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Differential Distribution of Corticotropin-Releasing Hormone Immunoreactive Axons in Monoaminergic Nuclei of the Human Brainstem

Mark C. Austin, Ph.D., James L. Rhodes, B.S., and David A. Lewis, M.D.

Corticotropin-releasing hormone (CRH) has been implicated in a variety of physiological and behavioral responses to stress, as well as in the pathophysiology of certain psychiatric disorders. Although studies in rodents support a neuromodulatory influence of CRH on monoamine neurotransmission in a number of brain regions, little information is available to support a similar role for CRH in the human brain. The present study used immunocytochemistry to characterize the anatomical organization of CRH-immunoreactive axons in the human brainstem. Substantial regional differences in the density and distribution of CRH-immunoreactive axons were found in the dopamine-, noradrenaline- and serotonin-containing cell body regions of the human brainstem. Dense networks of CRH-immunoreactive axons were found in the medial subnuclei of the ventral mesencephalon and in the dorsolateral region of the locus coeruleus. Moderate densities of CRH-positive fibers were located in the median and dorsal raphe, whereas lower numbers of CRH-labeled axons appeared in the substantia nigra pars compacta. In addition, differences in CRH innervation density were observed within each region. For example, the dorsal tier of the substantia nigra contained a greater density of CRH-labeled axons than the ventral tier. In all monoamine-containing nuclei, CRH-labeled axons exhibited numerous beaded varicosities and fine intervaricose segments. The differential distribution of CRH-containing axons across these human brainstem nuclei suggests that the influence of CRH on monoamine function may be neurotransmitter-specific. **[*Neuropsychopharmacology 17:326–341, 1997*]** *© 1997 American College of Neuropsychopharmacology. Published by Elsevier Science, Inc.*

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Corticotropin-releasing factor or hormone (CRH), a 41-amino acid peptide originally isolated from bovine hypothalamus, is involved in mediating and coordinating a variety of physiological, immunological, and behavioral responses associated with stress and other arousal-producing stimuli (for reviews, see Owens and Nemeroff 1991; De Souza 1995). CRH, the primary chemical regulator mediating the stress-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis, stimulates the secretion of adrenocorticotropin hormone from the anterior pituitary (Rivier and Vale 1983; Ono et al. 1985). Although the hormonal role of CRH is clearly established, a substantial body of evidence has accumulated supporting a neurotransmitter or neuromodulatory role for this peptide in a variety of extrahypothalamic brain regions (for review, see Owens and Nemeroff

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1991). For example, immunocytochemical studies in a variety of species have revealed a widespread distribution of CRH-containing neurons and axons in a number of brain regions including the cerebral cortex, nucleus accumbens, ventral pallidum, amygdala, cerebellum, brainstem, and spinal cord (Fischman and Moldow 1982; Merchenthaler et al. 1982; Cummings et al. 1983; Swanson et al. 1983; Foote and Cha 1988; Lewis et al. 1989; Pammer et al. 1990; Austin et al. 1995). Similarly, receptor autoradiography studies have shown that the distribution of CRH receptors generally corresponds with that of CRH fibers (De Souza et al. 1985; Millan et al. 1986; De Souza 1987).

These anatomical findings suggest that CRH may influence the activity of specific neural systems. For example, CRH-immunoreactive axons are located in the locus coeruleus, and the physiological effects of CRH on noradrenergic neurons in this nucleus have been well documented in both electrophysiological and biochemical studies. Acute intracerebroventricular (ICV) administration or direct application of CRH on locus coeruleus neurons increases the spontaneous discharge rate of these neurons, and this effect is blocked by central or local injection of the CRH antagonist, α-helical CRF_{9-41} (Valentino et al. 1983; Valentino and Foote 1987, 1988). Valentino and colleagues also reported that the electrophysiological effects of CRH on locus coeruleus neurons are mimicked by hemodynamic stress, and that these effects can be prevented by ICV pretreatment with α-helical CRF, but not by dexamethasone pretreatment (Valentino and Wehby 1988; Valentino et al. 1991). These findings suggest that the stimulatory effects of hypotensive stress on locus coeruleus neurons may be mediated, at least in part, by the release of CRH from terminals within the locus coeruleus, which then acts postsynaptically to increase locus coeruleus neuronal firing (Valentino et al. 1991). Supporting these electrophysiological findings, the ICV administration of CRH increases norepinephrine (NE) metabolism in several terminal field regions of the locus coeruleus (Dunn and Berridge 1987; Emoto et al. 1993), and recent microdialysis studies found that ICV or direct injection of CRH into the locus coeruleus stimulated NE release in the prefrontal or parietal cortex (Lavicky and Dunn 1993; Smagin et al. 1995; Schulz and Lehnert 1996). Furthermore, Melia and Duman (1991) reported that the increase in tyrosine hydroxylase (TH) protein levels in the locus coeruleus following intermittent footshock stress was antagonized by intra-locus coeruleus pretreatment with α-helical CRF. These investigators also found that rats administered CRH ICV twice daily for 5 days had elevated TH protein levels in the locus coeruleus. Given the supporting evidence of a stimulatory role of CRH on rat locus coeruleus neurons combined with the anatomical observations of dense CRH-containing axons in the human locus coeruleus (Pammer et al. 1990; Austin et al. 1995), it is conceivable that CRH in the human locus coeruleus may serve a similar function. These observations raise the question of whether CRH may also influence the function of other monoamine-containing nuclei in the human brainstem.

In addition to CRH and norepinephrine, both dopamine and serotonin also play a role in stress responses (for review, see Stanford 1993). Furthermore, each of these neurotransmitter systems has been implicated in the pathogenesis of a number of mental disorders. Understanding the anatomical basis for an interaction between CRH and these specific monoamine systems may provide insights into the pathophysiology of certain psychiatric disorders that are affected by stress. Consequently, in the present study, we used immunocytochemical techniques to characterize the anatomical organization of CRH-immunoreactive axons in the major monoamine-containing cell groups of the human pons and midbrain.

METHODS AND MATERIALS

Tissue Collection

Five human brainstems were collected in the course of routine autopsies. Neuropathological changes were excluded by macroscopic and microscopic examination of the brain. All subjects had negative toxicology screens for psychoactive or neurotoxic drugs and none of the subjects had a known history of neurologic or psychiatric disorders as determined by structured interviews with relatives. The demographics for each subject are presented in Table 1. Informed consent was obtained from surviving relatives, and all procedures were conducted in accordance with the Biomedical Institutional Review Board of the University of Pittsburgh.

Upon removal of the brain from the cranium, the brainstem was separated by a transverse cut rostral to the superior colliculi and the cerebellum was removed. The brainstem was then cut into coronal slabs of tissue consisting of the caudal pons, pons/midbrain, and rostral midbrain. The blocks of tissue were immersed in cold 4%

Table 1. Demographics of Human Subjects

Case	Sex	Age (years)	PMI (h)	Cause of Death
1	M	45	6.3	Aortic aneurysm
2	M	74	17.5	Accident
3	M	22	8.9	Homicide
4	M	25	5.3	Accident
5	M	45	7.0	ASCVD

ASCVD = atherosclerotic cardiovascular disease.

paraformaldehyde in phosphate buffer for 48 h and then washed with increasing concentrations of sucrose.

Immunocytochemistry

Brainstem tissue blocks were frozen and sectioned transversely (40 μm) using a cryostat. The tissue sections were stored in cryoprotectant (30% ethylene glycol, 30% glycerol in 0.02 mol-L phosphate buffer) at −20°C until processed. Sections were rinsed thoroughly in 0.01 mol-L phosphate-buffered saline (PBS, pH 7.6). Endogenous peroxidase was removed by bleaching in 1.0% H_2O_2 for 15 min. Non-specific binding was blocked by 1:20 normal goat and normal human serum in PBS with 0.3% Triton X-100. Tissue sections were incubated at 4°C for 48 h with primary antiserum to CRH (rabbit anti-CRF (human, rat), Pennisula Lab., Belmont, CA) diluted 1:20,000 in PBS containing 0.3% Triton X-100, 3.0% goat serum, and 0.05% bovine serum albumin. As reported previously, the CRH antiserum was preadsorbed to natural melanin before the immunocytochemical procedure to eliminate the cross-reactivity to the melanin-concentrating hormone precursor (Austin et al. 1995). Sections were then processed using the avidin-biotin peroxidase method. After rinsing in PBS, sections were incubated (60 min, 21°C on shaker) with biotin-labeled goat anti-rabbit IgG (1:200, Elite Rabbit Vectastain Kit, Vector Labs) and then with avidin and biotin-peroxidase (1:100, 60 min). The chromogen used for visualization of the bound peroxidase was 3'3' diaminobenzidine (0.001 g/ml in 0.1 mol-L sodium acetate). For some sections, the reaction was further intensified by the addition of 2.75% nickel ammonium sulfate or silver nitrate (Pucak et al. 1996).

The specificity of the CRH antiserum was determined by using several methods as previously described (Lewis et al. 1989). The primary antiserum was incubated at 4°C overnight with 10 nmol/ml of the following peptides: CRF (rat/human, Pennisula Labs), alpha-melanocyte-stimulating hormone (α-MSH, Calbiochem) or porcine peptide histidine-isoleucine (PHI, Sigma). Adjacent tissue sections were processed as described above with either the blocked or unblocked CRH antiserum. Preincubation of the CRH antiserum with rat/human CRH completely eliminated CRH-immunoreactivity (IR) in the human pons and midbrain. Preincubation with α-MSH or PHI produced no detectable change in the CRH staining pattern compared to unblocked antiserum. Also, replacing the primary antiserum with normal rabbit serum resulted in the complete absence of immunoreactivity.

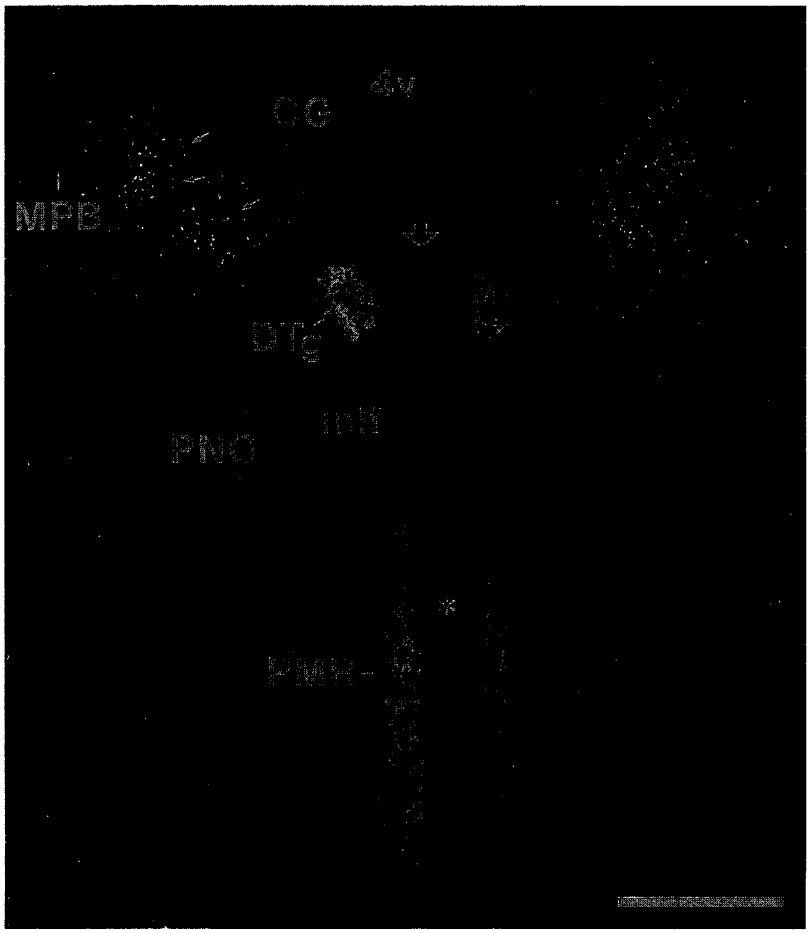

Figure 1. Darkfield photomicrograph illustrating the distribution of CRH immunoreactivity in the human pons. Note the very dense CRH fiber plexi in the dorsal tegmental nucleus (DTg) and paramedian raphe (PMR). CRH fibers are also present in the dorsolateral locus coeruleus; note that the locus coeruleus neurons (*arrows*) appear white as a result of the appearance of neuromelanin pigment under darkfield illumination. 4v = fourth ventricle; MPB = medial parabrachial nucleus; mlf = medial longitudinal fasciculus; PNO = oral pontine nucleus; CG = central gray; dorsal raphe = caudal part (*open arrow*); median raphe (*asterisk*). Calibration bar = 1.5 mm.

The regional distribution of CRH-IR in the human brainstem was determined by comparing labeled sections, examined under darkfield and brightfield illumination, with the cytoarchitectonic structures present in adjacent Cresyl-violet stained sections.

RESULTS

Noradrenaline-Containing Locus Coeruleus and Adjacent Pontine Structures

Figure 1 illustrates the distribution of CRH-IR axons in the human pons at the level of the noradrenergic neurons in the locus coeruleus. A high concentration of CRH fibers was present in the dorsolateral locus coeruleus region. By comparison, non-monoamine-containing pontine structures such as dorsolateral central gray region contained a moderate to high density of CRH-IR axons. A moderate density of CRH-IR fibers was also located in the medial parabrachial nucleus, whereas a very dense, oval-shaped CRH-IR fiber plexus was present bilaterally in the dorsal tegmental nucleus. An adjoining Nissl-stained tissue section illustrates the cytoarchitecture of the human pons (Figure 2).

Serotonin-Containing Nuclei

A moderate density of CRH-IR axons was present in the serotonin-containing median raphe nucleus (Figure 1). In contrast, a very dense column-like network of CRH-IR fibers was located bilaterally in the paramedian raphe nucleus, a region that borders the lateral aspect of the median raphe (Figure 1). Some CRH-IR axons extended medially from this zone into the median raphe nucleus. CRH-IR axons were sparsely scattered in the medial aspects of the central gray, and labeled fibers continued ventrally into the caudal region of the dorsal raphe nucleus (Figure 1). A low density of CRH-IR axons was scattered laterally throughout the oral pontine nucleus of the pontomesencephalic reticular formation.

The distribution of CRH-IR axons in the caudal midbrain at the level of the serotonin neurons in the dorsal raphe is illustrated in Figure 3, and the anatomical structures are revealed in the adjoining Nissl-stained section in Figure 4. A moderate density of CRH-IR axons was present in the dorsal and ventral subdivisions of the dorsal raphe nucleus. The density of CRH-IR axons was more abundant in the ventrolateral subdivision of the dorsal raphe. The CRH fiber innervation of the dorsal raphe was most prominent in the caudal to mid levels

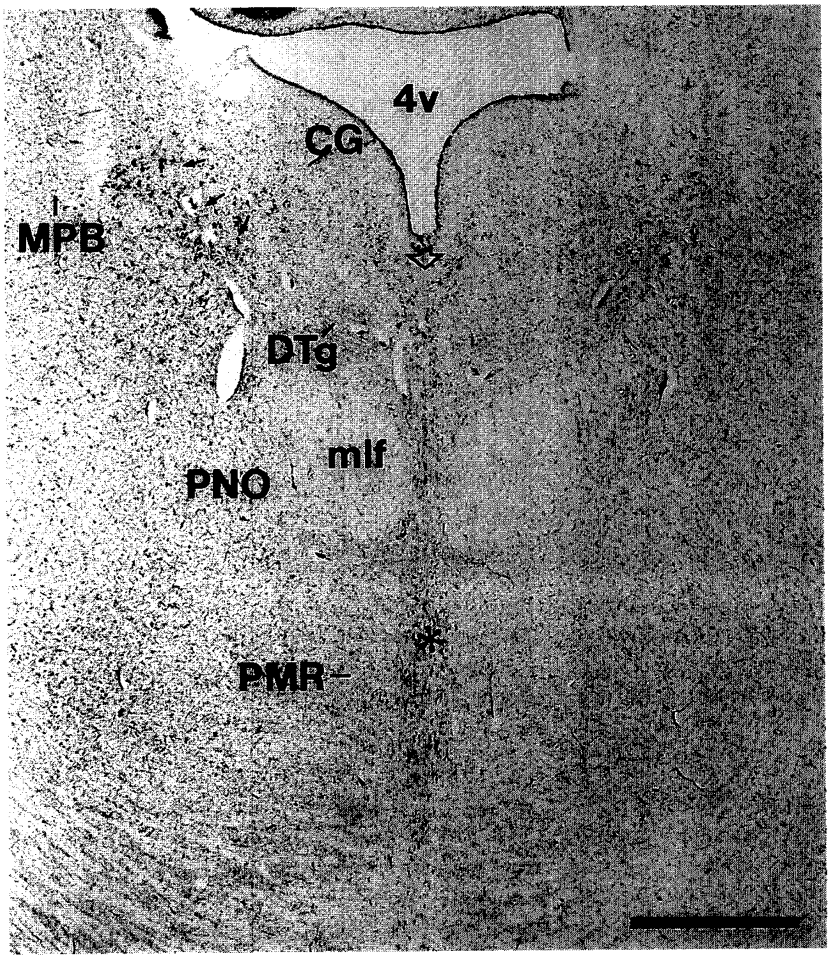

Figure 2. Brightfield photomicrograph of a Nissl-stained tissue section adjacent to Figure 1 illustrating the cytoarchitecture in the human pons. See Figure 1 legend for description of anatomical labels. Calibration bar = 1.5 mm.

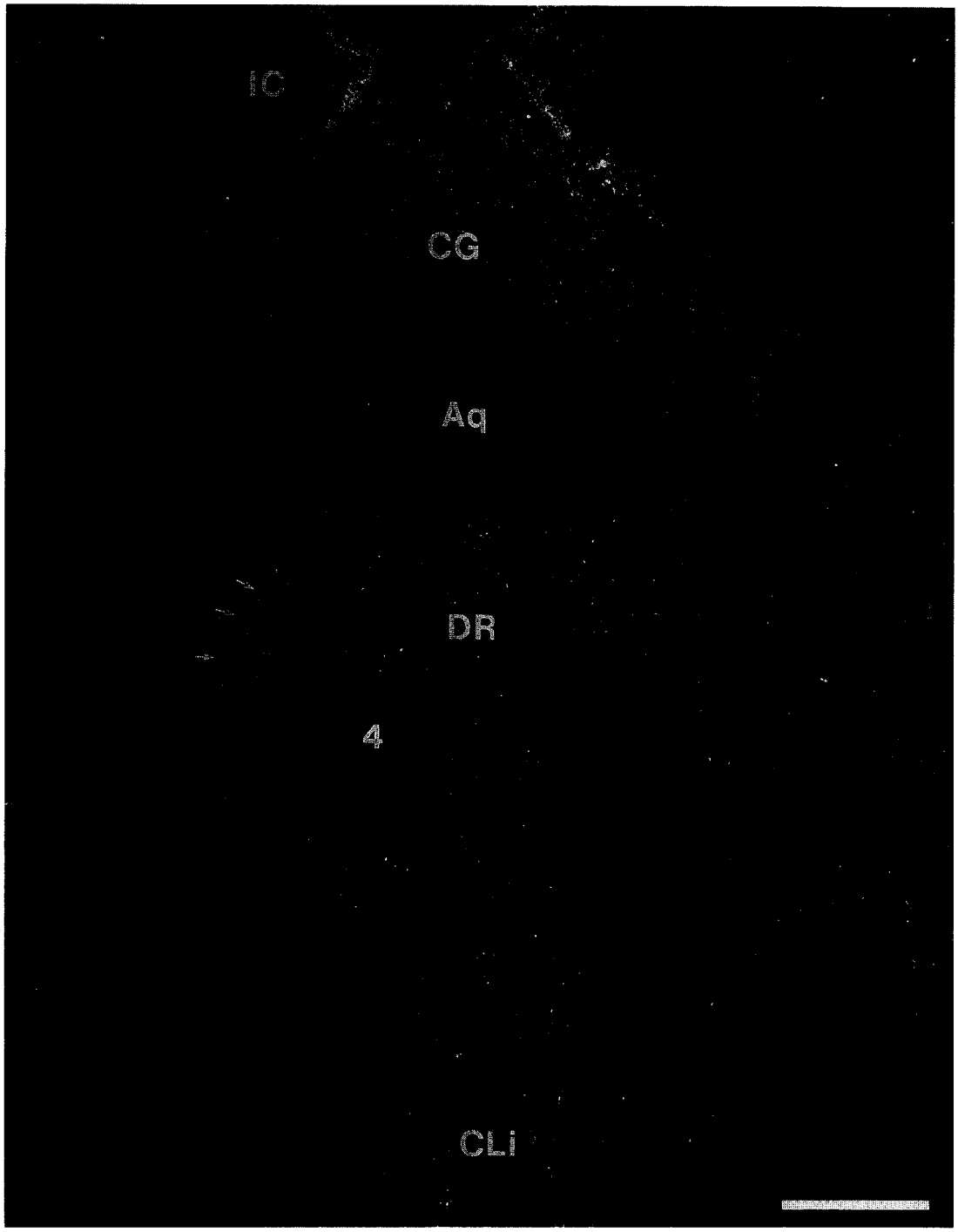

Figure 3. Darkfield photomicrograph of CRH immunoreactivity in the human midbrain at the level of the dorsal raphe (DR). Note the higher density of CRH fibers in the lateral (*arrows*) than in the medial portion of the DR. A dense population of CRH fibers are present in the dorsal area of the central gray (CG) and along the medial edge of the inferior colliculus (IC). A lower density of CRH fibers appear in the caudal aspect of the caudal linear nucleus (CLi). Aq = cerebral aqueduct; 4 = trochlear nucleus. Calibration bar = 1.5 mm.

and diminished in the more rostral levels of the midbrain. Also, a moderate density of CRH-IR axons appeared in the caudal aspect of the caudal linear nucleus. In adjacent non-serotonergic midbrain structures, a dense band of CRH fibers was evident along the medial edge of the inferior colliculi, which diminished along the ventrolateral edge of the inferior colliculi. The central gray region surrounding the cerebral aqueduct contained a moderate to high density of CRH-IR axons which was particularly dense in the dorsal and ventral aspects.

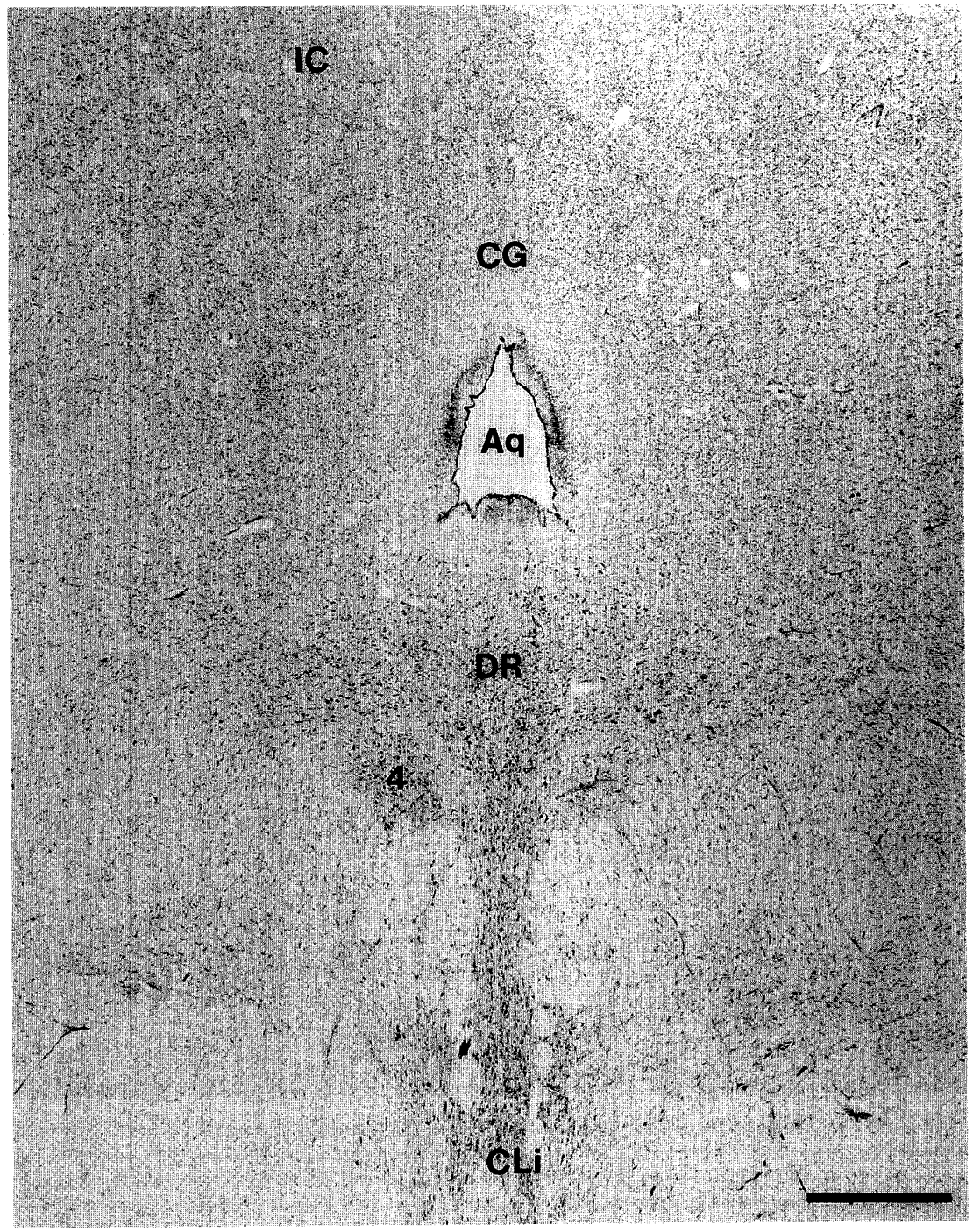

Figure 4. Brightfield photomicrograph of a Nissl-stained tissue section adjacent to Figure 3 illustrating the cytoarchitecture in the human midbrain. See Figure 3 legend for description of anatomical labels. Calibration bar = 1.5 mm.

Dopamine-Containing Nuclei

Figure 5 reveals the distribution of CRH-IR axons in the midbrain at the level of the dopamine neurons in the ventral mesencephalon. CRH-IR fibers were highly concentrated in the medial aspects of the ventral mesencephalon or the equivalent of the rodent A10 region. A very dense CRH fiber plexus was evident along the lateral edge of the interpeduncular nucleus. This dense network of CRH-IR axons continued dorsomedially into the interfascicular nucleus and caudal linear nucleus and laterally into the paranigral nucleus. The parabrachial pigmented nucleus and the retrorubal field (A8) also contained a moderate density of CRH-IR fi-

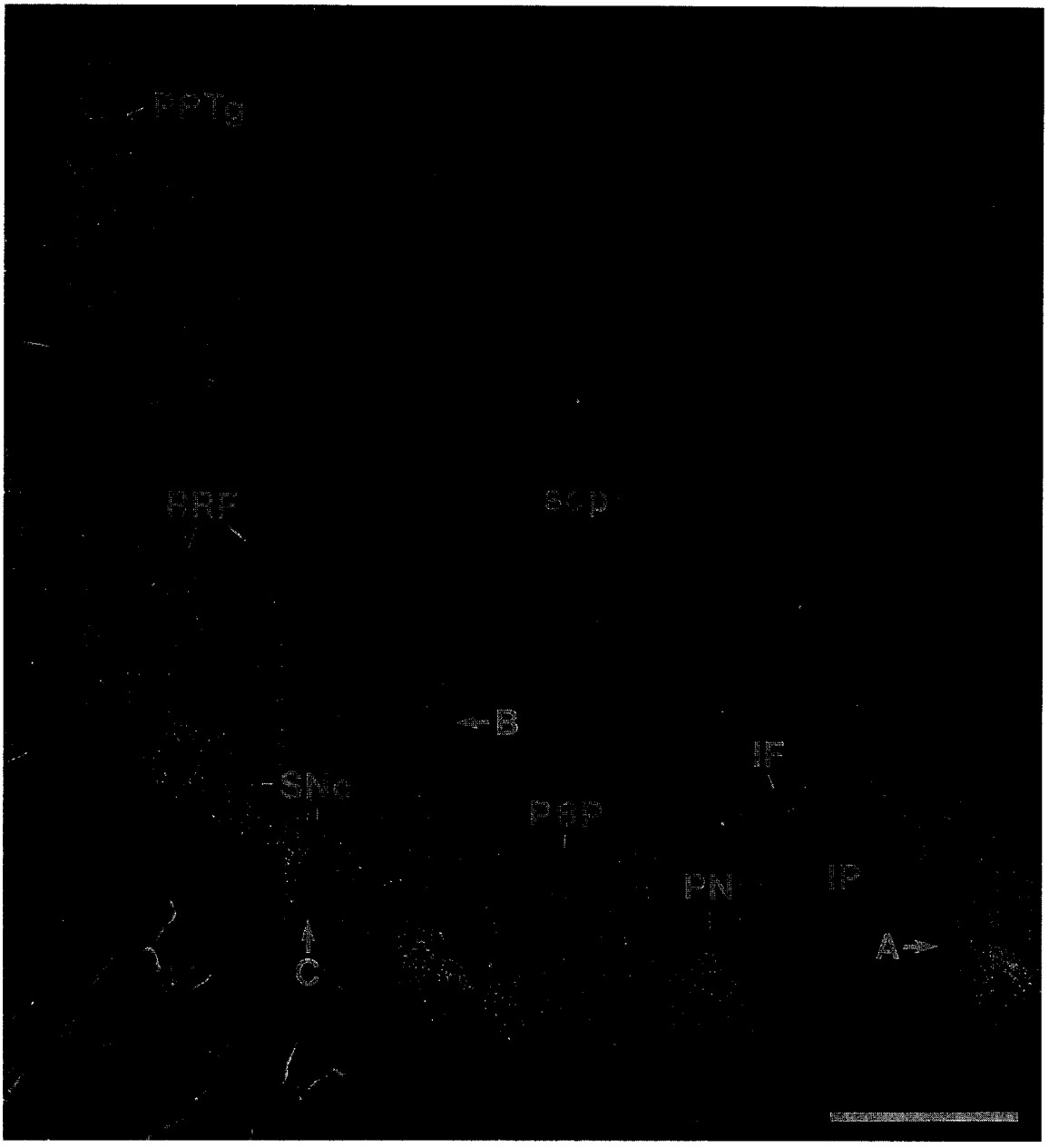

Figure 5. Darkfield photomicrograph of CRH immunoreactivity in the human midbrain at the level of the ventral mesencephalon. Note the very dense network of CRH fibers along the lateral edge of the interpeduncular nucleus (IP) and their distribution as they merge dorsomedially into the interfascicular nucleus (IF) and laterally into the paranigral nucleus (PN). The density of the CRH fibers diminishes laterally as they course ventral to the superior cerebellar peduncle (scp) through the parabrachial pigmented nucleus (PBP) and dorsolaterally along the dorsal tier of the substantia nigra pars compacta (SNc). Note the high density of CRH immunoreactivity in the pedunculopontine tegmental nucleus (PPTg). RRF = retrorubal field. Letters (**A**, **B**, and **C**) indicate the location of the brightfield photomicrographs shown in Figure 7. Calibration bar = 1.5 mm.

bers. The highest density of CRH-IR axons appeared to be restricted to the scattered dopamine-containing neurons located dorsal to the densely packed dopamine neurons of the substantia nigra pars compacta (A9) which contained sparsely distributed CRH fibers. The density of CRH-IR axons in the ventral mesencephalon was most prominent in the caudal levels of the midbrain and diminished rostrally. An adjoining Nissl-stained tissue section illustrates the cytoarchitecture of the rostral midbrain (Figure 6).

Figure 7 illustrates the differential distribution of CRH-immunoreactive axons across the subnuclei of the ventral mesencephalon with the highest densities found in the medial nuclei (A), moderate densities present in

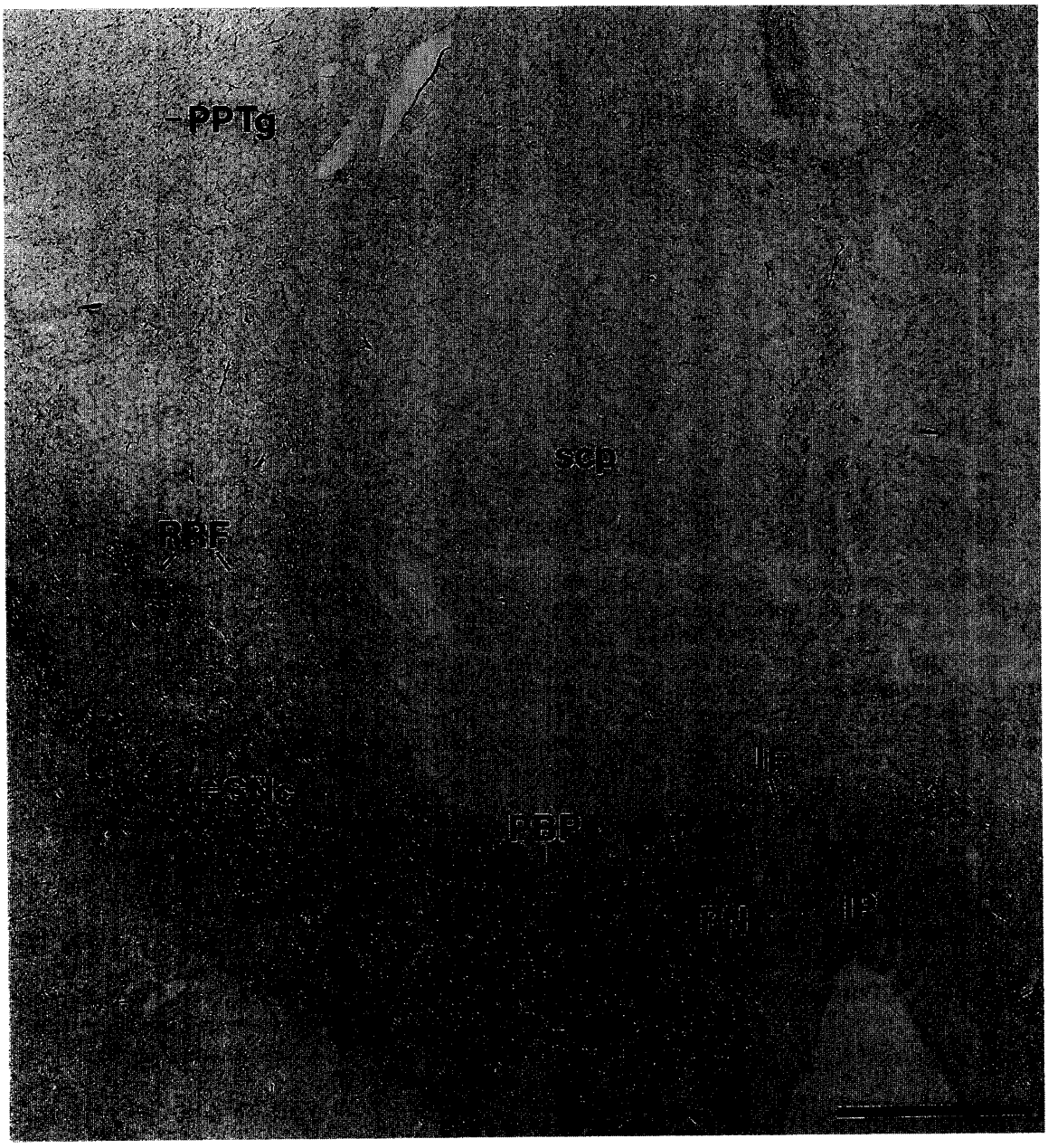

Figure 6. Brightfield photomicrograph of a Nissl-stained tissue section adjacent to Figure 6 illustrating the cytoarchitecture in the rostral human midbrain. See Figure 5 legend for description of anatomical labels. Calibration bar = 1.5 mm.

the dorsolateral region (B), and low densities in the ventrolateral nuclei (C).

Relative Density of CRH-IR Axons

A comparison of the relative densities of CRH-IR axons among the noradrenaline, serotonin and dopamine cell groups and adjacent areas is summarized in Table 2 and illustrated by representative photomicrographs in Figure 8. The highest densities of CRH-containing axons were present as dense plexi in the dorsal tegmental, paramedian raphe, and interpeduncular nuclei. In the regions composed of monoamine-containing cell bodies, a dense network of CRH-IR axons was present in the noradrenergic locus coeruleus (Figure 8A) and in the dopamine subnuclei consisting of the interfascicular, caudal linear and paranigral nuclei (Figure 8D), whereas the serotonin nuclei of the median and dorsal raphe contained a moderate density of CRH-IR axons (Figures 8B and C). A consistent observation regarding the localization of CRH-labeled axons in the monoamine nuclei was the presence of CRH-IR varicose segments adjacent to or surrounding neuromelanin-containing neurons in the catecholamine cell groups of the

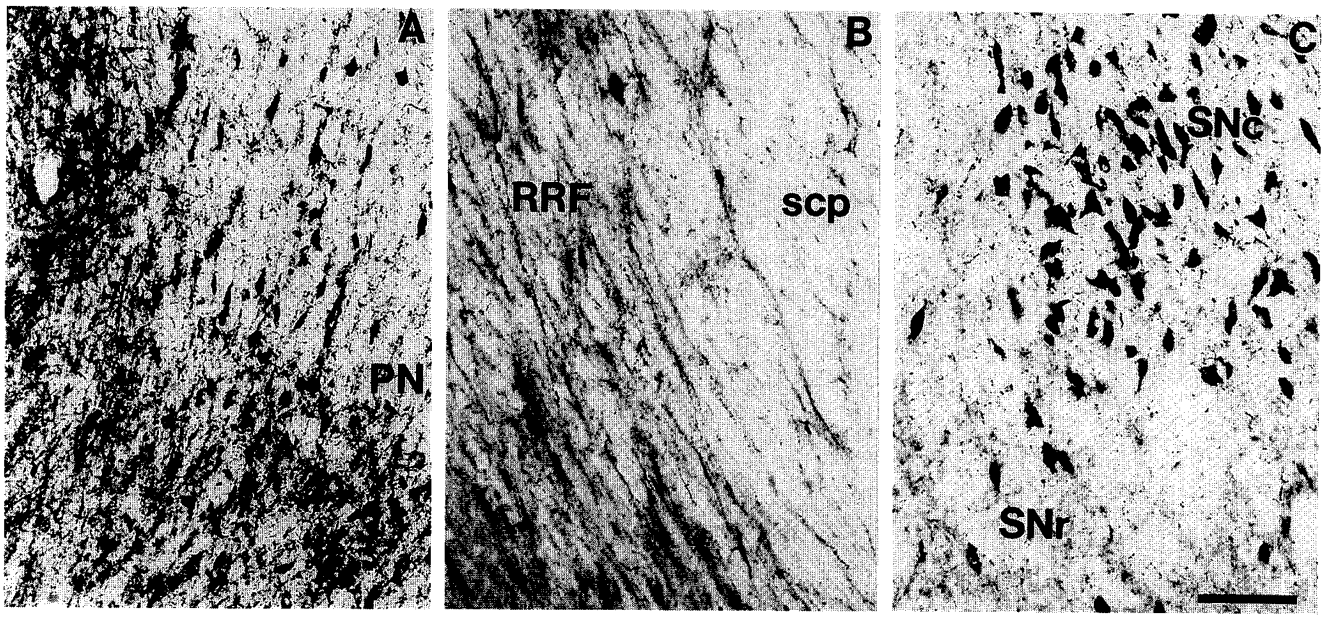

Figure 7. Brightfield photomicrographs illustrating the differential distribution of CRH-immunoreactive axons in the human mesencephalon. Among areas containing dopamine neurons, the density of CRH fibers is highest in the paranigral region (**A**), intermediate in the retrorubral field (**B**) and lowest in the substantia nigra pars compacta (**C**). In addition, each panel illustrates differences in CRH axon density between dopamine and non-dopamine-containing regions. For example, note the sharp border of labeled axons between the RRF and the scp (**B**). The location of each panel is illustrated by the corresponding letters in Figure 5. PN = paranigral nucleus; RRF = retrorubral field; scp = superior cerebellar peduncle; SNc = substantia nigra pars compacta; SNr = substantia nigra pars reticulata. Calibration bar = 150 μm.

locus coeruleus and ventral mesencephalon (Figures 8A and D), as well as in the vicinity of serotonin neurons in the median raphe and dorsal raphe (Figures 8B and C). The same distribution and relative densities of CRH-IR axons in the human brainstem nuclei were consistently observed in each of the cases examined and no age- or postmortem-related differences were apparent.

Morphology of CRH-IR Axons

The morphology of the CRH-IR axons in the locus coeruleus (Figure 9A), median raphe (Figure 9B), dorsal raphe, and A10 region was consistently characterized by numerous large, beaded varicosities with very fine intervaricose segments. Whereas all regions also exhibited punctate CRH immunoreactivity in the surrounding neuropil, it was particularly more abundant in the locus coeruleus and medial nuclei of the ventral mesencephalon (Figures 8A and D).

DISCUSSION

An earlier immunocytochemical study conducted in postmortem human brainstem tissue reported that the human locus coeruleus contains a dense population of CRH-IR fibers (Pammer et al. 1990). Previous studies in our laboratory confirmed and extended this observation, revealing both CRH-IR fibers in the dorsolateral locus coeruleus and CRH-IR perikarya in the ventromedial locus coeruleus region of the human brainstem (Austin et al. 1995). The findings of the present study have extended these observations by identifying CRH-IR axons in several brainstem structures, including the median and dorsal raphe nuclei and the subnuclei of the ventral mesencephalon, and by revealing a differential distribution of CRH axons across regions of the human pons and midbrain.

The distribution of CRH-IR axons in the human brainstem generally paralleled the findings previously reported in non-human primates (Foote and Cha 1988). The human brainstem nuclei that exhibited the highest density of CRH fibers included the dorsal tegmental nucleus, paramedian raphe, and the lateral region of the interpeduncular nucleus. Among the monoaminergic nuclei, the medial nuclei of the ventral mesencephalon, as well as the noradrenergic locus coeruleus all contained a dense collection of CRH-labeled axons. These observations are consistent with the previous findings of Foote and Cha (1988) who described a dense population of CRH fibers in the central and dorsolateral subdivisions of the interpeduncular nucleus and in the lateral aspects of the locus coeruleus in two primate species, the squirrel monkey (*Saimiri sciureus*) and cynomolgus monkey (*Macaca fascicularis*). Also consistent with the non-human primates, a moderate density of CRH fibers

Table 2. Relative Density of CRH Immunoreactive Axons in the Human Pons and Midbrain

Anatomical Region	Axon Density	Monoamine Neurotransmitter
Pontine level		
Locus coeruleus	+++	NE
Medial parabrachial nucleus	++	—
Dorsal tegmental nucleus	++++	—
Paramedian raphe	++++	5-HT
Median raphe	++	5-HT
Oral pontine nucleus	+	5-HT
Midbrain level		
Central gray	++	—
Dorsal raphe	++	5-HT
Interpeduncular nucleus (A10)	++++	DA/5-HT
Interfascicular nucleus (A10)	+++	DA
Paranigral nucleus (A10)	+++	DA
Caudal linear nucleus (A10)	+++	DA/5-HT
Parabrachial pigmented nucleus (A10)	++	DA
Retrorubral field (A8)	++	DA
Substantia nigra (A9)	+	DA

++++ = very dense; +++ = dense; ++ = moderate; + = low.
NE = norepinephrine; 5-HT = serotonin; DA = dopamine; — = non-monoaminergic.

was located in the median and dorsal raphe nuclei of the human brainstem. Lower densities of CRH-positive axons were found in the substantia nigra pars compacta and in the oral pontine nucleus. These immunocytochemical findings reveal that CRH-containing axons are heterogeneously distributed among the monoaminergic nuclei in the human brainstem.

CRH-labeled axons were located in immediate proximity to neuromelanin-containing cell bodies in both humans and monkeys (Foote and Cha 1988). This localization suggests that synaptic contacts may be present between CRH-IR axons and monoamine-containing cell soma or dendrites in the primate brainstem. Interestingly, a recent ultrastructural study reported that CRH-IR terminals formed synaptic contacts with tyrosine hydroxylase-IR dendrites in the rat locus coeruleus (Van Bockstaele et al. 1996). Based on this evidence, it is conceivable that a similar synaptic arrangement exists in the primate locus coeruleus as well as in the other monoaminergic nuclei. The presence of postsynaptic CRH receptors in the human locus coeruleus, raphe nuclei, or ventral mesencephalon would provide additional support for synaptic arrangements between CRH axons and monoaminergic soma or dendrites. Unfortunately, the receptor autoradiographic data on the distribution of CRH receptors in the primate brain are quite limited. The only study that examined brainstem regions found very low levels of CRH receptors in the locus coeruleus and substantia nigra and moderate densities in the inferior colliculus and dorsal parabrachial nucleus of *M. fascicularis* (Millan et al. 1986). A similar distribution of CRH receptors exists in the rodent brainstem with moderate densities located in the interpeduncular nucleus, ventral tegmental nucleus, dorsal tegmental nucleus, parabrachial nucleus, and inferior colliculus and low densities in the locus coeruleus and dorsal raphe (De Souza et al. 1985). In this study, De Souza and colleagues (1985) noted that in several instances the distribution of CRH-IR axons did not correlate with the distribution of CRH receptors in the rat brain. It was suggested that this disparity may reflect the existence of different CRH receptor subtypes. Indeed, recent cloning studies have identified two distinct CRH receptor subtypes (CRH_1, CRH_2; Chen et al. 1993; Lovenberg et al. 1995), which are differentially distributed in the rodent brain (Chalmers et al. 1995). Within the rat brainstem, a high density of both CRH_1 and CRH_2 receptor mRNAs were found in the interpeduncular nucleus, whereas a moderate density of CRH_2 receptor mRNA was located in the dorsal and median raphe, caudal linear nucleus and inferior colliculus (Chalmers et al. 1995). Interestingly, neither CRH receptor mRNA species was detected in rat locus coeruleus neurons (Chalmers et al. 1995). Given these observations in the rodent brain, it is possible that the previous receptor binding study in the primate brain detected only one CRH receptor subtype that exists at relatively low levels in the brainstem. Future investigations using molecular tools to examine specific CRH receptor subtypes may provide further insights into the cellular expression of CRH receptors in the primate central nervous system, as well as a means for elucidating the functional role of CRH on monoamine neurotransmission in the human brain.

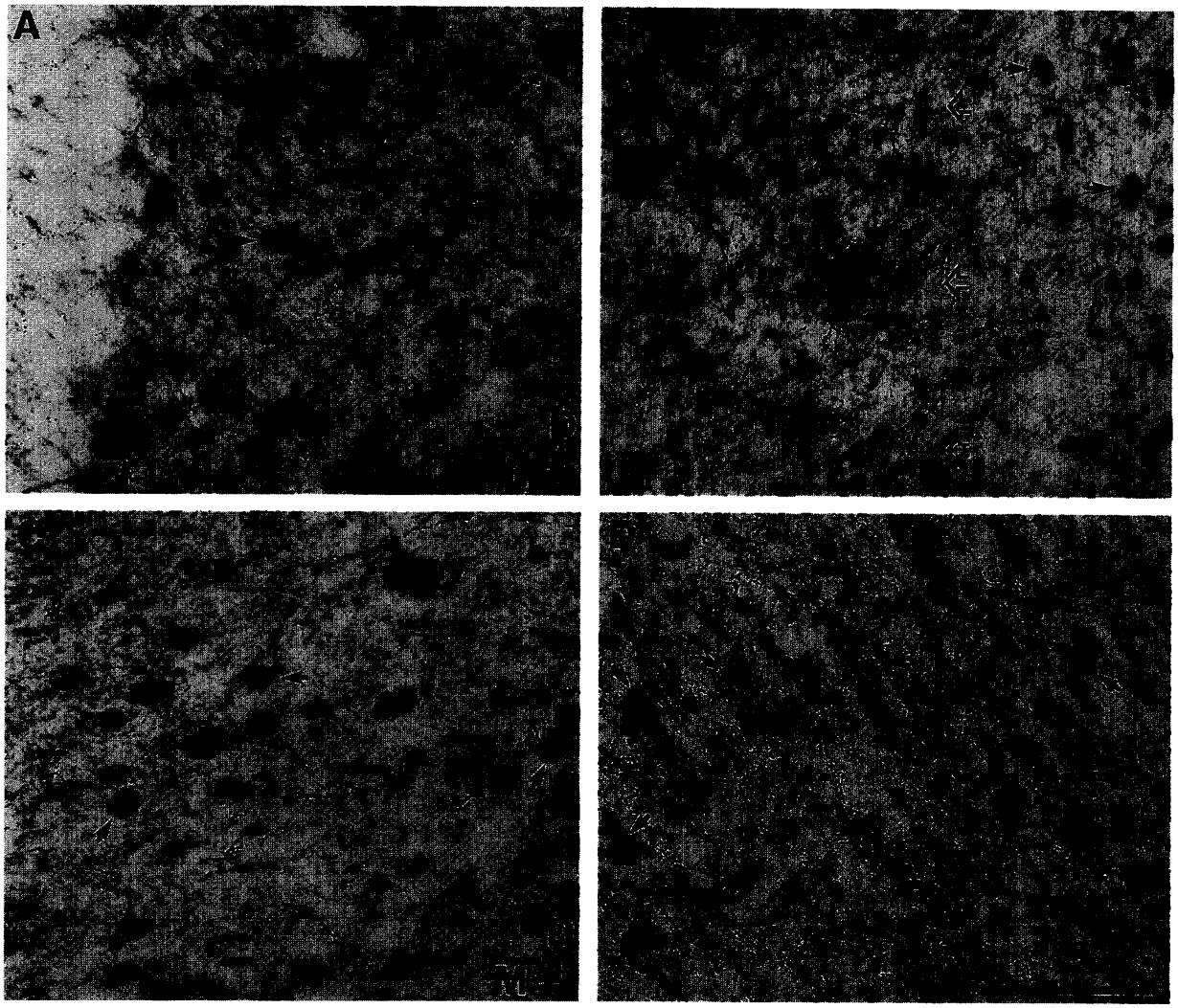

Figure 8. Brightfield photomicrographs illustrating the localization of CRH-immunoreactive axons in the locus coeruleus (**A**), paramedian/median raphe (**B**), dorsal raphe (**C**), and paranigral nucleus (**D**). Note the high density of CRH axons in the LC, PMR (*open arrows*) and PN and the lower density in the MR and DR. Punctate CRH immunoreactivity is also abundantly expressed in the LC and PN. Solid arrows in **A** and **D** identify neuromelanin-containing neurons in the LC and PN. Solid arrows in **B** and **C** identify presumably serotonergic neurons in the MR and DR. The orientation of the photomicrographs of **A** and **B** is illustrated in panel **A** and the orientation of **C** and **D** is illustrated in panel **C**. D = dorsal; L = lateral; M = medial. Calibration bar = 100 μm.

Differential Distribution of CRH-IR Axons: Functional Implications

Norepinephrine-Containing Neurons. The influence of CRH on locus coeruleus neurons and noradrenergic neurotransmission has been established by a number of electrophysiological, pharmacological, biochemical, and behavioral investigations in rodents. These studies generally support an excitatory action of CRH on locus coeruleus neurons (Valentino et al. 1983; Dunn and Berridge 1987; Valentino and Foote 1987, 1988; Butler et al. 1990; Melia and Duman 1991; Emoto et al. 1993; Koob et al. 1993; Lavicky and Dunn 1993; Smagin et al. 1995; Schulz and Lehnert 1996). The findings of the present and previous studies (Pammer et al. 1990; Austin et al. 1995) of a dense collection of CRH-IR axons in the dorsolateral locus coeruleus, predominantly localized in the mid to rostral levels of the nucleus, suggest that CRH may exert a similar influence on human noradrenergic neurons. Interestingly, tracing studies in rodents and monkeys have determined that locus coeruleus neurons exhibit a certain topographical organization with respect to their efferent projections (Bowden et al. 1978; Loughlin et al. 1986). For example, locus coeruleus neurons located rostrally project heavily to the hypothalamus (Loughlin et al. 1986), whereas cortical projections appear to originate from the caudal three-fifths of the locus coeruleus (Waterhouse et al. 1982). In addition, neu-

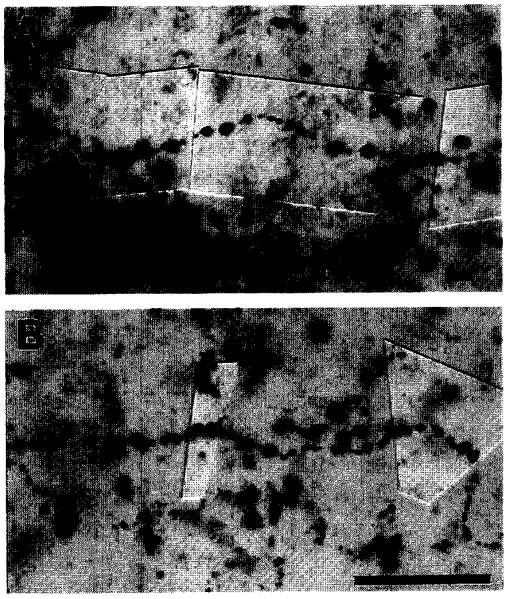

Figure 9. Brightfield photomicrographs revealing the morphology of CRH-immunoreactive axons in the LC (**A**) and MR (**B**). Note the characteristic large, beaded varicosities and fine intervaricose segments. Calibration bar = 20 μm.

rons in the dorsal portion of the locus coeruleus project to the hippocampal formation (Loughlin et al. 1986). Thus, our anatomical observations raise the possibility that CRH preferentially influences noradrenergic neurotransmission in the locus coeruleus neurons that project to the hypothalamus and hippocampus in the human brain.

Serotonin-Containing Neurons. The present study found that the density of CRH-IR axons varied widely across the serotonergic nuclei in the human brainstem. For example, the paramedian raphe and the caudal linear nucleus contained dense populations of CRH-IR axons, whereas the density of CRH-IR axons was lower in the median and dorsal raphe, and lower still in the oral pontine nucleus. This heterogeneous distribution of CRH-IR axons across the serotonergic nuclei suggests that CRH may play different functional roles within the human serotonin system.

However, in contrast to the locus coeruleus, studies investigating the functional significance of CRH in the raphe are quite limited. Singh and colleagues (1992) reported that ICV administration of CRH produced a dose-dependent increase in tryptophan hydroxylase (TPH) activity in the cortex and midbrain of rats. This effect resembled the activation of TPH after exposure of rats to acute sound stress or electrical stimulation of the dorsal raphe (Boadle-Biber et al. 1986, 1989). A subsequent study by these investigators (Boadle-Biber et al. 1993) extended these findings by demonstrating that intra-amygdala injections of CRH produced a dose-dependent increase in TPH activity in the midbrain and cortex, but did not influence the levels of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA). In addition, injections of the CRH antagonist, α-helical CRF, into the amygdala blocked both the CRH- and sound stress-induced increase in TPH activity. In contrast to the report of Singh and colleagues, Lavicky and Dunn (1993), using microdialysis, found that ICV or intraperitoneal (IP) injection of CRH significantly increased 5-HIAA concentrations in the medial prefrontal cortex and in the medial hypothalamus of rats. Although these studies provide a link between CRH and the serotonin system, the precise anatomical and neurotransmitter substrates involved in mediating such effects remain to be determined.

Dopamine-Containing Neurons. Similar to the serotonin system, substantial differences in the density of CRH-IR axons were found across the dopamine-containing nuclei in the human brainstem. A very dense CRH fiber plexus was present in the lateral region of the interpeduncular nucleus (A10). The interfascicular (A10), paranigral (A10), and caudal linear (A10) nuclei all contained a dense population of CRH-IR axons, whereas moderate densities of CRH fibers were present in the parabrachial pigmented nucleus (A10) and in the retrorubral field (A8), and only scattered CRH-IR axons were found in the substantia nigra (A9). Recent investigations have demonstrated that the A8–A10 groups of dopamine neurons in the primate ventral mesencephalon actually form separate dorsal and ventral tiers (Haber and Groenewegen 1989; Halliday and Törk 1991; Lynd-Balta and Haber 1994a). As a group, dorsal tier neurons comprise a continuous band that spans the entire medial-lateral extent of the ventral midbrain dorsal to the main body of the substantia nigra, including the A8 and A10 cell groups as well as the loosely scattered neurons in the dorsal substantia nigra. In contrast, ventral tier dopamine neurons include the dense band of cells in the substantia nigra pars compacta and the columns of dopamine-containing neurons that penetrate into the substantia nigra pars reticulata. In addition to their location, dorsal tier neurons differ from ventral tier neurons in a number of features including axon projection target, presence of the calcium binding protein, calbindin, and levels of expression of the mRNAs for the dopamine transporter and D_2 dopamine receptor (Lynd-Balta and Haber 1994 a,b; Haber et al. 1995). Given the apparent functional differences in these populations of dopamine neurons, it is interesting that CRH axons seem to preferentially innervate dorsal tier dopamine neurons. These neurons are known to provide projections to cortical and limbic areas in contrast to the striatal projections of the ventral tier neurons (for review, see Lewis and Sesack 1997). Thus, the greater density of CRH axons in the dorsal tier dopamine neurons might contribute to the greater stress-

responsiveness of these dopamine neurons (Roth et al. 1988), as opposed to those located in the ventral tier.

Consistent with this interpretation, pharmacological studies in rodents have revealed that ICV administration of CRH to mice produced behavioral activation and a "stress-like" increase in dopamine metabolism in the prefrontal cortex, as well as in the septum, hypothalamus and brainstem (Dunn and Berridge 1987). Direct injections of CRH into the ventral tegmental area produced a dose-dependent increase in locomotor activity, although this effect was not antagonized by the dopamine receptor blocker, haloperidol (Kalivas et al. 1987). Furthermore, a recent microdialysis study reported that ICV or IP administration of low doses of CRH increased dopamine and dopamine metabolite levels in the rat medial prefrontal cortex (Lavicky and Dunn 1993). Although these studies in rodents are consistent with an influence of CRH on dopamine neurons that provide corticolimbic projections, further studies are necessary to clarify the mechanisms of this effect.

Differential Distribution of CRH-IR Axons: Clinical Implications

The heterogeneous distribution of CRH-IR axons across the monoamine nuclei in the human brainstem may also have important clinical significance. Studies in rats have clearly demonstrated that CRH is involved in mediating the effects of stressors in several extrahypothalamic brain regions (for reviews, see Owens and Nemeroff 1991; Koob et al. 1993). Likewise, unequivocal evidence shows that a variety of stressors activate norepinephrine, serotonin, and dopamine neurotransmission (for review, see Stanford 1993). This evidence, in concert with our anatomical findings, raises the possibility that interactions of CRH with norepinephrine, serotonin, or dopamine neurons in the human brainstem may play a significant role in the pathophysiology of stress-induced neuropsychiatric disorders. Indeed, considerable evidence has accumulated supporting alterations in serotonin, norepinephrine, dopamine, and/or CRH neurotransmission in depressed patients and in postmortem brain of suicide victims (Van Praag 1980; Mann et al. 1986; Roy et al. 1986, 1989, 1992; Gold et al. 1986; Nemeroff et al. 1988; Widerlöv et al. 1988; Arató et al. 1989; Arora and Meltzer 1989; Roy 1993; Nordström et al. 1994; Ordway et al. 1994). In particular, the levels of 5-HIAA are reduced in the cerebrospinal fluid of patients with depression who attempt suicide (Roy et al. 1992; Nordström et al. 1994), and receptor-binding studies have documented elevated 5-HT_2 receptors in the cortex of suicide victims (Mann et al. 1986; Arora and Meltzer 1989). Viewed in the context of our anatomical observations, these clinical findings raise the possibility that stress-related alterations in serotonin neurotransmission in depression may be mediated, at least in part, by a dysfunctional CRH input to the serotonin neurons in the raphe nuclei.

Evidence has also accumulated suggesting that the pathophysiology of schizophrenia may be associated with altered neurobiological responses to stress (Breier et al. 1991). For example, chronic interpersonal stress and discrete stressful life events may precipitate relapses in schizophrenic patients (Ventura et al. 1992). Given the role of CRH and dopamine in stress responses, the findings of the present study raise the possibility that an interaction of these neurotransmitters in the ventral mesencephalon may play an important role in the stress-related exacerbations of this disorder. Interestingly, CSF CRH concentrations were reported to be significantly elevated in 18 of 21 schizophrenic patients after withdrawal of haloperidol treatment, and patients that relapsed tended to exhibit higher CSF CRH concentrations (Forman et al. 1994). In addition, intravenous administration of CRH to healthy human subjects produced a significant increase in plasma homovanillic acid levels 20 h after administration (Posener et al. 1994). Although other interpretations of these data are certainly possible, they may be consistent with a CRH-dopamine interaction in the pathophysiology of schizophrenia.

CONCLUSIONS

In summary, the differential localization of CRH axons in the cell body regions of the human locus coeruleus, raphe nuclei, and ventral mesencephalon provide anatomical evidence for a putative CRH afferent regulation of noradrenergic, serotonergic, and dopaminergic neurons in the human brainstem. In addition, the differences in density of CRH innervation across subpopulations of monoamine-containing neurons raises the possibility that CRH may play a role in the greater stress-responsivity of the dorsal tier dopaminergic neurons and the noradrenergic locus coeruleus as opposed to the ventral tier dopaminergic neurons and the serotonergic raphe nuclei. The potential interaction of CRH with these monoamine neurotransmitters may provide additional insight into the role of CRH in normal brain function, and in the pathophysiology of certain psychiatric disorders.

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