Life Sciences, Vol. 31, pp. 1351-1354 Pergamon Press
Printed in the U.S.A.

TRITIATED ETHYLKETOCYCLAZOCINE BINDING IN RAT BRAIN: DIFFERENTIAL DISTRIBUTION OF BINDING SITES ACROSS BRAIN REGIONS

William A. Hewlett, Huda Akil*, Walter Carlini, and Jack D. Barchas.

Nancy Pritzker Laboratory of Behavioral Neurochemistry, Stanford University School of Medicine, Stanford, CA 94305 and *Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109

(Received in final form June 14, 1982)

SUMMARY

In rat brain, ^{3}H-EKC shows a relative regional distribution of binding which parallels that of ^{3}H-morphine. Dynorphin(1-13) has a pattern similar to morphine and dissimilar to EKC in displacing the three labels. Dynorphin(1-13) is more potent against ^{3}H-morphine than against ^{3}H-EKC across brain regions while β-endorphin competes better against ^{3}H-EKC.

Several classes of opiate receptors exist in the mamalian CNS (1,2); the μ, δ, and k receptors have been best characterized by pharmacologic assays. The prototypical agonists for each of these receptors are morphine, [d-ala^2, d-leu^5]-enkephalin (DADLE), and ethylketocyclazocine (EKC) respectively. Our work to date (3) suggests that the μ and δ sites have different distributions in rat brain and that the enkephalins interact preferentially with the δ receptor while β-endorphin can interact at both the μ and δ sites, having a slightly greater preference for δ. The interaction of opioid peptides with the k site has not been well studied in CNS tissues, however, dynorphin reportedly interacts with the k receptor in guinea pig ilium and brain (4).

To characterize the interaction of the endogenous peptides with the different opiate receptors, particularly the k opiate receptor, we examine the ability of different opiates to inhibit the binding of ^{3}H-EKC, the prototypical k ligand, and compare that with the displacement of ^{3}H-DADLE and ^{3}H-morphine. Statements of differential potencies are based on relative potencies against all three ^{3}H-ligands in the same brain regional preparations. This approach allows us to characterize the different opioid peptides as being more morphine-like (μ), more enkephalin-like (δ), or more EKC-like (k).

METHODS

Brain regions of male Sprague-Dawley rats (160-200 g) were dissected on ice and homogenized by a polytron in cold 50 mM TRIS.HCL pH 7.4 at 50 mg/ml for 40 sec. Each homogenate was preincubated at 37° for 40 min, centrifuged at 30,000 g for 20 min, resuspended in buffer at 50 mg/ml, incubated for 40 min at 25°C for 2 hr with a 1 nM concentration of tritiated ligand and 3-7 concentrations of unlabeled ligand. Total incubation volumn was 500 μl. Homogenates were then diluted with 4.5 mls cold buffer, filtered, and rinsed with 4.5 mls cold buffer. Membrane bound radioactivity was assessed by scintillation counting. Specific binding for a given ^{3}H-ligand was defined as total binding minus binding in the presence of 1μM concentration of corresponding unlabeled ligand. Each estimate of specific displacement

0024-3205/82/121351-04$03.00/0

TABLE I.
REGIONAL BINDING - ^{3}H-EKC

	$\bar{X}$	SEM	N	$R(\frac{^{3}H\text{-DADLE}}{^{3}H\text{-EKC}})$	$R(\frac{^{3}H\text{-MOR}}{^{3}H\text{-EKC}})$
Striatum	2.9	.20	11	1.5	1.3
F. Cortex	2.3	.20	7	1.4	1.3
Hippocampus	1.7	.09	11	1.0	1.4
Hypothalamus	1.5	.07	12	0.6	1.1
Midbrain	1.6	.08	11	0.6	1.3
Whole Brain	1.3	.10	6	1.3	1.6

SPECIFIC BINDING OF 1 NM ^{3}H-EKC IN BRAIN REGIONS AND WHOLE BRAIN. Values represent the average specific binding in fmols/mg region (wet wt) for 1 nM ^{3}H-EKC incubated with 30 mg/ml concentration of membrane preparation at 4°C.

is the average of 2-4 replicates within a given experiment. Each experiment compares the displacement of at least two ^{3}H-ligands by at least two unlabeled ligands in the same regional preparations. In a given experiment 4-5 regions were tested simultaneously. IC_{50} values were determined by plots of percent control specific binding versus log concentration of cold ligand. Mean IC_{50} values and standard errors were computed using log transformed IC_{50} values from different experiments.

RESULTS

The ordering of regional binding for ^{3}H-EKC is the same as that seen in our studies with ^{3}H-morphine and ^{3}H-DADLE (3); striatum and frontal cortex contain the highest levels of ^{3}H-EKC binding followed by hippocampus, midbrain, and hypothalamus (Table I). Remaining brain regions as a whole contain only 0.6 fmol specific binding per mg tissue. Scatchard analysis of ^{3}H-EKC binding in whole brain yields a linear plot with an affinity (K_d) of 10 nM. While the ratio of ^{3}H-EKC to ^{3}H-DADLE binding varies by a factor of almost three over these five brain regions, the ratio of ^{3}H-EKC to ^{3}H-morphine binding remains almost constant across brain regions. In competition studies (Table II), DADLE is the least potent ligand in displacing ^{3}H-EKC binding in every region and is less than one-third as potent in hypothalamus as it is in hippocampus. SKF 10,047, dynorphin(1-13), and β-endorphin are the most potent ligands against ^{3}H-EKC. To determine whether dynorphin(1-13) is more μ-like or k-like in the rat CNS, we compare its potencies against the three ^{3}H-ligands with those of morphine and EKC (Figure 1). Unlabeled EKC shows remarkably similar potencies against all three labels across regions. Unlabeled dynorphin(1-13) shows a pattern of displacement of the three labels which is similar to that of unlabeled morphine. It is more potent against ^{3}H-morphine than against ^{3}H-EKC in each of the five regions and in whole brain. Like unlabeled morphine it is weakest against ^{3}H-DADLE across regions.

To more closely examine the preferences of the representative opioid peptides for the opiate receptor types, we calculate the ratio of potencies of the unlabeled peptides against 1 nM concentrations of ^{3}H-morphine and ^{3}H-EKC, and ^{3}H-DADLE and ^{3}H-EKC. The use of potency ratios has the effect of controlling for region-specific effects such as differential regional occupancy and regional breakdown. In our whole brain membrane preparation, dynorphin(1-13) is 1.4 times more potent against ^{3}H-morphine than ^{3}H-EKC. Figure 2a demonstrates that dynorphin(1-13) is more effective

TABLE II.
^{3}H-EKC
Log Average IC_{50} Values

	IC_{50}	LSEM	N	IC_{50}	LSEM	N	IC_{50}	LSEM	N
	Morphine			DADLE			Dynorphin(1-13)		
Striatum	12	.054	4	19	.041	5	2.9	.049	4
F. Cortex	4.8	.128	2	19	.033	2	1.8	.014	3
Hippocampus	4.6	.078	4	16	.081	3	2.3	.036	4
Hypothalamus	9.9	.097	4	50	.038	3	2.7	.048	5
Midbrain	11	.063	4	41	.126	3	2.7	.016	4
	β-Endorphin			EKC			SKF		
Striatum	2.4	.075	2	8.4	.056	4	3.4	.023	4
F. Cortex	2.1	.087	3	9.2	.066	4	3.0	.038	4
Hippocampus	1.6	.055	4	6.4	.110	4	2.5	.027	4
Hypothalamus	3.2	.094	3	12	.025	2	2.6	.125	4
Midbrain	3.4	.048	4	17	.080	4	4.6	.036	3

POTENCIES (NM) OF OPIATES AND OPIOID PEPTIDES AGAINST ^{3}H-EKC IN BRAIN REGIONS. ^{3}H-EKC (1nM) was incubated with 3-7 concentrations of unlabeled ligand. IC_{50} values were determined by plots of percent control specific binding versus log concentration of cold ligand. IC_{50} values given represent the log average of the IC_{50} values of (N) experiments and are expressed in nM units of concentration. Standard errors are expressed in log units (LSEM).

across brain regions against ^{3}H-morphine than against ^{3}H-EKC. In contrast, β-endorphin is more effective across brain regions against ^{3}H-EKC. When comparisons are made against ^{3}H-EKC and ^{3}H-DADLE (Figure 2b), dynorphin(1-13) shows a preference for ^{3}H-EKC labeled sites. β-endorphin is nearly equipotent against these two ^{3}H-ligands across regions.

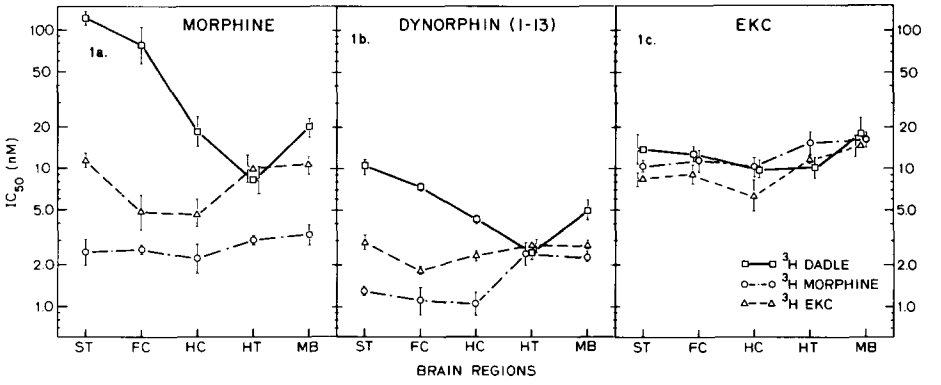

FIG. 1.
COMPARISON OF POTENCIES OF MORPHINE, DYNORPHIN(1-13), AND EKC AGAINST ^{3}H-OPIATES IN BRAIN REGIONS. Concentration curves of morphine, dynorphin(1-13), and EKC were run against 1 nM concentrations of the three ^{3}H-opiates in the same brain regional preparations.

DISCUSSION

Our purpose in these studies has been to determine the interaction of the opioid peptides with different opiate receptor types in the rat CNS and to

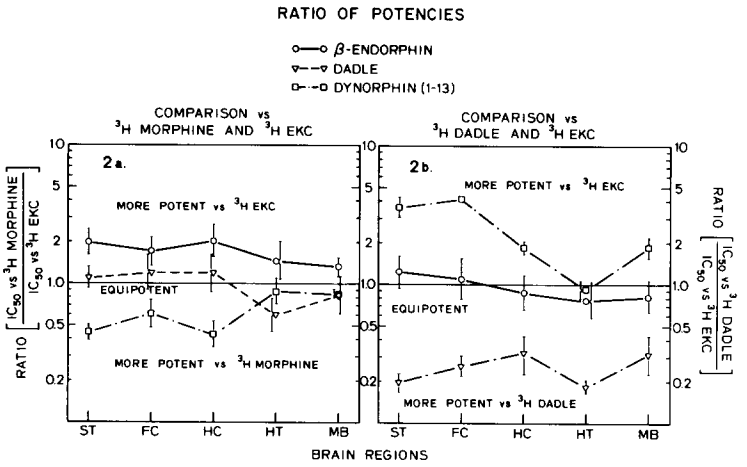

FIG. 2.
RATIOS OF POTENCIES OF OPIOID PEPTIDES AGAINST 1 NM ^{3}H-OPIATE LIGANDS. Concentration curves of DADLE, β-endorphin, and dynorphin(1-13) were run against 1 nM concentrations of ^{3}H-morphine and ^{3}H-EKC in (a), and ^{3}H-DADLE and ^{3}H-EKC in (b), both in the same brain region preparations.

seek evidence for a receptor with k properties in regional preparations of rat brain. We find, however, no evidence for a specific k receptor in these brain regions, but find that the k prototype ^{3}H-EKC shows a binding distribution which parallels the distribution of ^{3}H-morphine labeled sites. The putative k peptide, dynorphin(1-13), displaces the opiate labels with a pattern of inhibition which is similar to that of unlabeled morphine and different from that of unlabeled EKC. The μ-like character of dynorphin (1-13) is further demonstrated by its ratio of potencies against ^{3}H-morphine and ^{3}H-EKC. It is more potent against ^{3}H-morphine across brain regions while the other two peptides show a preference for ^{3}H-EKC labeled sites.

These data are consistent with the hypothesis that there are few k sites in the rat CNS, in particular, in these five brain regions. Thus, in the rat brain, ^{3}H-EKC may be predominately labeling μ and δ sites with a preference for μ sites. β-Endorphin, which also interacts well with both μ and δ sites, does better at displacing ^{3}H-EKC than do either dynorphin(1-13) or DADLE, which are weak at the δ site and μ site, respectively. Dynorphin(1-13) displaces ^{3}H-morphine more easily than ^{3}H-EKC since the latter binds to the δ site for which dynorphin has a low affinity. Whereas in the guinea pig CNS dynorphin may act as a k ligand, our data suggest that this same peptide may act predominately as a μ ligand in the central nervous system of the rat.

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Supported by NIDA grant DA-01207.