

Corticotropin-releasing Factor Receptors Are Widely Distributed within the Rat Central Nervous System: An Autoradiographic Study¹

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Abstract

Corticotropin-releasing factor (CRF) receptor-binding sites have been localized and quantified in the rat central nervous system (CNS) by autoradiography with an iodine-125-labeled analogue of ovine CRF substituted with norleucine and tyrosine at amino acid residues 21 and 32, respectively. High affinity and pharmacologically specific receptor-binding sites for CRF were found in discrete areas within the rat CNS. CRF receptors were highly concentrated in laminae 1 and 4 throughout the neocortex, the external plexiform layer of the olfactory bulb, the external layer of the median eminence, several cranial nerve nuclei in the brainstem including the facial, oculomotor, trochlear, vestibulocochlear, and trigeminal nuclei, the deep cerebellar nuclei, and the cerebellar cortex. Moderate concentrations of CRF receptors were present in the olfactory tubercle, caudate-putamen, claustrum, nucleus accumbens, nucleus of the diagonal band, basolateral nucleus of the amygdala, paraventricular nucleus of the hypothalamus, mammillary peduncle, inferior and superior olives, medullary reticular formation, inferior colliculus, and brainstem nuclei including tegmental, parabrachial, hypoglossal, pontine, cuneate, and gracilis nuclei, and in spinal cord. Lower densities of CRF binding were found in the bed nucleus of the stria terminalis, central and medial amygdaloid nuclei, and regions of the thalamus, hypothalamus, hippocampus, and brainstem. The distribution of CRF-binding sites generally correlates with the immunocytochemical distribu-

tion of CRF pathways and with the pharmacological sites of action of CRF. These data strongly support a physiological role for endogenous CRF in regulating and integrating functions in the CNS.

The primary role of a hypothalamic factor in regulating pituitary-adrenocortical secretion has been well established (Harris, 1948; Yasuda et al., 1982). In 1981, Vale and colleagues reported the isolation, characterization, sequence, synthesis, and biologic activity of a 41-amino acid hypothalamic ovine corticotropin-releasing factor (CRF) that fulfills many of the criteria of a physiological regulator of adrenocorticotropin (ACTH) secretion (Spiess et al., 1981; Vale et al., 1981). Synthetic CRF is a potent stimulus for the release of ACTH (Vale et al., 1981, 1983; Turkelson et al., 1981; Gibbs et al., 1982; Grossman et al., 1982; Hook et al., 1982; C. Rivier et al., 1982, 1983; Nussbaum et al., 1983; Orth et al., 1983; Gold et al., 1984), β -endorphin (Gibbs et al., 1982; C. Rivier et al., 1982; Nussbaum et al., 1983; Vale et al., 1983), and other pro-opiomelanocortin-derived peptides (Al-Noaemi et al., 1982; Meunier et al., 1982; Proulx-Ferland et al., 1982) from the pituitary gland in a variety of species including rats, sheep, monkeys, and humans. In addition to its endocrine activity in the pituitary gland, CRF has a wide variety of extrahypophysiotropic effects. Intracerebroventricular administration of CRF elicits autonomic (Brown et al., 1982a, b; Fisher et al., 1982, 1983; Kalin et al., 1983; Tache et al., 1983; Insel et al., 1984), electrophysiological (Aldenhoff et al., 1983; Eborly et al., 1983; Ehlers et al., 1983; Valentino et al., 1983), and behavioral (D. R. Britton et al., 1982, 1984; Morley and Levine, 1982; Sutton et al., 1982; Levine et al., 1983; Sahgal et al., 1983; Sirinathsinghji et al., 1983; Koob et al., 1984; Veldhuis and DeWeid, 1984) effects, suggesting that it may play a role in the organism's response to stress. Radioimmunoassay (Fischman and Moldow, 1982; Cote et al., 1983; Hashimoto et al., 1983; Palkovits et al., 1983) and immunocytochemical (Bloom et al., 1982; Bugnon et al., 1982; Olshchowska et al., 1982; Cummings et al., 1983; Lerenth et al., 1983; Merchenthaler et al., 1983; Paull and Gibbs, 1983; Swanson et al., 1983) studies have demonstrated that CRF-like immunoreactivity is distributed throughout the rat central nervous system (CNS). These pharmacological and immunohistochemical data suggest that CRF may function as a neurotransmitter or neuromodulator in the CNS.

Earlier we identified and characterized high affinity receptors for CRF in rat (De Souza et al., 1984a) and human (De Souza et al., 1985) anterior pituitary glands and in rat intermediate pituitary (De Souza et al., 1984a). More recently, we reported the autoradiographic identification of CRF receptors in selected regions of rat forebrain

Received February 7, 1985; Revised April 23, 1985;
Accepted April 26, 1985

¹We thank N. Taylor and R. Proctor for technical assistance and S. Wrightson and M. Flutka for manuscript preparation. We are especially grateful to Ron Emeson for his help in the iodination and high pressure liquid chromatography purification of corticotropin-releasing factor, to Dr. James Unnerstall for advice and help in preparing iodine-125-labeled standards, and to Dr. Paul Maclean for help in identifying some of the neuroanatomical structures. This study was supported by United States Public Health Service Grants MH25951 and MH00053 and by a grant from the McKnight Foundation. Research at the Peptide Biology Laboratory was supported in part by National Institutes of Health grants AM26741 and HD13527 and was conducted in part by the Clayton Foundation for Research, California. M. H. P. and W. W. V. are Clayton Foundation investigators.

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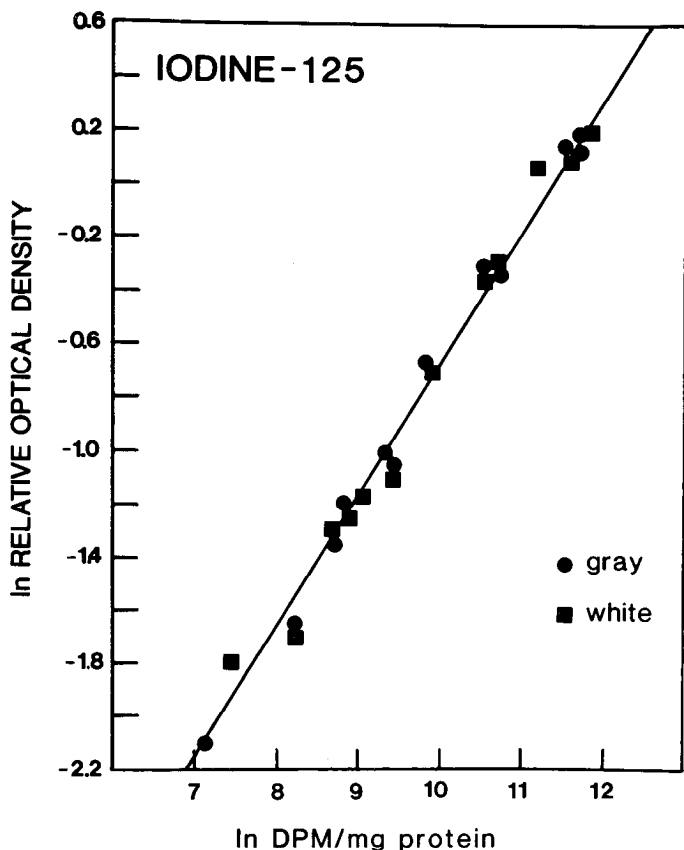


Figure 1. Relationship between the relative optical density in Ultrafilm and the concentration of iodine-125 as derived from the Loats computerized image analysis system for receptor autoradiograms. Tissue blocks of bovine caudate (gray matter) and bovine corpus callosum (white matter) were soaked in iodine-125-labeled compounds, and apposed to Ultrafilm. After exposure, the radioactivity and protein concentration in the standards were measured directly after scraping the tissue sections from the slides. The data are in "relative" optical density because the optical density measurements were not standardized in the image analysis. The regression line had a correlation coefficient of $r \geq 0.99$ ($p < 0.001$).

using an iodine-125-labeled ovine CRF analogue substituted with norleucine and tyrosine at amino acid residues 21 and 32, respectively (De Souza et al., 1984b). In this report, we describe in greater detail the autoradiographic distribution of CRF receptors in the rat CNS. These autoradiographic studies have been undertaken to indicate the anatomical loci at which CRF may produce its CNS effects.

Materials and Methods

Iodination and purification of Nle²¹, Tyr³²-oCRF. Nle²¹, Tyr³²-oCRF was iodinated by reaction of 2 μ g of peptide with 1.0 mCi of Na¹²⁵I (Amersham Corp., Arlington Height, IL) in the presence of one Iodobead (Pierce Chemical Co., Rockford, IL). The reaction was allowed to proceed for 5 min at room temperature and was stopped by addition of 5 μ l of 2-mercaptoethanol. The iodination mixture was diluted with 3.0 ml of solution A (20% acetonitrile in 0.1% trifluoroacetic acid) and cycled onto an ODS Sep-Pak cartridge (Waters Associates, Milford, MA) five times; the cartridge was then washed with 20 ml of solution A and eluted with six 0.5-ml aliquots of solution B (80% acetonitrile in 0.1% trifluoroacetic acid). The two most radioactive fractions were pooled and dried under a stream of N₂; the contents were then dissolved in 200 μ l of solution A. The iodine-125-labeled Nle²¹, Tyr³²-oCRF (Nle²¹, ¹²⁵I-Tyr³²-oCRF) was purified by reversed phase high pressure liquid chromatography (RP-HPLC) on a Waters C₁₈ (ODS) μ -Bondapak column (4.6 mm \times 30 cm) equilibrated with solution A at a flow rate of 1.0 ml/min; 10 min after sample injection, a linear gradient to 85% solution B over 65 min was initiated. Unlabeled Nle²¹, Tyr³²-oCRF was detected by absorbance at 220 nm.

Tissue preparation. Male Sprague-Dawley (Madison, WI) rats weighing 200 to 300 gm were anesthetized with pentobarbital and perfused intracardially with 500 to 700 ml of a mixture of equal parts of phosphate-buffered saline and 0.32 M sucrose (pH 7.4). The brains and spinal cords were then rapidly removed, embedded in homogenized brain paste, and frozen in powdered dry ice. The tissues were sectioned (8 μ m) using a microtome (Harris; N. Billerica, MA) at -16°C , thaw-mounted onto chrome alum/gelatin-subbed microscope slides, and stored at -20°C until used. In biochemical studies involving characterization of the receptor, we used slide-mounted coronal sections of rat forebrain through the diencephalon (one 10- μ m section per slide). Sections from at least 15 animals were subsequently used to generate autoradiograms.

Receptor labeling in slide-mounted brain sections. Slide-mounted tissue sections were brought to room temperature and incubated with 0.1 to 0.2 nM Nle²¹, ¹²⁵I-Tyr³²-oCRF in 50 mM Tris-HCl (pH 7.4) containing 5 mM MgCl₂, 2 mM EGTA, 0.1% bovine serum albumin (BSA), aprotinin (100 KIU/ml), and 0.1 mM bacitracin (Sigma Chemical Co., St. Louis, MO) at room temperature. Blanks were incubated in the same medium with the addition of 1 μ M Nle²¹,

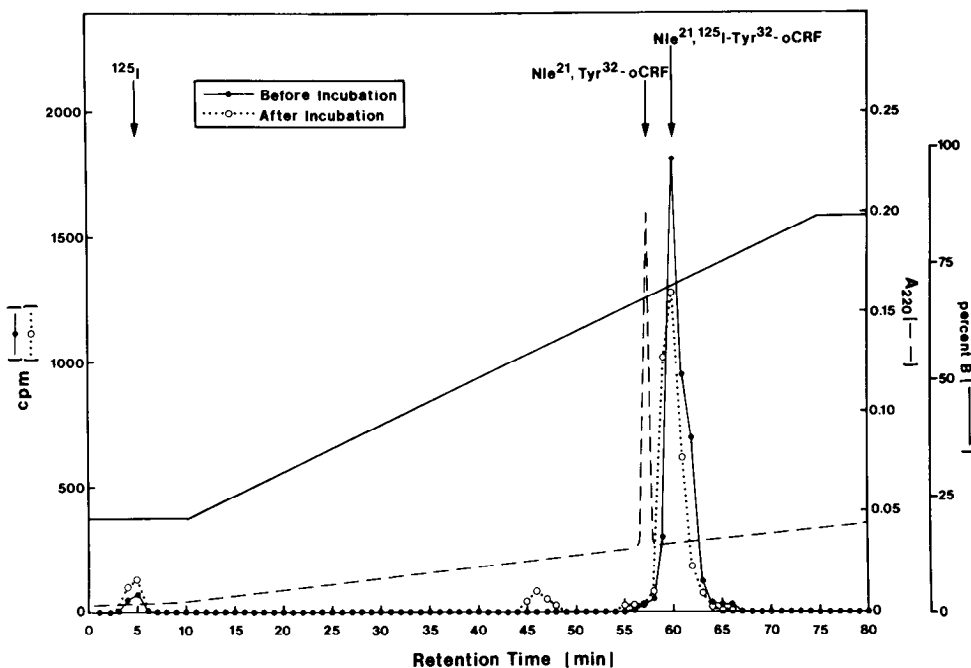


Figure 2. RP-HPLC profile of iodine-125-labeled Nle²¹, Tyr³²-oCRF: effects of incubation with brain tissue. Aliquots of Nle²¹, ¹²⁵I-Tyr³²-oCRF in the medium, before (●) and 120 min after (○) incubation with brain tissue were analyzed by RP-HPLC using conditions described under "Materials and Methods." The elution position of unlabeled Nle²¹, Tyr³²-oCRF (dashed line) was determined by absorbance at 220 nm.

Tyr³²-oCRF, oCRF, or rat (human) CRF. Before exposure to Nle²¹, ¹²⁵I-Tyr³²-oCRF, all tissue sections were incubated at room temperature for two 15-min periods in 50 mM Tris-HCl (pH 7.4) containing 5 mM MgCl₂ and 2 mM EGTA in order to displace endogenous CRF from its receptor. After incubation with Nle²¹, ¹²⁵I-Tyr³²-oCRF, tissue sections were washed in Dulbecco's phosphate-buffered saline containing 1% BSA (fraction V, Sigma), dipped in deionized water, and dried rapidly under a stream of cold, dry air. In preliminary nonautoradiographic studies to determine the time courses of dissociation and association, kinetics, and pharmacology of Nle²¹, ¹²⁵I-Tyr³²-oCRF binding to slide-mounted tissues sections, the tissue sections were dried and the tissue was scraped off the glass microscope slide, and the tissue-associated radioactivity was assayed in a gamma counter. In subsequent autoradiographic studies, the tissue sections were incubated with Nle²¹, ¹²⁵I-Tyr³²-oCRF for 60 or 90 min at room temperature and washed for two 5-min periods in Dulbecco's phosphate-buffered saline at 4°C.

Autoradiography. The dried, labeled, slide-mounted sections were apposed to either ³H-Ultrafilms (LKB Instruments, Gaithersburg, MD) (Palacios et al., 1981) or Kodak NTB 3 emulsion-coated glass (Young and Kuhar, 1979) or Aclar plastic coverslips, (De Souza et al., 1984b), and after 7 to 14 days of exposure at 4°C, the autoradiograms were developed and the tissue was stained with toluidine blue. Following the staining procedures, the sections were dried, and the coverslips were reapposed and set with Permount.

Data analysis. In autoradiograms prepared with NTB-3 emulsion, grain counts were made by eye using a × 100 oil immersion objective on a Zeiss microscope equipped with a grid-containing eyepiece. In autoradiograms prepared with tritium-sensitive film, optical density readings, construction of standard curves, and rapid quantification were carried out using a Loats PC-based computerized image analysis system (Loats Associates, Westminster, MD).

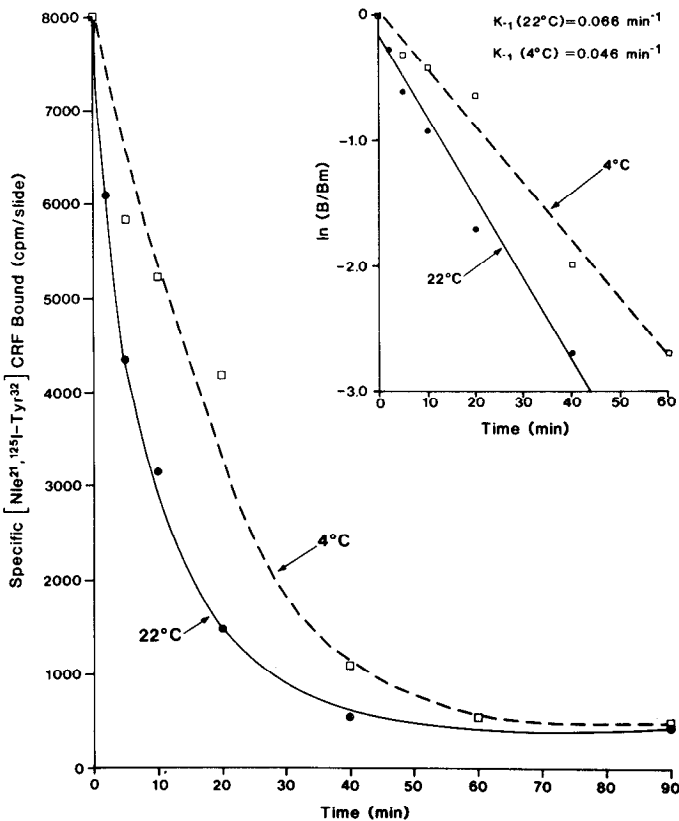


Figure 3. Time course of dissociation of the specific binding of Nle²¹, ¹²⁵I-Tyr³²-oCRF to slide-mounted tissue sections (10 μm) of rat forebrain as a function of temperature. All slide-mounted brain sections were first incubated with 0.2 nM Nle²¹, ¹²⁵I-Tyr³²-oCRF for 60 min at 22°C and then washed in Dulbecco's phosphate-buffered saline containing 1% BSA at 4°C (□) or at 22°C (●). Nonspecific binding was determined in the presence of 1 μM oCRF. The inset shows the dissociation rate of specific binding. B/B_m = specific binding per maximum specific binding. Each point is the average of three determinations which differed by less than 10%.

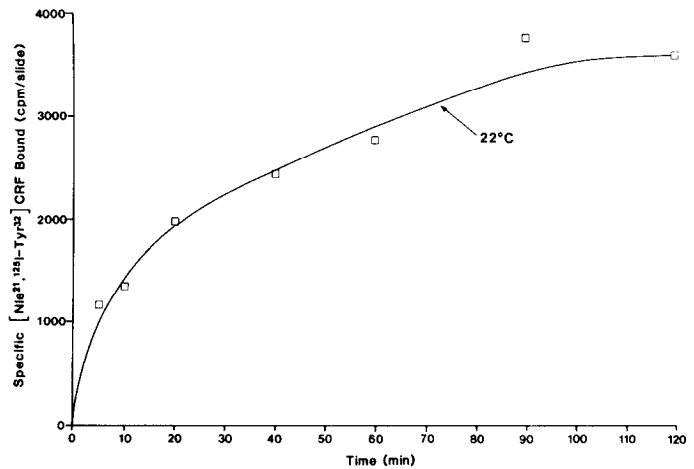


Figure 4. Time course of association of the specific binding of Nle²¹, ¹²⁵I-Tyr³²-oCRF to slide-mounted sections (10 μm) of rat forebrain at 22°C. Nonspecific binding was determined in the presence of 1 μM oCRF. Following the incubation period, all sections were washed for two 5-min periods in Dulbecco's phosphate-buffered saline containing 1% BSA at 4°C. Each point is the average of four determinations which differed by less than 10%.

Slide-mounted tissue sections (8 μm) of bovine caudate (gray matter) and bovine corpus callosum (white matter) were soaked for 10 min in increasing concentrations (10³ to 10⁶ cpm/100 μl of water) of iodine-125-labeled histamine at room temperature and dried rapidly under a stream of cold, dry air. The iodine-125-labeled gray and white matter standards were apposed to ³H-Ultrafilms along with the CRF-labeled brain sections. After exposure, the tissue sections were scraped from the slides, and the radioactivity and protein concentration in the gray and white matter standards were measured directly. The relationship between relative optical density in Ultrafilms and the concentration of iodine-125 in the gray and white matter standards is shown in Figure 1. We utilized a power function to describe the relationship between optical density and radioactivity since the Loats image analysis system indicated a best fit for the power function as opposed to a linear or log function. The regression line had a correlation coefficient of $r \geq 0.99$ ($p < 0.001$). No difference was found in the autoradiographic efficiency in gray and white matter labeled with iodine-125. By generating such a standard curve concomitantly with the Nle²¹, ¹²⁵I-Tyr³²-oCRF autoradiograms, the film optical density readings of the samples were related to the molar concentration of radioactivity.

Results

RP-HPLC analysis of iodine-125-labeled Nle²¹, Tyr³²-oCRF. The RP-HPLC profile of iodine-125-labeled and unlabeled Nle²¹, Tyr³²-oCRF is shown in Figure 2. Unlabeled Nle²¹, Tyr³²-oCRF eluted at 57.5 min and the iodine-125-labeled peptide eluted at 60 min.

To determine whether the incubation conditions used in labeling CRF receptors on slide-mounted tissue sections resulted in any appreciable degradation of the ligand, we ran analytical profiles of aliquots of the medium before and 120 min after incubation with tissue. As seen in Figure 2, 120 min after incubation, approximately 92% of Nle²¹, ¹²⁵I-Tyr³²-oCRF remained undegraded. The major degradation product (6.5% of total radioactivity) eluted at 45 to 48 min. In addition, minor peaks representing 3% (before incubation) and 4.5% (after incubation) of the total radioactivity eluted in the same position as iodine-125. These data demonstrate the stability of the incubation conditions for labeling CRF receptors in slide-mounted tissue sections.

Biochemical characterization of CRF receptors in slide-mounted tissue sections. We carried out a series of kinetic and pharmacologic studies to define the incubation parameters and assess the properties of CRF receptors in slide-mounted brain sections. First, the rate of dissociation of Nle²¹, ¹²⁵I-Tyr³²-oCRF binding was determined as a function of temperature (Fig. 3). Slide-mounted sections of rat brain through the diencephalon were first incubated with 0.2 nM Nle²¹, ¹²⁵I-Tyr³²-oCRF for 60 min at 22°C and then washed for various

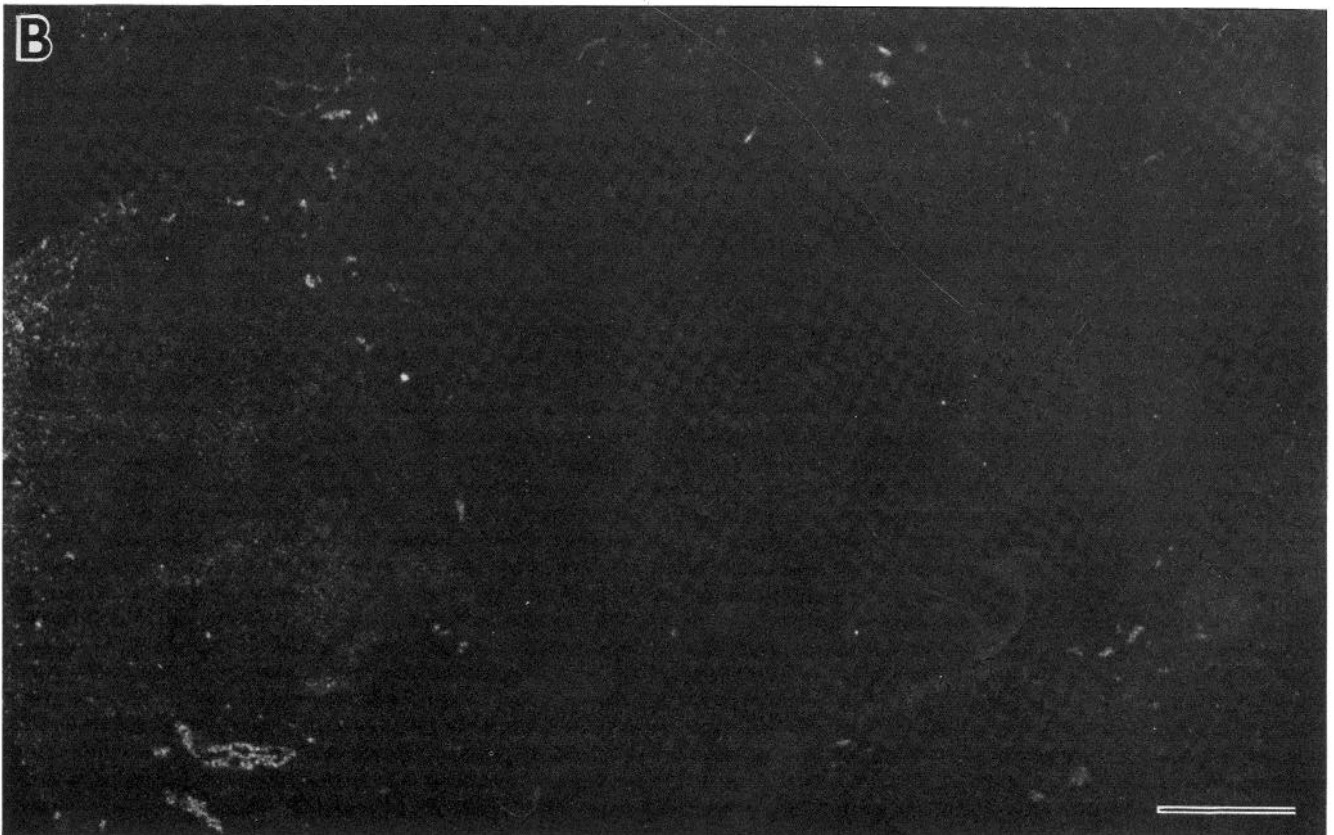


Figure 5. Autoradiographic distribution of CRF receptor-binding sites in rat brain, labeled with Nle²¹, Tyr³²-oCRF. A and B are darkfield photomicrographs (³H-Ultrofilm), showing the distribution of autoradiographic grains in sagittal sections of rat brain. In darkfield illumination the autoradiographic grains, i.e., binding sites, appear as white spots and the tissue is not visible. Thus, the brightest areas have the highest concentration of binding sites. A shows the "total" binding and B shows the absence of specific receptor binding when 1 μ M unlabeled Nle²¹, Tyr³²-oCRF is included in the incubation buffer (blank). Bar = 2 mm.

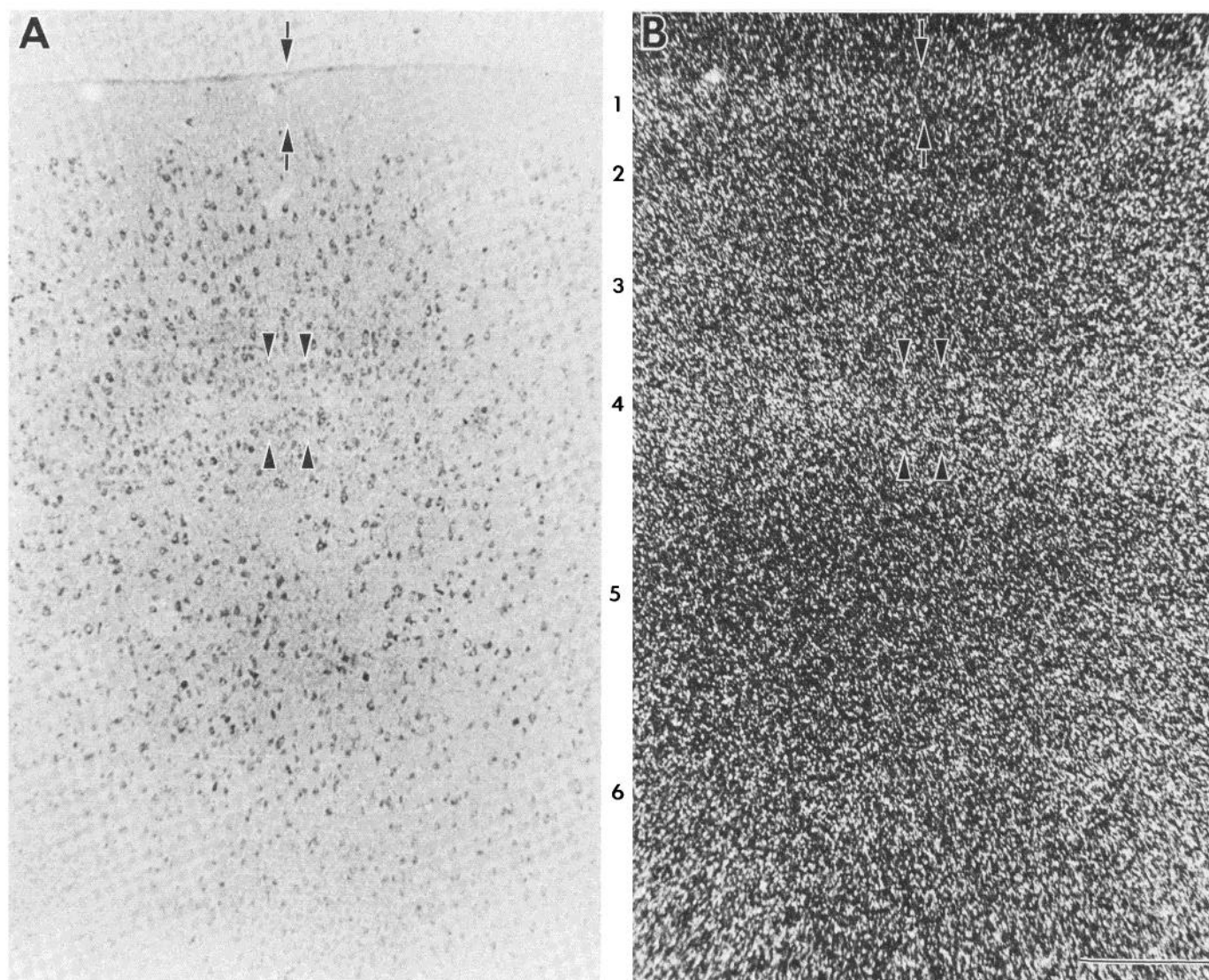


Figure 6. Autoradiographic localization of CRF receptors in the cerebral cortex (parietal region). *A*, Brightfield photomicrograph of a toluidine blue-stained section (coronal plane) showing the six layers (1 to 6) of the neocortex. *B*, Darkfield photomicrograph showing the autoradiographic grain distribution on emulsion-coated coverslips over the same area shown in *A*. In *B* note the high concentration of grains in lamina 1 (between arrows) and lamina 4 (between arrowheads) of the neocortex. Bar = 25 μm .

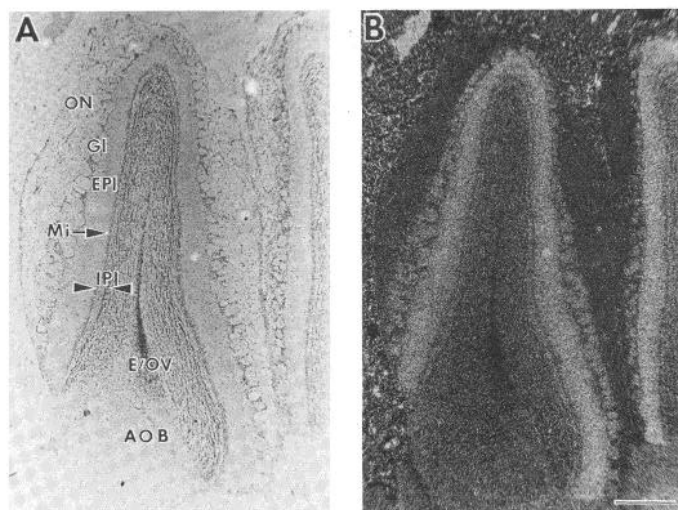


Figure 7. Autoradiographic localization of CRF receptors in rat olfactory bulb. *A*, Brightfield photomicrograph of a toluidine blue-stained tissue show-

ing the histology of the olfactory bulb sectioned in the horizontal plane. *B*, Darkfield photomicrograph showing the autoradiographic grain distribution on emulsion-coated coverslips over the same area shown in *A*. In darkfield illumination the autoradiographic silver grains appear as white spots and the tissue is not visible. In *B*, note the high concentration of grains in the external plexiform layer and glomerular layer with a somewhat lower concentration in the internal plexiform layer (arrowheads in *A*). Bar = 200 μm . AOB, accessory olfactory bulb; E/OV, ependymal olfactory ventricle; EPI, external plexiform layer; GI, glomerular layer; IPI, internal plexiform layer; Mi, mitral cell layer; ON, olfactory nerve layer.

times in Dulbecco's phosphate-buffered saline containing 1% BSA at either 4°C or at 22°C. Nonspecific binding was determined in the presence of 1 μM oCRF. The dissociation rates (K_{-1}) at 4°C and at 22°C were 0.046 and 0.066 min^{-1} , respectively.

Next, we examined the time course of association of Nle^{21} , ^{125}I -Tyr 32 -oCRF with receptors at 22°C in slide-mounted sections of rat forebrain (Fig. 4). Slide-mounted tissue sections were incubated with 0.2 nM Nle^{21} , ^{125}I -Tyr 32 -oCRF for various times at 22°C, rinsed for two 5-min periods at 4°C, and assayed for radioactivity. The specific binding increased with time during the first 60 min of incubation, after which there was no further significant increase. Based on these

ing the histology of the olfactory bulb sectioned in the horizontal plane. *B*, Darkfield photomicrograph showing the autoradiographic grain distribution on emulsion-coated coverslips over the same area shown in *A*. In darkfield illumination the autoradiographic silver grains appear as white spots and the tissue is not visible. In *B*, note the high concentration of grains in the external plexiform layer and glomerular layer with a somewhat lower concentration in the internal plexiform layer (arrowheads in *A*). Bar = 200 μm . AOB, accessory olfactory bulb; E/OV, ependymal olfactory ventricle; EPI, external plexiform layer; GI, glomerular layer; IPI, internal plexiform layer; Mi, mitral cell layer; ON, olfactory nerve layer.

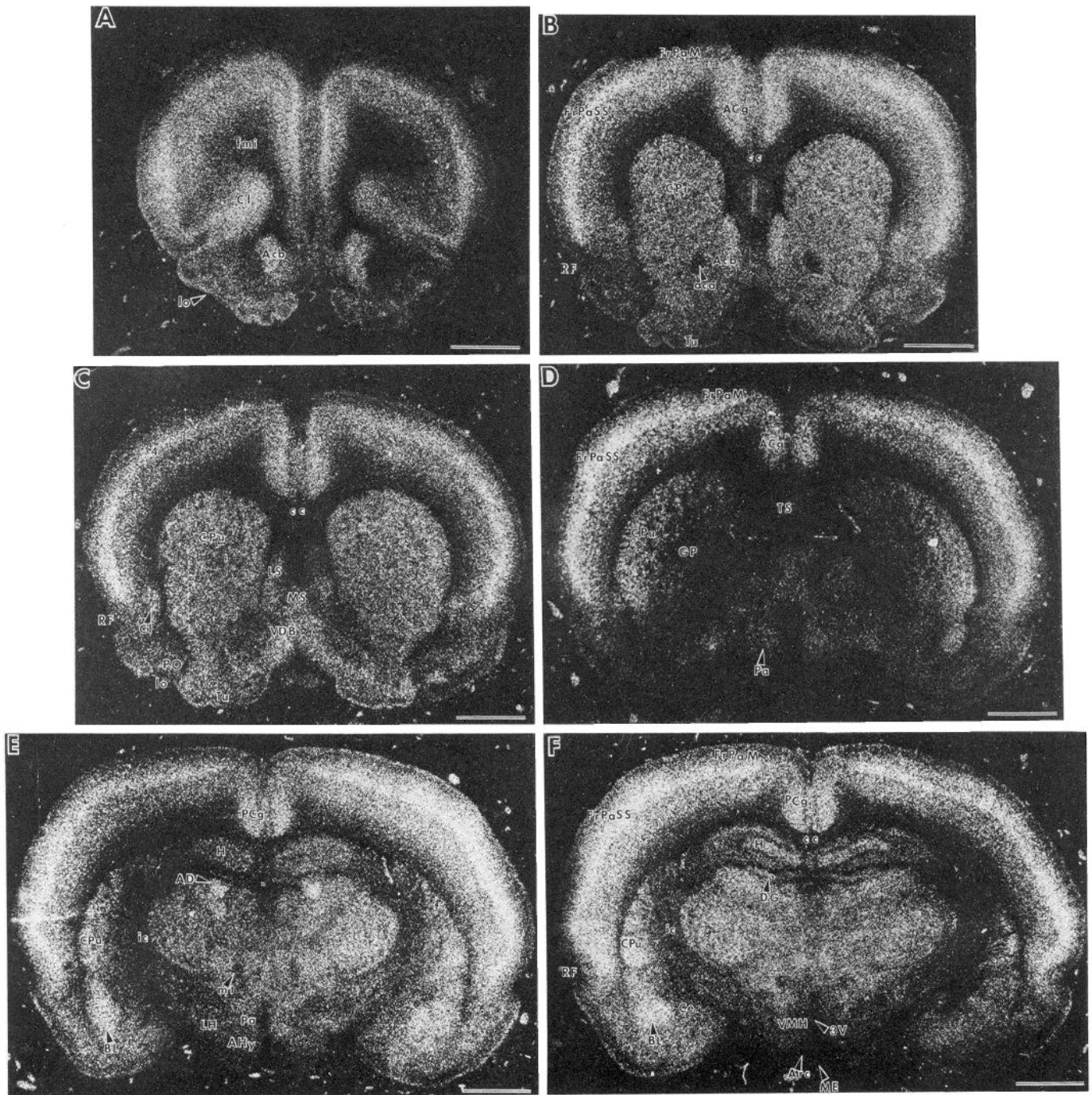


Figure 8.³ Autoradiographic mapping of the distribution of CRF receptor-binding sites in coronal sections of rat brain, labeled with Nle^{21} , $^{125}\text{I-Tyr}^{32}$ -oCRF. A to L are darkfield photomicrographs (tritium-sensitive Ultrafilm) showing the distribution of autoradiographic grains in coronal sections of rat forebrain (A to F) and brainstem (G to L). The autoradiograms pictured here are from one animal and are organized rostrocaudally. The anatomy of the CRF binding sites was confirmed in sections where autoradiograms were generated by apposition of emulsion-coated coverslips that were permanently affixed to the slides. A concentration of 0.2 nM Nle^{21} , $^{125}\text{I-Tyr}^{32}$ -oCRF was used to label these sections. Nonspecific binding, determined in the presence of 1 μM unlabeled Nle^{21} , $^{125}\text{I-Tyr}^{32}$ -oCRF was uniform and comparable to that shown in Figure 5B. See the text for details. Bar = 2 mm.

³ The abbreviations used in Figure 8 are: 3, principal oculomotor nucleus; 3V, third ventricle; 7, facial nucleus; 7n, facial nerve or root of facial nerve; 12, hypoglossal nucleus; aca, anterior commissure, anterior part; Acb, accumbens nucleus; ACg, anterior cingulate cortex; AD, anterodorsal thalamic nucleus; AHy, anterior hypothalamic area; Aq, cerebral aqueduct (Sylvius); Arc, arcuate hypothalamic nucleus; BL, basolateral amygdaloid nucleus; Cb, cerebellum; cc, corpus callosum; Ce, central amygdaloid nucleus; CG, central gray; Cl, claustrum; Co, cochlear nucleus; CPu, caudate-putamen; Cu, cuneate nucleus; DG, dentate gyrus; DpMe, deep mesencephalic nucleus; DR, dorsal raphe nucleus; DTg, dorsal tegmental nucleus (Gudzen); Ent, entorhinal cortex; Fl, flocculus; fmi, forceps minor of the corpus callosum; FrPaM, frontoparietal cortex, motor area; FrPaSS, frontoparietal cortex, somatosensory area; GP, globus pallidus; Gr, gracile nucleus; H, hippocampus; IC, inferior colliculus; ic, internal capsule; Int, interpositus cerebellar nucleus; IO, inferior olive; IP, interpeduncular nucleus; LatC, lateral

cervical nucleus; LC, locus ceruleus; LH, lateral hypothalamic area; ll, lateral lemniscus; lo, lateral olfactory tract; LS, lateral septal nucleus; Md, reticular nucleus of the medulla; ME, median eminence; MS, medial septal nucleus; MT, medial terminal nucleus of the accessory optic tract; Pa, paraventricular hypothalamic nucleus; PCg, posterior cingulate cortex; PFI, paraflocculus; Pn, pontine nuclei; PnO, pontine reticular nucleus, oral part; PO, primary olfactory (piriform) cortex; Pr5, principal sensory trigeminal nucleus; py, pyramidal tract; RF, rhinal fissure; RPn, raphe pontis nucleus; RSpI, retrosplenial cortex; S, subiculum; s5, sensory root of the trigeminal nerve; sol, solitary tract; sp5, spinal tract of the trigeminal nerve; Sp5C, nucleus of the spinal tract of the trigeminal nerve, caudal part; Str18a, striate cortex, area 18a; TS, triangular septal nucleus (interstitial nucleus of the ventral hippocampal commissure); Tu, olfactory tubercle; VDB, nucleus of the vertical limb of the diagonal band, dorsal part; Ve, vestibular nucleus; VMH, ventromedial hypothalamic nucleus.

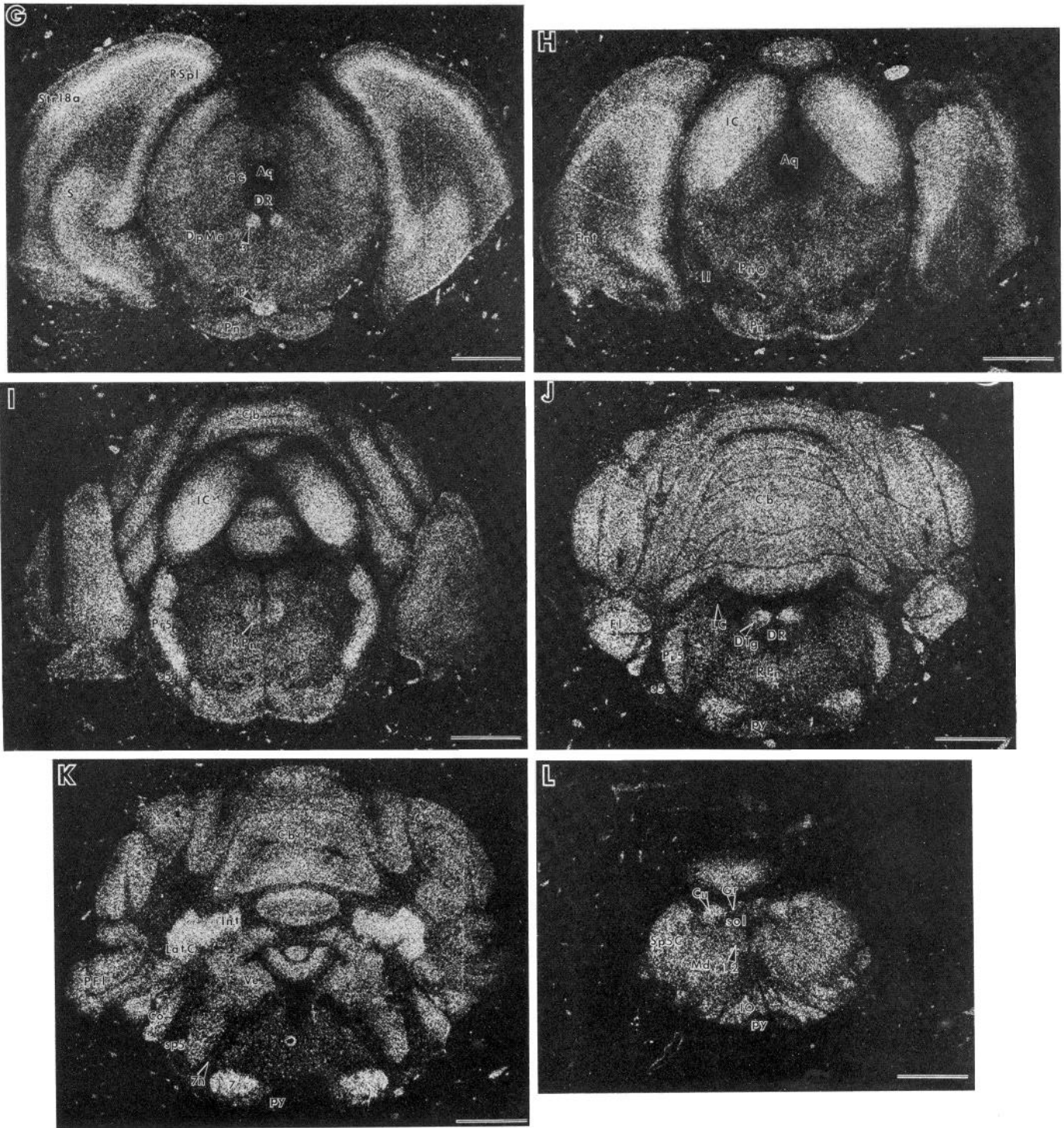


Figure 8, G-L

observations, all subsequent autoradiographic studies utilized a 60- to 90-min incubation at 22°C followed by two 5-min washes at 4°C.

In a previous study (De Souza et al., 1984b), we defined the characteristics of Nle²¹, ¹²⁵I-Tyr³²-oCRF binding in rat brain under conditions defined above. We incubated serial slide-mounted tissue sections with increasing concentrations of CRF-related and unrelated peptides. The binding of Nle²¹, ¹²⁵I-Tyr³²-oCRF in rat striatum was saturable and, on Scatchard analysis, revealed a high affinity component with an apparent K_d of 6.2 ± 0.8 nM and a low affinity binding site with a K_d of approximately 100 nM. In competition studies, 1 μ M

rat (human) CRF and ovine CRF inhibited 105% and 81%, respectively, of the specific Nle²¹, ¹²⁵I-Tyr³²-oCRF binding in rat striatum. Two fragments of oCRF, CRF(1-39) and CRF(1-22), which are weak in stimulating pro-opiomelanocortin-derived peptide secretion from the pituitary gland, displaced about 30%, and the unrelated peptide arginine vasopressin did not inhibit the binding.

Autoradiographic studies. The autoradiographic distribution of CRF-binding sites is shown in Figures 5 to 10 and quantified in Tables I to III. In general, nearly all of the Nle²¹, ¹²⁵I-Tyr³²-oCRF binding was to gray matter areas with nonsignificant levels present in white

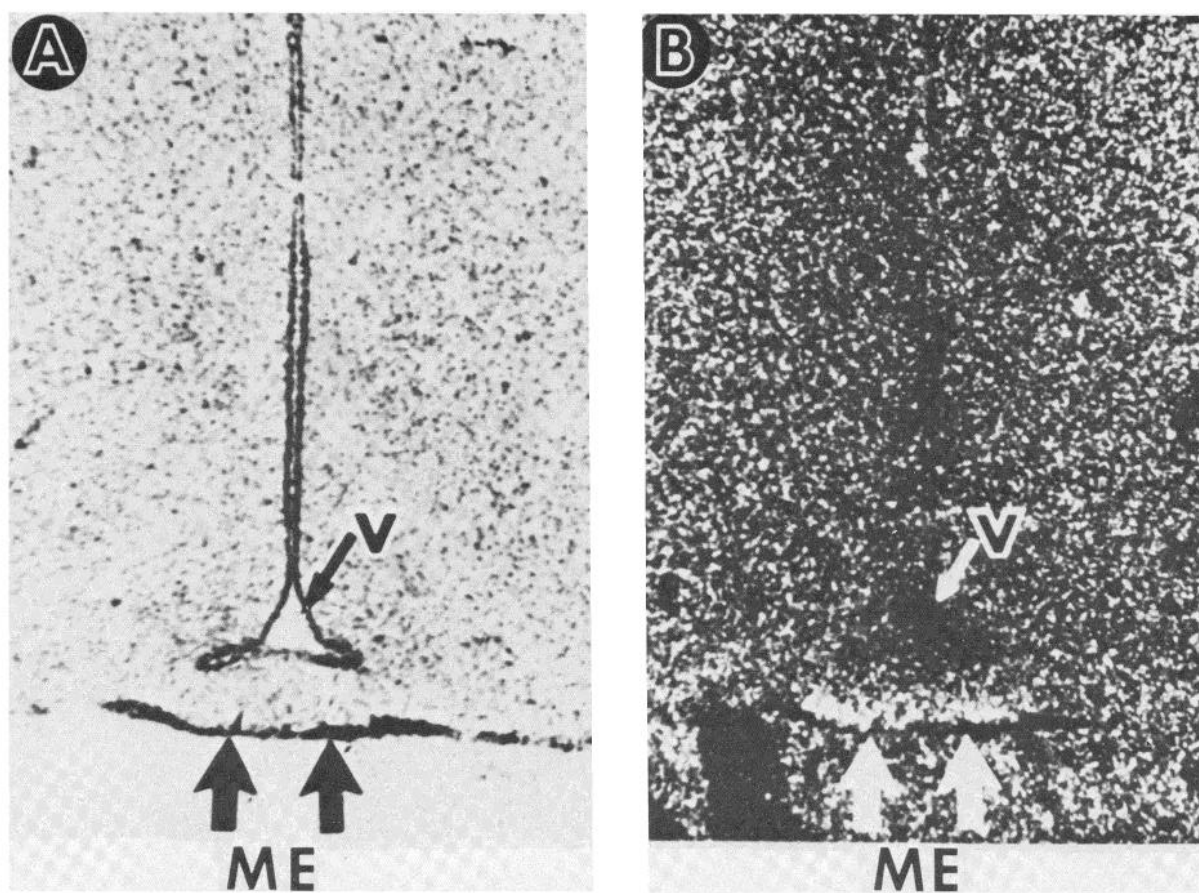


Figure 9. Binding of Nle^{21} , $^{125}\text{I-Tyr}^{32}$ -oCRF in the rat medial hypothalamus. A, Brightfield photomicrograph of a toluidine blue-stained tissue section showing a high power view of the median eminence of the hypothalamus (ME) at the base of the third ventricle (V). B, Darkfield photomicrograph showing the autoradiographic grain distribution on Kodak NTB-3 emulsion-coated coverslips over the same area shown in A. In B note that the concentration of grains in the external layer of the median eminence (arrows) is higher than in the remainder of the hypothalamus.

matter tracts such as corpus callosum, fornix, and optic chiasm. In describing the distribution of Nle^{21} , $^{125}\text{I-Tyr}^{32}$ -oCRF-binding sites which follows, qualitative expressions of receptor density will correspond to the relative binding values in Table III: very low levels, specific binding of 0 to 20 fmol/mg of protein; low levels, 20 to 40 fmol/mg of protein; moderate levels, 40 to 80 fmol/mg of protein; high levels, 80 to 100 fmol/mg of protein; and very high levels, >100 fmol/mg of protein. Nonspecific binding was consistently uniform in all brain areas (Fig. 5B) and was subtracted from the total binding to calculate specific binding.

CRF receptors were found throughout the neocortex. Within the neocortex, high grain densities were present in laminae 1 and 4 with moderate levels observed in lamina 2, 3, 5 and 6 (Fig. 6, Table II). Although this laminar distribution of Nle^{21} , $^{125}\text{I-Tyr}^{32}$ -oCRF-binding sites was maintained throughout the neocortex, on the average, more binding sites were found in somatosensory (see Fig. 8, B to F), striate (see Fig. 8H), and entorhinal (see Fig. 8G) cortex than in motor (see Fig. 8, B to F) and cingulate (see Fig. 8, B to F) cortex.

A change in the pattern of CRF binding was seen at the transition of the neocortex and the pyriform cortex near the rhinal fissure (see Fig. 8, B and C). In the pyriform cortex, a high density of binding sites was present only in lamina I, with much lower levels found in the remaining laminae. A high grain density was found in the external plexiform layer of the olfactory tubercle, whereas low density binding was observed in the pyramidal and polymorphic layers of the tubercle as well as in the islands of Calleja (see Fig. 8C). No specific CRF binding was present in the lateral olfactory tract (see Fig. 8, A to C). In the olfactory bulb (Fig. 7, Table I), Nle^{21} , $^{125}\text{I-Tyr}^{32}$ -oCRF-binding sites were highly localized to the external plexiform layer

and the adjacent glomerular layer. Low to negligible concentrations of receptors were found in the remainder of the bulb including the nerve fiber, internal plexiform, mitral cell body, and internal granule layers of the olfactory bulb.

In the corpus striatum, moderate levels of CRF receptors were observed in the caudate-putamen (Fig. 8, B to F). The adjacent globus pallidus (Fig. 8D) had a low density of CRF-binding sites. Moderate grain densities were observed over the claustrum (Fig. 8, A and C).

Moderately high levels of specific binding were associated with the nucleus accumbens (Fig. 8A), with slightly higher grain densities observed over the medial than the lateral parts of this nucleus (Fig. 8B). Low to moderate grain densities were observed over both the vertical and horizontal limbs of the nucleus and tract of the diagonal band (Fig. 8C). Low levels of CRF binding were present in the areas of medial and lateral septal nuclei (Fig. 8C) and triangular septal nucleus (Fig. 8D), and slightly higher levels were present in the bed nucleus of the stria terminalis. In the amygdala, low concentrations of receptors were observed in the medial and central nuclei, whereas a moderate concentration of CRF receptors was present in the basolateral nucleus (Fig. 8, E and F).

There was a fairly uniform, low to moderate level of CRF-binding sites throughout the thalamus with a slightly higher concentration of grains observed in the lateral compared to the medial thalamus (Fig. 8, E and F). In the dorsal thalamic complex, a higher concentration of receptors was associated with the anterodorsal nucleus (Fig. 8E). The habenular nuclei of the epithalamus contained a moderate level of CRF-binding sites.

In general, the hypothalamus contained a low density of autora-

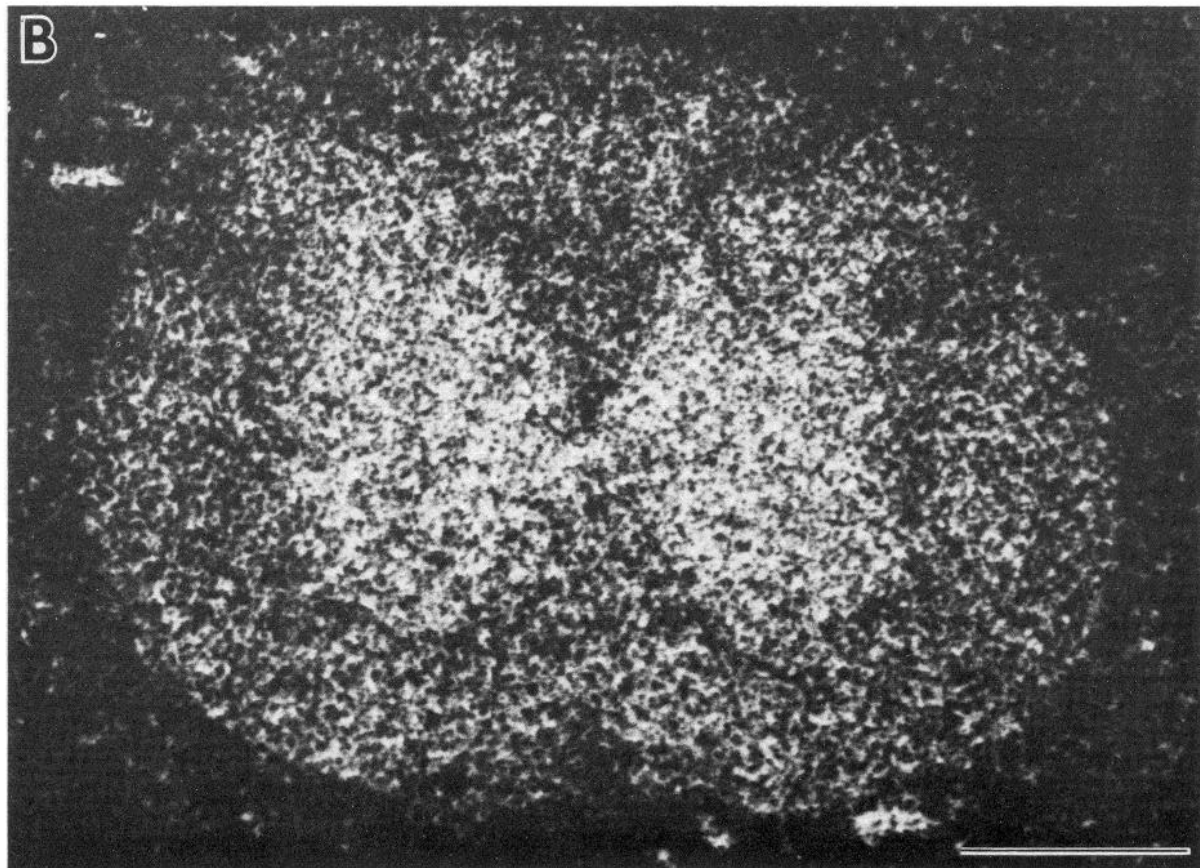
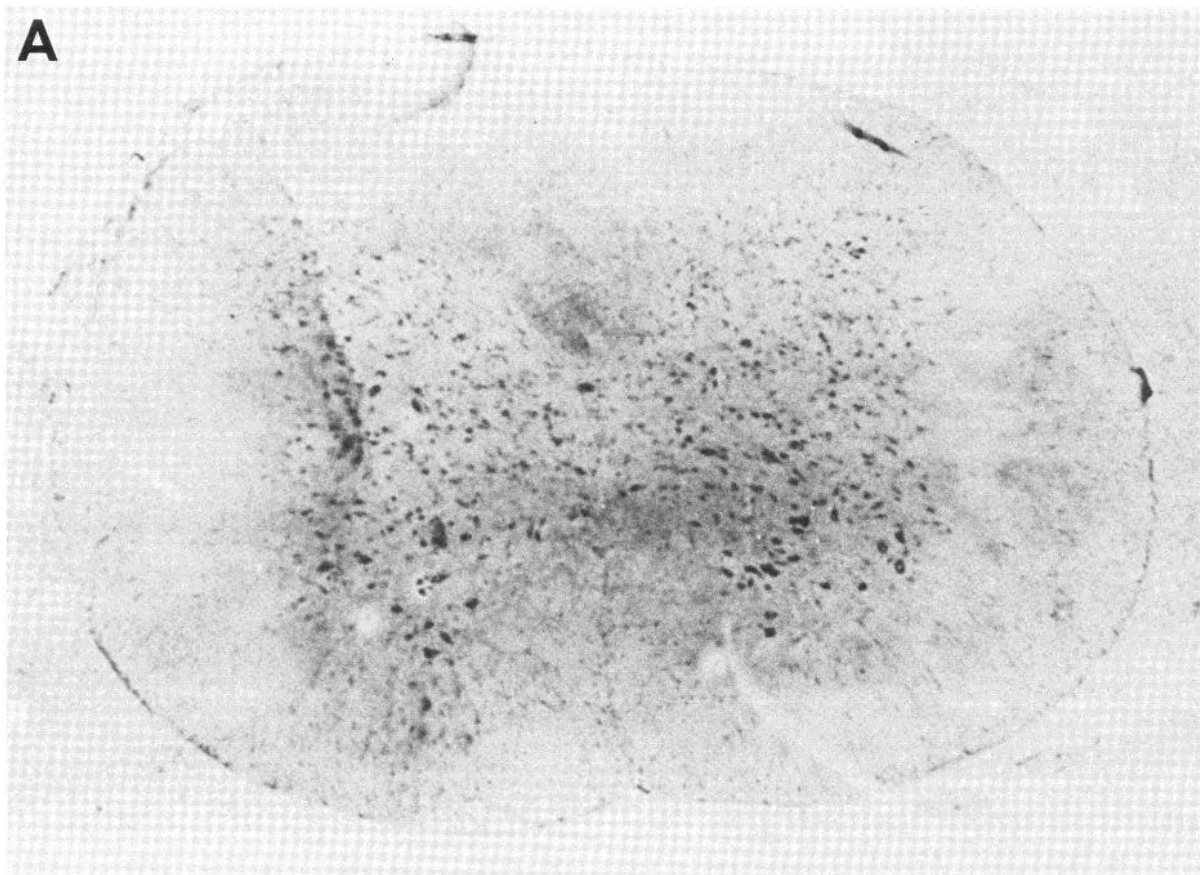


Figure 10. Distribution of CRF receptors in rat spinal cord. *A*, Brightfield photomicrograph of a toluidine blue-stained section of the cervical spinal cord. *B*, Darkfield photomicrograph showing the autoradiographic grain distribution on tritium-sensitive Ultrafilm over the same area shown in *A*. In *B*, note the lower concentration of grains in Rexed layers 1 to 3 of the dorsal horn than in the rest of the dorsal horn or the entire ventral horn. *Bar* = 500 μm .

TABLE I

Distribution of CRF-binding sites in the rat olfactory bulb

Data represent the total density of autoradiographic silver grains per 500 μm^2 as visualized over a given locus and are reported as mean \pm SEM. Nonspecific binding as defined by the density of grains in the presence of 1 μM Nle²¹, Tyr³²-oCRF was 81.9 \pm 2.4 grains/500 μm^2 and was uniform over the entire bulb. These data are semiquantitative since the relationship between grain density and tissue radioactivity is not linear (Kuhar and Unnerstall, 1985). Therefore, the nonspecific binding was not subtracted from the above values. The mean grain counts were determined in six 475- μm^2 areas from three horizontal sections through the olfactory bulb.

Area	Total Nle ²¹ , ¹²⁵ I-Tyr ³² -oCRF-binding Sites (grains/500 μm^2)
Nerve fiber layer	82.0 \pm 3.4
Glomerular layer	172.1 \pm 4.2
External plexiform layer	187.4 \pm 6.3
Mitral cell body layer	116.7 \pm 4.8
Internal plexiform layer	148.4 \pm 4.8
Internal granule cell layer	100.7 \pm 4.7
Ependyma/olfactory ventricle	81.2 \pm 2.3
Accessory olfactory bulb	91.0 \pm 2.9

TABLE II

Laminar distribution of CRF-binding sites in the cerebral cortex

Data represent the total density of autoradiographic silver grains per 500 μm^2 ; values are mean \pm SEM. Nonspecific binding as defined by the density of grains in the presence of 1 μM Nle²¹, Tyr³²-oCRF was 90.8 \pm 2.6 grains/500 μm^2 and was uniform over the entire cortex. These data are semiquantitative since the relationship between grain density and tissue radioactivity is not linear (Kuhar and Unnerstall, 1985). Therefore, the nonspecific binding was not subtracted from the above values. The mean grain counts were determined in six 475- μm^2 areas from three horizontal sections through the parietal cortex.

Lamina	Total Nle ²¹ , ¹²⁵ I-Tyr ³² -oCRF-binding Sites (grains/500 μm^2)
1	164.4 \pm 5.6
2	122.9 \pm 6.6
3	136.2 \pm 4.8
4	208.2 \pm 5.9
5	142.9 \pm 4.6
6	136.6 \pm 4.3

diographic grains (Fig. 8, D to F). Two exceptions included the paraventricular nucleus (Fig. 8, D and E) and mammillary peduncle where moderate levels of receptors were present. A very high concentration of grains was found in the external layer of the median eminence (Fig. 9). No specific binding was observed in the arcuate nucleus.

Overall, the hippocampus contained a low concentration of CRF receptors (Fig. 8, E to G). Within the hippocampus, the highest concentrations of binding sites were present in the subiculum (Fig. 8G), the molecular layer of the dentate gyrus, and the CA1 region (Fig. 8F). Lower concentrations of binding sites were found in the remaining areas of this brain region.

In the midbrain a moderate level of CRF receptors was seen in the inferior colliculus (Fig. 8, H and I). Within the inferior colliculus, the ventrolateral aspects had higher grain densities than did the dorsomedial portion. Fairly dense labeling of CRF receptors was found in the trochlear nuclei and in the interpeduncular nucleus (Fig. 8G). Low levels of CRF receptors were present in the periaqueductal gray, dorsal raphe nucleus (Fig. 8G), and superior colliculus, and very low concentrations were seen in the substantia nigra.

In the pons, the cranial nerve nuclei including the facial (Fig. 8, J and K), trigeminal (Fig. 8, I to K), cochlear (Fig. 8K), and vestibular nuclei (Fig. 8K) had very high concentrations of autoradiographic

grains. In fact, the highest concentration of CRF-binding sites in the rat CNS was found in the facial nucleus. The medial portions of the vestibular nucleus had a much higher concentration of CRF-binding sites, whereas the lateral and superior aspects of this nucleus had moderate to low concentrations of autoradiographic grains. High grain densities were found in the lateral cervical nucleus (Fig. 8K) and at the point of entry of the eighth nerve. All portions of the nucleus of the spinal tract of the trigeminal nerve (Fig. 8, I to K) had comparable high levels of CRF receptors. Moderate numbers of grains were associated with the hypoglossal (Fig. 8L), lateral reticular, paragigantocellular reticular, parabrachial and dorsal (Fig. 8J), and ventral tegmental nuclei. The pontine nuclei (Fig. 8, G and H) themselves and the raphe pontis nucleus (Fig. 8, I and J) contained moderate to low numbers of grains, whereas the pontine reticular nuclei (Fig. 8H) had a very low density of CRF receptors. Very low levels of receptors were found in the locus ceruleus (Fig. 8J).

In the medulla, the highest receptor densities were found in the cuneate and gracilis nuclei (Fig. 8L). High grain densities were also found in the inferior olive (Fig. 8L). The concentration of CRF receptors was variable (average of low to moderate) in the nucleus of the solitary tract, the solitary tract (Fig. 8L), and throughout the extent of the pyramids (Fig. 8, K and L).

In the cerebellum, CRF receptors were present in all parts of the cerebellar cortex, with highest concentrations in the granular layer (Fig. 8, J and K). The medial nucleus and the interpositus nucleus (Fig. 8K) had high receptor levels.

In the cervical spinal cord, moderate levels of receptors were seen in the gray matter with lower levels found in the dorsal horn layers 1 to 4 (Fig. 10).

Discussion

Biochemical receptor binding studies in slide-mounted forebrain sections using conditions identical to those used in autoradiographic experiments have identified high affinity and pharmacologically specific binding sites for Nle²¹, ¹²⁵I-Tyr³²-oCRF in rat brain. The kinetic characteristics and peptide specificity of the Nle²¹, ¹²⁵I-Tyr³²-oCRF-binding site in rat brain were comparable to those for the CRF receptor in the anterior lobe of rat (Wynn et al., 1983; De Souza et al., 1984a; Holmes et al., 1984), human (De Souza et al., 1985), and bovine (De Souza and Kuhar, 1985) pituitary glands and in the rat intermediate pituitary gland (De Souza et al., 1984a). These data substantiate earlier suggestions (Peterfreund and Vale, 1983; Valentino et al., 1983; D. R. Britton et al., 1984) that some structural requirements for CRF activity are shared by brain and pituitary receptors.

The densities of CRF receptors in the rat CNS are generally consistent with the relative distribution of CRF-immunoreactive (CRF-IR) terminals (Bloom et al., 1982; Bugnon et al., 1982; Olschowka et al., 1982; Cummings et al., 1983; Joseph and Knigge, 1983; Lerenth et al., 1983; Merchenthaler et al., 1983; Paull and Gibbs, 1983; Swanson et al., 1983). For example, CRF receptors are present in most of the hypothalamus with the highest concentrations of binding sites present in the external layer of the median eminence, where the most intense concentration of CRF fibers is also found. Also, CRF cell bodies in the neocortex are concentrated in laminae 2 and 3, with projections to laminae 1 and 4, areas rich in CRF receptors. Additional areas of good correspondence between the distribution of CRF-IR nerve terminals and Nle²¹, ¹²⁵I-Tyr³²-oCRF binding sites include the olfactory tubercle, caudate-putamen, nucleus of the diagonal band of Broca, medial and lateral septal nuclei, the cranial nerve nuclei in the brainstem—in particular, the facial, principal oculomotor, trochlear, trigeminal, and hypoglossal nuclei, and in other brainstem regions including the central gray, inferior olive, reticular formation, and cuneate and parabrachial nuclei.

There are areas in the rat CNS in which the distributions of CRF-IR nerve fibers and CRF receptors do not correlate as well. Some of these areas include the olfactory bulb, amygdala, cerebellum, and spinal cord. In the amygdala, CRF cell bodies and nerve terminals appear confined primarily to the central nucleus (Olschowka et al.,

TABLE III

Distribution of CRF-binding sites in the rat CNS

The data are based on observations from two (no SEM) or three animals. The anatomical terminology is derived from Paxinos and Watson (1982). CRF-binding sites were visualized using 0.7 nM Nle²¹, ¹²⁵I-Tyr³²-oCRF. At this concentration of ligand, approximately 10% of the total number of binding sites are occupied. Autoradiograms of rat brain, rat spinal cord, and iodinated gray (bovine caudate) and white (bovine corpus callosum) sections (all 8 μm) were generated with tritium-sensitive Ultrafilm (LKB). An analysis of Nle²¹, ¹²⁵I-Tyr³²-oCRF binding in the different regions of rat CNS was performed by computerized image analysis densitometry, and the film optical density was related to the molar concentration of radioactivity by use of the standard curve generated and analyzed concomitantly with the autoradiograms.

Region	Specific Binding fmol/mg of protein ± SEM
Frontoparietal Cortex (somatosensory)	
Laminae I and II	68.7 ± 6.2
Laminae III and IV	98.6 ± 6.4
Laminae V and VI	71.2 ± 5.5
Frontoparietal Cortex (motor)	
Laminae I and II	48.3 ± 3.9
Laminae III and IV	74.3 ± 3.9
Laminae V and VI	58.0 ± 4.1
Striate Cortex (area 18a)	
Laminae I and II	35.3 ± 7.4
Laminae III and IV	82.6 ± 6.5
Laminae V and VI	53.9 ± 5.5
Cingulate Cortex (all laminae)	43.6 ± 4.1
Entorhinal Cortex (all laminae)	76.1 ± 9.3
Olfactory Tubercle	65.6 ± 11.6
Basal Ganglia	
Caudate-putamen	47.2 ± 3.7
Globus pallidus	16.5 ± 0.5
Clastrum	78.8 ± 13.3
Septal Area	
Nucleus accumbens	65.2 ± 8.0
Nucleus of the diagonal band (Broca)	34.5 ± 2.6
Lateral septal nucleus	19.6 ± 2.6
Medial septal nucleus	24.3 ± 4.2
Triangular septal nucleus	10.0
Bed nucleus of the stria terminalis	21.5
Amygdala	
Basolateral nucleus	50.5 ± 3.8
Central nucleus	21.6 ± 2.8
Medial nucleus	25.6 ± 2.7
Thalamus	
Lateral	37.5 ± 4.5
Medial	21.2 ± 3.7
Anterodorsal thalamic nucleus	29.7
Habenula (medial plus lateral)	33.5 ± 3.3
Hypothalamus	
Lateral	24.0 ± 4.8
Medial	24.5 ± 4.5
Anterior	27.8 ± 1.1
Paraventricular nucleus	32.9 ± 1.6
Arcuate nucleus	0
Mammillary peduncle	54.7 ± 5.9
Hippocampus (whole)	19.9 ± 4.1
Dentate gyrus	22.4 ± 4.9
Subiculum	31.3

TABLE III—continued

Region	Specific Binding fmol/mg of protein ± SEM
Midbrain	
Inferior colliculus	43.1 ± 9.2
Dorsal raphe nucleus	8.2 ± 6.2
Central gray	22.4 ± 1.6
Medulla-Pons	
Inferior olive	75.8 ± 8.4
Superior olive	71.3 ± 13.4
Lateral reticular nucleus	61.8 ± 9.2
Paragigantocellular reticular nucleus	49.7
Nucleus of the spinal tract of the trigeminal nerve	96.1 ± 14.1
Cuneate nucleus	88.1 ± 6.7
Gracile nucleus	86.1 ± 10.1
Nucleus of the solitary tract	42.4 ± 23.1
Solitary tract	58.6 ± 10.3
Lateral vestibular nucleus	29.1
Medial vestibular nucleus	73.9 ± 4.7
Facial nucleus	150.6 ± 28.4
Lateral cervical nucleus	92.1 ± 17.4
Vestibulocochlear nucleus	107.4 ± 24.2
Cochlear nucleus	83.9
Dorsal tegmental nucleus	43.5 ± 13.0
Ventral tegmental nucleus	61.1
Parabrachial nucleus	59.4
Pontine nucleus	33.2
Hypoglossal nucleus	65.4 ± 6.9
Pyramids	44.9 ± 20.6
Locus Ceruleus	9.1 ± 7.1
Cerebellum (all lobules)	98.5 ± 12.7
Interpositus nucleus	71.3
Medial nucleus	92.2
Paraflocculus	118.1 ± 20.0
Spinal Cord (cervical)	
Dorsal horn (Rexed 1-4)	23.5 ± 09
Dorsal horn (Rexed 4-6)	44.3
Ventral horn	40.3 ± 1.4
White Matter Tracts	
Corpus callosum	7.5 ± 4.9
Forceps minor corpus callosum	6.3 ± 4.7
Fornix	0
Anterior commissure	25.0 ± 2.7
Optic chiasm	0

1982; Cummings et al., 1983; Merchenthaler et al., 1983; Swanson et al., 1983), whereas the highest concentration of CRF receptors is present in the basolateral nucleus. The cerebellum provides a very striking example of a disparity between the anatomical distribution of Nle²¹, ¹²⁵I-Tyr³²-oCRF-binding sites and CRF-IR fibers. In the rat cerebellum, moderate (Merchenthaler et al., 1983; Cummings et al., 1983), low (Olschowka et al., 1982), and undetectable (Swanson et al., 1983) densities of CRF-IR fibers have been reported. In contrast, the concentration of cerebellar binding sites is one of the highest in the rat CNS. In the spinal cord, laminae I and II of the dorsal horn contained the highest numbers of CRF-IR fibers (Olschowka et al., 1982; Schipper et al., 1983), areas of low CRF binding site density.

Similar mismatches between the levels of receptor and neurotransmitter have been noted in previous studies (Palacios and Kuhar, 1980; Palacios et al., 1980; Wamsley et al., 1982; Zarbin et al., 1983; Goedert et al., 1984; Mantyh et al., 1984), and the "mismatch

problem" has been the topic of a recent report (Kuhar, 1985). There should not be a match between receptor and neurotransmitter at the light microscopic level in whole sections. Neurotransmitter is contained throughout a neuron, whereas the receptor is contained in the postsynaptic neuron which has a different spatial distribution. Perhaps only when both neurons are interneurons and contained within the same region would a match be expected. Technical limitations in both autoradiographic and immunocytochemical experiments may prevent the complete and appropriate identification of receptor and neurotransmitter molecules, respectively. Subtypes of the CRF receptor may exist which only bind under an appropriate set of conditions such as ligand concentration, salts, and guanyl nucleotides. Also, agonist ligands similar to the radiolabeled CRF analogue used in the present study primarily label high affinity states of the receptor which may not be found in constant proportions in all regions of the CNS. Immunohistochemistry, in contrast, is dependent on the specificity of the antisera used. Most of the reports (see above) on the immunohistochemical distribution of CRF in rat brain have utilized primary antibodies directed against ovine CRF. Different distributions of CRF immunoreactivity in the rat CNS were recently reported using a variety of antibodies directed against rat and ovine CRF (Skofitsch and Jacobowitz, 1984). Future studies utilizing more specific antisera in combination with better radioligands may minimize the discrepancies between the distribution of CRF receptors and of CRF immunoreactivity.

Low to moderate concentrations of CRF-binding sites are present in several regions of the rat CNS known to contain primary groups of CRF-IR cell bodies such as the paraventricular nucleus of the hypothalamus, the central nucleus of the amygdala, the bed nucleus of the stria terminalis, and the nucleus of the solitary tract. These CRF-binding sites may represent presynaptic autoreceptors or receptors for internuclear communication which may play important roles in regulating peptide release originating from these perikarya.

CRF-binding sites are present in high concentrations in the median eminence. We are presently investigating the pharmacological characteristics of these CRF-binding sites in the median eminence. Several alternatives exist to explain the nature of the CRF binding in the median eminence. The binding sites may be presynaptic autoreceptors regulating the release of CRF into the portal vasculature. The CRF-binding sites in the median eminence may also represent interactions of CRF with proteins associated with ependymal tanyocytes which may function to transport CRF from the cerebrospinal fluid to the hypophysial portal vasculature (Zimmerman et al., 1973). Another possibility is that the CRF binding in the median eminence may be to CRF receptors on blood vessels similar to those described for vasopressin (Schiffrin and Genest, 1983). Although the precise role of these CRF-binding sites is unclear, similar distributions of receptors in the median eminence have been reported for other hypothalamic hypophysiotropic neurotransmitters such as vasopressin (Brinton et al., 1984) and dopamine (Cronin and Weiner, 1979).

There is relatively good correspondence within the rat CNS between areas containing CRF receptors and anatomic loci which are electrophysiologically responsive to CRF. Ionophoretic application of CRF excited in the cortex and hypothalamus and inhibited in the thalamus and lateral septal area spontaneously occurring action potentials (Eberly et al., 1983). CRF has also been demonstrated to produce excitation of hippocampal pyramidal cells in an *in vitro* hippocampal slice preparation (Aldenhoff et al., 1983). Direct microapplication of CRF to parabrachial neurons increased the discharge rate of these cells (Valentino et al., 1983). Intracerebroventricular administration of low doses of CRF in rats produced changes in electroencephalographic activity suggestive of increased arousal, whereas high doses of CRF elicited seizures that appeared electroencephalographically to originate in the amygdala (Ehlers et al., 1983). In contrast to the good correlation between the presence of CRF receptors and electrophysiologically responsive cells described above, CRF has been shown to directly activate noradrenergic neurons of the locus ceruleus (Valentino et al., 1983), an area with a low concentration of CRF-binding sites.

A role has been proposed for CRF in the control of food intake (Morley and Levine, 1982; D. R. Britton et al., 1982, 1984; Gosnell et al., 1983; Levine et al., 1983). The medial and lateral hypothalamus represent important brain areas that are involved in regulating food intake (see Stevenson 1969; Grossman, 1975). The hypothalamus may represent a primary site of action for integrating some endocrine and behavioral effects of CRF. Microinfusion of CRF into the arcuate-ventromedial area of the hypothalamus suppresses sexual behavior (Sirinathsinghji et al., 1983). In addition, intracerebroventricular injections of CRF have been shown to have antipyretic effects (Bernardini et al., 1984), to inhibit the release of growth hormone (Ono et al., 1984; Rivier and Vale, 1984b), luteinizing hormone (One et al., 1984; Rivier and Vale, 1984a), vasopressin, and oxytocin (Plotsky et al., 1984), and to have deleterious actions on reproductive functions (Rivier and Vale, 1984a). CRF can stimulate the secretion of somatostatin by cultured rat hypothalamic and cerebral cortical cells (Peterfreund and Vale, 1983).

The distribution of CRF receptors in the rat olfactory system is striking. Within the olfactory bulb, the CRF-binding sites are discretely localized in the glomerular layer where the primary olfactory afferent axons terminate (Sheppard, 1979) and in the adjacent external plexiform layer and in the mitral cell body layer where the olfactory output neurons originate (Sheppard, 1979). There is evidence in the literature suggesting that sexual, reproductive, social, and feeding behaviors of most mammalian species are mediated by olfactory substances and pheromonal agents acting through the olfactory system (see Whitten and Bronson, 1970). CRF influences both feeding behavior and sexual activity (see above). Although the hypothalamus is the primary anatomical locus regulating sexual activity and feeding behavior, hypothalamic neuronal activity can be modulated by olfactory impulses relayed through the circuit involving the olfactory bulb, pyriform cortex, amygdala, and subiculum (Lohman, 1963; Powell et al., 1965; Cain and Bindra, 1972; Dreifuss, 1972; Krettek and Price, 1978a, b); moderate to high densities of CRF receptors are present throughout the circuits.

CRF injected intracerebroventricularly produced dose-dependent locomotor activation (Sutton et al., 1982; D. R. Britton et al., 1982, 1984; Koob et al., 1984). High concentrations of CRF receptors were detected in several brain areas subserving motor function. These structures include the caudate-putamen, the cerebellum, and the facial and hypoglossal motor cranial nerve nuclei. Our localization of CRF-binding sites in the ventral horn of the spinal cord may demonstrate another potential site for CRF to centrally affect motor activity.

CRF acts within the CNS to elicit changes in the activity of the autonomic nervous system similar to those changes produced by many forms of stress. The autonomic effects of CRF are evident in a variety of species including rats (Brown et al., 1982a, b, c, 1984; Fisher et al., 1982, 1983; Tache et al., 1983, 1984; Fisher and Brown, 1984; Hamel and Tache, 1984), dogs (Brown et al., 1982c; Tache et al., 1984), sheep (Scoggins et al., 1984), and monkeys (Kalin et al., 1983; Insel et al., 1984), and include increased plasma concentrations of epinephrine, norepinephrine, glucose, glucagon, cardiovascular parameters such as heart rate and mean arterial pressure, and decreased gastric acid secretion. The present study provides an anatomical basis for some of the observed effects of CRF on autonomic function. CRF may produce some of its effects via receptors in the parabrachial nucleus, medullary reticular formation, nucleus of the solitary tract, or other brainstem areas known to participate in autonomic regulation. CRF-binding sites are also present in several forebrain regions that exert a powerful influence on autonomic outflow. These regions include the hypothalamus, especially the paraventricular nucleus, bed nucleus of the stria terminalis, amygdala, septum and hippocampus, and cingulate cortex. The autonomic effects of CRF administration may also be mediated by receptors in the spinal cord as suggested by effects of intrathecal injections of CRF at the lumbar level to inhibit gastric acid secretion (Hamel and Tache, 1984).

The immunohistochemical, electrophysiological, behavioral, phar-

macological, and receptor studies provide compelling evidence that endogenous CRF may function as a neurotransmitter in the CNS. The data discussed above suggest a key role for this neuropeptide in the endocrine, autonomic, and behavioral responses to stress that are characteristic of Selye's "general adaptation syndrome." There are some preliminary studies that support a role for endogenous CRF in stress. Footshock stress increased the CSF levels of CRF immunoreactivity in rats (K. T. Britton et al., 1984). Central administration of a CRF receptor antagonist in rats produced dose-dependent inhibition of the plasma epinephrine response to ether stress- or insulin-induced hypoglycemia (Brown et al., 1984).

In summary, we have used the radioiodinated ovine CRF analogue, Nle²¹, ¹²⁵I-Tyr³²-oCRF to map CRF-binding sites in the rat CNS using *in vitro* labeling autoradiographic techniques. The areas of distribution of CRF-binding sites are correlated well with the immunohistochemical distribution of CRF pathways and pharmacological sites of action of CRF. These data strongly support a physiological role for endogenous CRF in regulating and integrating CNS activity and suggest the importance of this neuropeptide in regulating endocrine and visceral functions and behavior, especially in response to stressful stimuli. The exact mechanisms by which CRF can modulate these complex functions remain unknown. Studies to characterize CRF receptors and CRF-containing pathways in the brain provide a means for better understanding the various functions of this neuropeptide in different areas of the CNS.

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