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κ_1 Receptor mRNA Distribution in the Rat CNS: Comparison to κ Receptor Binding and Prodynorphin mRNA

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Three opioid receptor types have been identified in the CNS and periphery that are referred to as μ, δ, and κ. The present study examines the mRNA distribution of the κ_1 receptor in the rat brain and compares it to the distribution of κ receptor-binding sites and prodynorphin mRNA using a combination of *in situ* hybridization and receptor autoradiographic techniques. κ_1 receptor mRNA was localized with a cRNA probe generated with a *Bam*HI–*Hin*dIII cDNA fragment of the rat κ_1 receptor and corresponds to the last 45 bp of the protein coding region and 728 nucleotides of the 3′ untranslated region. Prodynorphin mRNA was localized with a cRNA probe corresponding to a 733-bp *Bam*HI–*Hinc*II fragment of prodynorphin. κ receptor-binding sites were labeled in one of two ways: [^{3}H]U69,593 or [^{3}H]bremazocine in the presence of a 300-fold excess of DAMGO and DPDPE. A high degree of correspondence between the κ_1 receptor mRNA and κ receptor binding was observed in several brain regions, including the endopiriform nucleus, claustrum, nucleus accumbens, olfactory tubercle, bed nucleus of the stria terminalis, medial preoptic area, paraventricular, supraoptic, suprachiasmatic, dorsomedial and ventromedial hypothalamic nuclei, basolateral, medial and cortical amygdaloid nuclei, midline thalamic nuclei, periaqueductal grey, parabrachial nucleus, locus coeruleus, and the nucleus of the solitary tract. Differences in the localization of κ_1 receptor mRNA and binding and the relationship between the distribution of κ_1 receptor and prodynorphin mRNAs are discussed.

INTRODUCTION

The existence of at least three opioid receptor types is now well established (1–3). These receptor types are referred to as μ, δ, and κ and each have distinct pharmacological profiles (4) and distributions in the central nervous system (5–8). The κ receptors, which are the main focus of this study, have been implicated in a number of behaviors and functions, including analgesia (9), electrolyte maintenance (10–13), eating and drinking (14, 15), hormonal regulation (16–18), and the modulation of reward and aversion (19). Benzomorphans, such as ethylketazocine and arylacetamides U50,488 and U69,593, have high affinity for these receptors and are of particular clinical interest because of their ability to modulate these functions in conjunction with a comparatively low abuse liability.

The κ receptors can be further differentiated into subtypes that differ in their pharmacology and in their anatomical localization (20–24). At present, there is no agreement on the number and precise pharmacological properties of these κ receptor subtypes, but there is some consensus regarding the κ_1 subtype. The κ_1 receptor subtype is defined as having a high affinity for U50,488 and U69,593 as well as the endogenous opioid peptide dynorphin A1–17, while having poor affinity for the μ selective ligand DAMGO (D-Ala^2-MePhe^4,Gly-ol^5-enkephalin) and the δ selective ligand DPDPE (D-Pen^2,D-Pen^5-enkephalin). κ_1 receptor binding levels are low in many rodents (3, 7, 22), but are relatively high in the guinea pig CNS and in the brains of higher primates, including man (25, 26). In the rat, κ_1 receptor binding sites are relatively enriched in the nucleus accumbens, olfactory tubercle, endopiriform nucleus, claustrum, several hypothalamic nuclei, paraventricular thalamic nucleus, several amygdaloid nuclei, periaqueductal grey, and the nucleus of the solitary tract (22, 24, 25).

Agreement concerning the properties and distribution of the additional κ receptor subtypes has been more difficult. These sites have a high affinity for benzomorphans and several prodynorphin peptides, but generally lack a good affinity for U50,488 and related compounds. Pharmacological profiles and anatomical distributions for the non-κ_1 receptors vary across laboratories, suggesting either the presence of multiple κ sites or technical and species differences between studies. These differences include the presence or absence of NaCl in the binding buffer and incubation temperature (20, 21, 23, 24).

One factor that has complicated the characterization of these non-κ_1 receptors has been the lack of a selective ligand to label these sites. To visualize these binding sites investigators have used nonselective opioid ligands such

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as bremazocine and ethylketazocine to presumably label all opioid sites, while selectively blocking μ and δ receptors with an excess of unlabeled ligands (3). This approach is effective if the nonselective opioid ligand only labels μ, δ, and κ opioid receptor sites. However, additional opioid sites, referred to as λ (27) and ϵ (28), as well as nonopioid sites may potentially be labeled using this approach.

Thus far, the vast majority of information concerning the pharmacological properties and anatomical distribution of the κ receptors has come from homogenate binding and receptor autoradiographic studies. The recent cloning of the mouse (29) and the rat (30) κ_1 receptor has opened new avenues of research on the structure, distribution, and regulation of these receptors. The rat κ receptor (KOR_1) is a 380-amino acid protein with 59% protein identity to the δ receptor (31, 32) and has a high affinity for dynorphin A1–17 and U50,488. Hydrophobicity analysis suggests it has seven hydrophobic transmembrane (TM) domains and is a member of the seven TM family of G-protein-coupled receptors.

Given the recent cloning of the κ_1 receptor, the three main goals of the present study are as follows: (i) to provide the first detailed description of the cellular distribution of the κ_1 receptor mRNA in the rat CNS; (ii) to compare the κ_1 receptor mRNA distribution to the distribution of κ receptor binding as defined by either the selective κ_1 ligand [^{3}H]U69,593 or the nonselective ligand [^{3}H]bremazocine in the presence of μ and δ receptor blockers; and (iii) to compare the distributions of the κ_1 mRNA to prodynorphin mRNA.

Parallels in the distribution of κ_1 receptor mRNA and [^{3}H]U69,593 and [^{3}H]bremazocine receptor binding provide a more accurate description of the κ_1 receptor localization in the brain and highlight regions of possible local receptor synthesis. Similar analyses in other transmitter receptors suggest that the correspondence between receptor mRNA and binding should be good, but not perfect, likely reflective of receptor synthesis and transport (33). Regions demonstrating a similar distribution of κ_1 mRNA and [^{3}H]U69,593 binding but differing from [^{3}H]bremazocine receptor binding provide insights into the distribution of non-κ_1 receptor sites.

Prodynorphin peptides have a high affinity for κ receptors (34, 35), and a codistribution with κ_1 receptor mRNA would suggest possible local opioid circuits, and perhaps in some cases an autoreceptor function. As in the previous set of comparisons, the correspondence in the distribution of κ_1 receptor mRNA and prodynorphin mRNA is expected to be informative, but a perfect correspondence is not expected. Some prodynorphin-containing cells may have distant projections, making a comparison of κ_1 receptors and prodynorphin on the mRNA level difficult. In addition, prodynorphin peptides also have good affinity for μ and δ receptors (36) and may interact with these receptors in specific brain areas.

MATERIALS AND METHODS

Tissue Preparation

Adult Sprague–Dawley rats (Charles River, 250–300 g) were sacrificed by decapitation and their brains and pituitaries were dissected and frozen. Brains were frozen in liquid isopentane (−30°C) for 30 s and transferred to dry ice, while pituitaries were placed in Lipshaw (Detroit, MI) embedding matrix and frozen on dry ice. Brains and pituitaries were stored at −80°C until sectioning on a Bright cryostat (15 μm). Tissue sections were thaw-mounted on polylysine-subbed microscope slides and stored at −80°C.

In Situ Hybridization

Frozen brain and pituitary sections were removed from storage at −80°C and placed into 4% formaldehyde for 60 min (22°C) prior to processing for *in situ* hybridization (33). Following three 5-min rinses in 2× SSC (300 m*M* sodium chloride, 30 m*M* sodium citrate, pH 7.2), sections were treated with proteinase K (1 μg/ml in 100 m*M* Tris, pH 8.0, 50 m*M* EDTA) for 10 min at 37°C. Slides were then rinsed in water, followed by 0.1 *M* triethanolamine, pH 8.0, and treated with a mixture of 0.1 *M* triethanolamine, pH 8.0, and acetic anhydride (400:1, v/v) with stirring for 10 min. The sections were rinsed again in water and dehydrated through graded alcohols and allowed to air dry.

Brain and pituitary sections were hybridized with S^{35}-UTP and S^{35}-CTP labeled riboprobes generated to either the rat κ_1 receptor or prodynorphin. A *Bam*HI–*Hin*dIII fragment of the κ_1 cDNA clone that corresponds to the last 45 nucleotides of the protein coding region and 728 nucleotides of the 3′ untranslated region was used to prepare a cRNA probe for the κ_1 receptor (30). The prodynorphin cRNA was prepared with a 733-bp *Bam*HI–*Hin*cII fragment of prodynorphin (37). cRNA probes were diluted in hybridization buffer (75% formamide, 10% dextran sulfate, 3× SSC, 50 m*M* Na_2PO_4, pH 7.4, 1× Denhardt's, 0.1 mg/ml yeast tRNA, 10 m*M* dithiothrietol) to result in a final concentration of $1–2 \times 10^6$ cpm/50 μl. Volumes of 50 μl of diluted probe were applied to coronal brain sections and pituitary sections. Glass coverslips were placed over sections to keep hybridization buffer in contact with tissue. The slides were then transferred to sealed chambers containing 50% formamide and hybridized overnight at 55°C.

The next day the slides were rinsed in 2× SSC (5 min) and treated with RNase A (200 μg/ml in 100 m*M* Tris, pH 8.0, and 0.5 *M* NaCl) for 60 min at 37°C. Subsequently, the sections were rinsed in 2× SSC for 5 min (22°C) and 0.1× SSC for 60 min (65°C). Following the low salt wash, sections were rinsed in water and dehydrated through graded alcohols and air dried. Sections were apposed to Kodak XAR-5 X-ray film for 1–11 days or dipped in NTB2 film emulsion. Sections used to visualize κ_1 receptor

mRNA were developed following a 19-day exposure to NTB2 emulsion, while those used for localizing prodynorphin mRNA were developed following a 10-day exposure.

In Situ Hybridization Specificity Controls

Several controls were performed to test the specificity of the *in situ* hybridization results: (i) *In situ* hybridization studies were performed with a cRNA probe generated to a different region of the rat κ_1 receptor (transmembrane III–VII) to determine whether the results were the same as those derived from probes generated to the 3′ untranslated region of the κ_1 receptor; (ii) a "sense"-strand cRNA control was performed using a series of paired, adjacent sections that were divided into two sets: One set was treated according to the *in situ* hybridization protocol described above, while the second set was treated similarly except a sense-strand RNA probe was used that corresponded to transmembrane III–VII; and (iii) an RNase control, where sections were fixed in 4% formaldehyde and rinsed in 2× SSC as described above, but prior to treatment with proteinase K, were incubated with RNase A (200 μg/ml) for 60 min at 37°C. The sections were then processed using the *in situ* hybridization protocol described above.

κ Receptor Autoradiography

After being warmed to room temperature, the unfixed brain sections were placed in incubation chambers designed to maintain ambient temperature (22°C) and humidity (60–80%) and incubated with 200 μl of tritiated ligand in a 50 m*M* Tris buffer (pH 7.4, 22°C). κ receptor binding sites were labeled with one of two tritiated ligands: (i) [^{3}H]bremazocine (0.58 n*M*, New England Nuclear, 35 Ci/mmol) in the presence of 200 n*M* DAMGO and 200 n*M* DPDPE, selective μ and δ agonists, respectively; or (ii) [^{3}H]U69,593 (1.2 n*M*, New England Nuclear, 58.0 Ci/mmol). The labeling concentrations chosen correspond to approximately three times the K_d value of each ligand to their high-affinity site as determined by saturation studies on slide-mounted rat brain sections (unpublished observation). The slides were incubated (22°C) for 60 min, drained, and washed for either four consecutive 4-min ([^{3}H]bremazocine) or 2-min ([^{3}H]U69,593) 50 m*M* Tris washes (pH 7.0, 4°C). The slides were then rinsed in water (4°C), dried under a stream of cool air, and apposed to tritium-sensitive hyperfilm (Amersham) for 378 days. Nonspecific binding was evaluated by treating a parallel set of slides with the same concentration of tritiated ligands with a 1 μ*M* final concentration of unlabeled bremazocine.

RESULTS

Figure 1 provides the results of the *in situ* hybridization controls, while Figs. 2–6 provide low magnification darkfield images comparing the κ_1 receptor mRNA distribution to prodynorphin mRNA and κ receptor binding defined either by [^{3}H]bremazocine or [^{3}H]U69,593. Figures 7–10 provide high-resolution cellular images of κ_1 receptor mRNA and prodynorphin mRNA expressing cells in selected brain areas using emulsion-dipped sections. A similar analysis is difficult to perform with [^{3}H]bremazocine and [^{3}H]U69,593 binding as these ligands cannot be efficiently fixed to the binding sites, making it impossible to dip into NTB2 emulsion without substantial loss of tritiated ligand.

The anatomical nomenclature is that of Paxinos and Watson (38), except in the case of the hypothalamus, where the subdivisions of the paraventricular nucleus conform to that of Sawchenko and Swanson (39). The anatomical description that follows is qualitative, and is designed to provide relevant comparisons across distributions. Any reference to density of binding sites or levels of mRNA expression is only within a particular ligand or mRNA distribution. Regions that are described as having high amounts of [^{3}H]U69,593 binding, for example, should be viewed as having high levels compared to [^{3}H]U69,593 binding in other areas of the CNS and not directly compared to high levels of [^{3}H]bremazocine binding. This is clearly evident from the relatively weak autoradiographic signal generated with [^{3}H]U69,593 as compared to [^{3}H]bremazocine, despite a 1-year exposure. In some cases, in fact, where [^{3}H]U69,593 is low, the apparent verbal description of the binding may not be immediately apparent in the photomicrograph and only seen in the original hyperfilm image. Similarly, references to high κ_1 receptor mRNA expression cannot be directly and quantitatively compared to prodynorphin mRNA levels in the same regions.

In Situ Hybridization Controls

In situ hybridization with cRNA probes generated to different regions of the κ_1 receptor produced the same anatomical distribution. Compare, for example, the *in situ* hybridization image in Fig. 1A that was produced with the 3′ untranslated probe of the κ_1 receptor to that produced with a cRNA probe generated to transmembrane III–VII in Fig. 1B. The κ_1 receptor mRNA distributions are indistinguishable regardless of which cRNA probe was used. In the remaining figures, the 3′ untranslated cRNA probe was utilized to label the κ_1 receptor mRNA. Brain and pituitary sections hybridized with the sense-strand RNA probe to the rat κ_1 receptor failed to show any specific *in situ* hybridization (compare Figs. 1B and 1C). Similarly, prior RNase treatment resulted in a complete loss of specific *in situ* signal in sections hybridized with the 3′ untranslated antisense κ_1 receptor cRNA probes (data not shown). Taken together, the results suggest that the mRNA distribution that follows represents specific hybridization to the κ_1 receptor. Similar controls were

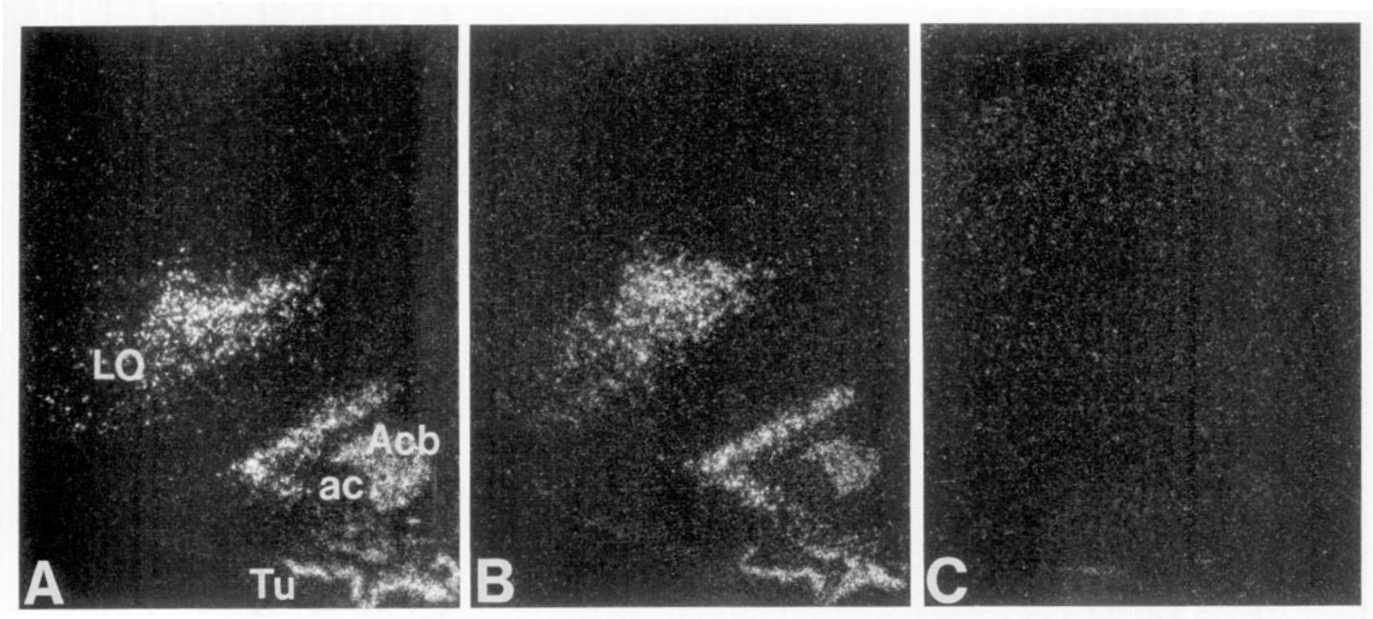

FIG. 1. *In situ* hybridization controls. Darkfield autoradiograms demonstrating a similar κ_1 receptor mRNA distribution in the lateral orbital cortex (LO), nucleus accumbens (Acb), and olfactory tubercle (Tu) when antisense cRNA probes generated to either the 3′ untranslated region (A) or transmembrane III–VII (B) of the rat κ_1 receptor are used. No specific *in situ* hybridization is observed when a "sense" RNA probe is hybridized to an adjacent brain section (C).

previously performed for the prodynorphin cRNA probe and were not repeated here.

Telencephalon

In the olfactory bulb κ_1 receptor mRNA is restricted to the internal granular layer, where a few widely scattered cells are observed. These cells, while few in number, express high levels of κ_1 receptor mRNA. No cells expressing κ_1 receptor mRNA are seen in the other layers of the rat olfactory bulb, including the exterior and interior plexiform and glomerular layers. κ receptor binding in the olfactory bulb varies dramatically with tritiated ligand. [^{3}H]Bremazocine labels sites most prominently in the internal plexiform layer, with moderate levels in the external plexiform and glomerular layers and a lower density of sites in the internal granular layer. [^{3}H]U69,593 binding, in contrast, is undetectable in the layers of the olfactory bulb. Similarly, no prodynorphin mRNA is detected in the olfactory bulb.

κ_1 receptor mRNA distribution in the cerebral cortex is complex, with high levels of cellular expression that extend across neo- and allocortical divisions and that vary dramatically, both between and within cortical lamina. Rostrally, κ_1 receptor mRNA expression is high in the deep portions of the lateral and ventrolateral orbital cortex (Figs. 1A and 1B) with scattered cells seen in more superficial layers. This pattern of cortical expression extends caudally to the level of the nucleus accumbens and dorsally to include the insular and parietal cortex (Figs. 2A and 7A). Within the insular cortex, κ_1 receptor mRNA expression is primarily in the deeper layers and in the parietal cortex it is limited to layer VI with scattered cells in layer V. Within the parietal cortex, κ_1 receptor mRNA expressing cells are restricted only to the ventral extent of these lamina and generally do not extend dorsally beyond the level of the caudate–putamen (Figs. 2A, 3A, 4A, and 5A). The same ventral distribution within layers V and VI extend caudally and continuously to the midbrain with a similar expression pattern observed in the temporal and occipital cortex (Fig. 6A). κ_1 receptor mRNA expression is also high in the entorhinal cortex, but undetectable in the frontal, cingulate, and piriform cortex.

κ receptor binding in the cortex parallels the κ_1 receptor mRNA distribution with the highest density of [^{3}H]bremazocine and [^{3}H]U69,593 binding sites in the deep layers of the lateral and ventrolateral orbital cortex, insular cortex, and in the ventral extents of layers V and VI of the parietal, temporal, and occipital cortex (Figs. 2–6, B and D). Moderate densities of [^{3}H]bremazocine and [^{3}H]U69,593 receptor binding are also seen in the entorhinal cortex. However, in contrast to the κ_1 receptor mRNA distribution, κ receptor binding defined by both [^{3}H]bremazocine and [^{3}H]U69,593 is more widespread with lower densities of binding sites also seen in more superficial layers II–III of the parietal, temporal, and occipital cortex and extending dorsally in layers V and VI (Figs. 2–6, B–D). Similarly, a low level of [^{3}H]bremazocine and [^{3}H]U69,593 binding is observed in the frontal and cingulate cortex (e.g., Figs. 2B and 2D) regions where κ_1 receptor mRNA expressing cells are undetectable.

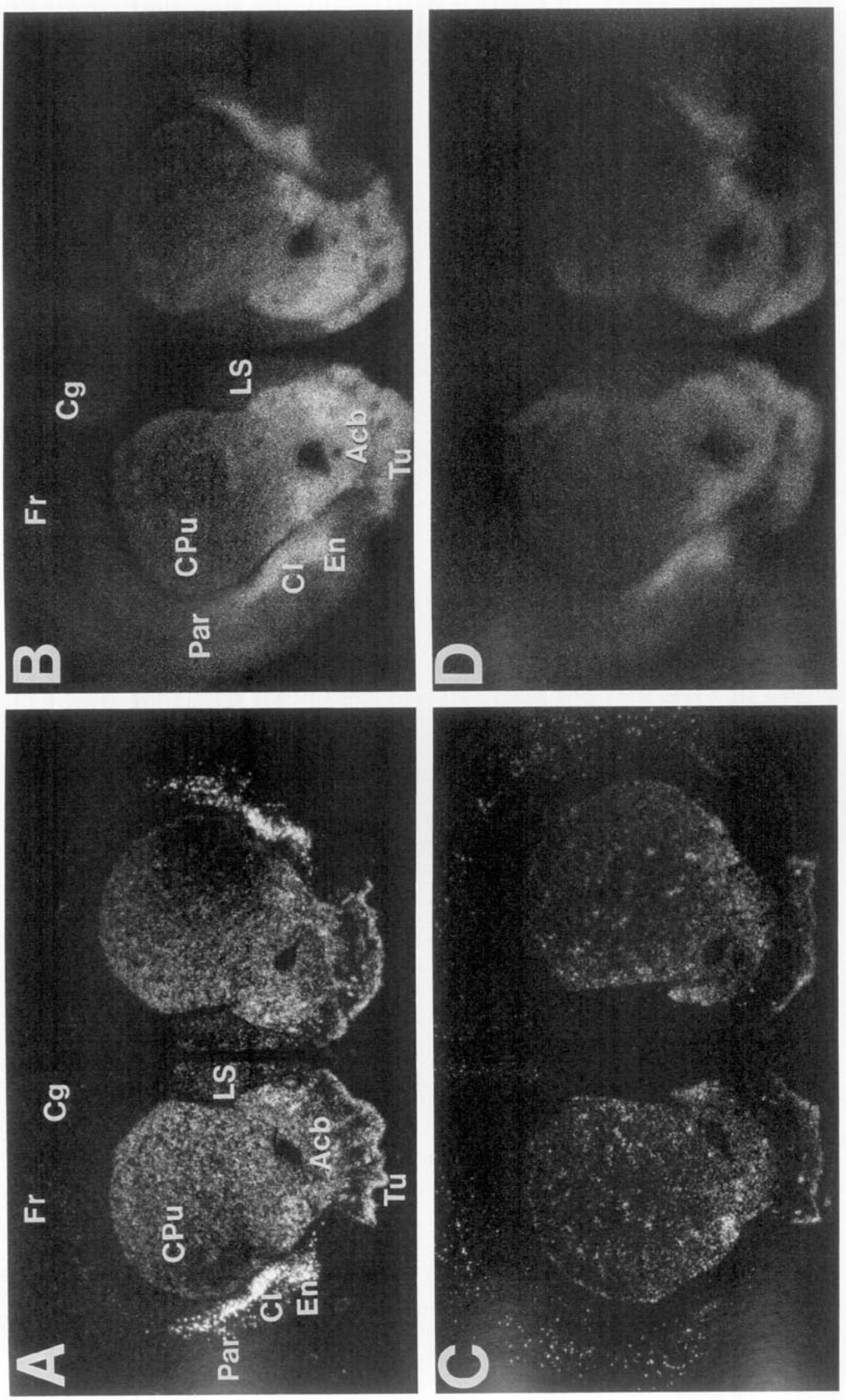

FIG. 2 Comparative distribution of κ_1 receptor mRNA (A), [^{3}H]bremazocine receptor binding (B), prodynorphin mRNA (C), and [^{3}H]U69,593 receptor binding (D) at the level of the striatum. Note the high correspondence between the distribution of the κ_1 receptor mRNA and [^{3}H]bremazocine and [^{3}H]U69,593 receptor binding in the claustrum (Cl), endopiriform (En), nucleus accumbens (Acb), olfactory tubercle (Tu), caudate-putamen (CPu), parietal cortex (Par), and lateral septum (LS). Marked differences are observed in the distribution of cells expressing prodynorphin mRNA in the pariatal, frontal, and cingulate cortex and in the patchy and subcallosal distribution in the caudate-putamen. Cells expressing prodynorphin mRNA do appear to be distributed similarly to those expressing κ_1 receptor mRNA in the nucleus accumbens.

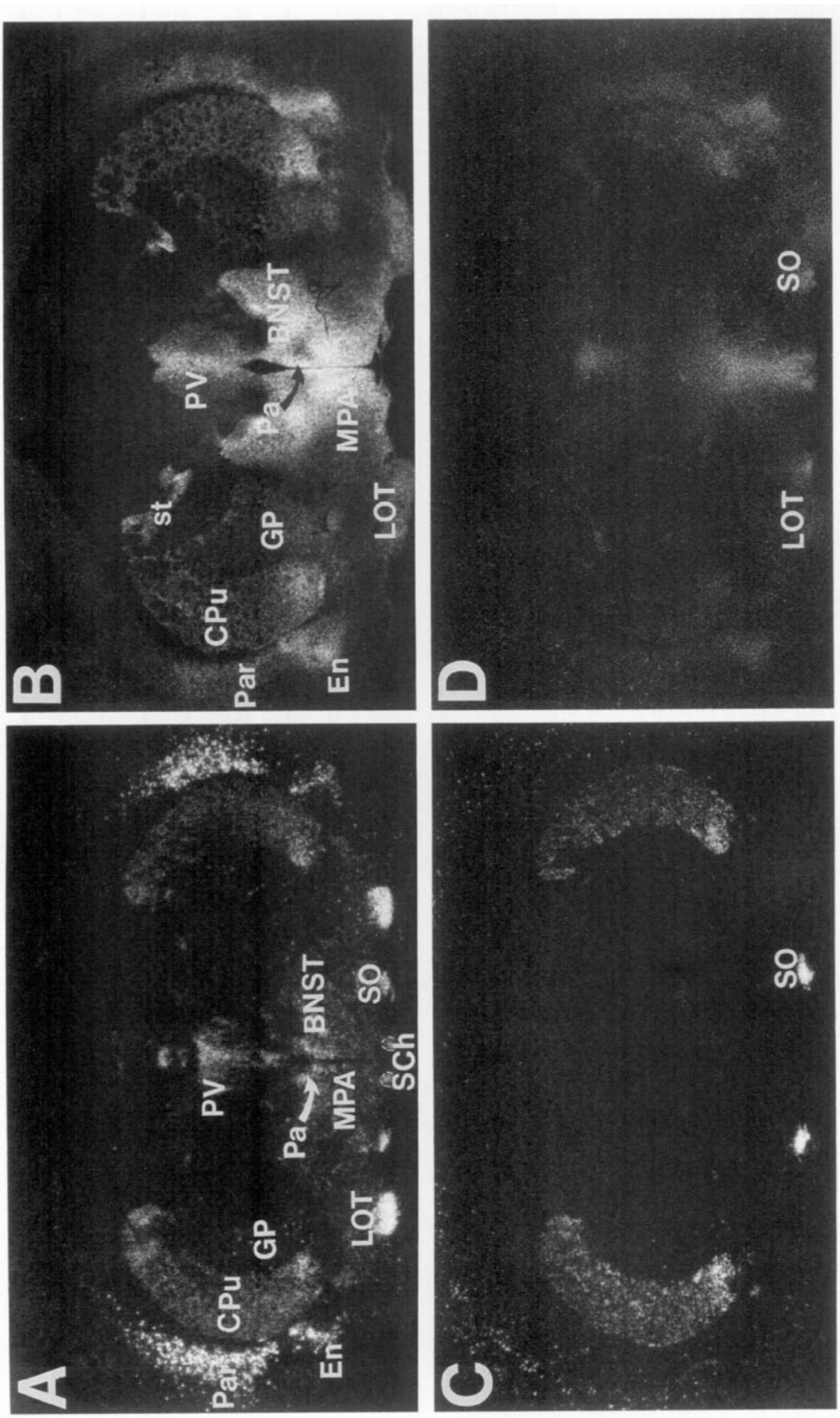

FIG. 3. Comparative distribution of κ_1 receptor mRNA (A), [^{3}H]bremazocine receptor binding (B), prodynorphin mRNA (C), and [^{3}H]U69,593 receptor binding (D) at the level of anterior hypothalamus. A good correspondence between the distributions of κ_1 receptor mRNA and [^{3}H]bremazocine and [^{3}H]U69,593 receptor binding is observed in the paraventricular hypothalamus (Pa), medial preoptic area (MPA), bed nucleus stria terminalis (BNST), supraoptic (SO), and suprachiasmatic (SCh) nuclei of the hypothalamus, paraventricular thalamus (PV), the nucleus of the lateral olfactory tract (LOT), caudate–putamen (CPu), and globus pallidus (GP). High levels of prodynorphin mRNA expression are seen in the supraoptic nucleus of the hypothalamus.

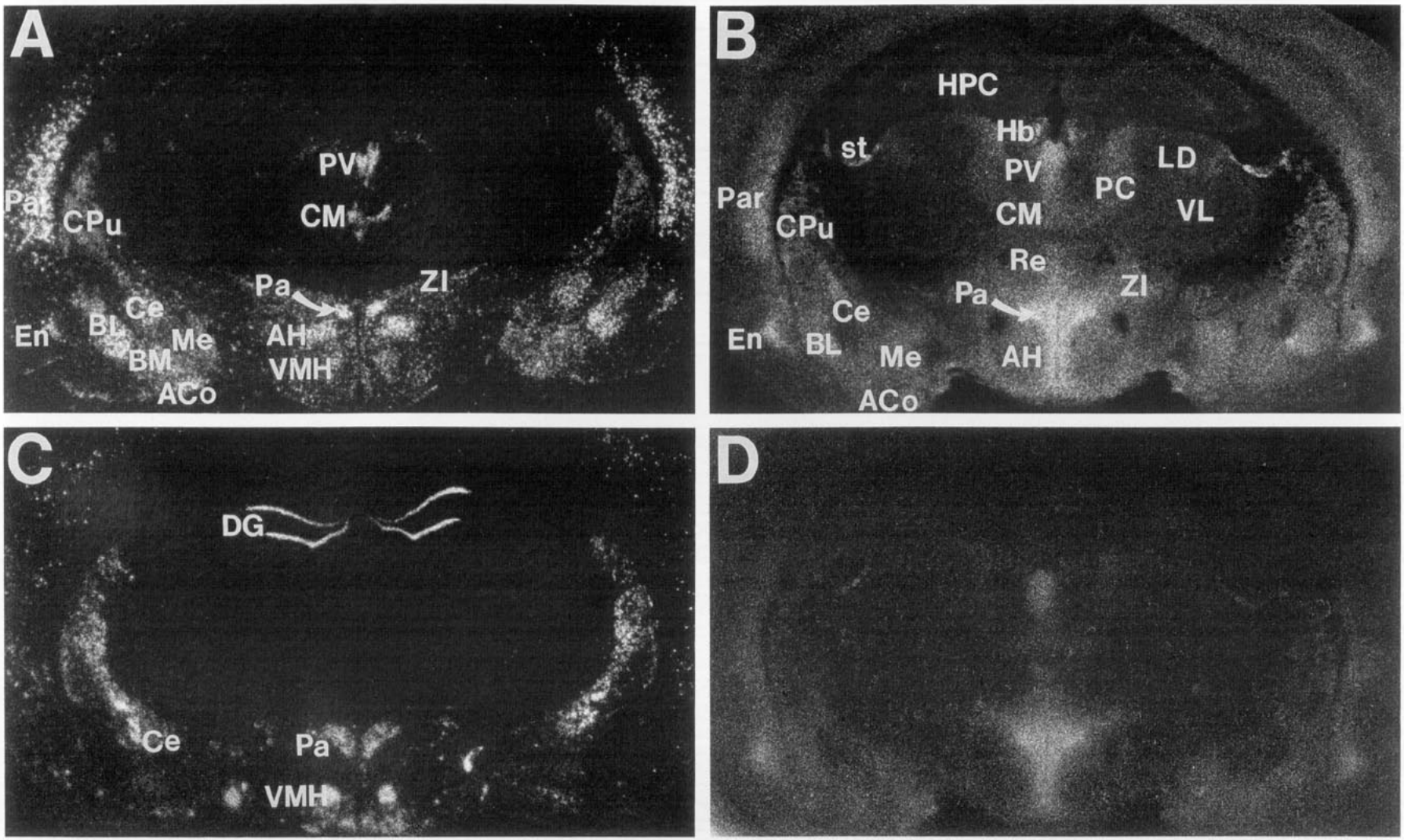

FIG. 4. Comparative distribution of κ_1 receptor mRNA (A), [^{3}H]bremazocine receptor binding (B), prodynorphin mRNA (C), and [^{3}H]U69,593 receptor binding (D) in coronal rat brain sections at the level of the midhypothalamus. A good correspondence between the distribution of κ_1 receptor mRNA and [^{3}H]bremazocine and [^{3}H]U69,593 receptor binding is observed in the basolateral (BL), basomedial (BM), medial (Me), and anterior cortical (ACo) amygdala, paraventricular (Pa), anterior (AH), and ventromedial (VMH) hypothalamus, zona incerta (ZI), paraventricular (PV), and centromedial (CM) thalamus. [^{3}H]Bremazocine receptor binding differs from [^{3}H]U69,593 in the hippocampus (HPC) and several thalamic nuclei, including the medial habenula (Hb). Prodynorphin mRNA expression is high in the dentate gyrus (DG), a region not expressing κ_1 receptor mRNA, and the central nucleus of the amygdala, paraventricular, and ventromedial hypothalamus. This level of the paraventricular nucleus of the hypothalamus is further examined in Fig. 9.

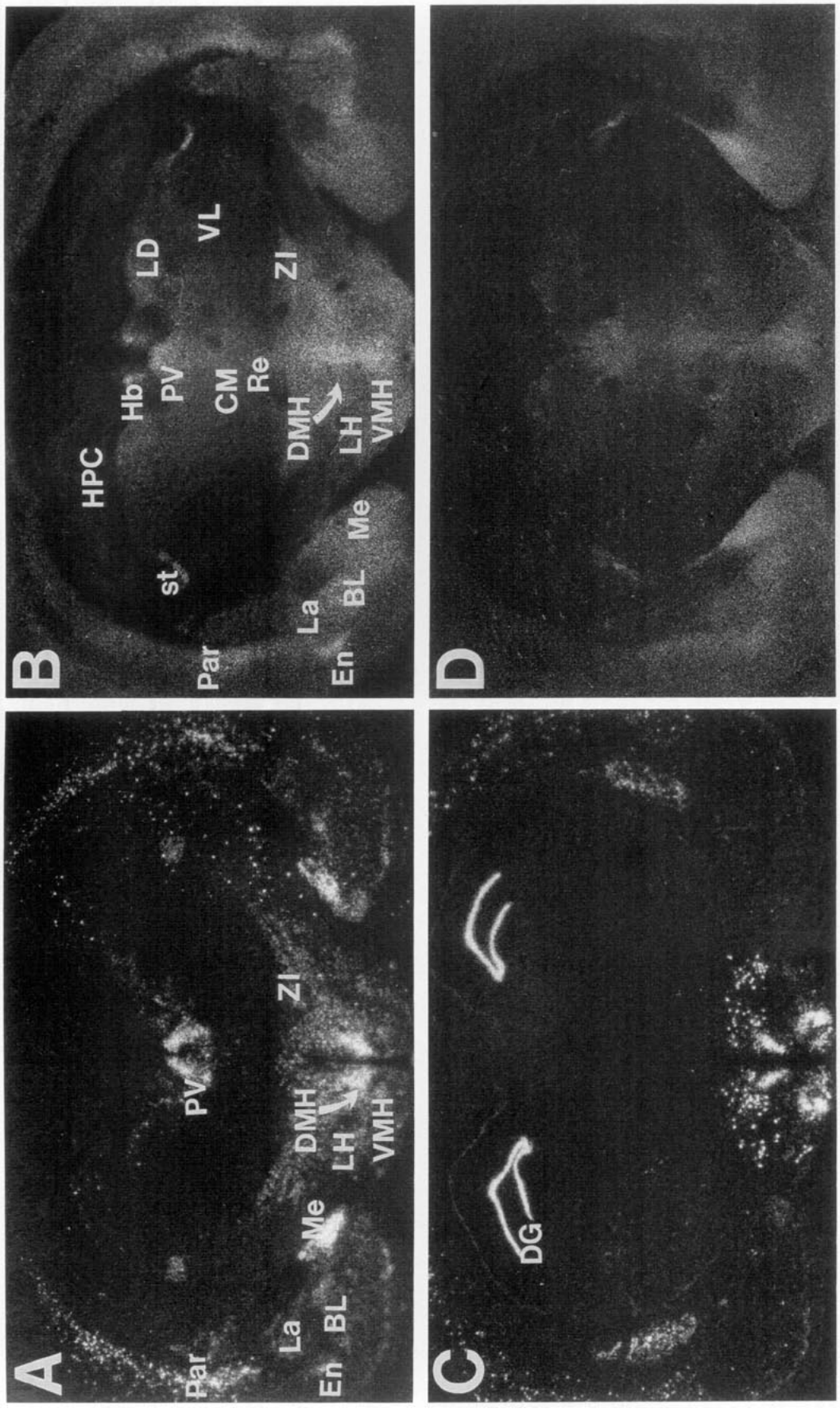

FIG. 5. Comparative distribution of κ_1 receptor mRNA (A), [^{3}H]bremazocine receptor binding (B), prodynorphin mRNA (C), and [^{3}H]U69,593 receptor binding (D) at the level of the caudal hypothalamus. A good correspondence between the distribution of κ_1 receptor mRNA and [^{3}H]bremazocine and [^{3}H]U69,593 binding is seen at the lateral (La), basolateral (BL), and medial (Me) amygdala, dorsomedial (DMH), lateral (LH), and ventromedial (VMH) hypothalamus, zona incerta (ZI), and paraventricular thalamus (PV). [^{3}H]Bremazocine receptor binding differs from [^{3}H]U69,593 in the hippocampus (HPC) and medial habenula (Hb). Prodynorphin mRNA expression is high in the dentate gyrus (DG), dorsomedial and ventromedial hypothalamus, and in scattered cells of the lateral hypothalamus.

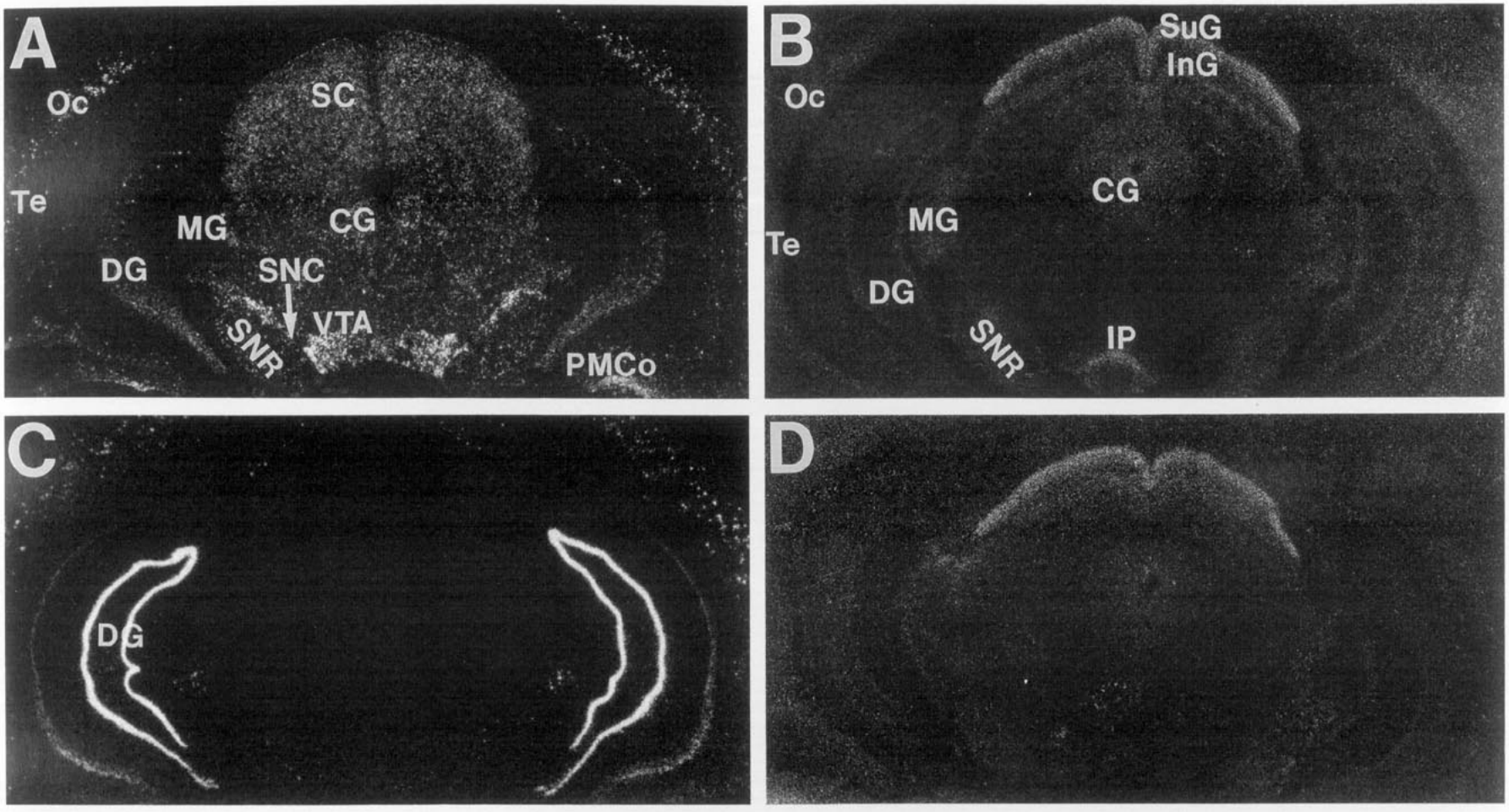

FIG. 6. Comparative distribution of κ_1 receptor mRNA (A), [^{3}H]bremazocine receptor binding (B), prodynorphin mRNA (C), and [^{3}H]U69,593 receptor binding (D) at the level of the rat midbrain. Note the lack of correspondence in the distribution of κ_1 receptor mRNA and [^{3}H]bremazocine and [^{3}H]U69,593 binding in the ventral tegmental area (VTA), substantia nigra, pars compacta (SNC), and the interpeduncular nucleus (IP). κ_1 receptor mRNA, [^{3}H]bremazocine, and [^{3}H]U69,593 receptor binding is observed in the substantia nigra, pars reticulata (SNR), superior colliculus (SC), central grey (CG), medial geniculate (MG), and ventral dentate gyrus (DG).

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In contrast to the relatively restricted κ_1 receptor mRNA distribution in cortex, cells expressing prodynorphin mRNA are more widespread and display a somewhat complementary pattern to the cells expressing κ_1 receptor mRNA. Within the parietal, temporal, and occipital cortex, prodynorphin expressing cells are scattered, with highest density in layers II–IV (Figs. 2C–6C) and a reduced number of cells in layers V and VI. A moderate number of widely scattered prodynorphin expressing cells are also observed in the lateral and ventrolateral orbital cortex (Fig. 7E), insular cortex (Fig. 7B), and the frontal and cingulate cortex (Fig. 2C). In the case of the orbital cortex, prodynorphin cells are localized in more superficial layers, external to cortical layers demonstrating κ_1 receptor expressing cells. This is evident by comparing the distributions of cells in the lateral orbital and insular cortex that express prodynorphin and κ_1 receptor mRNAs in Figs. 7A and 7D. Prodynorphin mRNA expressing cells are not detected in the piriform and entorhinal cortex.

The claustrum and endopiriform nucleus are among the nuclei that demonstrate the highest levels of κ_1 receptor expression in the CNS (Figs. 2–5). High levels of κ_1 receptor mRNA and binding defined by either [^{3}H]bremazocine and [^{3}H]U69,593 are demonstrated in these regions. A high-resolution image showing the density and level of cellular expression of κ_1 receptor mRNA in the claustrum is provided in Fig. 7B. In contrast, no detectable prodynorphin mRNA expressing cells are observed in these regions.

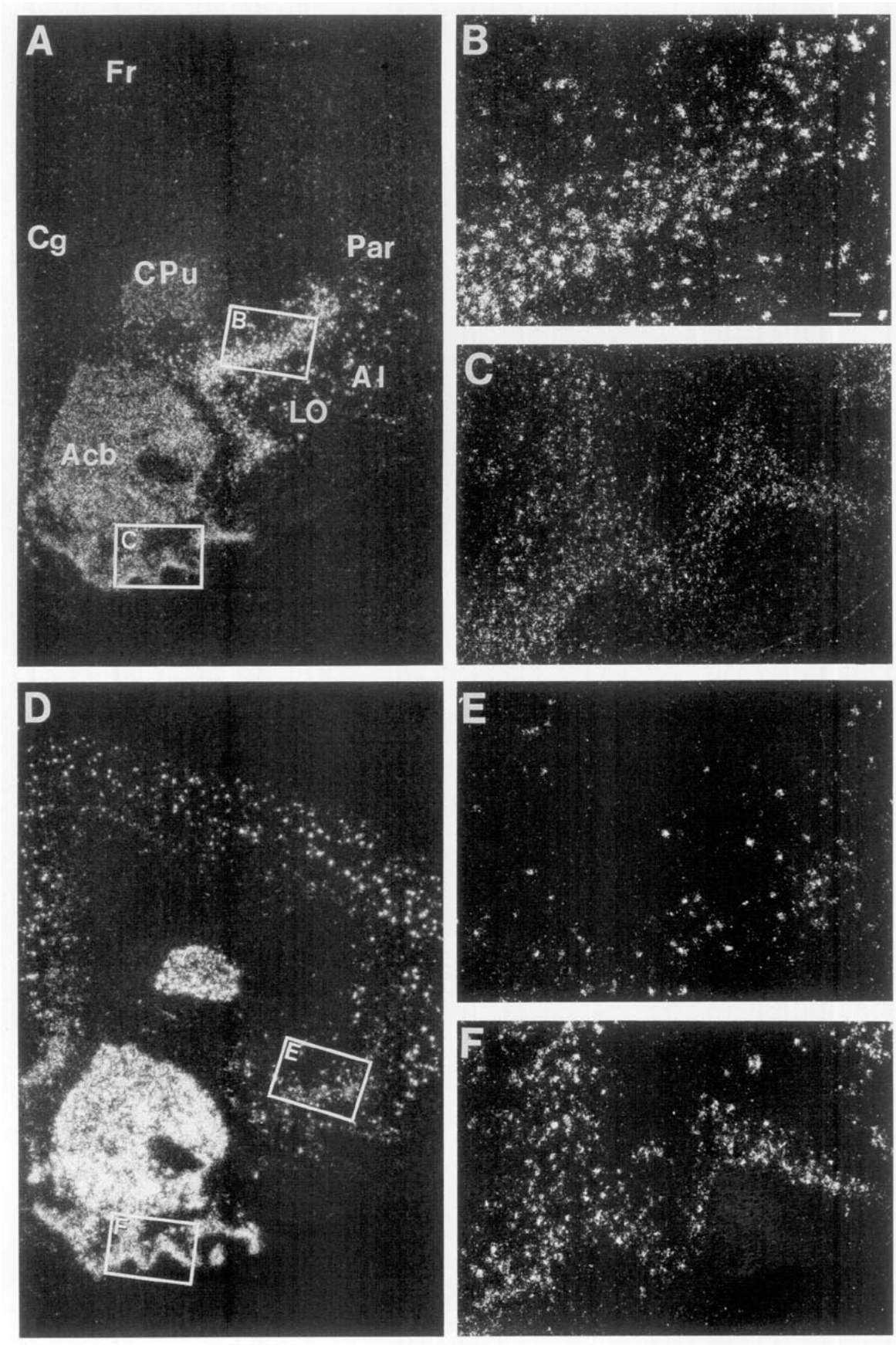
A high degree of correspondence between κ_1 receptor mRNA and binding is also observed in the striatum, where κ_1 receptor expression is highest in the nucleus accumbens and olfactory tubercle (Figs. 2 and 7). Within the nucleus accumbens κ_1 receptor mRNA and binding defined by both [^{3}H]bremazocine and [^{3}H]U69,593 is highest in accumbens shell and in the septal pole, with somewhat lower expression in the accumbens core (Figs. 2 and 7A). Prodynorphin mRNA distribution corresponds well in the ventral striatum with higher levels of expression in accumbens shell and septal pole (Figs. 2C and 7D). Comparisons of the high level of cellular expression of prodynorphin mRNA and κ_1 receptor mRNA in the olfactory tubercle are provided in Figs. 7F and 7C. In the dorsal striatum, κ_1 receptor mRNA expression and [^{3}H]U69,593 binding is highest in the ventral and medial portions of the caudate–putamen (Figs. 2A and 2D), with the lateral portion showing little or no κ_1 receptor mRNA expression and [^{3}H]U69,593 binding. [^{3}H]Bremazocine labels a similar distribution of sites in the dorsal striatum as [^{3}H]U69,593, but a few patches in the lateral caudate–putamen are observed (Fig. 2B). The relative density of [^{3}H]bremazocine binding in the caudate–putamen compared to the nucleus accumbens is also higher than that observed with [^{3}H]U69,593 (Figs. 2B and 2D), making the dissociation between dorsal and ventral striatum less distinct with [^{3}H]bremazocine. Prodynorphin mRNA cellular expression in the caudate–putamen is distinctly different, with a patch–matrix and subcallosal distribution. In the caudal caudate–putamen higher levels of κ_1 receptor mRNA, receptor binding, and prodynorphin mRNA expression are observed in fundus striati, medial to the endopiriform nucleus (Fig. 3). Higher κ_1 receptor mRNA expression is also observed in the dorsal portion of the caudal caudate–putamen (Fig. 3) which is thought to be the rodent equivalent of the caudate nucleus.

Low levels of κ_1 receptor mRNA expression are observed in the lateral septum, a region demonstrating a low to moderate density of κ binding sites defined by either [^{3}H]bremazocine or [^{3}H]U69,593 (Fig. 2). Prodynorphin mRNA expressing cells are not found in most of the lateral septum, but a few cells are localized in the dorsal division.

Both the ventral pallidum and globus pallidus show widely scattered cells expressing κ_1 receptor mRNA (Fig. 3); however, the level of expression per cell is higher in the ventral pallidum. This corresponds well with the low levels of [^{3}H]bremazocine and [^{3}H]U69,593 binding (Fig. 3) and the scattered prodynorphin expressing cells found in the globus and ventral pallidum.

κ_1 receptor mRNA and receptor binding is expressed in all the nuclear subdivisions of the amygdala (Figs. 4 and 5). Rostrally, the highest levels of κ_1 receptor mRNA expression are in the central and basolateral nuclei with somewhat lower levels in the lateral, medial, basomedial, and anterior cortical nuclei (Fig. 4A). There appears to be some heterogeneity in the κ_1 receptor mRNA expression within these nuclear divisions. Compare, for example, the relatively lower level of κ_1 receptor mRNA expression in the anterior portion of the medial and cortical amygdaloid nuclei (Fig. 4A) to these same nuclei posteriorly (Figs. 5A and 6A). This pattern of receptor expression is also observed at the level of receptor binding, with comparatively higher levels of [^{3}H]bremazocine and [^{3}H]U69,593 binding in the basolateral nucleus and the posterior part of the medial nucleus, while moderate amounts are observed in the lateral, anterior medial, basomedial, and cortical amygdaloid nuclei (Figs. 4–5, B and D). The central nucleus demonstrates a dissociation between κ_1 receptor mRNA expression and κ receptor binding, with high levels of κ_1 receptor mRNA and relatively low levels of binding (Figs. 4A, 4B, and 4D). Prodynorphin mRNA expression is predominantly localized in the central nucleus, with some cellular expression in the basomedial and medial nuclei (Fig. 4C).

Rostral–caudal differences in κ_1 receptor mRNA expression are also observed in the hippocampal formation and dentate gyrus. Rostrally, no κ_1 receptor mRNA is detected in the CA1–3 pyramidal fields or in the granular cells of the dentate gyrus (Figs. 4A and 5A). However, caudally at the level of the midbrain, low levels of κ_1 receptor mRNA expression are observed in the ventral dentate gyrus (Fig. 6A). This mRNA distribution parallels the receptor binding distribution defined by [^{3}H]U69,593,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where no binding is detected in the dorsal hippocampus and dentate gyrus (Figs. 4D and 5D), but low levels of binding are seen in the ventral dentate gyrus (Fig. 6D). [^{3}H]Bremazocine binding is markedly different in these structures, with a moderate density of sites in the stratum oriens and lacunosum-moleculare of the dorsal hippocampus and in the molecular layer of the dentate gyrus (Figs. 4B and 5B). The density of [^{3}H]bremazocine sites also increases in the caudal CA1–3 region and in the ventral dentate gyrus (Fig. 6C).

Unlike the κ_1 receptor mRNA expression in the hippocampus and dentate gyrus, dramatically high levels of prodynorphin mRNA expression are observed in the dorsal and ventral dentate gyrus (Figs. 4C, 5C, and 6C). A few scattered prodynorphin cells are seen in the dorsal CA2–CA3 pyramidal cells, with a higher level of prodynorphin mRNA expression in the CA1–CA2 pyramidal fields of the caudal hippocampus at the level of the midbrain.

Cells of the bed nucleus of the stria terminalis and the medial preoptic area demonstrate a comparatively high level of κ_1 receptor mRNA expression (Fig. 3A). κ_1 receptor mRNA expression is highest in the anterior medial preoptic area, a level more rostral than that shown in Fig. 3. κ receptor binding defined by [^{3}H]bremazocine and [^{3}H]U69,593 is also relatively high in both the bed nucleus stria terminalis and the medial preoptic area (Figs. 3B and 3D), but as can be seen from Fig. 3 there is more extensive labeling in these regions by [^{3}H]bremazocine compared to [^{3}H]U69,593, suggesting the binding to non-κ_1 sites. [^{3}H]Bremazocine and [^{3}H]U69,593 receptor binding extends within the stria terminalis itself (Figs. 3–5, B and D), which may serve to transport κ opioid receptors to and from the amygdala. Small clusters of κ_1 receptor mRNA expressing cells are localized in the stria terminalis, which may also contribute to the κ receptor binding observed in the stria terminalis. Prodynorphin mRNA cells are scattered in the bed nucleus of the stria terminalis and in the medial preoptic area, where a moderate level of expression is observed.

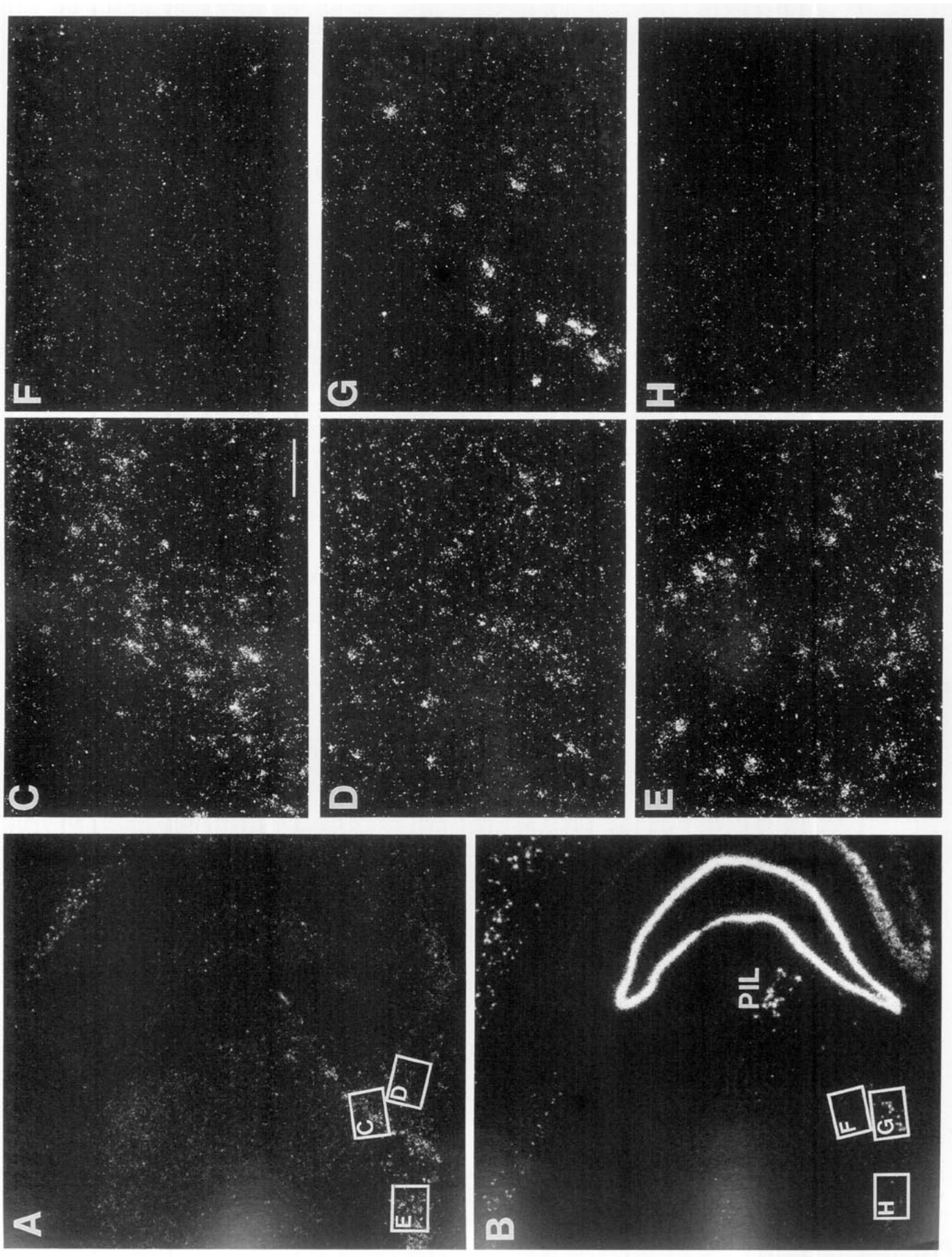
Another region of interest in the telencephalon is the nucleus of the lateral olfactory tract which expresses high levels of κ_1 receptor mRNA, moderate densities of [^{3}H]bremazocine and [^{3}H]U69,593 binding, and no detectable prodynorphin mRNA (Fig. 3). The nucleus of the lateral olfactory tract is thought to be part of the "olfactory" amygdala and is consistent with the high levels of κ_1 receptor mRNA expression and receptor binding found in other amygdaloid nuclei. Similarly, in the horizontal limb of the diagonal band there is a scattering of cells expressing κ_1 receptor mRNA, a moderate density of [^{3}H]bremazocine and [^{3}H]U69,593 receptor binding, and no detectable prodynorphin expressing cells.

Diencephalon

Within the thalamus, κ_1 receptor mRNA is found predominantly in the midline nuclei. Highest κ_1 receptor mRNA expression is observed in the paraventricular nucleus (Figs. 3A–5A), followed by somewhat lower levels of expression in the centromedial nucleus (Fig. 4A). Scattered cells expressing κ_1 receptor mRNA are also observed in the zona incerta (Figs. 4A and 5A) and medial geniculate body (Fig. 6A). This pattern corresponds well to [^{3}H]U69,593 receptor binding which is highest in the paraventricular nucleus, with lower levels in the centromedial, laterodorsal, paracentral, reuniens, rhomboid, zona incerta, and the medial geniculate nucleus (Figs. 4D–6D). No specific [^{3}H]U69,593 receptor binding is detected in the medial and lateral habenula, and the ventrolateral and ventromedial nuclei of the thalamus (Figs. 4D and 5D). In contrast, while [^{3}H]bremazocine binding is also predominant in midline nuclei (paraventricular, centromedial, paracentral, rhomboid, and reuniens), it has a wider distribution in the thalamus with lower levels of binding in the zona incerta, mediodorsal, ventromedial and ventrolateral nuclei, as well as high levels in the medial habenula (Figs. 4B and 5B). By comparison, no prodynorphin mRNA expressing cells could be detected in most of the thalamus. Only a few scattered prodynorphin cells are observed in the posterior intralaminar thalamic nucleus (Fig. 8B), which does not demonstrate any cells expressing κ_1 receptor mRNA.

Several hypothalamic nuclei express κ_1 receptor mRNA with the highest levels observed in the anterior hypothalamic area, paraventricular, suprachiasmatic, supraoptic, and dorsomedial nuclei (Figs. 3A–5A). Cells expressing κ_1 receptor mRNA are also seen along the third ventricle in the periventricular nucleus (Fig. 3A), and as scattered cells more caudally in the ventromedial and lateral hypothalamus (Figs. 4A and 5A). Relatively few κ_1 receptor mRNA expressing cells are observed in the rostral arcuate nucleus. Of particular interest is the heterogeneity of κ_1 receptor mRNA expression within particular hypothalamic nuclei. For example, in the rostral paraventricular nucleus, κ_1 receptor mRNA expressing cells are localized predominantly in the anterior parvocellular division (Fig.

FIG. 7. Darkfield autoradiogram comparing the cellular distribution of κ_1 receptor mRNA (A) and prodynorphin mRNA (D) in the striatum and cortex. While the κ_1 receptor and prodynorphin mRNA distributions are similar in the nucleus accumbens (Acb) and olfactory tubercle, they differ dramatically in the claustrum and lateral orbital (LO), agranular insular (AI), parietal (Par), frontal (Fr), and cingulate (Cg) cortex. B and C are higher magnifications of areas outlined in A and correspond to cells expressing κ_1 receptor mRNA in the claustrum (B) and olfactory tubercle (C). E and F are higher magnifications of areas highlighted in D and correspond to cells expressing prodynorphin mRNA in the lateral orbital and insular cortex (E) and olfactory tubercle (F). Size bar in B = 100 μm and applies to all high magnification images.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4A). More caudally as can be seen from Fig. 9C, κ_1 receptor mRNA expression is predominantly in the ventral portion of the medioparvocellular paraventricular nucleus of the hypothalamus, with little or no expression in the magnocellular or the dorsal parvocellular divisions where prodynorphin is expressed (Fig. 9E). Similarly, in the supraoptic nucleus only the cells immediately adjacent to the optic chiasm express κ_1 receptor mRNA (Fig. 9D), while prodynorphin expressing cells extend more laterally from the optic tract or chiasm (Fig. 9F), suggesting that the κ_1 receptor expressing cells are at best only a subpopulation of the prodynorphin expressing cells found in this nucleus. Finally, within the suprachiasmatic nucleus (Fig. 3A), only cells in the lateral portion of the nucleus express κ_1 receptor mRNA. Prodynorphin expressing cells are also seen in the dorsomedial and ventromedial hypothalamus (Figs. 4C and 5C), the arcuate nucleus, periventricular nucleus (Fig. 4C), and as scattered intensely labeled cells of the lateral hypothalamus (Fig. 5C).

κ receptor binding defined by [^{3}H]bremazocine or [^{3}H]U69,593 appears similar in the hypothalamus and largely parallels the κ_1 receptor mRNA distribution. κ receptor binding is seen in the zona incerta, paraventricular, periventricular, supraoptic, suprachiasmatic, dorsomedial, and the ventromedial nuclei of the hypothalamus, with a lower density of binding in the anterior and lateral hypothalamic areas and in the arcuate nucleus (Figs. 3–5, B and D). In contrast, the lateral mammillary and supramammillary nuclei, as well as the median eminence show high levels of κ receptor binding and no κ_1 receptor mRNA expression.

The subthalamic nucleus demonstrates the converse situation, with high levels of κ_1 receptor mRNA expression, a very low density of [^{3}H]bremazocine receptor binding, and no detectable [^{3}H]U69,593 receptor binding. No detectable prodynorphin mRNA expressing cells are found in the subthalamic nucleus.

Mesencephalon

Within the midbrain marked differences are observed in the distributions of the κ_1 receptor mRNA and κ receptor binding in the substantia nigra and ventral tegmental area. High levels of κ receptor mRNA expression are observed in the cells of the ventral tegmental area and in the substantia nigra, pars compacta and pars reticulata (Figs. 6A, 8A, 8C–8E). Within the substantia nigra, pars compacta, not all cells appear to express κ_1 receptor mRNA. The highest number of cells is found medially, suggesting they may be localized in a subpopulation of pars compacta cells. In contrast to the κ_1 receptor mRNA distribution, only a low density of [^{3}H]bremazocine and [^{3}H]U69,593 sites is observed in the ventral tegmental area and substantia nigra. The pars reticulata is the primary localization of [^{3}H]bremazocine and [^{3}H]U69,593 (Figs. 6B and 6D). Prodynorphin expressing cells are not detectable in the ventral tegmental area (Fig. 8H), and in the substantia nigra only a few scattered cells are observed, primarily in the pars reticulata (Figs. 8B and 8G).

In the periaqueductal grey (CG), a region thought to be involved in opioid analgesia, κ_1 receptor mRNA expressing cells are observed with their highest density in the ventral and lateral subregions (Fig. 6A). Cells expressing κ_1 receptor mRNA are found throughout the rostro–caudal extent of the CG from the rostral midbrain to the pontine central grey, where the highest level of expression is observed. [^{3}H]Bremazocine and [^{3}H]U69,593 receptor binding parallels this distribution with binding sites localized around the aqueduct in the rostral CG, ventral and lateral to the aqueduct at the level of the inferior colliculi, and extend into the pontine central grey, where the density of κ binding sites is highest. Scattered prodynorphin cells are observed in the lateral and ventral CG at the levels of the superior and inferior colliculi, with cellular expression extending to the pontine central grey.

Scattered cells in the dorsal and caudal linear raphe and more caudally in the raphe magnus show κ_1 receptor mRNA expression which is paralleled by [^{3}H]bremazocine and [^{3}H]U69,593 receptor binding in these nuclei. No prodynorphin mRNA expressing cells were detected in the dorsal raphe, with a few scattered cells in the caudal linear raphe, raphe magnus, and raphe obscurus.

In contrast to the low levels of κ_1 mRNA expression in the interpeduncular nucleus, [^{3}H]bremazocine binding is high in most of the interpeduncular complex with κ receptor binding observed in the rostral, lateral, and central divisions (Fig. 6B). A similar binding distribution is observed in the interpeduncular complex with [^{3}H]U69,593, but the density of binding sites is low (Fig. 6D). Only a few scattered prodynorphin expressing cells are seen in the rostral interpeduncular nucleus.

Both the superior and inferior colliculi express κ_1 receptor mRNA. A moderate level of κ_1 mRNA expression is observed across all the layers of the superior colliculus (Fig. 6A) with a somewhat higher level of expression in the internal grey layer. κ receptor binding defined by either

FIG. 8. Darkfield autoradiograms comparing the cellular distribution of κ_1 receptor mRNA (A) to prodynorphin mRNA (B) in the midbrain. Boxes in A are shown as high magnification images in adjacent panels and correspond to cells expressing κ_1 receptor mRNA in the substantia nigra, pars compacta (C), substantia nigra, pars reticulata (D), and ventral tegmental area (E). Similar regions have been outlined in B and appear as adjacent high magnification images to demonstrate the lack of prodynorphin mRNA expression in substantia nigra, pars compacta (F) and ventral tegmental area (H). A few scattered prodynorphin mRNA expressing cells are, however, seen in the substantia nigra, pars reticulata (G). Size bar in C = 100 μm and applies to all high magnification images.

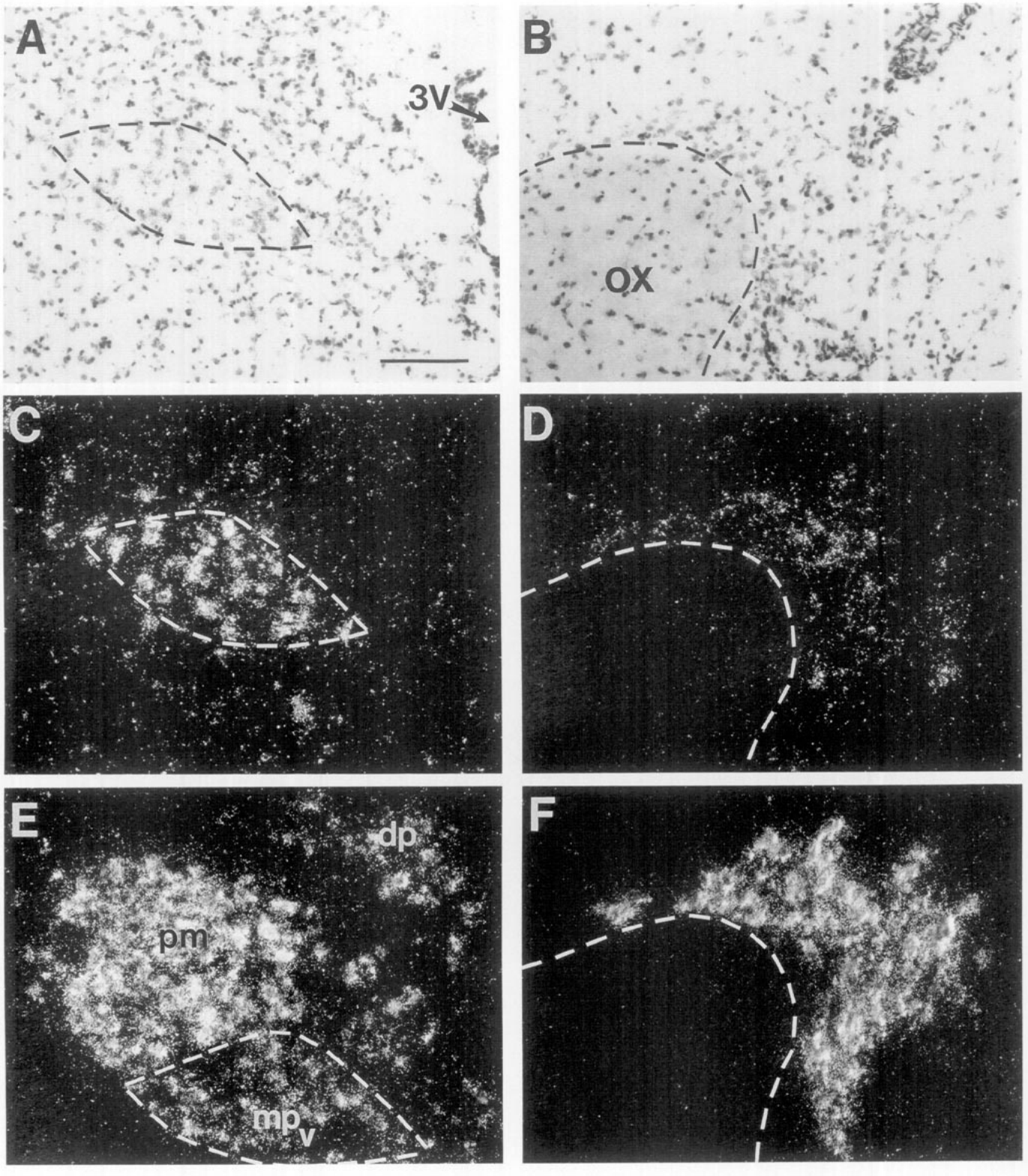

FIG. 9. Comparative cellular distributions of κ_1 receptor mRNA and prodynorphin mRNA in the paraventricular (left column) and the supraoptic nucleus (right column) of the hypothalamus. Cells expressing prodynorphin mRNA are found in posterior magnocellular (pm), the dorsal parvocellular (dp), and the ventral portion of the medial parvocellular (mpv) divisions of the paraventricular nucleus (E), while cells expressing κ_1 receptor mRNA are primarily restricted to the ventral portion of the medial parvocellular group (A,C). Cells expressing κ_1 receptor mRNA in the mpv of the paraventricular nucleus of the hypothalamus are outlined in the brightfield image in A and the darkfield image in C. Bright- (B) and darkfield (D) images of cells expressing κ_1 receptor mRNA in the supraoptic nucleus demonstrate that these cells are primarily localized immediately surrounding the optic chiasm (OX) and are not as laterally distributed as cells expressing prodynorphin mRNA (F). Also, note the markedly higher level of prodynorphin mRNA expression compared to the κ_1 receptor mRNA expression. Future studies are necessary to determine whether κ_1 mRNA is colocalized with prodynorphin in the cells of the paraventricular and supraoptic nuclei. Size bar in A = 100 μm.

[^{3}H]bremazocine or [^{3}H]U69,593, on the other hand, shows a highly laminated distribution with the greatest density of binding sites in the superficial grey layer with low to moderate densities in the internal grey layer. Within the inferior colliculus cells expressing κ receptor mRNA are localized predominantly in the external cortex, with comparatively few cells in the central nucleus of the inferior colliculus. [^{3}H]Bremazocine binding is prominent in both the central and the external inferior colliculus, with the highest density of receptor-binding sites in the external cortex. Only a low level of [^{3}H]U69,593 binding is observed in the inferior colliculus, and is restricted to the external cortex, consistent with κ_1 receptor mRNA distribution. In contrast, no prodynorphin expressing cells are observed in the superior or inferior colliculus.

Met- and Mylencephalon

The distribution of [^{3}H]U69,593 and [^{3}H]bremazocine receptor binding differs in the pontine nuclei. Low levels of [^{3}H]bremazocine receptor binding are observed in these nuclei, yet no [^{3}H]U69,593 or κ_1 receptor mRNA are detected. Similarly, no cells expressing prodynorphin mRNA are found.

Cells in the parabrachial nucleus and locus coeruleus express κ_1 receptor mRNA (Fig. 10), which is paralleled by the [^{3}H]bremazocine and [^{3}H]U69,593 receptor binding observed in these nuclei. As can be seen in Fig. 10F, prodynorphin mRNA expressing cells are also localized in the parabrachial nucleus where the cellular expression is far higher than that observed for cells expressing κ_1 receptor mRNA (Fig. 10E). Within the locus coeruleus, only a small proportion of cells express prodynorphin and κ_1 receptor mRNA (Figs. 10A–10D). However, as is the case for the parabrachial nucleus, the level of expression of prodynorphin mRNA per cell in the locus coeruleus is far higher than that of κ_1 receptors mRNA (Figs. 10B and 10D vs 10A and 10C).

Within the trigeminal nuclei, scattered cells expressing low levels of κ_1 receptor mRNA are found in the sensory, motor, and spinal nuclei. Similarly, comparatively little [^{3}H]bremazocine and [^{3}H]U69,593 receptor binding is seen in the sensory and motor nuclei, but the spinal trigeminal shows moderate to high levels of κ receptor binding. Scattered cells expressing prodynorphin mRNA are found in the rostral sensory and motor nuclei and in the spinal trigeminal nucleus.

κ_1 receptor expression in the hypoglossal nucleus is particularly interesting, as it is limited to the prepositus subdivision. Cells expressing κ_1 receptor mRNA and [^{3}H]bremazocine and [^{3}H]U69,593 receptor binding are seen only in the prepositus division of the hypoglossal with the rest of the nucleus showing no κ receptor expression. In contrast, no prodynorphin mRNA expressing cells are detected within any region of the hypoglossal nucleus.

Further caudally, cells in the rostral and caudal nucleus of the solitary tract express κ_1 receptor mRNA. This corresponds well to the relatively high levels of [^{3}H]bremazocine and [^{3}H]U69,593 binding, as well as the large number of intensely labeled cells expressing prodynorphin mRNA in nucleus of the solitary tract.

No cells expressing κ_1 receptor mRNA are detected in the nucleus cuneatus and nucleus gracilis. This is in marked contrast with the relatively high level of prodynorphin mRNA expression seen in the deep and external cuneate nucleus. Interestingly, no prodynorphin expressing cells are observed in the nucleus gracilis.

Large cells expressing relatively high levels of κ_1 receptor mRNA were also scattered in the pontine reticular formation and more caudally in the gigantocellular reticular and the gigantocellular reticular α formation in the brainstem. Scattered prodynorphin expressing cells were also localized in the pontine reticular formation, but more caudally were found in the pontine gigantocellular and intermediate reticular formation. Because of the widely scattered nature of these κ_1 receptor and prodynorphin expressing cells, they were difficult to associate with regions of specific κ receptor binding.

Cells in the cerebellum demonstrate no detectable κ_1 receptor mRNA or prodynorphin mRNA which is paralleled by the lack of specific [^{3}H]U69,593 binding. Low levels of [^{3}H]bremazocine receptor binding are observed in the cerebellum.

Pituitary

No detectable κ_1 receptor mRNA is detected in the anterior, intermediate, or neural lobes of the rat pituitary. In contrast, high levels of prodynorphin mRNA expression are observed in the cells of the intermediate lobe, with a somewhat lower level of prodynorphin mRNA expression in scattered cells of the anterior lobe of the pituitary. No κ receptor binding was performed in the pituitary in this study.

DISCUSSION

Analysis of the *in situ* hybridization and receptor autoradiographic results suggests there is a high degree of correspondence between the κ_1 receptor mRNA and κ receptor binding distributions. Both are localized in ventral and midline structures, including the endopiriform nucleus, claustrum, nucleus accumbens, olfactory tubercle, bed nucleus stria terminalis, medial preoptic area, paraventricular, supraoptic, suprachiasmatic, dorsomedial, and ventromedial hypothalamus, basolateral, medial and cortical amygdala, midline thalamic nuclei, periaqueductal grey, superior and inferior colliculus, parabrachial nucleus, locus coeruleus, and the nucleus of the solitary tract. These findings are the first description of the cellular distribution of the κ_1 receptor mRNA in the rat brain, and in conjunction with the κ receptor binding and prodynorphin mRNA results, provide insight into κ receptor anatomy, circuitry, and function.

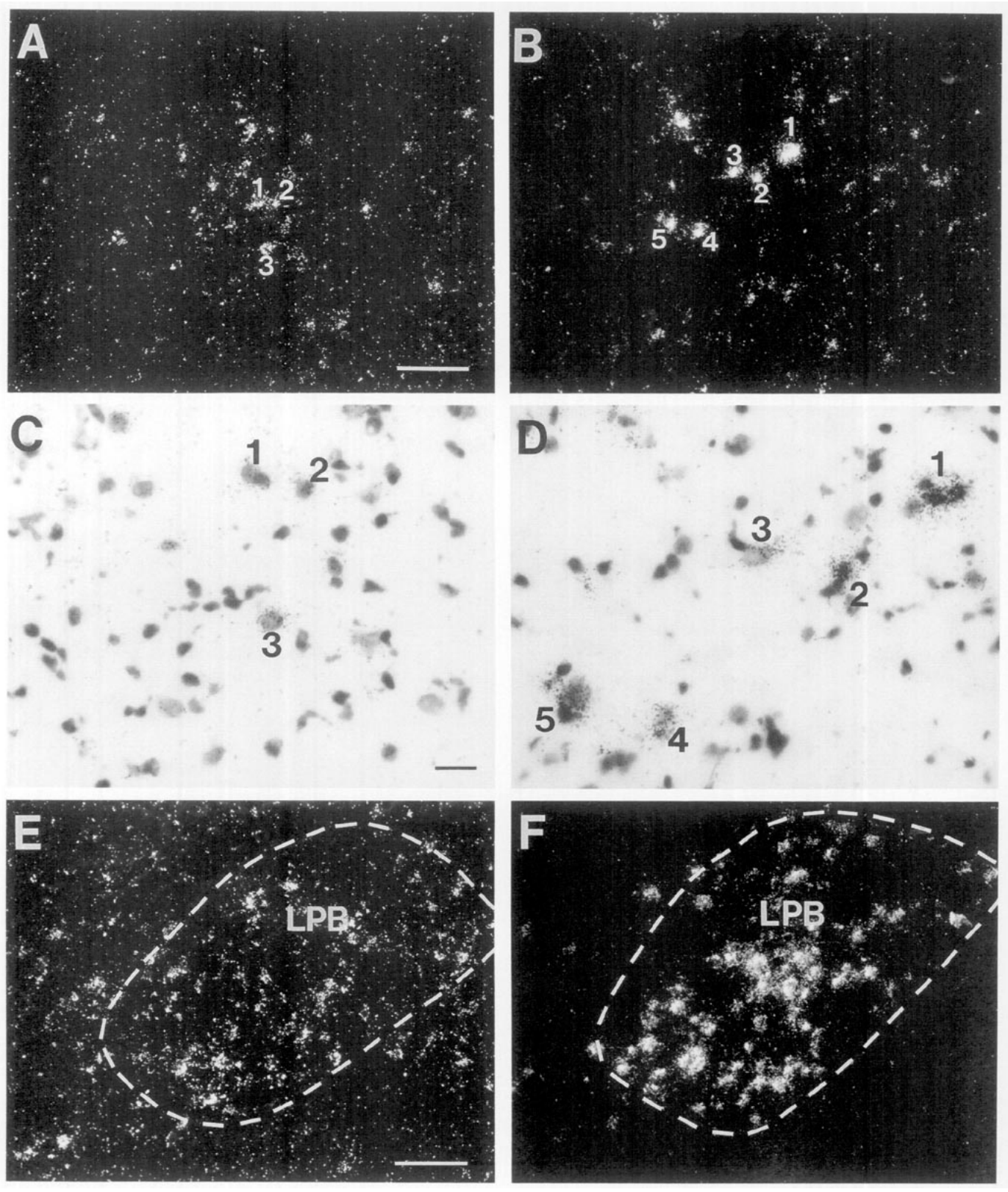

FIG. 10. Comparison of the κ_1 receptor mRNA (left column) and prodynorphin mRNA (right column) expression in locus coeruleus (A–D) and lateral parabrachial nucleus (LPB) (E,F). Cells in the locus coeruleus expressing κ_1 receptor mRNA are identified in the darkfield image (A) and magnified in the brightfield image (C) to demonstrate the specific *in situ* hybridization grains observed over the cells. Similarly, cells expressing prodynorphin mRNA in the darkfield image (B) are magnified in the brightfield image (D). As can be seen from E and F, cells in the parabrachial nucleus express κ_1 receptor mRNA (E) and prodynorphin mRNA (F). Future studies are necessary to determine whether κ_1 mRNA and prodynorphin are colocalized in the locus coeruleus and parabrachial nucleus. Size bars in A and E = 100 μm and apply to corresponding darkfield images, while the size bar in C = 10 μm and applies to brightfield images (C and D).

While there is a high correspondence between the distribution of κ_1 receptor mRNA and κ receptor binding defined by either [^{3}H]bremazocine or [^{3}H]U69,593, it is by no means perfect. A number of brain regions demonstrated either the presence of κ_1 receptor mRNA and no receptor binding or the reverse of κ receptor binding and no κ_1 receptor mRNA. In either case, these observations represent mismatches and are as interesting, if not more so, than the areas of convergence. One factor that clearly affected the degree of correspondence between the receptor mRNA and binding distributions was the choice of tritiated ligand. Overall, there was a better correspondence between the κ_1 receptor mRNA distribution and the receptor-binding distribution defined by [^{3}H]U69,593. This is evident in such regions as the olfactory bulb, dorsal hippocampus, dentate gyrus, medial habenula, ventromedial and ventrolateral thalamus, pontine nuclei, and the cerebellum, where [^{3}H]bremazocine receptor binding is easily measured, yet no [^{3}H]U69,593 binding or κ_1 receptor mRNA can be detected. The assay conditions chosen for [^{3}H]bremazocine, room temperature incubation with no NaCl in the presence of a 300-fold excess of DAMGO and DPDPE, result in a receptor distribution that closely parallels that of [^{3}H]U69,593. Far greater differences in the total number and distribution of benzomorphan-labeled sites have been reported when the assays are performed at 4°C in the presence of 100 m*M* NaCl (22, 24). However, even under the present conditions, where less dramatic differences in [^{3}H]U69,593 and [^{3}H]bremazocine binding distributions were observed, it is clear that [^{3}H]U69,593 labels only a subpopulation of receptor binding sites as compared to [^{3}H]bremazocine, and these binding sites represent only a small proportion of the total opioid receptor sites in the rat as evidenced by the weak autoradiographic signals and long film exposure time. Whether the additional binding sites labeled by [^{3}H]bremazocine represent multiple κ subtypes, residual binding to μ and δ receptors, or the binding to other opioid receptors such as λ or ϵ is unclear.

A second factor that needs to be considered in examining the differences between the κ_1 mRNA and binding distributions is the selectivity of the *in situ* hybridization and receptor autoradiographic conditions. The use of high stringency hybridization and wash conditions, the finding that cRNA probes generated to different regions of the κ_1 receptor produce identical anatomical distributions, and the lack of specific hybridization with the sense-strand and RNase controls argue that the *in situ* hybridization results were specific for the κ_1 receptor mRNA. Further, the κ_1 receptor mRNA distribution described here is markedly different from the μ and δ opioid receptor mRNA distributions in the CNS (40, 41). Whether the κ_1 mRNA distribution may also represent some cross-hybridization to an unidentified opioid receptor subtype is impossible to know at present and needs to be considered in the future. With regards to the receptor autoradiographic conditions, both the selective benzenacetamide [^{3}H]U69,593 and the benzomorphan [^{3}H]bremazocine were used to label the κ receptors and its only regions of overlap that differed from the κ_1 receptor mRNA distribution which are considered as mismatches. Some investigators have suggested that even selective ligands, such as U50,488 and U69,593, may be labeling subtypes of κ_1 receptors (20). If that is the case, it could also affect the match between the κ_1 mRNA and receptor binding distributions.

A third and more anatomically relevant factor influencing the lack of correspondence between the localization of κ_1 receptor mRNA and κ binding is that of receptor synthesis and transport. This is evident, for example, in the hypothalamus and pituitary where κ agonists have profound effects on hormonal regulation and water balance (10–13). κ receptor binding sites are present in the paraventricular, suprachiasmatic, and supraoptic nuclei of the hypothalamus, as well as the neural lobe of the pituitary (42, 43). It is unclear from binding studies, however, whether the κ sites found in the neural lobe are on pituicytes, or on fibers originating from the magnocellular cells of the hypothalamus (13, 42). The localization of κ_1 receptor mRNA in the suprachiasmatic and supraoptic nuclei and not in the pituitary suggests that the κ_1 receptor binding observed in the neural lobe is indeed localized on hypothalamic fibers. Similarly, the presence of κ_1 receptor binding in the median eminence and the lateral mammillary nuclei where no κ_1 receptor mRNA can be detected suggests that this binding may also be localized primarily on neuronal fibers or terminals. κ receptors on the fibers of the median eminence may have profound effects on the release of hypothalamic hormones into the hypophysial portal system, influencing a host of hormonally driven behaviors.

The localization of κ receptor binding and little κ_1 receptor mRNA is not limited to the hypothalamus and is evident in the interpeduncular nucleus and in the stria terminalis. The lack of κ_1 receptor mRNA in the stria terminalis is not surprising, as this is a major fiber pathway containing reciprocal projections from the nuclei of the amygdala and bed nucleus. The presence of κ_1 receptor mRNA in the amygdala and bed nucleus with corresponding receptor binding in these regions, as well as in the stria terminalis, suggests that the stria terminalis may serve to transport κ receptors. κ receptors are likely then to be synthesized in either the bed nucleus or the nuclei of the amygdala, with some portion transported via the stria terminalis. The presence of κ_1 receptor mRNA expression in small clusters of cells in the stria terminalis may also contribute to the κ receptor binding observed in the stria terminalis.

In the nigrostriatal system, the presence of κ_1 receptor mRNA in the cells of the ventral tegmental area (VTA) and the medial substantia nigra, pars compacta (SNC), in relation to relatively little κ receptor binding in these

regions suggests that κ receptors may be synthesized in the VTA and SNC and transported, most likely, to the striatum, where they are localized on presynaptic terminals. High levels of κ_1 receptor mRNA expression in the nucleus accumbens and caudate–putamen suggest that some portion of the κ receptor binding is also postsynaptic. Taken together, these observations suggest that the κ_1 receptor binding observed in the striatum is from at least two sources: (i) presynaptic receptor sites on fibers originating from the midbrain; and (ii) postsynaptic receptors synthesized by cells intrinsic to the striatum. This suggestion is consistent with lesion studies in the nigrostriatal system demonstrating that opioid receptor binding sites are on both pre- and postsynaptic elements in the striatum (7, 44). Pharmacological studies have similarly demonstrated that κ agonists can inhibit release of dopamine from terminals by a presynaptic mechanism (45–47), as well as postsynaptically by their direct actions on the striatal neurons (48). The lack of both κ_1 receptor mRNA expression and [^{3}H]U69,593 binding in the lateral caudate–putamen, a region thought to be important for motor integration, is consistent with the lack of locomotor activation observed with κ agonists.

The distribution of cells expressing κ_1 receptor mRNA in the substantia nigra, pars reticulata, is suggestive of a colocalization with GABA and a possible role for κ_1 receptors in the control of transmission from the substantia nigra. It is presently unclear whether the κ receptor binding observed in the pars reticulata originates from intrinsic substantia nigra, pars reticula (SNR) cells expressing the κ_1 receptor mRNA or on fibers originating from the striatum or subthalamic nucleus that project to the SNR. The subthalamic nucleus contains primarily neurons that project to the globus pallidus and substantia nigra, so it is not entirely surprising to find κ_1 receptor mRNA and no [^{3}H]U69,593 receptor binding in this nucleus. κ receptors originating in the subthalamic nucleus may serve to further regulate striatal circuits.

The localization of κ_1 receptor mRNA in the locus coeruleus and raphe nuclei suggests that κ receptors may also regulate noradrenergic and serotonergic systems. This is consistent with the findings that selective κ agonist can inhibit norepinephrine release in the cortex (49) and the dependence on serotonergic systems for κ receptor-mediated analgesia (50). The presence of [^{3}H]U69,593 and [^{3}H]bremazocine binding sites in the cingulate, frontal, and in portions of the parietal and temporal cortex in relation to an absence of κ_1 receptor mRNA, suggests that some of the receptor binding in the mismatched areas of cortex are on fiber terminals, perhaps originating from monoaminergic cells in the midbrain and brain stem. Sedation, a common effect observed with the administration of κ receptor agonists (2) may, in fact, be related to modulation of monoamine release in these cortical areas.

With regard to the relationship between κ_1 receptors and prodynorphin, overlaps were observed in their mRNA distributions in a number of brain regions, including the nucleus accumbens, olfactory tubercle, central amygdala, globus pallidus, paraventricular, supraoptic, dorsomedial and ventromedial hypothalamus, locus coeruleus, parabrachial, spinal trigeminal, and the nucleus of the solitary tract. Regions of anatomical overlap are suggestive of local opioid circuits and in some brain areas may be indicative of an autoreceptor function. For example, the same pattern of cellular expression of prodynorphin and κ_1 mRNA in the nucleus accumbens shell and septal pole, as well as the medial supraoptic nucleus, is suggestive of a possible cellular colocalization and a κ autoreceptor function. Future studies will, however, be necessary to determine if κ_1 receptors are indeed colocalized with prodynorphin and whether they serve to regulate prodynorphin release.

Despite the codistribution in some brain areas, the vast majority of brain regions examined failed to show a good correspondence between the distribution of cells expressing prodynorphin and κ_1 receptor mRNA. This is most obvious in the dorsal dentate gyrus, where there are high levels of prodynorphin mRNA expression and no detectable κ_1 receptor mRNA, and in the cortex, where prodynorphin cells are scattered in the cingulate, frontal, and parietal cortex, while cells expressing κ_1 receptor mRNA are restricted to the deep layers of parietal cortex. Similarly, in the caudate–putamen, cells expressing prodynorphin mRNA have a patch–matrix distribution and extend to the dorsolateral portion of this nucleus, while cells expressing κ_1 receptor mRNA are localized in the medial and ventral caudate–putamen and are not distributed in patches. Other areas expressing κ_1 receptor mRNA and no prodynorphin include the endopiriform, claustrum, lateral septum, suprachiasmatic nucleus, lateral, basolateral and cortical amygdala, thalamus, substantia nigra, pars compacta, ventral tegmental area, superior and inferior colliculus, and the hypoglossal nucleus. This apparent mismatch between the distribution of prodynorphin mRNA and κ_1 receptor mRNA should be viewed with some caution, as this study does not localize prodynorphin fibers and terminals that are ultimately the cellular elements that will synapse on the opioid receptive cells. For technical reasons, such questions of receptor–peptide colocalization are better addressed with dual immunohistochemical studies, but antibodies to the κ_1 receptor are not presently available. With this word of caution in mind, it is likely in certain brain regions such as the cortex, caudate–putamen, and dentate gyrus that prodynorphin peptides do not bind to κ receptors, but may interact with μ or δ binding sites. This agrees with receptor binding studies demonstrating that prodynorphin peptides also have high affinity for μ and δ receptors, depending on the cleaved fragment (36).

Another finding that has become clear by comparing the κ_1 receptor and prodynorphin mRNA distributions, particularly in the hypothalamus, is that κ_1 expressing cells are localized within specific divisions of the hypo-

thalamic nuclei. For example, in the paraventricular nucleus, κ_1 expressing cells are observed predominantly in the anterior parvocellular and ventral portion of the medial parvocellular groups, the latter of which project to the brain stem and spinal cord, and are integral in coordinating autonomic responses (39). Cells expressing prodynorphin mRNA have a wider distribution in the paraventricular hypothalamus, with prodynorphin expression also in the posterior magnocellular and dorsal parvocellular divisions. Similarly, cells expressing κ_1 receptor mRNA are localized primarily in the lateral suprachiasmatic and the medial supraoptic nucleus which has important implications in terms of their possible colocalization with vasopressin and oxytocin (51) and κ receptor regulation of water intake and lactation. Cells expressing prodynorphin mRNA in the supraoptic nucleus of the hypothalamus extend more laterally than those expressing κ_1 receptor mRNA, suggesting a different colocalization with the neurophysins.

Of particular clinical importance is the involvement of κ opioid receptors in analgesia. The localization of cells expressing κ_1 mRNA and κ receptor binding in the nucleus of the solitary tract, the rostral–caudal extents of the central grey, and the thalamus is consistent with this functional role. While κ agonists are potent analgesics, their dysphoric effects have limited their clinical usefulness (52). Some investigators have suggested that these effects may be related to a decreased activity in the mesolimbic dopamine system (46).

In summary, the present study provides a detailed description of the cellular distribution of κ_1 receptor mRNA in the rat brain and compares it to κ receptor binding and prodynorphin mRNA. Such an analysis provides a better understanding of the anatomy, circuitry, and function of the κ opioid receptors and lays the foundation for future regulatory and mRNA colocalization studies. Similar analyses with the μ and δ receptors and the development of receptor–specific antibodies will further our understanding of these receptor systems at a cellular level and within neuronal circuits.

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