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Receptor-Mediated Actions of Corticotropin-Releasing Factor in Pituitary Gland and Nervous System

Greti Aguilera, Peter C. Wynn, James P. Harwood, Richard L. Hauger, Monica A. Millan, Charles Grewe, Kevin J. Catt

Endocrinology and Reproduction Research Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Md., USA

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Abstract. High-affinity corticotropin-releasing factor (CRF) receptors which mediate the actions of the hypothalamic peptide on adrenocorticotropic hormone (ACTH) release have been identified in the rat anterior pituitary gland. Occupancy of the pituitary receptor by CRF agonists stimulates ACTH release via activation of adenylate cyclase and cyclic adenosine monophosphate dependent protein kinase. In the regulation of ACTH secretion, the effects of CRF on the corticotroph are integrated with the stimulatory actions of cyclic adenosine monophosphate-independent stimuli such as angiotensin II, vasopressin and norepinephrine, and the inhibitory effects of glucocorticoids and somatostatin. In contrast to the major importance of the inhibitory effect of glucocorticoid feedback on ACTH secretion, somatostatin has relatively little effect on CRF-stimulated ACTH release in the normal rat corticotroph. Following adrenalectomy, the progressive elevation of plasma ACTH levels is accompanied by a concomitant decrease in pituitary CRF receptors. The postadrenalectomy loss of CRF receptors, which is prevented by dexamethasone treatment, is caused by a combination of occupancy and processing of the pituitary sites during increased secretion of the hypothalamic peptide. Recently, specific receptors for CRF have been localized in the rat and monkey brain and adrenal medulla, where they are also coupled to adenylate cyclase. Brain CRF receptors are most abundant in the cerebral and cerebellar cortices and in structures related to the limbic system and control of the autonomic nervous system. The actions of CRF on the central and peripheral nervous systems, as well as on the pituitary gland, emphasize the role of CRF as a key hormone in the integrated response to stress.

The secretion of adrenocorticotropic hormone (ACTH) from the corticotroph cells of the anterior pituitary gland is influenced by several peptide hormones, of which the hypothalamic neuropeptide, corticotropin-releasing factor (CRF), serves as the primary regulator [31]. The normal corticotroph has specific receptors for CRF and several other regulatory ligands, including vasopressin, angiotensin II, and catecholamines. While the latter hormones are less effective than CRF as stimuli of ACTH release, they show synergism with CRF and are probably of physiological importance as regulators of ACTH secretion in the intact animal [3, 10, 12, 22]. Certain mouse pituitary tumor cells (AtT-20 cells) respond to CRF and other stimuli of ACTH release in vitro and also contain somatostatin (SRIF) receptors through which SRIF exerts an inhibitory effect on ACTH secretion [4]. In vivo, the stimulatory effects of CRF and other peptide regulators of ACTH secretion are modulated by the inhibitory feedback actions of glucocorticoids upon the corticotroph, a physiological effect that is also demonstrable in vitro [25, 31, 37].

The presence of cell surface receptors for the several putative regulators of ACTH release in the corticotroph provides for a complex and multifactorial control system in which the actions of cyclic adenosine monophosphate (cAMP) dependent regulators, notably CRF, are integrated with those of calcium-dependent stimuli such as vasopressin, angiotensin II, and catecholamines. In the course of defining the actions of CRF in this system, we have characterized the binding and functional properties of CRF receptor sites of the pituitary gland and the brain, and the postreceptor mechanisms through which the hypothalamic peptide controls ACTH secretion.

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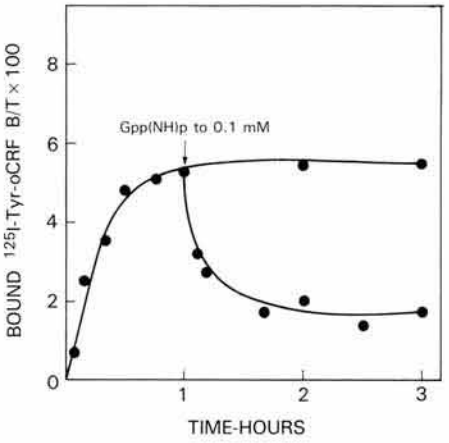

Fig. 1. Steady state binding of ^{125}I-Tyr-oCRF to anterior pituitary membrane-rich fractions and its dissociation by the guanosine triphosphate analog Gpp(NH)p.

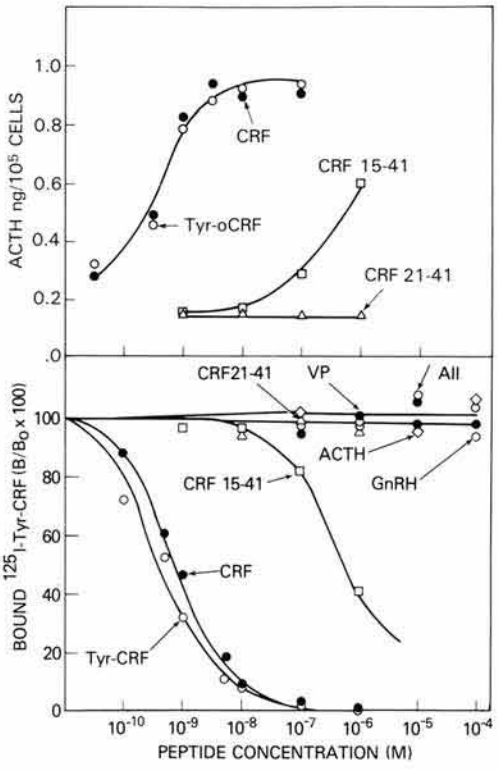

Fig. 2. Dose-response curves for the stimulation of ACTH release from dispersed pituitary cells incubated with oCRF, Tyr-oCRF, oCRF 15–41, and oCRF 21–41 (upper panel) and binding inhibitory activities of oCRF peptides on the binding of ^{125}I-Tyr-oCRF to pituitary particles. VP = Vasopressin; AII = angiotensin II.

Characterization of the Pituitary CRF Receptor

Although there is abundant evidence of the importance of CRF as a major regulator of ACTH secretion, identification and characterization of the CRF receptors have been impeded by problems in the preparation of biologically active tracers and the lability of the receptor itself. Using lactoperoxidase radioiodination of Tyr-oCRF, we have obtained tracers with binding activities of up to 30%, with which it has been possible to study the properties and regulation of the CRF receptor [34]. The specific binding of ^{125}I-Tyr-oCRF to anterior pituitary membrane-rich fractions was time and temperature dependent and reached a steady state within 30–40 min at 22 °C. Scatchard analysis of the equilibrium binding data showed a single class of CRF receptors with a Kd of 10^{-9} *M*. The number of sites after correction for the binding activity of the tracer was 349 ± 31 fmol/mg. The binding was enhanced by several divalent cations (Mg, Ca, and Mn; 2–5 m*M*) and was unaffected by sodium concentrations up to 140 m*M*. Addition of dithiothreitol (up to 1 m*M*), aprotinin (100 KIU/ml), and EGTA (2 m*M*) also increased CRF binding, presumably by inhibiting tracer and/or receptor degradation. Binding was substantially decreased by freezing and thawing of the pituitaries or by keeping tissue or membranes at 4 °C for more than 2 h before the binding assay.

The binding of CRF to pituitary membranes was markedly influenced by guanyl nucleotides. Addition of the nondegradable guanosine triphosphate analog Gpp(NH)p inhibited the binding of ^{125}I-Tyr-CRF by 60% with an ID_{50} of 3 μ*M*. This effect of Gpp(NH)p on CRF receptor affinity was also manifested during kinetic studies on CRF binding, when addition of the guanyl nucleotide caused rapid dissociation of the bound peptide (fig. 1). This effect of guanyl nucleotides is characteristic of receptors that are coupled to adenylate cyclase by a nucleotide regulatory unit, as appears to be the case for the CRF receptor.

The specificity of the binding sites for ^{125}I-Tyr-oCRF was indicated by the ability of CRF peptides to inhibit tracer binding, with potencies similar to their known bilogical activities. Thus, oCRF, rat CRF, and Tyr-oCRF were equipotent, with ID_{50} values of about 1 n*M*, while the CRF 15–41 fragment was 1,000 times less potent, and CRF 21–41, vasopressin, angiotensin II, ACTH, and gonadotropin-releasing hormone (GnRH) were completely inactive (fig. 2, lower panel).

Correlation of CRF Binding and Activation of ACTH Secretion

Validation of a peptide hormone binding site as a receptor which mediates hormone action requires that receptor occupancy is correlated with a characteristic biological re-

sponse of the cell. In the corticotroph, such a relationship was sought by comparison of the binding activities of the different CRF peptides with their ACTH-releasing potencies. In cultured pituitary cells, both CRF and Tyr-CRF stimulated ACTH release eightfold, with half-maximum effective concentrations (ED_{50}) of 3.2 and $2.8 \times 10^{-10} M$, respectively (Fig. 2). Consistent with previous observations [3], the 15–41 CRF fragment was a weak agonist with full intrinsic activity (ED_{50} $5 \times 10^{-7} M$), whereas the 21–41 CRF fragment was completely inactive. The ACTH response to CRF was maximal when receptor occupancy reached about 50%, indicating the presence of a proportion of spare receptors. Such excess or spare CRF receptors in the corticotroph could be relevant to the effects of adrenalectomy, when high rates of ACTH secretion are maintained in the presence of marked downregulation of CRF receptors (see below).

The concentrations of CRF reported in portal blood [8] are in the range of the pituitary CRF receptor affinity, supporting the view that the binding sites detected by radioligand assay represent the functional receptors through which CRF regulates ACTH secretion.

Mode of Action of CRF

The responses of target cells to peptide hormones require that the interaction of the hormone with its specific plasma membrane receptor triggers a chain of cellular events including the generation of a signal or second messenger, followed by protein phosphorylation and activation of metabolic and biosynthetic pathways [5]. The possibility that cAMP mediates the action of CRF was suggested by the stimulatory effects of phosphodiesterase inhibitors and cAMP analogs on ACTH release and by observations such as the ability of hypothalamic extracts to cause parallel increases in cAMP and ACTH in ectopic ACTH-producing tumors [14, 30]. The recent availability of synthetic CRF has permitted a more complete evaluation of the mechanisms involved in ACTH release. We and others have found that CRF causes a rapid increase in cAMP formation which precedes the increases in ACTH release [3, 11]. In dose-response studies, there is a close relationship between the concentrations of CRF that stimulate cAMP production and ACTH release. The role of cAMP as the second messenger for CRF action was emphasized by the ability of isobutylmethylxanthine to potentiate cAMP production and ACTH release and the lack of additivity between the stimulatory effects of CRF on ACTH release and those of 8-bromo-cAMP or cholera toxin. The stimulation of cAMP by CRF was due to increased synthesis of the nucleotide, as shown by the ability of CRF to activate adenylate cyclase in pituitary homogenates and membrane-rich fractions. The role of cAMP in the stimulation of ACTH release by CRF was also indicated by the ability of the hypothalamic peptide to stimulate cAMP-dependent protein kinase at doses similar to those required to activate adenylate cyclase in pituitary homogenates and ACTH release in cultured pituitary cells [3].

Stimulation of the corticotroph by CRF is also calcium dependent, as shown by the attenuation of the ACTH responses to CRF in low-calcium media or in the presence of cobalt [32]. Further studies in cultured pituitary cells to analyze the nature of the calcium requirement in CRF action have shown that increasing the calcium concentration of incubation media from 0 to 2.5 mM increased the magnitude of the ACTH response to 1 nM CRF from 1.6- to 3.3-fold. In dose-response studies, reduction of calcium concentration decreased the magnitude and the sensitivity of the ACTH response to CRF, with ED_{50} of 0.2, 0.6, and 1 nM with 1.5, 0.5, and 0 mM calcium, respectively (fig. 3, upper panel). In contrast to the impaired ACTH response, only the maximal cAMP response was reduced in the absence of calcium (fig. 3, lower panel). Since the generation of small amounts of cAMP is sufficient to elicit maximum ACTH responses, it is likely that the requirement for extracellular calcium is mainly at a site beyond cAMP formation.

The dihydropyridine derivative, nifedipine, is known to inhibit calcium influx by blockade of the voltage-dependent calcium channel. Increasing concentrations of nifedipine up to 100 μM caused a slight reduction in the magnitude of the ACTH response to 1 nM CRF, with a significant decrease in the sensitivity to the response (fig. 4). Nifedipine had no significant effect in the cAMP dose-response to CRF. In contrast, the calmodulin antagonist pimozide caused a marked decrease in both sensitivity and maximum stimulation of the ACTH response by CRF. Consistent with the recognized requirement of calmodulin for the activation of adenylate cyclase, pimozide also inhibited the stimulation of cAMP by CRF (not shown).

Regulation of Pituitary CRF Receptors

The interaction of peptide hormones with the target cell is frequently accompanied by regulatory changes in their specific receptors [6]. In many cases, changes in receptors parallel the changes in responsiveness of the cell to the hormone, and receptor regulation has an important role in the modulation of the cell response to hormones. To determine whether CRF receptors undergo such changes during fluctuations in ACTH secretion, we analyzed the CRF receptors in pituitaries of adrenalectomized rats, in which ACTH levels and responsiveness to CRF are increased [36]. Following adrenalectomy, pituitary CRF binding was reduced by 48, 80, 78, and 79% after 1, 2, 3, or 4 days, respectively. As shown by Scatchard analysis, this was due to a decrease in receptor concentration with no change in binding affin-

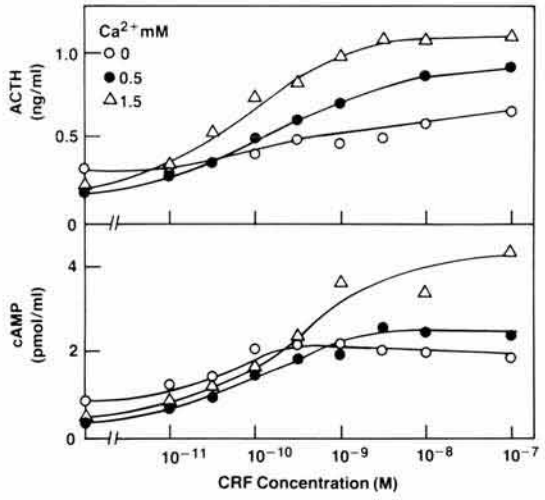

Fig. 3. Effects of extracellular calcium concentration on CRF-stimulated ACTH release (upper panel) and cAMP production (lower panel) in enzyme-dispersed, 48-hour cultured rat pituitary cells.

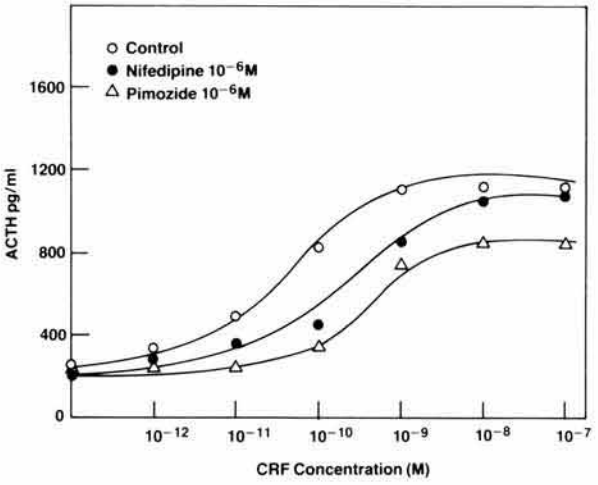

Fig. 4. Effect of the calcium channel antagonist nifedipine and pimozide on CRF-stimulated ACTH release in cultured rat pituitary cells.

ity. This decrease may be partially due to receptor occupancy, since infusion of CRF (500 μg/min) for 30 min prior to pituitary membrane preparation reduced CRF-binding sites by about 40%. However, it is likely that the decrease in receptors after acute CRF infusion reflects rapid internalization of hormone-receptor complexes, as suggested by the autoradiographic demonstration of CRF uptake in the lysosomes and Golgi apparatus 5 min after CRF injection [15]. The reduction of CRF receptors after adrenalectomy was accompanied by a 60% decrease in CRF-stimulated adenylate cyclase activity at 24 h, with no further change at later times. The decreases in CRF receptors and adenylate cyclase activity following adrenalectomy were prevented by dexamethasone treatment. Consistent with the inhibition of CRF-activated adenylate cyclase, CRF-stimulated cAMP production was significantly reduced in cultured pituitary cells from adrenalectomized rats. However, in contrast to the decreases in CRF receptors and cAMP production, there was a threefold increase in CRF-stimulated ACTH release with no change in sensitivity to CRF. Such increased ACTH release from corticotrophs with reduced CRF receptors and impaired activation of adenylate cyclase indicates that elevated ACTH secretion can be maintained by occupancy of few receptors and generation of small amounts of cAMP. It is also possible that the synergistic interactions between CRF and other ACTH secretagogues may contribute to the sustained increase in ACTH secretion that follows adrenalectomy (see below).

Interaction of CRF with Other Regulators of ACTH Secretion

In addition to CRF, a number of hormonal factors have been shown to influence ACTH secretion by the pituitary corticotroph. These include inhibitory hormones such as glucocorticoids and somatostatin and stimulatory factors such as catecholamines, VIP, vasopressin, and angiotensin II [2, 4, 7, 9, 16, 26, 33]. In contrast to the established role of glucocorticoid feedback in the regulation of ACTH secretion, the physiological importance of somatostatin is less certain. In experiments to determine the relative effects of somatostatin on the secretion of several pituitary hormones, the effects of growth hormone releasing factor and thyrotropin-releasing hormone upon secretion of growth hormone and thyrotropin were completely inhibited by somatostatin, with only minor attenuation of prolactin and ACTH secretion. In four such experiments, the basal ACTH production was unchanged, and the CRF-stimulated ACTH release was reduced by only $14 \pm 2\%$ by 10 n*M* somatostatin (fig. 5). Such a small degree of inhibition renders it unlikely that somatostatin has a physiological role in the control of ACTH secretion. In contrast, somatostatin has prominent inhibitory actions upon CRF-stimulated ACTH and cAMP production in AtT-20 mouse pituitary tumor cells [4]. However, although this cell line secretes only ACTH, it may not be representative of the normal corticotroph. Recent studies have shown that AtT-20 cells release

ACTH in response to growth hormone releasing factor, indicating the presence of growth hormone releasing factor receptors, sites which have a recognized functional relationship with the somatostatin receptor [19]. Such results indicate the need for caution in extrapolating results obtained in this tumor cell line to the physiological regulation of the normal corticotroph.

Other secretagogues such as norepinephrine, VIP, vasopressin, and angiotensin II, are much less effective than CRF in stimulating ACTH release in vitro. Whereas CRF and VIP stimulate ACTH release via adenylate cyclase activation, vasopressin, norepinephrine, and angiotensin II exert their actions through a cAMP-independent mechanism [3]. Beta-adrenergic agonists have been also shown to stimulate ACTH release through increases in cAMP formation in AtT-20 cells [4]. In addition to their minor direct stimulatory effects on ACTH release, vasopressin and norepinephrine have been shown to potentiate the stimulatory effect of CRF on ACTH and cAMP formation.

Angiotensin II also has the ability to potentiate CRF stimulation of ACTH in vitro. Although this effect is not obvious during static incubations, it is prominent when pituitary cells are stimulated during column perifusion (fig. 6). In three experiments, perifusion with 10 p*M* angiotensin II during stimulation with increasing concentrations of CRF decreased the ED_{50} for CRF from 0.48 ± 2.3 to 0.05 ± 0.04 n*M*, a notable increase in sensitivity to the hypothalamic hormone. In addition, in 5 of 7 experiments, simultaneous stimulation with 10 p*M* angiotensin II and 1 n*M* CRF significantly increased the duration of the ACTH response to CRF. Although it is likely that angiotensin II has an important role in the regulation of CRF release at the hypothalamic level [1, 24], its potentiating effect on CRF action upon the pituitary gland may also contribute to the control of ACTH release.

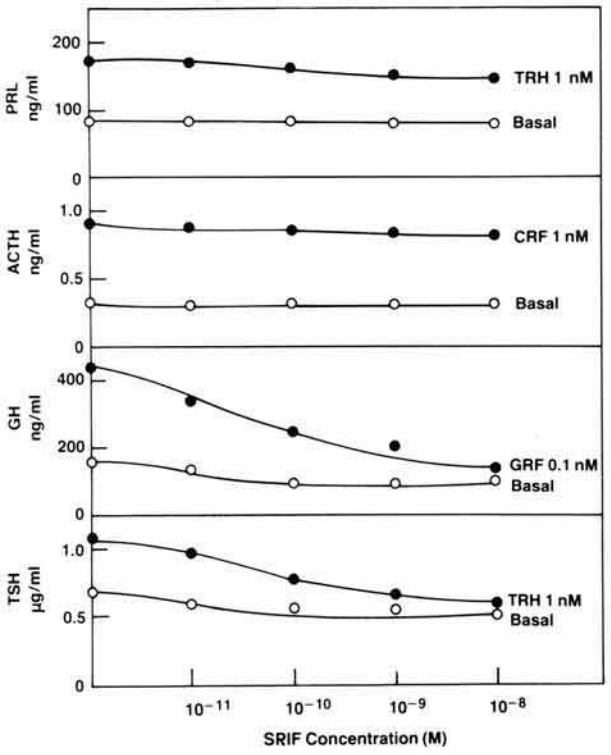

Fig. 5. Effects of somatostatin on basal and ligand-stimulated prolactin (PRL), ACTH, growth hormone (GH), and thyrotropin-stimulating hormone (TSH) release in 48-hour cultured rat pituitary cells. TRH = Thyrotropin-releasing hormone: (GRF = growth hormone releasing factor.

CRF Receptors in the Brain

The term CRF was applied to the neuropeptide in accordance with its release from the hypothalamus into the portal system and its major actions upon ACTH release in the anterior pituitary gland [31]. However, as with other hypothalamic peptides, its biological role is not limited to the hypophysis, and recent studies have shown marked visceral and behavioral effects of CRF injected intraventricularly or into the brain [31]. In addition, immunofluorescence and radioimmunoassay techniques have revealed considerable quantities of CRF in the central nervous system at locations unrelated to its action on the anterior pituitary, suggesting a more general role for CRF as a neuropeptide. To further elucidate the mechanisms and pathways by which the peptide exerts its effects, we performed a series of studies to characterize and localize the CRF receptors.

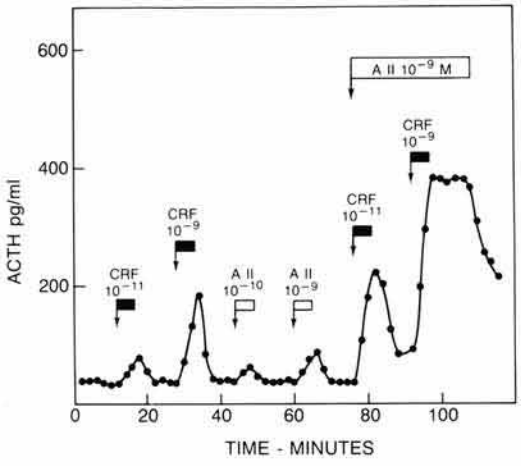

Fig. 6. Potentiation of CRF-stimulated ACTH production by angiotensin II (A II) in column-perifused rat pituitary cells.

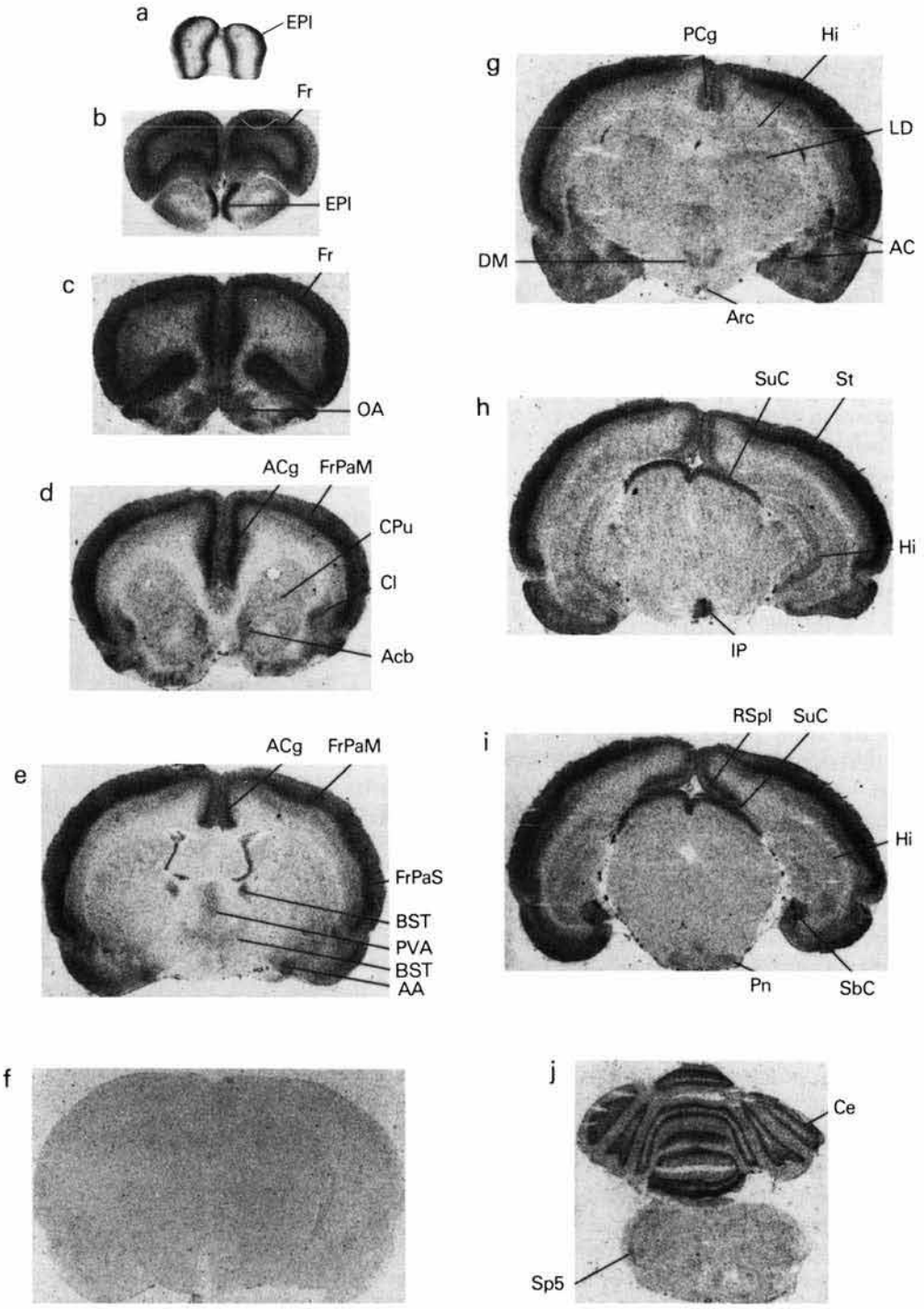

Fig. 7. Autoradiographic analysis of ^{125}I-Tyr-oCRF binding to rat brain sections. AA = Anterior amygdala; AC = amygdala complex; Acb = nucleus accumbens; ACg = anterior cingulate cortex: Arc = arcuate nucleus; BST = bed nucleus stria terminalis; Ce = cerebellum; Cl = claustrum; CPu = caudate putamen; DM = dorsomedial hypothalamic nucleus; EPI = external plexiform layer olfactory bulb; Fr = frontal cortex; FrPaM = frontal parietal cortex (motor); FrPaS = frontal parietal cortex (sensory); Hi = hippocampus; IP = interpeduncular nucleus; LD = laterodorsal thalamic nucleus; OA = anterior olfactory nucleus; PCg = posterior cingulate cortex; Pn = pontine nucleus; PVA = paraventricular thalamic nuclei anterior: SbC = subiculum complex; Sp5 = spinal trigeminal tract; St = striate cortex; SuC = superior colliculus; RSpl = retrosplenial cortex; [from ref. 35, with permission].

Using autoradiographic mapping in brain sections [35], CRF receptors were initially identified in several areas including the olfactory bulb, amygdala, and cerebral cortex. Therefore, membrane-rich fractions of these areas were utilized for characterization of the brain CRF recptors. After homogenization in 50 m*M* Tris-HCl buffer (pH 7.4), aliquots of the 100–30,000 *g* fraction (200–400 μg of protein) were incubated with 0.1 n*M* ^{125}I-Tyr-oCRF in 0.3 ml of the same buffer containing 2 m*M* $CaCl_2$, 5 m*M* $MgCl_2$, 0.1 m*M* phenylmethylsulfonylfluoride, 100 KIU aprotinin, and 1 m*M* dithiothreitol. Nonspecific binding measured in the presence of 1 μ*M* oCRF was less than 2% of the added radioactivity. The properties of brain CRF receptors were similar to those in the pituitary, with high affinity and specificity for the CRF peptides. Scatchard analysis of the binding data revealed a single site with a Ka of $3.9 \pm 1.2 \times 10^8$ M^{-1} (n = 10) and a concentration of 52.1 ± 8.3 fmol/mg.

To determine whether brain CRF-binding sites were coupled to an activation process we analyzed the effects of CRF on adenylate cyclase activity in brain areas including amygdala and prefrontal cortex. Tissues were homogenized in 10 vol. of phosphate-buffered saline by six strokes of a glass homogenizer and centrifuged at 1,500 *g*. After three washes with phosphate-buffered saline the pellet was resuspended to give a protein concentration of 100–200 μg in 20 μl. The adenylate cyclase activity was determined as previously described [3] using 20 μl of protein suspension and addition of 25 U/ml adenosine deaminase. In the presence of 10 μ*M* guanosine triphosphate, basal and stimulated adenylate cyclase activities were linear during incubation up to 15 min. At 10 min, the adenylate cyclase activity was increased by 150 ± 12% with 10 m*M* NaF, 183 ± 8% with 0.1 m*M* forskolin, 57 ± 6% with 0.1 m*M* norepinephrine, and 30 ± 5% with 1 μ*M* CRF. Only slight stimulation by CRF was observed in the absence of guanosine triphosphate. The demonstration that the CRF-binding sites are coupled to adenylate cyclase in brain tissue emphasizes the importance of such sites as functional receptors which mediate the actions of the peptide in the central nervous system.

The anatomical distribution of the areas containing CRF receptors within the brain was determined by autoradiographic analysis in 20-μm slide-mounted frozen sections [23]. Measurements of binding affinity and specificity performed on the sections were similar to those determined in brain membranes (Ka 8.7×10^8 M^{-1}). As shown in figure 7, CRF receptors were located mainly in two functionally distinct systems: the cerebral and cerebellar cortex and a series of structures associated with the limbic system. The receptors were concentrated throughout the cerebral cortex, with relatively higher densities in the anterior cingulate cortex, frontoparietal (motor-sensory) area, and temporal cortex (auditory area). The highest receptor concentration was found in the cortical layer IV followed by layers I–III. Other cortical-related areas containing CRF receptors were the hippocampus, the subiculum, and the claustrum.

Variable concentrations of CRF receptors were found throughout the brain in a number of structures related to the limbic system and the control of the autonomic nervous system. In the basal telencephalon, from highest to lowest densities, binding was found in the external plexiform layer of the olfactory bulb, amygdala complex, bed nucleus of the stria terminalis, lateral intermediate and medial septal nuclei, nucleus accumbens, and caudate-putamen.

In the diencephalon the receptors were located in the dorsomedial hypothalamic nucleus, supramammillary nuclei, dorsolateral thalamic nuclei, arcuate nucleus, and paraventricular thalamic nuclei anterior. No receptors were found in the paraventricular and periventricular hypothalamic nuclei or the median eminence, sites related to the release of the peptide to the portal blood and containing high concentrations of immunoreactive CRF.

In the brain stem, the highest densities were found in the interpeduncular and pontine nuclei followed by the superior colliculus, inferior olive, dorsal tegmental nucleus, spinal trigeminal tract, locus ceruleus, and nucleus of the solitary tract. In the cerebellum the binding was associated with granular layer of the cortex.

It is noteworthy that cell bodies and fibers containing immunoreactive CRF have been shown in most of the areas in which CRF receptors have now been localized by radioligand mapping in brain sections [17, 20, 28]. The coexistence of CRF and its receptors in specific areas of the brain supports a role for the peptide as a neurotransmitter within the central nervous system. In contrast to the marked decreases in anterior pituitary CRF receptors after adrenalectomy, CRF receptors in the brain and intermediate pituitary were unchanged following removal of the adrenals [35]. In three experiments, no significant differences in CRF receptor concentration or affinity were observed in olfactory bulb particles (36.0 and 37.8 fmol/mg in sham-operated and adrenalectomized rats, respectively). Similarly, autoradiographic analysis of CRF binding to brain and pituitary sections revealed no difference in the optical densities in the prefrontal cortex (0.52 and 0.51), frontal parietal cortex (0.34 and 0.41), basolateral amygdaloid nuclei (0.35 and 0.30), lateral amygdaloid nucleus (0.23 and 0.22), and bed nucleus stria terminalis (0.21 and 0.23). In contrast, the receptor density was markedly decreased in the anterior pituitary of adrenalectomized rats (0.06 ± 0.01 compared with 0.21 ± 0.01 in the sham-operated controls, n = 15, $p < 0.001$).

It is interesting to note that in the intermediate lobe, which is less influenced by negative glucocorticoid feedback, the optical density was unchanged after adrenalectomy (0.21 ± 0.02 and 0.23 ± 0.02, n = 9). This suggests that the regulatory mechanisms in the intermediate lobe and brain differ from those in the anterior pituitary. It is likely that CRF secretion into the portal circulation is increased after adrenalectomy, and elevated CRF levels have been

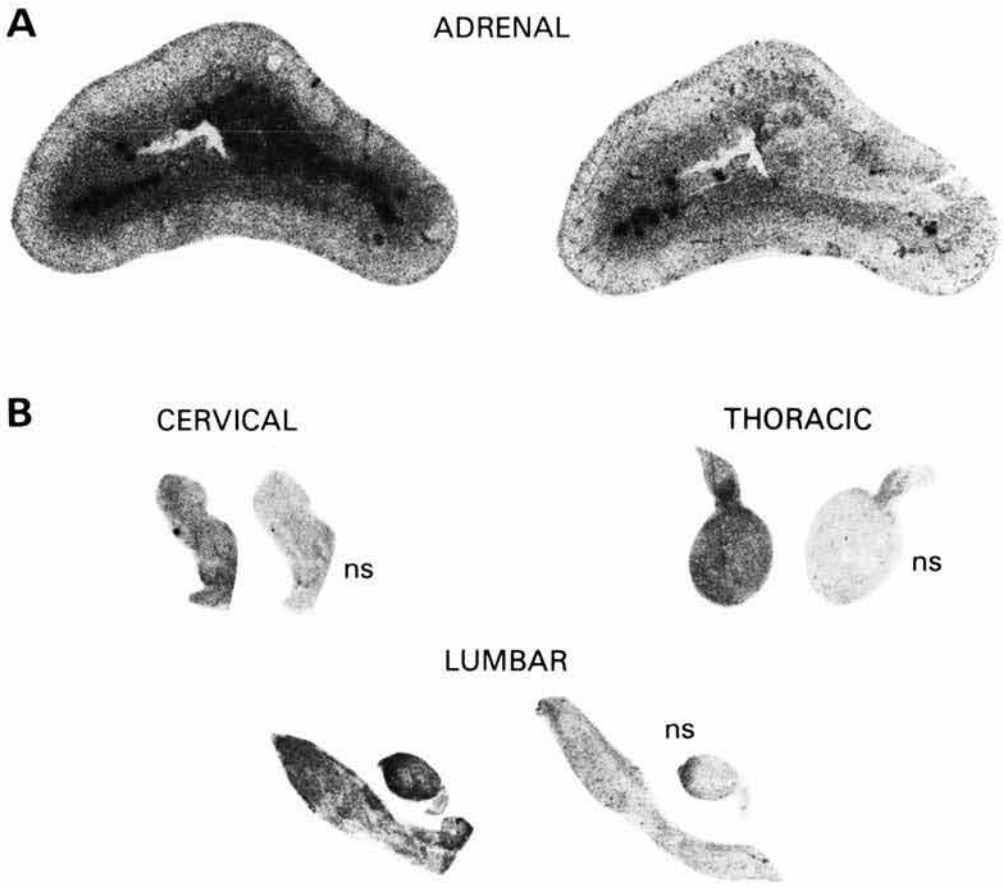

Fig. 8. Autoradiographic analysis of ^{125}I-Tyr-oCRF binding to 20-μm frozen monkey adrenal (**A**) and sympathetic ganglia sections (**B**). Slide-mounted sections were incubated for 1 h at 22 °C with 0.2 n*M* ^{125}I-Tyr-oCRF in the absence and in the presence of 1 μ*M* unlabeled oCRF for nonspecific (ns) binding. The slides were washed four times in CRF-free incubation buffer, dried, and exposed in cassettes (Wolf X-ray) to LKB ultrafilm for 6 days at room temperature.

shown to contribute to the decrease in anterior pituitary CRF receptors. However, the effects of glucocorticoids upon CRF-mediated brain functions are not known, and it is possible that extrahypothalamic CRF levels are not changed by decreases in glucocorticoid secretion. Alternatively, the interaction of CRF with its brain receptor may not involve processing of the receptor-hormone complex, in contrast to the endocytosis and receptor downregulation in the anterior pituitary gland.

Recent studies in our laboratory have demonstrated the presence of CRF receptors in the monkey brain. In membrane-rich particles from the amygdala and frontal brain cortex of the cynomolgus monkey, CRF receptors were of high affinity (1 n*M*), specific for CRF-related peptides, and, as in the rat, they were coupled to adenylate cyclase. Autoradiographic studies in frozen brain sections indicate a topographic distribution of the receptors similar to that observed in the rat brain with preferential localization in the brain cortex and limbic system components [18].

CRF Receptors in the Peripheral Sympathetic Nervous System

A number of studies have demonstrated the presence of immunoreactive CRF in several tissues in the periphery, including pancreas, gut, and chromaffin tissue in the adrenal medulla [13, 21, 27]. Since the peptide is known to centrally modulate the activity of the autonomic nervous system, the presence of CRF in the adrenal medulla suggests that CRF is locally released and may regulate the activity of the sympathetic system in the periphery. Therefore, we investigated the presence of CRF receptor in the adrenal medulla and sympathetic ganglia and the actions of the peptide in chromaffin cell function [29].

Binding studies of ^{125}I-Tyr-oCRF to 30,000 *g* membrane-rich fractions from monkey adrenal revealed specific and high-affinity CRF receptors in the adrenal medulla with a Kd of 3 n*M*. As previously demonstrated for CRF receptors in other tissues, adrenal medulla CRF receptors were also

coupled to adenylate cyclase. In experiments in membrane-rich particles CRF stimulated the conversion of [^{32}P]-adenosine triphosphate to [^{32}P]-cAMP by 30%. This effect was obtained with concentrations of the peptide in the range of the CRF-binding affinity for the adrenal medullary receptors.

Binding sites for ^{125}I-Tyr-oCRF in the adrenal medulla were also evident by autoradiographic analysis in slide-mounted frozen sections of rhesus monkey adrenal glands. Consistent with the results in adrenal membranes, CRF receptors were confined to the adrenal medulla, with no specific autoradiographic staining in the adrenal cortex (fig. 8A). Autoradiographic analysis also demonstrated specific CRF binding in all sympathetic ganglia studied including lumbar, thoracic, cervical (fig. 8B), and celiac ganglia (not shown). Analysis of the autoradiograms by computerized densitometry indicates optical densities of 0.69 ± 0.05 and 0.23 ± 0.03, respectively, in the absence or presence of excess CRF. In all sympathetic ganglia, complete inhibition of the specific binding was observed with 10 n*M* CRF, indicating the high affinity of the binding sites.

To determine whether CRF receptor activation in the adrenal medulla results in changes in secretory activity of the cells, we studied the effect of CRF on catecholamine and enkephalin release in cultured bovine chromaffin cells. Short-term incubation of the cells with CRF had no effect on catecholamine secretion. However, after 24 h culture of the cells with CRF there was a dose-dependent stimulation of epinephrine, norepinephrine, and methionine-enkephalin release to the media.

Although the physiological role of CRF in the autonomic nervous system will require further study, the presence of the peptide and its functional receptors in the brain, adrenal medulla, and sympathetic ganglia suggests that CRF can regulate the activity of the autonomic nervous system at both central and peripheral sites. These findings support the view that the peptide acts as a neurotransmitter in the central nervous system and mediates the responses to stress via CRF receptors in the brain, as well as regulating the peripheral secretion of stress hormones via its actions in the central nervous system and the anterior pituitary gland.

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Greti Aguilera, MD,
National Institutes of Health,
NICHD/ERRB,
9000 Rockville Pike,
Bldg. 10/Room 8C-407,
Bethesda, MD 20892 (USA)