Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 5703–5707, September 1982
Neurobiology

κ opiate receptors localized by autoradiography to deep layers of cerebral cortex: Relation to sedative effects

(enkephalin/endorphins/ethylketocyclazocine/morphine/bremazocine)

ROBERT R. GOODMAN AND SOLOMON H. SNYDER*

Departments of Neuroscience, Pharmacology and Experimental Therapeutics, Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, Maryland 21205

Contributed by Solomon H. Snyder, June 4, 1982

ABSTRACT **κ opiate drugs differ from other opiates in their unique sedative actions and lack of cross-tolerance. We have visualized κ opiate receptors by *in vitro* autoradiography using the κ drugs [^{3}H]ethylketazocine ([^{3}H]EKC) and [^{3}H]bremazocine. Though these ligands also label μ and δ opiate receptors, their binding is rendered κ specific by coincubation with morphine and [D-Ala^2, D-Leu^5]enkephalin (DADL-Enk) to displace μ and δ interactions, respectively. Labeling patterns with [^{3}H]EKC and [^{3}H]bremazocine are the same and differ markedly from localizations of μ and δ opiate receptors visualized with [^{3}H]dihydromorphine and [^{3}H]DADL-Enk, respectively. The highest density and most selective localization of putative κ receptors occurs in layers V and VI of the cerebral cortex. In these layers cells are localized which project to the thalamus regulating sensory input to the cortex. Receptors in these layers could account for the unique sedative and possibly analgesic effects of κ opiates.**

The diverse actions of opiates imply the existence of multiple receptors. Martin *et al.* (1) suggested that the unique sedative actions of drugs such as ethylketazocine (EKC) and bremazocine are mediated by a unique class of opiate receptors designated κ. Though κ-specific drugs are analgesic, the opiate antagonist naloxone is less effective in blocking their effects than those of morphine-like or μ opiates. Although morphine can suppress the symptoms of κ drug abstinence, κ drugs fail to suppress morphine abstinence and do not precipitate abstinence symptoms in morphine-dependent animals (1). κ drugs produced substantially greater sedation than other opiates and have been evaluated as anesthetic agents.

κ opiates can be distinguished from other opiates in pharmacological studies in animals. EKC-induced analgesia occurs more at the spinal cord level, whereas morphine analgesia appears to be predominantly supraspinal (2). κ opiate drugs can be distinguished from others by selective development of tolerance in the guinea pig ileum (3) and selective inhibition of electrically induced contractions of the rabbit vas deferens (4).

Opiate receptor-binding studies clearly differentiate two receptors—μ sites for which morphine has selective high affinity and δ sites where certain enkephalin analogues are selectively potent (5). The differential localization of μ and δ opiate receptors in autoradiographic studies explains differential pharmacologic actions of μ- and δ-specific drugs (6, 7). For instance, the selective localization of μ receptors to areas involved in pain perception (6) and the relatively greater analgesic potencies of μ-selective enkephalin analogues suggest that μ receptors are specifically involved in mediating analgesic effects of opiates.

Though the pharmacologic actions of κ drugs in dogs suggest that they should not interact with the same sites as morphine, in opiate receptor-binding studies EKC and other κ drugs are quite potent inhibitors of [^{3}H]naloxone binding associated largely with μ opiate receptors (8). Direct binding studies with [^{3}H]EKC indicate extensive labeling of μ opiate receptors (9, 10, 11). Kosterlitz *et al.* (12) found evidence for labeling by [^{3}H]EKC of receptors in guinea pig brain which could be distinguished from μ and δ opiate receptors by drug specificity and in selective protection experiments. Subsequently, other investigators identified selective [^{3}H]EKC binding in rat brain membranes (13, 14, 15) and neuroblastoma hybrid cells (16).

In the present study we have visualized κ opiate receptors by autoradiographic analysis of [^{3}H]EKC and [^{3}H]bremazocine disposition alone or in the presence of substances that displace binding of these agents to μ and δ opiate receptors. We find κ receptors highly localized to the deep layers (V and VI) of the guinea pig cerebral cortex.

MATERIALS AND METHODS

Male Hartley guinea pigs (200–400 g) were used for all experiments. Preliminary homogenate-binding studies of brain membrane were conducted as reported (17). Tritium-labeled compounds used (New England Nuclear) included [^{3}H]EKC (15 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels), [^{3}H]dihydromorphine ([^{3}H]H_2-morphine; 72 and 86 Ci/mmol), and [^{3}H][D-Ala^2, D-Leu^5]enkephalin ([^{3}H]DADL-Enk; 32 Ci/mmol). [^{3}H]Bremazocine (27 Ci/mmol) was obtained from Sandoz Pharmaceutical (Basel, Switzerland). Reaction mixtures were incubated at 25°C for 40 min (final volume, 2.0 ml) and contained tissue suspension (final concentration, 10 mg/ml), ^{3}H-labeled ligand (at concentrations indicated by *Results*), and either an unlabeled ligand (at varying concentrations) or buffer. Nonspecific binding was defined as cpm remaining in the presence of 1 μM levallorphan.

The autoradiographic procedure involves the labeling of opiate receptors in intact, slide-mounted tissue sections (18). Guinea pigs (anesthetized with pentobarbital) were perfused with 100–200 ml of 0.1% formaldehyde in phosphate-buffered saline (pH 7.4, isotonic), the brains were rapidly removed and frozen on microtome chucks, and thin sections (8 or 10 μm) were cut and thaw-mounted on gelatin-coated microscope slides. After a 15-min preincubation with 50 μM GTP and 100 mM NaCl (to remove endogenous opioids) and a 5-min wash, both at room temperature in 0.17 M Tris·HCl at pH 7.6, the slide-mounted tissue sections were transferred to the same buffer containing the ^{3}H-labeled ligand and any unlabeled compound

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: EKC, ethylketazocine; H_2-morphine, dihydromorphine; DADL-Enk, [D-^{2}Ala, D-Leu^5]enkephalin.

* To whom reprint requests should be addressed.

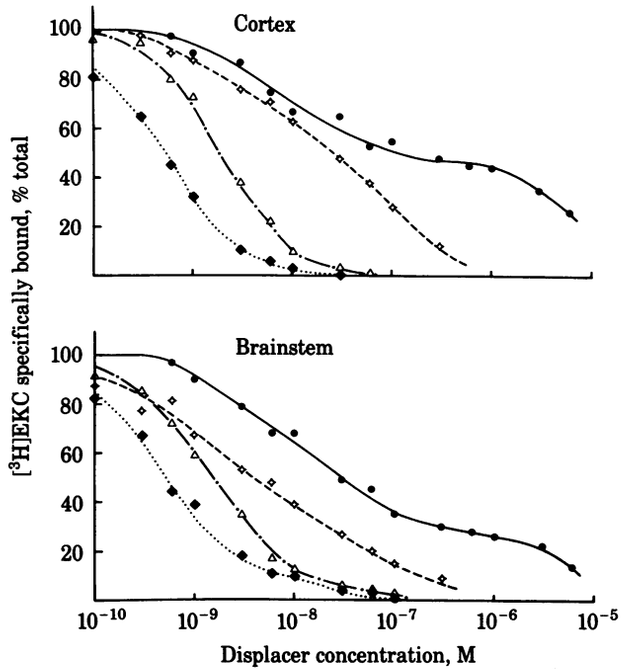

FIG. 1. Displacement by opiates of [^{3}H]EKC binding to guinea pig brain homogenates. Each point represents the mean of triplicates. The cerebral cortex (cortex) was removed and homogenized, separately from the remaining brain minus the cerebellum (brainstem), and the two tissues were used in parallel assays. Binding is reported as the percentage of specific cpm (total binding minus cpm in the presence of 1 μM levallorphan). ●, DADL-Enk; ◇, morphine; △, etorphine; ◆, bremazocine. The experiment was replicated twice with results varying <10%.

desired, at varying concentrations. After two consecutive 5-min washes in buffer at ice-water temperatures, the sections were either wiped off the slides (two 10-μm forebrain sections per slide) and their radioactivity assayed by liquid scintillation (in biochemical studies) or slides were placed on an iced metal plate and dried under a stream of cold dry air (for autoradiographic studies). Adjacent sections were incubated in different conditions to allow comparison of different receptor-subtype distributions and nonspecific binding was determined by coincubation with 1 μM levallorphan. Autoradiograms were generated by apposing a tritium-sensitive film (^{3}H-Ultrofilm, LKB) to the dry tissue sections (for 15–25 wk) and were analyzed by microdensitometry (Gamma Scientific, New York, NY) (19, 20).

RESULTS

Biochemical Conditions for Selective Labeling of κ Receptors. μ and δ opiate receptor binding-sites can be distinguished by using [^{3}H]H_2-morphine and [^{3}H]DADL-Enk (21). [^{3}H]H_2-Morphine labels μ sites selectively with less than a 1% labeling of δ sites. [^{3}H]DADL-Enk labels both μ and δ receptors, and its interactions with μ receptors can be eliminated by small concentrations of morphine, which is μ selective (21). [^{3}H]EKC labels μ sites well, δ sites to a very limited extent, and a third site which is defined as the κ receptor-binding site. The κ-selective drug [^{3}H]bremazocine has binding characteristics similar to [^{3}H]EKC (22).

In guinea-pig cerebral cortical membranes, DADL-Enk displacement of [^{3}H]EKC binding is distinctly biphasic with a high affinity component displaying an IC_{50} of $\approx$10 nM, whereas the low affinity component has an IC_{50} of 10 μM (Fig. 1). The lower affinity component is greater in the cerebral cortex than in the brainstem (which includes all noncortical brain areas). Taken together with other findings (12), these data indicate that about half of the [^{3}H]EKC binding in the cerebral cortex involves putative κ binding sites for which DADL-Enk has extremely low affinity, whereas the other half predominantly involves μ sites with some δ contribution.

Morphine also has a shallow displacement curve for [^{3}H]EKC, suggesting that morphine displaces [^{3}H]EKC with higher affin-

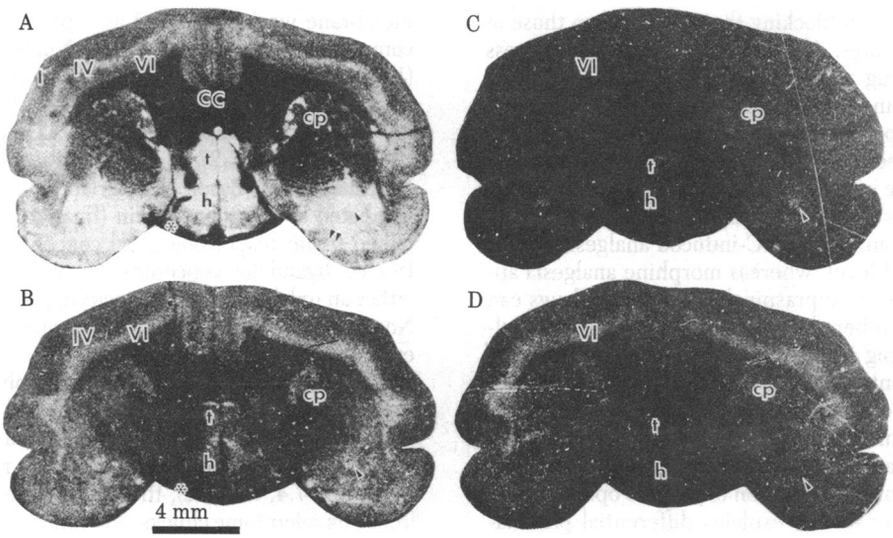

FIG. 2. Darkfield micrographs of [^{3}H]H_2-morphine and [^{3}H]EKC binding in coronal sections of guinea pig brain. Sections were labeled *in vitro* and tritium-sensitive film was apposed for 15–25 wk before development. Tissue was stained with cresyl violet to verify localization of grain densities. (*A*) Section (8 μm) labeled by [^{3}H]H_2-morphine (1.8 nM). Note high grain densities (white areas) in layer IV of the cerebral cortex (IV), some clusters in the caudate-putamen (cp), amygdala (arrowhead indicates central nucleus; double arrowhead indicates cortical nucleus), thalamus, and hypothalamus, low densities in layers V and VI of the cerebral cortex (VI), and virtual absence of grains in white matter areas such as the corpus callosum (CC) and optic tract (*). (*B*) Adjacent thin section labeled by [^{3}H]EKC (1.6 nM). Note highest grain densities in layers V and VI of the cerebral cortex, with low densities (relative to [^{3}H]H_2-morphine in layer IV, the caudate-putamen, amygdala, thalamus, and hypothalamus. The addition of 30 nM morphine to [^{3}H]H_2-morphine in an adjacent section (*C*) eliminates virtually all specific labeling, whereas its addition to [^{3}H]EKC (*D*) eliminates binding in the amygdala, thalamus, and hypothalamus without significantly altering labeling in layers V and VI of the cortex or in the caudate-putamen. These results were replicated in duplicate sections from three different animals.

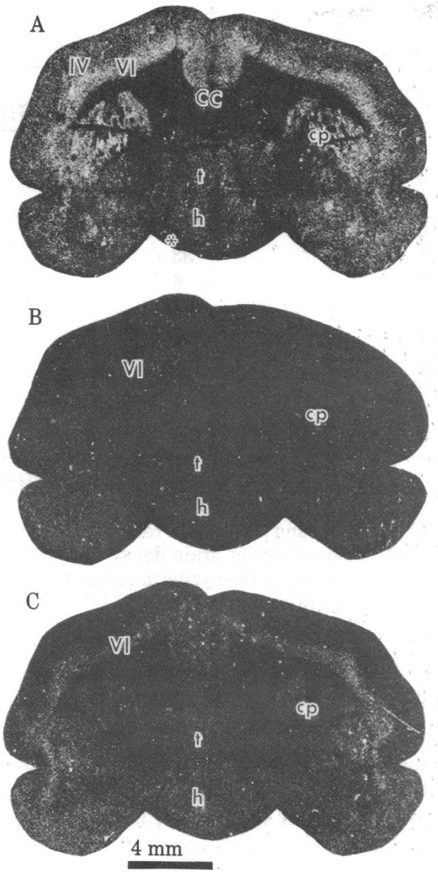

FIG. 3. Darkfield micrographs of [^{3}H]DADL-Enk and [^{3}H]EKC in adjacent thin sections (8 μm) of guinea pig brain. These sections are adjacent to those shown in Fig. 2 and labeled as described. (*A*) Section labeled by [^{3}H]DADL-Enk (3.8 nM). Note highest grain densities in layers V and VI of the cerebral cortex (VI) and diffuse labeling in the caudate-putamen, with moderate densities more superficially in the cortex, amygdala, thalamus, and hypothalamus. The addition of 30 nM morphine and 100 nM DADL-Enk (*B*) eliminates essentially all specific labeling. In contrast, addition of morphine (30 nM) and DADL-Enk (100 nM) to [^{3}H]EKC (1.6 nM) (*C*) leaves significant labeling in the deep layers of cortex, as seen in Fig. 2 *B* and *D*. These results were replicated in duplicate with sections from two different animals. Addition of 1 μM levallorphan to [^{3}H]EKC in adjacent sections yields low densities similar to those seen in white matter areas here.

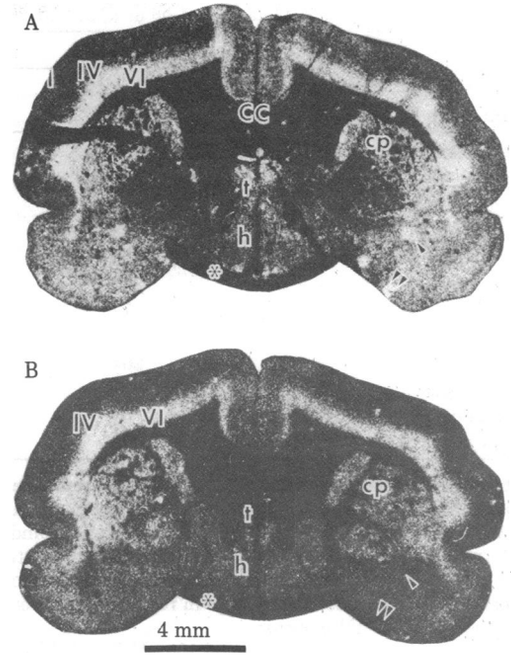

FIG. 4. Darkfield micrographs of [^{3}H]bremazocine (0.5 nM) in adjacent thin sections (8 μm) of guinea pig brain. These sections are adjacent to those shown in Fig. 2 and labeled as described. (*A*) Note similarity to [^{3}H]EKC (Fig. 2*B*) with highest densities in layers V and VI of the cerebral cortex and low densities in layer IV. Moderate densities are seen in the caudate-putamen (cp), thalamus (t), hypothalamus (h), and central (arrowhead) and cortical (double arrowhead) nuclei of the amygdala. (*B*) Adjacent section labeled by [^{3}H]bremazocine in the presence of 30 nM morphine. Note the elimination of labeling in the μ receptor-rich areas (thalamus, hypothalamus, and amygdala nuclei) with minimal reduction of labeling in layers V and VI of the cerebral cortex and in the caudate-putamen. These results were replicated in duplicate sections from two different animals. CC, corpus callosum; *, optic tract.

ity from μ sites and with lower affinity from apparent κ sites. Both morphine and DADL-Enk are less potent in displacing [^{3}H]EKC binding from cerebral cortex than from brainstem, suggesting a higher density of putative κ receptors in the cortex than in the brainstem. Etorphine, which is thought to have similar affinities for μ, δ, and κ receptors, has a much less shallow displacement curve in both cortex and brainstem, as is the case with the κ agonist bremazocine. In parallel binding experiments with [^{3}H]bremazocine (data not shown), displacement by DADL-Enk, morphine, etorphine, bremazocine, and EKC is essentially the same as observed with [^{3}H]EKC.

Based on these displacement data, we conclude that [^{3}H]EKC and [^{3}H]bremazocine may label κ and μ receptors to a similar extent with a small degree of labeling of δ receptors. For autoradiographic experiments, 30 nM morphine should inhibit the labeling of μ receptors by [^{3}H]EKC or [^{3}H]bremazocine, whereas 100 nM DADL-Enk should inhibit labeling of δ receptors by these ^{3}H-labeled ligands.

Autoradiographic Studies. Prior to autoradiographic studies, the characteristics of [^{3}H]EKC and [^{3}H]bremazocine binding to guinea-pig brain thin sections was examined by using previously described procedures (18) (see *Materials and Methods*). The K_d values for [^{3}H]EKC and [^{3}H]bremazocine are essentially the same in slices as in homogenates, about 1–2 nM. With both [^{3}H]EKC and [^{3}H]bremazocine about 85% of total binding is specific, being displaced by 1 μM levallorphan. Displacement curves for morphine and DADL-Enk are similar in slices as in homogenates. [^{3}H]EKC localization is clearly different from that of [^{3}H]H_2-morphine (Fig. 2). In the absence of displacers, both ^{3}H-labeled ligands label deep zones in the cerebral cortex, areas of the caudate-putamen, thalamus, hypothalamus, and amygdala. However, whereas [^{3}H]H_2-morphine labels layers IV and VI of the cerebral cortex with negligible labeling of layer V, [^{3}H]EKC labels primarily layers V and VI with very little labeling in layer IV. Moreover, whereas caudate-putamen, amygdala, thalamus, and hypothalamic labeling by [^{3}H]H_2-morphine is much more intense than labeling in the cerebral cortex, the reverse is true for [^{3}H]EKC and [^{3}H]bremazocine.

To eliminate labeling of μ receptors, slices were incubated with 30 nM morphine (Fig. 2). This treatment abolishes all receptor labeling with [^{3}H]H_2-morphine. It blocks fully the labeling by [^{3}H]EKC of thalamus and hypothalamus and greatly decreases labeling in the amygdala. In the caudate-putamen the morphine treatment seems to abolish labeling of clusters by [^{3}H]EKC, whereas diffuse labeling is retained. Receptor clusters in the caudate reflect μ sites, whereas diffuse labeling is associated with δ receptors (6). Strikingly, labeling of layers V and VI of the cerebral cortex is not affected by morphine treat-

Table 1. Microdensitometric quantification of κ, μ, and δ opiate receptors in different areas of guinea pig brain

Brain areas	Densities					
	[^{3}H]EKC	[^{3}H]EKC*	κ [^{3}H]EKC†	μ [^{3}H]H_2-morphine	δ [^{3}H]DADL-Enk‡	[^{3}H]DADL-Enk
Cerebral cortex						
Layer I	23	5	0	5	15	3
II/III	6	0	0	2	21	5
IV	15	63	0	16	5	3
V and VI	43	254	92	2, 12§	33	9
Pyriform cortex	14	243	100	42	6	5
Hippocampus	15	77	—	10	44	10
Caudate nucleus	16	200	11	21	45	30
Nucleus accumbens	80	264	11	65	—	37
Medial thalamus	52	56	—	76	30	23
Hypothalamus	22	63	—	27	30	44
Mamillary body	100	100	—	100	100	100

Thin brain sections labeled by the *in vitro* autoradiographic technique were used to expose tritium-sensitive film. The densities of [^{3}H]H_2-morphine (μ receptors) binding, [^{3}H]EKC binding in the absence (μ, δ, and κ receptors) and presence (δ and κ receptors) of 30 mM morphine or 30 nM morphine and 100 nM DADL-Enk (κ receptors), and [^{3}H]DADL-Enk binding in the absence (μ and δ receptors) and presence (δ receptors) of 30 nM morphine were determined by microdensitometry by utilizing known standards. Results are reported as percentage of grain density relative to that found in the mamillary body in the same labeling condition, except for the κ receptor labeling condition in which the pyriform cortex is set equal to 100%. Values are means of at least 10 readings in representative sections from two different guinea pig brains and varied by <10%.

* In the presence of 30 nM morphine.

† In the presence of 30 nM morphine and 100 nM DADL-Enk.

‡ In the presence of 30 nM morphine.

§ For μ receptors, layers V and VI were discriminated with respective densities of 2 and 12.

ment, whereas labeling in the superficial layers of the cerebral cortex is eliminated by morphine. These results suggest that [^{3}H]EKC interacts with μ receptors, but that this labeling can be abolished by morphine. In addition, [^{3}H]EKC uniquely labels the deep layers of the cerebral cortex in a fashion that is not affected by morphine.

To compare [^{3}H]EKC localization with δ receptors, we evaluated [^{3}H]DADL-Enk binding, using sections adjacent to those employed in Fig. 2 (Fig. 3). As previously reported (21) [^{3}H]-DADL-Enk labels both μ and δ receptors, with preferential labeling of δ receptors (Fig. 3). Thus, there is diffuse labeling throughout the cerebral cortex but some greater density in the deep layers, both diffuse and patch-like labeling in the caudate-putamen, and limited labeling in the thalamus, hypothalamus, and amygdala. Treatment with 30 nM morphine and 100 nM DADL-Enk, to eliminate labeling of μ and δ receptors, abolishes all [^{3}H]DADL-Enk binding (Fig. 3). By contrast, similar morphine and DADL-Enk treatment does not eliminate [^{3}H]EKC binding. Labeling in the deep cerebral cortex and some in the caudate-putamen is retained, whereas labeling in the thalamus, hypothalamus, and amygdala is largely eliminated. This result suggests that κ sites labeled selectively by [^{3}H]EKC occur predominantly in the deep cerebral cortex, but some also exist in the caudate-putamen.

Do the [^{3}H]EKC binding sites localized by autoradiography represent the sites responsible for the pharmacologic effects of κ opiates? To evaluate this question, we conducted autoradiographic localization of the binding of [^{3}H]bremazocine, a drug which differs chemically from EKC, but which has the same characteristic pharmacology (22). As with [^{3}H]EKC, [^{3}H]bremazocine alone displays a pattern resembling that of [^{3}H]EKC alone, with similarities to μ receptor disposition as well as some differences (Fig. 4). Treatment with 30 nM morphine abolishes the labeling of μ receptors, leaving a dense band of [^{3}H]bremazocine sites in layers V and VI of the cerebral cortex.

In the brainstem and spinal cord we have also analyzed the localization of [^{3}H]EKC and [^{3}H]bremazocine binding sites by autoradiography (data not shown). In these areas specific κ labeling is identified by detecting autoradiographic grains that persist after treatment with 30 nM morphine and 100 nM DADL-Enk. κ receptor localization in brain stem and spinal cord resembles closely that of μ opiate receptors as we have described in rat brain (6).

To verify the localizations obtained by visual inspection of the autoradiographs, we have conducted microdensitometric analysis of the autoradiograms obtained with tritium-sensitive film (Table 1). Grain quantification reveals essentially the same results as obtained by visual inspection. In the absence of drug displacement, [^{3}H]EKC binding shows a disposition somewhat similar to that of μ receptors. Displacement of μ and δ binding with 30 nM morphine and 100 nM DADL-Enk results in a selective concentration of [^{3}H]EKC grains in the deep layers (V and VI) of the cerebral cortex and in the pyriform cortex.

DISCUSSION

A major finding of the present study is the unique localization of κ receptor-binding sites. Interpretation of this localization depends on the selective labeling of these sites by the ligands employed, [^{3}H]EKC and [^{3}H]bremazocine. The biochemical data we have obtained indicate that both in homogenates and on slides the sites labeled are the same as those described by Kosterlitz *et al.* (12) and probably by Chang *et al.* (13). Various data, including drug specificity and selective protection experiments, indicate that these binding sites are distinct from μ and δ opiate receptors and are labeled preferentially by drugs whose pharmacologic properties are characteristic of κ opiate drugs (12). Our observation of selective localizations for [^{3}H]EKC and [^{3}H]bremazocine supports the conclusion that these apparent κ receptor-binding sites are distinct from μ and δ receptors.

Though the κ receptor-binding sites are unique for κ opiates, discrepancies between binding data and *in vivo* pharmacology argue for caution in equating the biochemically defined binding sites and pharmacologic κ receptors. For instance, etorphine has similar affinities for κ, μ, and δ opiate receptors, but its pharmacology is that of a pure μ opiate agonist. Conceivably a modestly greater affinity of etorphine for μ than for κ recep-

tors would cause the μ effects to take precedence pharmacologically. In dogs the prototypic κ agonist EKC does not suppress morphine abstinence and does not precipitate abstinence in morphine-dependent dogs, suggesting that it should not display affinity for morphine-preferring μ receptors. However, in binding studies EKC has almost as great an affinity for μ receptors as for the κ binding sites. Of course, it is conceivable that μ opiate binding-sites cannot be equated directly with pharmacologic μ effects. In dogs, morphine suppresses abstinence symptoms elicited with EKC withdrawal, suggesting that morphine had high affinity for κ receptors. However, morphine is very weak at the κ binding sites.

Despite these reservations, the selective labeling of unique sites by κ-specific opiates suggests that these binding sites are relevant to the unique pharmacologic actions of these drugs. κ agonists cause analgesia and a pronounced "apathetic" sedation. These sedative effects are apparent with all opiates that have κ activity and are readily distinguished from the modestly sedating or euphoric effects of morphine (1). These unique behavioral effects are interesting in light of the highly selective localization of κ receptors to a dense band in layer VI and to a certain extent in layer V of the cerebral cortex. The cerebral cortical projection to the thalamus takes origin exclusively in layer VI. Thus, κ receptors in this region would be uniquely suited to influence this projection, which regulates sensory input from thalamus to cortex. Influences here might well account for κ-mediated analgesia and for the uniquely sedative effects of κ drugs. Pyramidal cells in layer VI also project to the brain stem whose reticular activating system might also play a role in the level of alertness influenced by κ drugs.

We thank Lynda Hester for superb technical assistance and Nancy Bruce for excellent manuscript preparation. This research was supported by U.S. Public Health Service Grant DA 00266 and Research Scientist Award DA 00074 to S.H.S., Grant GM 07309 to R.R.G., and a grant from the McKnight Foundation.

1. Martin, W. R., Eades, C. G., Thompson, J. A., Huppler, R. E. & Gilbert, P. E. (1976) *J. Pharmacol. Exp. Ther.* **197**, 517–532.
2. Wood, P. L., Rackham, A. & Richard, J. (1981) *Life Sci.* **28**, 2119–2125.
3. Schulz, R., Wuster, M., Rubini, P. & Herz, A. (1981) *J. Pharmacol. Exp. Ther.* **219**, 547–550.
4. Oka, T., Negishi, K., Suda, M., Matsumiya, T., Inazu, T. & Veki, M. (1981) *Eur. J. Pharmacol.* **73**, 235–236.
5. Lord, J. A. H., Waterfield, A. A., Hughes, J. & Kosterlitz, H. W. (1977) *Nature (London)* **267**, 495–499.
6. Goodman, R. R., Snyder, S. H., Kuhar, M. J. & Young, W. S., III (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6239–6243.
7. Duka, T., Schubert, P., Wuster, M., Stoiber, R. & Herz, A. (1981) *Neurosci. Lett.* **21**, 119–124.
8. Pert, C. B., Snyder, S. H. & May, E. L. (1976) *J. Pharmacol. Exp. Ther.* **196**, 316–322.
9. Snyder, S. H. & Goodman, R. R. (1980) *J. Neurochem.* **35**, 5–15.
10. Hiller, J. M. & Simon, E. J. (1979) *Eur. J. Pharmacol.* **60**, 389–390.
11. Pasternak, G. W. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3691–3694.
12. Kosterlitz, H. W., Paterson, S. J. & Robson, L. E. (1981) *Br. J. Pharmacol.* **73**, 939–949.
13. Chang, K.-J., Hazum, E. & Cuatrecasas, P. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4141–4145.
14. Wolozin, B. L., Nishimura, S. & Pasternak, G. W. (1982) *J. Neurosci.* **2**, 708–713.
15. Pfeiffer, A. & Herz, A. (1982) *Mol. Pharmacol.* **21**, 266–271.
16. McLawhon, R. W., West, R. E., Jr., Miller, R. J. & Dawson, G. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4309–4313.
17. Childers, S. R., Creese, I., Snowman, A. M. & Snyder, S. H. (1979) *Eur. J. Pharmacol.* **55**, 11–18.
18. Young, W. S., III, & Kuhar, M. J. (1979) *Brain Res.* **179**, 255–270.
19. Palacios, J. M., Niehoff, D. L. & Kuhar, M. J. (1981) *Neurosci. Lett.* **25**, 101–105.
20. Penney, J. B., Frey, K. & Young, A. B. (1981) *Eur. J. Pharmacol.* **72**, 421–422.
21. Kosterlitz, H. W. & Paterson, S. J. (1980) *Proc. R. Soc. London, Ser. X* **210**, 113–122.
22. Romer, D., Buscher, H., Hill, R. C., Maurer, R., Petcher, T. J., Welle, H. B. A., Bakel, H. C. C. K. & Akkerman, A. M. (1980) *Life Sci.* **27**, 971–978.