illness.

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