Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 4061–4065, June 1988
Neurobiology

Characterization and visualization of rat and guinea pig brain κ opioid receptors: Evidence for κ_1 and κ_2 opioid receptors

(opioid receptor subtypes/quantitative autoradiography/comparative neurochemistry/κ opioid analgesics/dynorphin)

R. SUZANNE ZUKIN, MAHBOUBEH EGHBALI, DIANE OLIVE, ELLEN M. UNTERWALD, AND ANN TEMPEL

Departments of Neuroscience and Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461

Communicated by Michael V. L. Bennett, January 5, 1988

ABSTRACT **κ opioid receptors (κ receptors) have been characterized in homogenates of guinea pig and rat brain under *in vitro* binding conditions. κ receptors were labeled by using the tritiated prototypic κ opioid ethylketocyclazocine under conditions in which μ and δ opioid binding was suppressed. In the case of guinea pig brain membranes, a single population of high-affinity κ opioid receptor sites (κ sites; K_d = 0.66 nM, B_{max} = 80 fmol/mg of protein) was observed. In contrast, in the case of rat brain, two populations of κ sites were observed—high-affinity sites at low density (K_d = 1.0 nM, B_{max} = 16 fmol/mg of protein) and low-affinity sites at high density (K_d = 13 nM, B_{max} = 111 fmol/mg of protein). To test the hypothesis that the high- and low-affinity κ sites represent two distinct κ receptor subtypes, a series of opioids were tested for their abilities to compete for binding to the two sites. U-69,593 and Cambridge 20 selectively displaced the high-affinity κ site in both guinea pig and rat tissue, but were inactive at the rat-brain low-affinity site. Other κ opioid drugs, including U-50,488, ethylketocyclazocine, bremazocine, cyclazocine, and dynorphin (1–17), competed for binding to both sites, but with different rank orders of potency. Quantitative light microscopy *in vitro* autoradiography was used to visualize the neuroanatomical pattern of κ receptors in rat and guinea pig brain. The distribution patterns of the two κ receptor subtypes of rat brain were clearly different. The pattern of rat high-affinity κ sites paralleled that of guinea pig in the caudate-putamen, midbrain, central gray substance of cerebrum, and substantia nigra; interspecies differences were apparent throughout most of the rest of the brain. Collectively, these data provide direct evidence for the presence of two κ receptor subtypes; the U-69,593-sensitive, high-affinity κ_1 site predominates in guinea pig brain, and the U-69,593-insensitive, low-affinity κ_2 site predominates in rat brain.**

Opiates and opioid peptides produce their pharmacological actions by interactions with the μ, δ, κ, and σ opioid receptors (μ, δ, κ, and σ receptors; for a review, see ref. 1). Pharmacological studies have established that ketocyclazocine-like opioids produce their antinociceptive and unique sedative actions through an interaction with κ receptors (2). These drugs effect a more pronounced sedation than do other opioids and have been evaluated as anesthetic agents. κ opioid drugs neither suppress morphine abstinence nor induce abstinence in morphine-dependent monkeys (3). The endogenous opioid peptide dynorphin also interacts with high selectivity at κ receptors.

Evidence for a separate κ receptor distinct from the morphine (μ) and enkephalin (Enk; δ) receptors has been provided by pharmacological (2, 4), electrophysiological (5, 6), binding (4, 7, 8), and solubilization and purification (9–11) studies. *In vitro* autoradiography was used to visualize κ receptors in rat (12) and guinea pig brain (13); guinea pig κ receptors were shown to be selectively localized in the deep layers of the neocortex. Rat brain κ receptors were not characterized biochemically. Because most neurophysiological, behavioral, and biochemical studies of the endogenous opioid systems have been undertaken in rat, we have carried out a study of the properties and anatomical distribution of κ receptors in these two species. The present study provides direct evidence for the presence of two κ receptor subtypes: the high-affinity κ_1 receptor site (κ_1 site) predominates in guinea pig brain, and the low-affinity κ_2 site predominates in rat brain.

MATERIALS AND METHODS

Binding Assay. Rats (male, Sprague–Dawley, 150–200 g) or guinea pigs (male, Hartley, 300–400 g) were decapitated, and brains were immediately removed. Brain tissue preparation and binding assays were carried out as described (14). Aliquots (1 ml; 0.8–0.9 mg/ ml of protein) of freshly prepared homogenate from whole brain were incubated in triplicate with tritiated ethylketocyclazocine ([^{3}H]EKC; 0.1–25 nM) in 50 mM Tris·HCl buffer (pH 7.4) at 4°C for 60 min in the absence or presence of 10 μM nonradioactive EKC. For blockade of μ and δ receptors, the binding assay was carried out in the presence of 100 nM [D-Ala^2,MePhe^4,Glyol^5]Enk in which MePhe is *N*-methylphenylalanine and Glyol is glycinol and 100 nM [D-Ala^2,D-Leu^5]Enk. [^{3}H]Bremazocine was not used in these studies because it labeled an additional site in brain not labeled by [^{3}H]EKC and not displaceable by μ, δ, κ, or σ opioids. Scatchard data were analyzed by computer-assisted linear regression analysis (in the case of monophasic curves) and by the program LIGAND (15) (in the case of biphasic curves). Each experiment was carried out a minimum of three times. Competition analyses were carried out with 2 nM [^{3}H]EKC in guinea pig brain assays to ensure specific binding to κ_1 receptors (K_d = 0.7 nM) or 10 nM [^{3}H]EKC in the presence of 1 μM U-69,593 for rat brain assays to ensure specific binding to κ_2 sites (K_d = 13 nM).

Autoradiography. Autoradiographic studies were carried out with tritium-sensitive film as described (16). Male Sprague–Dawley rats and male Hartley guinea pigs were sacrificed by decapitation; brains were rapidly removed, and coronal sections (20 μm) were prepared. Brain sections were incubated for 1 hr at 4°C in 50 mM Tris·HCl buffer (pH 7.4) with either 10 nM [^{3}H]EKC in the presence of 100 nM [D-Ala^2, MePhe^4,Glyol^5]Enk and 100 nM [D-Ala^2,D-Leu^5]Enk (to label total κ receptors) or in 10 nM [^{3}H]U-69,593 {tritiated (5α,7α,8β)-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro-(4,5)dec-8-yl]benzeneacetamide} (to label κ_1 receptors). Adjacent sections were incubated under the same conditions in a solution containing radiolabeled ligand in the presence of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: [^{3}H]EKC, [^{3}H]ethylketocyclazocine; Glyol, glycinol; Enk, enkephalin; μ, δ, κ, and σ receptor(s), μ, δ, κ, and σ opioid receptor(s); κ sites, κ receptor sites.

1000-fold excess (10 μM) of unlabeled levorphanol or U-69,593 to assess nonspecific binding. Nonspecific binding in every case was <8 ± 2% of total binding. Adjacent brain sections were stained with cresyl violet for histological verification of structures, and the anatomical structures of interest were identified by reference to the rat atlas of Paxinos and Watson (17). The optical density of each structure was determined, and receptor densities were computed as described in the legend to Table 3.

Drugs. [^{3}H]EKC (45.95 Ci/mmol; 1 Ci = 37 GBq) was provided by the National Institute on Drug Abuse. [D-Ala^2,MePhe^4,Glyol^5]Enk and haloperidol were purchased from Sigma; [^{3}H]U-69,593 (42.1 Ci/mmol) from New England Nuclear; and [D-Ala^2,D-Leu^5]Enk and dynorphin (1–17), from Peninsula Laboratories (San Carlos, CA). EKC and cyclazocine were given by Sterling-Winthrop (Rensselaer, NY); U-50,488H {*trans*-(+)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamidemethanesulfonate} and U-69,593, by Upjohn; Cambridge 20, by J. Hughes of Parke, Davis; tifluadom, by Anaquest (Murry Hill, NJ) and bremazocine, by H. B. A. Welle of ACF Chemiefarma (The Netherlands).

RESULTS

Pharmacological Characterization of κ_1 and κ_2 Opioid Binding Sites. Scatchard analysis of specific [^{3}H]EKC binding (unblocked) to guinea pig brain membranes revealed a biphasic curve suggesting binding to be at least two classes of binding sites (Fig. 1). Computer-assisted nonlinear regression analysis (15) of the data afforded a best fit for a curve calculated for two binding components. The first was characterized by a K_d = 0.38 ± 0.1 nM and a B_{max} = 86 ± 8 fmol/mg of protein; and the second, by a K_d = 21 ± 9 nM and a B_{max} = 349 ± 27 fmol/mg of protein. Biphasic curves were also observed for unblocked [^{3}H]EKC binding to rat brain membranes (Fig. 2). The density of the lower affinity binding component computed from the Scatchard plot was substantially greater in guinea pig than in rat brain (B_{max} 2 = 349 vs. 173 fmol/mg of protein).

κ receptors were specifically labeled in preparations of rat and guinea pig brain membranes by using the κ opioid [^{3}H]EKC in the presence of saturating concentrations (100 nM) of nonlabeled [D-Ala^2,MePhe^4,Glyol^5]Enk (μ ligand) and [D-Ala^2,D-Leu^5]Enk (δ ligand) to block binding of the radioligand to μ and δ receptors (1, 7, 12). In the case of guinea pig membranes (Fig. 1), a linear Scatchard plot was observed (K_d = 0.66 ± 0.09 nM and B_{max} = 80 ± 7 fmol/mg of protein). In contrast, in the case of rat brain membranes with μ and δ receptor ligands (Fig. 2), the Scatchard plot remained biphasic (K_d1 = 1.0 nM, B_{max}1 = 16 fmol/mg of protein; K_d2 = 13.1 nM, B_{max}2 = 111 fmol/mg of protein). Inclusion of U-69,593 (5 μM), a specific κ receptor ligand (Fig. 2), or Cambridge 20 (data not shown) selectively eliminated the higher affinity component of the Scatchard plot obtained for rat brain membranes with μ and δ receptor blockers; in contrast, U-50,488 displaced [^{3}H]EKC binding from both sites. Increasing the concentration of μ and δ receptor blockers to 1 μM resulted in data indistinguishable from that obtained at a concentration of 100 nM. Table 1 summarizes κ_1 and κ_2 receptor affinities and densities obtained for the two species by Scatchard analysis. Together,

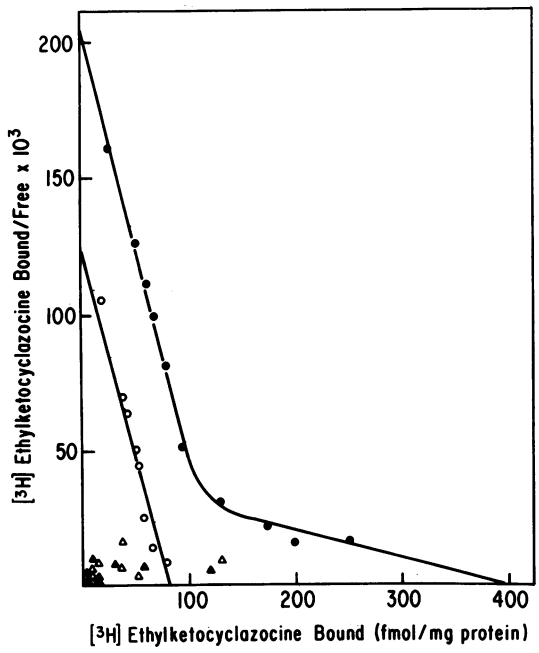

FIG. 1. Scatchard analyses of [^{3}H]EKC binding to guinea pig brain membranes. ●, Total specific binding, defined as the total binding of [^{3}H]EKC minus binding in the presence of 10 μM nonlabeled EKC; ○, specific binding to κ receptors, defined as binding of [^{3}H]EKC in the presence of 100 nM [D-Ala^2,MePhe^4,Glyol^5]Enk (μ receptor blocker) and 100 nM [D-Ala^2,D-Leu^5]Enk (δ receptor blocker) minus binding in the presence of 10 μM nonlabeled EKC and the μ and δ blockers at 100 nM; ▲, specific binding to κ_2 receptors, defined as binding of [^{3}H]EKC to κ receptors, except that 5 μM U-69,593 was included in all samples; and △, binding of [^{3}H]EKC in the presence of 100 nM [D-Ala^2,MePhe^4,Glyol^5]Enk, 100 nM [D-Ala^2,MePhe^4,Glyol^5]Enk, and 5 μM U-50,488h minus binding in the presence of 10 μM EKC and all blockers. Data are from one experiment, performed in triplicate, which was repeated two times. Data as shown were analyzed by a computer program for nonlinear least-squares regression analysis.

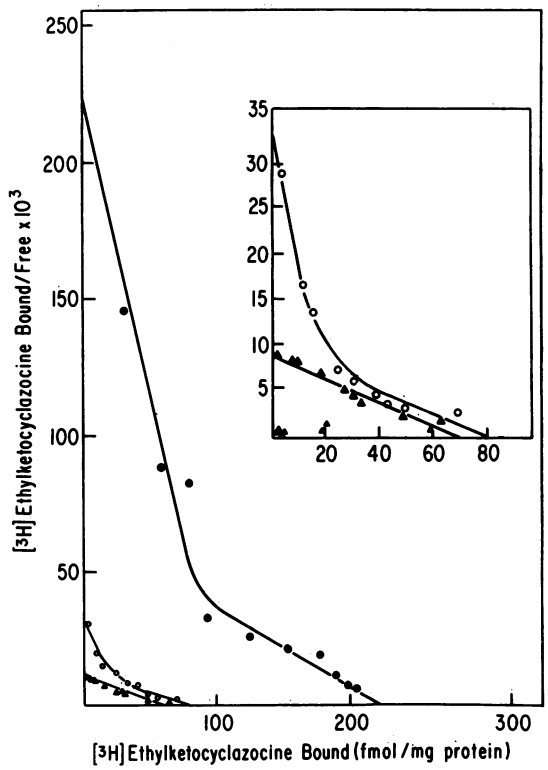

FIG. 2. Scatchard analyses of [^{3}H]EKC binding to rat brain membranes. ●, Total specific binding; ○, specific binding to κ receptors; ▲, specific binding to κ_2 receptors; and △, binding of [^{3}H]EKC in the presence of 100 nM [D-Ala^2,MePhe^4,Glyol^5]Enk, 100 nM [D-Ala^2,D-Leu^5]Enk, and 5 μM U-50,488h. Receptor definitions and data analysis were as described in the legend to Fig. 1. Data are the means from a representative experiment, which was repeated two times.

Table 1. Binding parameters for [^{3}H]EKC binding to rat and guinea pig brain κ receptors

	Parameters for κ receptors			
	κ_1		κ_2	
Brain	K_d, nM	B_{max}, fmol/mg of protein	K_d, nM	B_{max}, fmol/mg of protein
Rat	1.0 ± 0.5	16 ± 8	13 ± 4	111 ± 16
Guinea pig	0.66 ± 0.1	80 ± 7	ND	ND

Whole rat or guinea pig brain minus cerebellum was prepared and binding was carried out as described. Values reported are the means ± SEM of three independent experiments. ND, not detected.

these results document the presence of high-affinity κ_1 receptors in guinea pig brain and both high- and low-affinity κ_2 sites, the latter predominating, in rat brain.

To further characterize the different κ receptor subtypes in rat and guinea pig brain membranes, a series of drugs were tested for their abilities to compete for κ receptor binding in the two tissues (Table 2). The rank order of potency observed for κ_2 receptors in rat brain [dynorphin > bremazocine >> (−)EKC > cyclazocine >> U-50,488h; U-69,593 was inactive] is clearly different from that observed for κ_1 receptors in guinea pig brain [(−)EKC > bremazocine > U-50,488h > cyclazocine > U-69,593]. In particular, U-50,488h was much more potent at the guinea pig κ_1 site than at the rat brain κ_2 site, and U-69,593 was specific for the κ_1 receptor subtype that predominates in guinea pig brain. That both sites are likely to be relevant κ receptors and not nonspecific sites is suggested by their selectivity for κ opioids (Table 2) and their sensitivity to heat and protease treatment (data not shown). Competition analyses showed that U-69,593 displaced only about 85% of κ receptor binding in guinea pig membranes (data not shown). The residual binding could result from a small population of κ_2 receptors. In contrast, U69,593 displaced only 15% of κ receptor binding in rat brain tissue (data not shown). Moreover, nonlabeled EKC competed for the binding of [^{3}H]EKC to rat brain membranes with an K_i of 44 nM when the radioligand concentration was 10 nM (favoring binding to the low-affinity site) and with an K_i of 0.5 nM when the radioligand was 1 nM (favoring binding to the high-affinity site) (data not shown). A comparison of the drug potency

Table 2. Relative potencies of opioids in inhibiting [^{3}H]EKC binding to rat and guinea pig brain κ receptors

	Parameters for κ receptors			
	Guinea pig brain κ_1		Rat brain κ_2	
Drug	K_i, nM	Relative potency*	K_i, nM	Relative potency*
(−)-EKC	0.7 ± 0.15	1	44 ± 5†	1
Dynorphin	6.7 ± 8.0‡	0.1	1.7 ± 1.2	26
Bremazocine	0.9	0.8	3.9 ± 0.4	11
Tifluadom	2.7 ± 0.4‡	0.26	39 ± 26	1.1
Cyclazocine	4.3	0.16	65 ± 20	0.68
Cambridge 20	5.1 ± 3.4‡	0.14	151 ± 27	0.29
U-50,488h	2.4 ± 2.0‡	0.29	484 ± 110	0.09
U-69,593	20.8 ± 9.6	0.03	>10,000	<0.01
Normorphine	>10,000	<0.0001	>10,000	<0.01
[D-Pen^{2,5}]Enk	>10,000	<0.0001	>10,000	<0.01
Haloperidol	>10,000	<0.0001	>10,000	<0.01
TCP	>10,000	<0.0001	>10,000	<0.01

Membranes of whole rat or guinea pig brain minus cerebellum were prepared and binding was carried out as described. Each determination represents the mean ± SEM of three independent experiments. TCP, *N*-[1-(2-thienyl)cyclohexyl] piperidine; Pen, penicillamine.

*Potencies relative to (−)-EKC in the same tissue.

†Calculated from the K_i determined for (±)-EKC, assuming that (+)-EKC is inactive.

‡Determinations represent the means ± SD of two independent experiments.

profiles for the predominant receptor in the two species revealed a good fit to a two-site model (correlations $r = 0.88$ and $r = 0.94$ for the two sites; data not shown). Collectively, these data suggest that there are two distinct κ binding sites, a U-69,593-sensitive high-affinity κ_1 receptor that predominates over a small fraction of low-affinity sites in guinea pig brain and a U-69,593-insensitive low-affinity κ_2 receptor that predominates over a small fraction of high-affinity sites in rat brain.

Neuroanatomical Distribution of Rat and Guinea Pig κ Receptors. To visualize neuroanatomical patterns of κ receptors, quantitative light-microscopy autoradiography was carried out on thaw-mounted sections of frozen rat and guinea pig brain (Fig. 3–5 and Table 3). At the level of the anterior

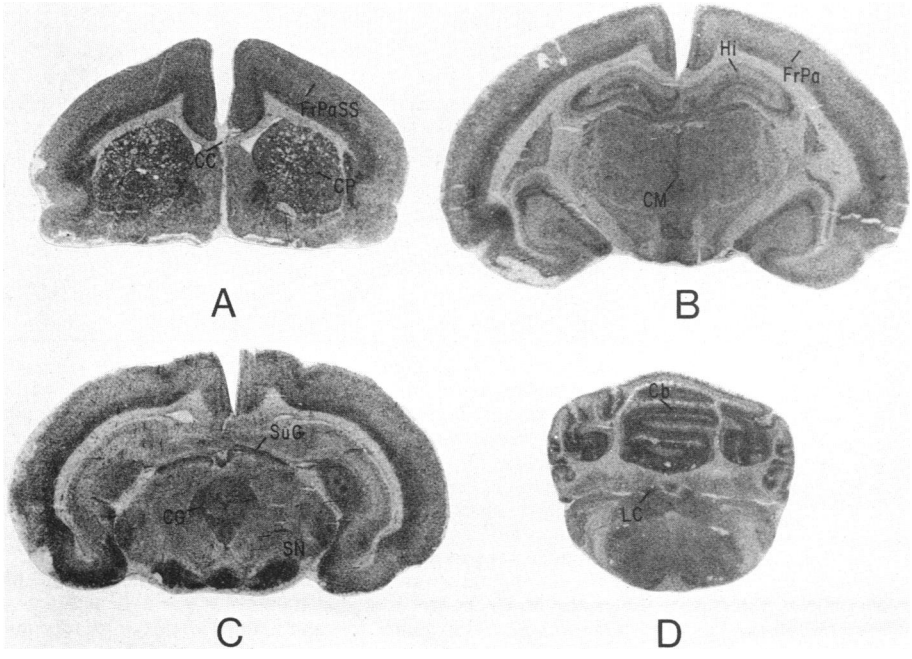

FIG. 3. Autoradiograms of selected coronal guinea pig brain sections labeled with [^{3}H]EKC in the presence of 100 nM [D-Ala^2,MePhe^4,Glyol^5]Enk and 100 nM [D-Ala^2,D-Leu^5]Enk to visualize κ_1 receptors. FrPaSS, frontoparietal cortex, somatosensory area; CP, caudate-putamen; cc, corpus callosum; FrPa, frontoparietal cortex; Hi, hippocampus; CM, central medial nucleus of the thalamus; VP, ventroposterior nucleus of the thalamus; BL, basolateral nucleus of the amygdala; SuG, superficial gray layer of the superior colliculus; CG, central gray; SN, substantia nigra reticulata; Cb, cerebellum; LC, locus coeruleus.

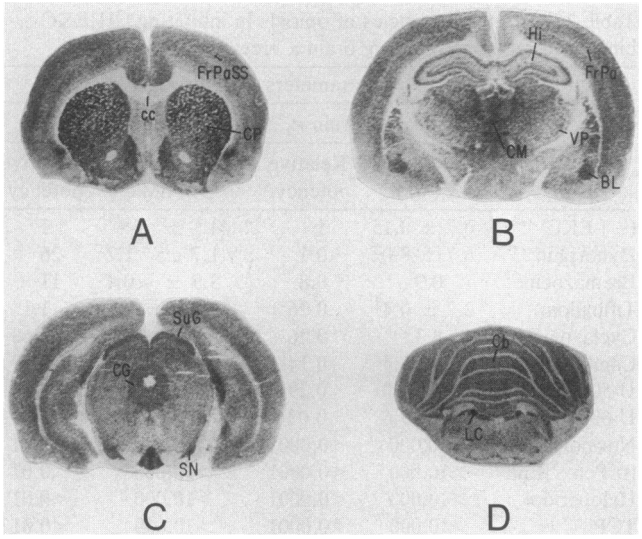

FIG. 4. Autoradiograms of selected coronal rat brain sections labeled with [^{3}H]EKC in the presence of 100 nM [D-Ala^2,MePhe^4, Glyol^5]Enk and 100 nM [D-Ala^2,D-Leu^5]Enk to label κ_1 and κ_2 receptors. For abbreviations, see Fig. 3.

commissure (Fig. 3*A*), κ_1 receptors of guinea pig were selectively localized at a moderate density in the surrounds of the striatum and were absent in the striasomes. Guinea pig κ_1 receptors were also of moderate density in the bed nucleus of the stria terminalis. Rat brain κ_1 sites (Fig. 5*A*) had patterns similar to that of guinea pig κ_1 sites in the caudate-putamen; rat κ_2 sites (total minus κ_1) occurred at a particularly high density in the bed nucleus of the stria terminalis and at moderate density in the striasomes.

κ_1 receptors of guinea pig were selectively localized in the deep layers V and VI of the neocortex (Fig. 3 *A* and *B*). Total κ receptors of rat were nearly uniform throughout the layers of the neocortex with the exception of layer III, where they were not detected (Fig. 4 *A* and *B*); boundaries corresponding to the known cytoarchitectural layers were not visible. In contrast, rat κ_1 receptors (Fig. 5 *A* and *B*) were of higher density in layers IV–VI.

At the level of the guinea pig diencephalon (Fig. 3*B*), κ_1 sites occurred at high density in the molecular layer of the

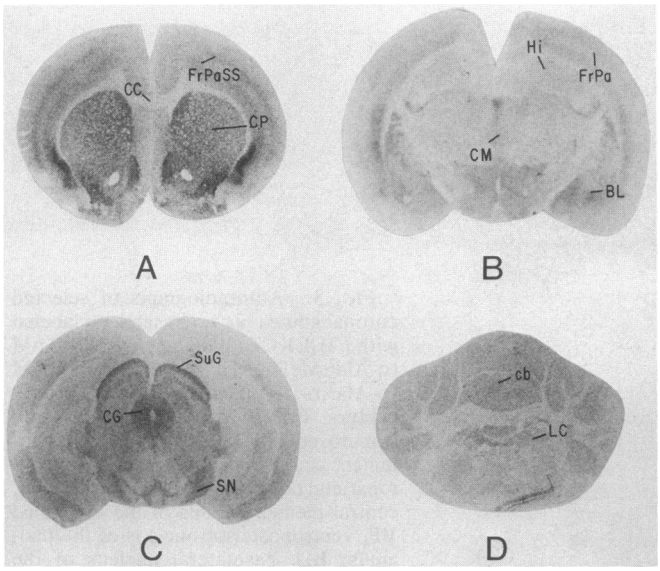

FIG. 5. Autoradiograms of selected coronal sections from rat brain labeled with [^{3}H]U-69,593 to visualize κ_1 receptors. For abbreviations, see Fig. 3.

Table 3. Regional distribution of κ opioid receptor densities in sections of rat and guinea pig brain as determined by quantitative autoradiography

Region	κ opioid receptor densities		
	Guinea pig brain	Rat brain	
	κ_1, fmol of [^{3}H]EKC per mg of tissue*	$\kappa_1 + \kappa_2$, fmol of [^{3}H]EKC per mg of tissue*	κ_1, fmol of [^{3}H]U-69,593 per mg of tissue
Neocortex			
Layers I and II	25.5 ± 0.5	41.9 ± 2.1	10.3 ± 2.1
Layer III	25.5 ± 0.5	ND	10.3 ± 2.1
Layer IV	25.5 ± 0.5	41.9 ± 2.1	14.3 ± 2.4
Layers V and VI	54.6 ± 2.7	41.9 ± 2.1	9.2 ± 1.8
Striatum			
Patches	30.9 ± 0.9	71.0 ± 2.3	ND
Surrounds	30.9 ± 0.9	45.5 ± 2.3	17.5 ± 2.0
Nucleus accumbens	49.1 ± 1.9	58.2 ± 3.5	30.9 ± 1.8
Bed nucleus of the stria terminalis	61.9 ± 4.3	81.9 ± 7.4	18.8 ± 1.6
Claustrum	61.3 ± 1.9	87.8 ± 8.2	25.5 ± 2.4
Endopiriform nucleus	ND	ND	24.7 ± 2.1
Fundus striati	ND	ND	42.0 ± 1.6
Hippocampus			ND
Molecular layer	56.4 ± 3.4	28.6 ± 4.3	
Pyramidal cell layer	52.8 ± 3.2	41.9 ± 1.7	
Granular cell layer	ND	51.0 ± 2.6	
Thalamic nuclei (in total)	7.3 ± 0.1†		
Paraventricular nucleus	ND	ND	21.2 ± 1.6
Laterodorsal nucleus	ND	61.3 ± 1.4	ND
Posterior nuclear group	ND	72.8 ± 2.9	ND
Centrolateral nucleus	ND	83.7 ± 3.4	10.6 ± 0.8
Intermediodorsal nucleus	ND	98.3 ± 4.9	12.5 ± 0.1
Rhomboid nucleus	ND	92.8 ± 9.3	ND
Gelatinosus	ND	56.4 ± 0.2	ND
Medial geniculate nucleus	34.6 ± 1.0	ND	9.7 ± 1.8
Hypothalamic nuclei			
Dorsal hypothalamic area	9.1 ± 0.1	51.0 ± 1.0	24.1 ± 2.2
Ventromedial nucleus	9.1 ± 0.1	56.4 ± 3.4	19.3 ± 2.0
Zona incerta	ND	53.3 ± 2.3	22.6 ± 1.9
Amygdaloid nuclei	ND	92.3 ± 8.8	18.5 ± 1.5
Substantia nigra reticulata	58.2 ± 1.2	51.0 ± 1.3	14.3 ± 2.2
Interpeduncular nucleus	63.7 ± 1.9	72.8 ± 2.2	14.8 ± 2.8
Superficial gray layer of superior colliculus	76.4 ± 3.1	78.3 ± 3.1	20.7 ± 3.0
Cerebellum	56.4 ± 3.4	32.8 ± 1.0	ND
Central gray substance	45.5 ± 0.9	56.4 ± 1.1	19.8 ± 2.0
Locus coeruleus	47.3 ± 5.2	78.3 ± 8.6	ND
Spinal tract of trigeminal nerve	29.1 ± 0.9	43.7 ± 1.8	ND
Corpus callosum	3.6 ± 0.0	7.3 ± 0.1	ND

Sections of rat or guinea pig brain were labeled *in vitro* with radioligands specific for κ_1 or total κ receptors. Corrected densitometric readings (in optical density units) were averaged and converted to receptor density values (fmol/mg) by reference to a standard curve for brain tissue, computed by using tritium standards (Amersham). Receptor density values are reported as means ± SEM of averaged values from the corresponding sections of three rats or guinea pigs after correction for the contributions caused by nonspecific binding and to background film density. ND, not detected.

*[^{3}H]EKC binding was carried out in the presence of 100 nM [D-Ala^2,MePhe^4,Glyol^5]Enk and 100 nM [D-Ala^2,D-Leu^5]Enk.

†Guinea pig brain exhibited low and uniform density of κ receptors in the thalamic nuclei.

dentate gyrus and in the pyramidal cell layer of the hippocampal formation. In contrast, rat κ_1 receptors were absent from the hippocampus, while κ_2 receptors were of high density in the pyramidal cell layer as well as the CA_1, CA_2, and CA_3 areas (of the hippocampus); and the granular layer of the dentate gyrus (Fig. 4*B*).

In the thalamic and hypothalamic areas, striking differences were seen between the two species and between the two receptor subtypes. In the case of guinea pig brain (Fig. 3*B*), κ_1 receptors were selectively localized in the medial geniculate nucleus of the thalamus. κ_1 receptors of rat were undetectable in the thalamus except in the paraventricular nucleus and the medial geniculate and (at low levels) in the centrolateral and intermediodorsal nuclei (Fig. 5*B*). κ_2 receptors occurred in the posterior nuclear group and the medial and midline nuclear groups, including the centrolateral nucleus, the intermediodorsal nucleus, and the rhomboid and gelatinosus nuclei (Fig. 4*B*). Both κ_1 and κ_2 receptors of rat were of moderate density in the dorsal hypothalamus and ventromedial nucleus of the hypothalamus.

At the level of the midbrain, guinea pig κ_1 receptors were at high density in the central gray area, the substantia nigra, the interpeduncular nucleus, and the superior colliculus (Fig. 3*C*). Rat κ_2 receptors (Fig. 4*C*) paralleled guinea pig κ_1 receptors at this level; high levels occurred in the periaqueductual gray area (primarily the dorsal area), the substantia nigra reticulata, and the interpeduncular nucleus. Particularly striking was the high density of κ receptors in the superficial gray layer of the superior colliculus. Rat κ_1 receptors exhibited a distribution similar to that of total κ sites but were lower in density by a factor of $\approx$5 (Fig. 5*C*). At the level of the brainstem (Fig. 3*D*), guinea pig κ_1 receptors were at high density in the cerebellum and central gray of the pons and at moderate density in the locus coeruleus, and the spinal tract of the trigeminal nerve. Rat κ_2 receptors were of high density in the cerebellum, the locus coeruleus, and the central gray area of the pons (Fig. 4*D*) and of moderate density in the spinal tract of the trigeminal nerve (fifth nerve). Rat κ_1 receptors were not detectable in the locus coeruleus, cerebellum, or spinal tract of the trigeminal nerve (Fig. 5*D*).

DISCUSSION

The present study shows, on the basis of both *in vitro* binding assays and quantitative receptor autoradiography at the level of the light microscope, the presence of κ receptor sites in both guinea pig and rat brain. An important difference between the two species is the presence of a population of high-affinity κ sites in guinea pig brain that is found at low density in rat brain and the presence at a high density of a lower affinity site in rat brain that is at low density (or absent) in guinea pig brain. Several findings indicate that the κ sites that predominate in the two species represent different κ receptor subtypes. First, the two sites have different affinities (for the κ_1 subtype, K_d = 1 nM; for the κ_2 subtype, K_d = 13 nM). Second, the two sites exhibit different ligand selectivity profiles. In particular, U-50,488h and Cambridge 20 are much more potent at the guinea pig (κ_1) site than at the predominant rat brain (κ_2) site, and U-69,593 is specific for κ_1 (inactive at the κ_2 site). Third, the two receptor sites exhibit different neuroanatomical distribution patterns, as evidenced by autoradiography on sections of rat brain.

What might be the functional significance of two κ receptor subtypes in rat brain? In analogy to M_1 and M_2 muscarinic receptors, it is possible that κ_1 and κ_2 opioid receptors couple directly to different channel types or couple to the same or different channel types through different second-messenger systems. A recent electrophysiological study by Shen and Crain showed that at nanomolar concentrations, dynorphin and U-50,488h produced prolongation of the action potential duration in some mouse dorsal root ganglion neurons and shortening in others (18). Our finding of κ receptor subtypes is consistent with this observation.

Our finding of two κ receptor subtypes in rat brain is not surprising in view of the recent finding of at least four functional muscarinic receptor genes in this tissue (19). Moreover, several other laboratories have reported evidence for κ receptor subtypes. For example, Castanas *et al.* (20) reported the presence of κ_1, κ_2, and κ_3 binding sites in adrenal medulla on the basis of multiphasic binding isotherms. Iyengar *et al.* (21) provided physiological evidence for κ receptor heterogeneity by determining differential sensitivity of κ opioids to WIN 44,441-3 antagonism in two neuroendocrine systems—(*i*) the hypothalamic–pituitary–adrenal axis and (*ii*) release of plasma thyroid-stimulating hormone. Our study extends these previous studies by identifying ligands specific or highly selective for each of the two κ receptor subtypes and by identifying tissues highly enriched in each site.

Our autoradiographic studies clearly indicate that κ_1 and κ_2 receptors have different distributions in rat brain. These studies also show that, although the κ sites of rat and guinea pig brain exhibit similar distributions in the midbrain and hindbrain, they exhibit strikingly different patterns in several forebrain structures such as the striatum, nucleus accumbens, neocortex, and hippocampal formation. The most striking differences between the species and subtypes are seen in the thalamic and hypothalamic areas. The differences in neuroanatomical distribution support the finding from our receptor binding studies of heterogeneity in the κ receptor system.

This work was supported by National Institutes of Health Grants DA 04439 (to R.S.Z.) and NS 21973 (to A.T.). R.S.Z. is the recipient of a Research Career Development Award DA 00069 from the National Institute on Drug Abuse. E.M.U. is a postdoctoral trainee funded by National Institutes of Health Training Grant MH 1578.

1. Zukin, R. S. & Maneckjee, R. M. (1986) *Methods Enzymol.* **124,** 172–190.
2. Martin, W. R., Eades, C. G., Thompson, J. A., Huppler, R. E. & Gilbert, P. E. (1976) *J. Pharmacol. Exp. Ther.* **197,** 517–532.
3. Villareal, J. E. & Seevers, M. H. (1972) *Bull. Probl. Drug Depend.* **34** (Addendum 7), 1040–1053.
4. James, I. F., Chavkin, C. & Goldstein, A. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7570–7574.
5. Cherubini, E. & North, R. A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1860–1863.
6. Werz, M. A. & Macdonald, R. L. (1985) *J. Pharmacol. Exp. Ther.* **234,** 49–54.
7. Kosterlitz, H. W., Paterson, S. J. & Robson, L. E. (1981) *Br. J. Pharmacol.* **73,** 939–949.
8. James, I. F. & Goldstein, A. (1984) *Mol. Pharmacol.* **25,** 337–342.
9. Chow, T. & Zukin, R. S. (1983) *Mol. Pharmacol.* **24,** 203–212.
10. Demoliou-Mason, C. D. & Barnard, E. A. (1984) *FEBS Lett.* **170,** 378–382.
11. Itzhak, Y., Hiller, J. M. & Simon, E. J. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4217–4221.
12. Morris, B. J. & Herz, A. (1986) *Neuroscience* **19,** 839–846.
13. Goodman, R. R. & Snyder, S. H. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 5703–5707.
14. Vaysse, P., Gardner, E. L. & Zukin, R. S. (1987) *J. Pharmacol. Exp. Ther.* **241,** 534–539.
15. Munson, P. J. & Rodbard, D. (1980) *Anal. Biochem.* **107,** 220–239.
16. Tempel, A. & Zukin, R. S. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 4308–4312.
17. Paxinos, G. & Watson, C. (1982) *The Rat Brain in Stereotaxic Coordinates* (Academic, New York).
18. Shen, S.-F. & Crain, S. M. (1987) *Soc. Neurosci. Abstr.* **13,** 768.
19. Bonner, T. I., Buckley, N. J., Young, A. C. & Brann, M. R. (1987) *Science* **237,** 527–532.
20. Castanas, E., Bourhim, N., Giraud, P., Boudouresque, F., Cantau, P. & Oliver, C. (1985) *J. Neurochem.* **45,** 688–699.
21. Iyengar, S., Kim, H. S. & Woods, P. L. (1986) *Life Sci.* **39,** 637–644.