MOLECULAR PHARMACOLOGY, 26:484–488

Benzomorphan Sites Are Ligand Recognition Sites of Putative ϵ-Receptors

K.-J. CHANG, S. G. BLANCHARD, AND P. CUATRECASAS

Department of Molecular Biology, The Wellcome Research Laboratories, Research Triangle Park, North Carolina 27709

Received May 8, 1984; Accepted August 29, 1984

SUMMARY

The binding characteristics of benzomorphan sites of rat brain membranes are compared with those of κ-sites of human placenta and guinea pig brain membranes. Enkephalins and the stable analog [D-Ala^2,D-Leu^5]enkephalin, which are virtually inactive at κ-sites, possess moderate binding affinity at benzomorphan sites. In contrast, a dynorphin analog, PL017-dynorphin A(6–17), binds well to κ-sites but poorly to benzomorphan sites. Among all opioid peptides tested, β_h-endorphin, which is essentially inactive at the κ-receptor sites, is the most potent ligand at benzomorphan sites. The potencies of β_h-endorphin and its fragments at ϵ-receptors of the rat vas deferens correlate well with their binding affinities of benzomorphan sites but not of μ- and δ-sites. These data, as well as the data which show the distinct distribution of benzomorphan sites in rat brain as compared with the distribution of μ- and δ-sites of rat brain and of κ-sites of guinea pig brain, suggest that benzomorphan sites of rat brain are the ligand-binding sites of ϵ-receptors.

INTRODUCTION

Opioid receptors are now believed to be heterogeneous. Based on the distinct pharmacologic effects produced by different types of opioids, at least four types of opioid receptors were postulated (see recent review in ref. 1). These are designated as μ-, δ-, κ-, and ϵ-receptors. The existence of μ-, δ-, and κ-receptors has now been confirmed by the biochemical receptor binding studies (2–7). The anatomically distinct localization of μ-, δ, and κ-binding sites in brain further establishes their existence and suggests possible distinct physiologic functions (8–10). The synthesis of various opioid ligands highly selective for μ-, δ-, and κ-receptors substantiates the above hypothesis and provides useful tools for studying the pharmacologic effects and physiologic role of endogenous opioids (11–16).

ϵ-Receptors have been postulated to account for the unique characteristic of opioid receptors in rat vas deferens (17). In this system, β-$_h$-endorphin is the most potent opioid peptide; DADLE[1] has about 1 to 10% activity of β_h-endorphin (18, 19). The classical narcotic μ-agonist, morphine, behaves as a partial agonist of low potency (20). The κ-agonists such as EKC and bremazocine behave as competitive antagonists (21) of moderate potency. Although biochemical and anatomical evidence supporting the existence of distinct ϵ-receptors is lacking, some binding studies employing [^{3}H]β_h-endorphin suggest their presence in rat brain. β_h-Endorphin was found to be more potent than enkephalins and opioid alkaloids in competition for the binding of [^{3}H]β_h-endorphin (22).

We have also described a specific benzomorphan-binding site in rat brain membranes using [^{3}H]diprenorphine, a universal opioid antagonist, after suppressing μ- and δ-sites with highly selective μ- and δ-agonists (23). Although this binding site resembles the κ-site described in guinea pig brain (5, 6, 14), several discrepancies exist. 1) The affinity of many benzomorphan drugs for benzomorphan sites is about an order of magnitude lower than that for κ-sites in guinea pig brain membranes (5) and human placenta membranes (7). 2) The δ-ligand DADLE, which is virtually inactive in κ-receptors of rabbit vas deferens (24) and possesses essentially no affinity for κ-sites of guinea pig brain (5) and human placenta membranes (7), has significant agonist activity in rat vas deferens (19, 20) and binding affinity in benzomorphan sites of rat brain membranes (23). 3) A peptide which is composed of Tyr-Pro-*N*Me-Phe-D-Pro at the amino terminus and dynorphin A(6–17) sequence at the carboxy terminus was found to be a potent κ-ligand in human placental membranes, but is inactive at benzomorphan sites (25). 4) Furthermore, β_h-endorphin, which is virtually inactive at κ-receptors (26), is the most potent ligand known for benzomorphan sites (27). These data also suggest that benzomorphan sites may be the ligand recognition sites of putative ϵ-receptors. In this report, we describe the evidence supporting this concept. The biological activities of a series of β_h-endorphin fragments, enkephalin and opioid alkaloids, in rat vas deferens correlate well with their binding potencies at the benzomorphan sites, thus suggesting the so-called benzomor-

[1] The abbreviations used are: DADLE, [D-Ala^2,D-Leu^5]enkephalin; EKC, ethylketocyclazocine.

0026-895X/84/060484-05$02.00/0

phan sites of rat brain are likely to be ε-receptor-binding sites.

MATERIALS AND METHODS

β_h-Endorphin, dynorphin, FK33824 [Tyr-D-Ala-Gly-*N*Me-Phe-Met(*O*)ol] and PLO17-dynorphin(6–17) were obtained from Peninsula Laboratories, San Carlos, CA. [^{3}H]Diprenorphine (9 Ci/mmol) was purchased from Amersham. The β-endorphin fragments were generous gifts of Dr. N. Ling, Salk Institute, Los Angeles, CA.

Human placental membranes were prepared by differential centrifugation in isotonic solution containing 0.25 M sucrose, 10 mM Tris·HCl (pH 7.7). The nuclei fractions were removed by centrifugation at 600 × *g* for 15 min, the mitochondria were removed by centrifugation at 10,000 × *g* for 15 min. The crude membrane fractions were then pelleted at 40,000 × *g* for 30 min.

The membrane particulates were then washed twice with 50 mM Tris·HCl (pH 7.7), suspended in 2 volumes of buffer, and stored at −70°. Rat (Sprague-Dawley) brain membranes were prepared as described previously (2). The binding assay for benzomorphan sites was described previously (23). In a typical experiment, total binding was about 800 cpm and the nonspecific binding determined in the presence of 1 μM diprenorphine was generally ∿25% of the total. All samples were counted for 5–10 min so that counting error was 3% or less. Data points were determined in duplicate and the variation between duplicate values was less than 7%. The number of experiments performed for each compound is indicated in Table 1. The μ- and δ-binding assays were performed with ^{125}I-labeled FK33824 and DADLE, respectively (2, 3). It should be noted that slight cross-reactivity (about 15%) of ^{125}I-DADLE (δ-ligand) to μ-binding sites occurred at the concentration selected (2). Fifty μg/ml of bacitracin was included in all assays to prevent peptide degradation. The apparent dissociation constant (K_i) values were calculated according to Cheng's and Prusoff's equation (28). In all studies with β-endorphin, its fragments and the dynorphins, the tubes were pretreated with 1% bovine serum albumin to prevent the absorption of peptides by glass.

RESULTS

Opioid peptides that lack activity at κ-sites have good affinity at benzomorphan sites. Human placenta membranes which contain apparently homogenous κ-sites (7) serve as a useful tool for examining the binding affinity of opioids for κ-sites. The saturation binding isotherm of [^{3}H]diprenorphine is shown in Fig. 1. The Scatchard plot indicates a single binding site for [^{3}H]diprenorphine with an apparent dissociation constant (K_d) value of 0.48 nM and a B_{max} value of 230 fmol/tube. The prototype κ-agonist EKC and the endogenous κ-agonist dynorphin A(1–13) potently inhibit the binding of [^{3}H]diprenorphine (Fig. 2), but the competition curves exhibit a biphasic nature and extend into 4 log units. Diprenorphine also potently inhibits the binding of [^{3}H]diprenorphine. The potency of morphine is much lower than that of EKC or dynorphin (Fig. 2). DADLE is virtually inactive in this κ-binding assay; the IC_{50} value, the concentration which inhibits the binding by 50%, is greater than 10 μM. These observations are consistent with the characteristics of κ-sites described previously in human placenta membrane by Porthe *et al.* (7) and in guinea pig brain membranes (5).

Guinea pig brain membranes were shown by Corbett *et al.* (24) to contain large quantity of κ-binding sites which can be studied after quenching the μ- and δ-sites

TABLE 1

Correlation of binding affinities of β_h-endorphin fragments to their bioactivities in ε-receptor of rat vas deferens

Values are mean ± standard deviation.

	ED_{50}*		Binding site K_i		
			Benzomorphan	μ	δ
	nM		*nM*		
β_h-Endorphin	89	90	9.8 ± 3.1 (3)	0.65	1.0
β_p-LPH$_{61-89}$	142	150			
β_p-LPH$_{61-87}$		81	98 ± 32 (2)	1.3	6.0
β_p-LPH$_{61-86}$	256	150	64 ± 14 (3)	0.5	1.0
β_p-LPH$_{61-83}$	500	150	321 ± 95 (4)	4.0	9.0
β_p-LPH$_{61-81}$	6070	3000	262 ± 160 (4)	6.0	30.0
β_p-LPH$_{61-79}$	>15,000	50,000	480 ± 115 (3)	10.0	18.0
β_p-LPH$_{61-77}$		>50,000	>1,500 (3)	30.0	100.0
β_p-LPH$_{61-76}$	>15,000		>2,000 (3)	50.0	140.0
β_p-LPH$_{61-65}$	>15,000	>100,000	800 ± 53 (3)	8.0	4.4
Leu-Enkephalin		>100,000	780 ± 53 (2)	20.0	3.0
DADLE	1,036	8,000	390 ± 100 (3)	4	1.6
FK-33.824	128	246	170 ± 40 (3)	1.2	14.0
Etorphine	20	4	2.9 ± 3.1 (3)	0.15	0.3

*These data are obtained from refs. 18 and 19. The apparent affinities at benzomorphan-binding sites were determined according to the method described in Chang *et al.* (23). The affinities at μ- and δ-binding sites were determined according to Chang *et al.* (2, 3). The numbers in the parentheses are the number of experiments performed.

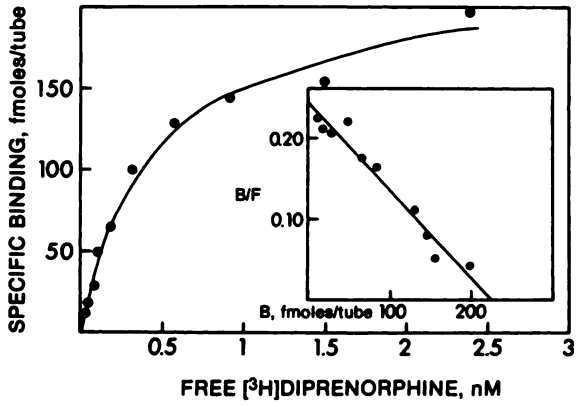

FIG. 1. *Saturation binding curves of [^{3}H]diprenorphine in human placenta membranes*

The binding was carried out at 24° for 60 min in 2 ml of solution. The inset shows a Scatchard plot of the data. The calculated K_d and B_{max} are 0.48 nM and 230 fmol/tube, respectively. The protein concentration was 1 mg/ml.

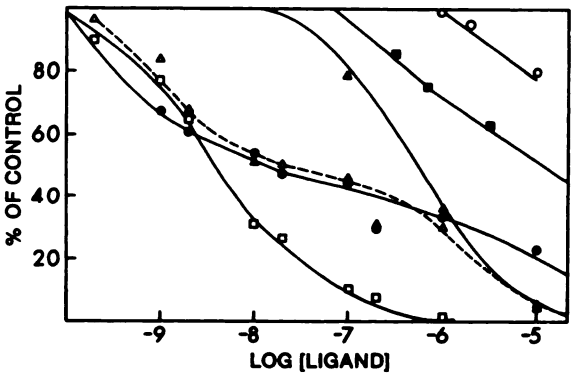

FIG. 2. *Competition curves of dynorphin A(1–13) (△), diprenorphine (□), (−)EKC (●), morphine (▲), β_h-endorphin (■), and DADLE (○) against the binding of [^{3}H]diprenorphine (0.5 nM) to human placenta membrane*

with saturating concentrations of selective μ- and δ-ligands. When guinea pig brain membrane in the presence of μ- and δ-ligands is used as κ-sites source and [^{3}H] diprenorphine as labeled ligand, EKC is a potent competitor but DADLE is virtually inactive and the IC_{50} value is about 10 μM (Fig. 3). Again, these data are consistent with the data reported by Corbett *et al.* (24) that DADLE is virtually inactive as a κ-ligand.

The ϵ-agonist β_h-endorphin is also a poor ligand for the κ-sites of either human placenta membranes or guinea pig brain membrane (Figs. 2 and 3). The IC_{50} value in competing with the binding of [^{3}H]diprenorphine to human placenta membrane is about 10 μM. The IC_{50} value in competing with the binding of [^{3}H]diprenorphine to guinea pig brain membrane after quenching μ- and δ-sites is about 2 μM. This result is consistent with data that show the lack of activity of β_h-endorphin in κ-receptors of rabbit vas deferens (26). In contrast, β_h-endorphin is an excellent ligand at benzomorphine sites of rat brain membranes (Fig. 4). The calculated apparent K_i value is about 10 nM.

An opioid peptide that lacks activity at benzomorphan site possesses good affinity at κ-sites. We have recently synthesized a dynorphin analog, PL017-dynorphin A(6–17), in which the amino terminal, Leu-enkephalin sequence is replaced by the sequence of a morphiceptin analog (PL017, Tyr-Pro-*N*MePhe-D-Pro-NH) (25). This analog surprisingly has good affinity for κ-sites in human placenta (Fig. 4) and guinea pig brain membranes (not shown); the calculated apparent K_i value is 25 nM. In contrast, it binds to benzomorphan sites of rat brain membranes very poorly (Fig. 4). The IC_{50} value is greater than 5 μM.

Correlation of ϵ-receptor activity to the binding affinity of benzomorphan sites. Since β_h-endorphin is an ϵ-agonist in the rat vas deferens and the most potent ligand for benzomorphan sites, it is logical to speculate that benzomorphan sites are, in fact, the ligand recognition sites of ϵ-receptors. To substantiate this, we examined the binding potency of various β_p-endorphin fragments and opioids at μ-, δ- and benzomorphan sites. These binding affinities were then compared with their biological activities in rat vas deferens reported by two laboratories

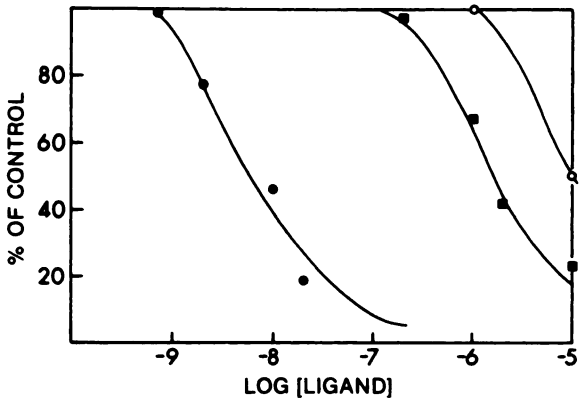

FIG. 3. *Competition curves of (−)EKC (●), β_h-endorphin (■), or DADLE (○) against the binding of [^{3}H]diprenorphine (0.5 nM) to guinea pig brain membranes in the presence of 100 nM DADLE and 1 μM [D-Pro^4]morphiceptin to quench δ- and μ-sites*

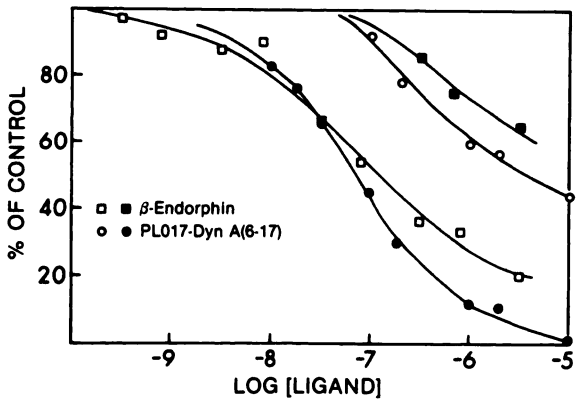

FIG. 4. *Differential potency of β_h-endorphin (□, ■) and PL017-dynorphin A(6–17) (○, ●) at benzomorphan sites of rat brain membranes (□, ○) and κ-sites of human placenta membranes (■, ●)*

The competitive binding curves for benzomorphan sites were conducted with [^{3}H]diprenorphine (0.5 nM) in the presence of 100 nM DADLE and 1 μM [D-Pro^4]morphiceptin. The κ-sites were determined with [^{3}H]diprenorphine (0.5 nM) in human placenta membranes. A similar competition curve for PL017-dynorphin A(6–17) was obtained at κ-sites of guinea pig brain membranes.

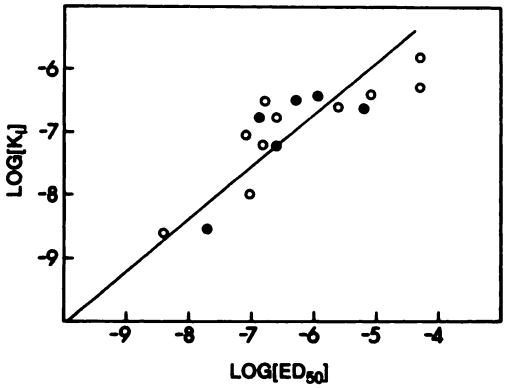

FIG. 5. *Correlation between the potencies in rat vas deferens and the binding affinities at benzomorphan sites for β_h-endorphin, β_p-endorphin fragments, etorphine, DADLE, and FK33824*

These data are from Table 1. ○ and ● are from refs. 18 and 19, respectively. Linear regression analysis gave log (K_i) = −1.65 + 0.84 log (ED_{50}) (r = 0.81, $p < 0.01$) and log (K_i) = −3.01 + 0.62 log (ED_{50}) (r = 0.81, $p < 0.01$) for ● and ○, respectively.

(Table 1). All small peptides including stable enkephalin analogs that have very high affinities at μ- and δ-sites show very low potency at rat vas deferens. Thus, it is very unlikely that μ- and δ-sites are the functional receptors in rat vas deferens.

A very good correlation can be obtained between the binding potency at benzomorphan sites and the biological activity in rat vas deferens (Table 1, Fig. 5). Regression analysis reveals a linear relation for both sets of data shown with log (K_i) = −1.65 + 0.84 log (ED_{50}) (r = 0.81, $p < 0.01$) and log (K_i) = −3.01 + 0.62 log (ED_{50}) (r = 0.81, $p < 0.01$). All small peptides (α-endorphin, γ-endorphin, Met-enkephalin and Leu-enkephalin) that have affinity less than 500 nM show virtually no bioactivity in rat vas deferens and are not included in the analysis of Fig. 5. However, it was recently demonstrated that the bioactivity of Met- and Leu-enkephalin is greatly increased if a peptidase inhibitor cocktail is in-

cluded to inhibit the peptide degradation. The activity is increased from virtual inactivity to moderate activity with ED_{50} value of about 0.5 μM (29). These values are very similar to the value of the stable analog DADLE and their binding affinities at benzomorphan sites.

DISCUSSION

The present data provide biochemical evidence supporting the presence of ϵ-receptors in rat brain. The benzomorphan sites originally detected in rat brain membranes by [^{3}H]diprenorphine after quenching μ- and δ-sites are likely to be the ligand recognition sites of ϵ-receptors postulated in the rat vas deferens (16–18) rather than κ-sites described in guinea pig brain, human placenta membranes (5–7) and rabbit vas deferens (26). The evidence for this conclusion reported by us and others is as follows.

1) The affinities of many benzomorphan drugs for benzomorphan sites are about 10 times lower than the affinity for κ-binding sites in guinea pig ileum and human placenta membranes (23), and are, in fact, very similar to their antagonist potency (K_e) in rat vas deferens (21).

2) DADLE, which has virtually no affinity to κ-sites, still binds to benzomorphan sites with K_i values of about 0.5 μM, which is similar to its potency (ED_{50}) in the ϵ-receptor of rat vas deferens.

3) PL017-dynorphin A(6–17) binds well to κ-sites and shows very little affinity at benzomorphan sites.

4) β_h-Endorphin, which is shown to have virtually no activity at κ-receptors of rabbit vas deferens (26) and very low affinity for κ-sites of human placenta and guinea pig brain membranes, binds extremely well to benzomorphan sites of rat brain. In fact, β_h-endorphin is the most potent peptide among all known endogenous opioid peptides. A good correlation between the potencies of β-endorphin fragments in the rat vas deferens bioassay and their binding affinities to the benzomorphan sites is obtained.

5) Similar binding sites were described in the spinal cord of guinea pig which are designed as κ_2-sites (30), human amygdala (3), bovine retina (32), human pheochromocytoma and bovine adrenal medulla (33) after suppression of μ- and δ-sites. Interestingly, the adrenal medulla and the pheochromocytoma bear large numbers of these benzomorphan sites.

6) The direct binding of [^{3}H]β_h-endorphin is displaced more potently by native β_h-endorphin than by prototype opiates (e.g., morphine) or enkephalins. The binding of labeled opiates and enkephalins is inhibited less well by β_h-endorphin than by the corresponding unlabeled ligands (22).

7) *In vitro* receptor autoradiography reveals that the distribution of benzomorphan sites in rat brain differs from the distribution of μ- and δ-sites in rat brain as well as κ-sites in guinea pig brain (34).[2] The benzomorphan sites are densely localized in the habenular nuclei, the dorsal medial hypothalamus and the interstitial nucleus of stria terminalis. The difference in the distribution of benzomorphan sites in rat brain and κ-sites in guinea pig brain may be attributable to a species difference. However, the clear difference in the receptor binding characteristics between benzomorphan sites and κ-sites makes this possibility very unlikely.

It should be noted that K_i values determined in Tris·HCl buffer alone are lower than their bioassay ED_{50} values obtained from studies in the isolated tissue. Similar results have also been reported for other types of opiate receptors, including the latest reports on κ-receptors (24). The binding affinity of opioid agonist is known to be reduced by the presence of sodium ions (35) and GTP (36, 37) which are also known to be required for the biological effects (i.e., the inhibition of adenylate cyclase) (38). Under physiologic conditions, where sodium ions are in the medium and GTP is likely present inside the cells, the affinity of agonists is likely to be lower than their affinities determined in Tris·HCl alone. However, the relative potency or the correlation between these two assays should remain the same for a given series of agonists provided that the intrinsic efficacy for all compounds is similar. Some discrepancy between δ-receptor bioassay and benzomorphan-binding assay is also noticed. This seems to be attributed to the larger errors encountered in the bioassay. About 2- to 3-fold difference in ED_{50} for some compounds is found between the two laboratories cited despite the same source of β-endorphin fragments. For instance, the ED_{50} value of β_p-LPH_{61-83} reported by Schülz *et al.* (18) is only slightly higher than that of β_h-endorphin. In contrast, the value reported by Huidobro-Toro *et al.* (19) is about 6-fold higher than for β_h-endorphin. The later value correlates with our K_i value better than the former. An ED_{50} value of 19 nM for β_h-endorphin has also been reported by others (29), which is about 4-fold less than those mentioned in Table 1. Recently, it was reported that β-endorphin(1–27) behaves as an antagonist against the analgesic effect of β-endorphin(1–31) *in vivo* (39). It is conceivable that various β_p-endorphin fragments may have different intrinsic efficacy in eliciting the biological effects. This could account for the discrepancy between the ED_{50} values and K_i value observed for β_p-LPH_{61-81}. In light of the good correlation between the binding affinity at benzomorphan sites and the potency at δ-receptors of rat vas deferens, as well as the fact that β-endorphin is the most potent endogenous opioid peptide at both systems, we propose that benzomorphan sites in rat brain membranes are ligand recognition sites of ϵ-receptors.

Although [^{3}H]diprenorphine binds well to benzomorphan sites, the binding of [^{3}H]diprenorphine to guinea pig brain membranes after suppression of μ- and δ-sites is not readily displaced by β_h-endorphin and DADLE below micromolar concentrations, suggesting that there are few benzomorphan or ϵ-sites in guinea pig brain. The majority of [^{3}H]diprenorphine binding in guinea pig brain membrane after suppression of μ- and δ-sites is readily displaced by κ-ligands (Fig. 3), suggesting that κ-sites predominate in guinea pig brain. In contrast, the binding of [^{3}H]diprenorphine to benzomorphan sites of rat brain membrane is relatively insensitive to PL017-dynorphin A(6–17), an active κ-ligand in human placenta membrane (25) and guinea pig brain membranes. This

[2] Unpublished observation.

suggests that there are very few κ-sites in rat brain, although it is difficult to rule out that about 10 to 20% of the benzomorphan sites may be κ-sites. This conclusion is consistent with observation of Gillian *et al.* (21) that the content of κ-sites in rat brain is very low (21).

In summary, the evidence presented here and previously provided by us and others supports the existence of μ-, δ-, and κ-, and ε-opioid receptors. Human β-endorphin is a nonselective agonist for μ- and δ- as well as ε-receptors and virtually has no activity at κ-receptor sites. Dynorphins are relatively potent κ-agonists with significant cross-reactivity to μ- and δ-receptors. Both Leu- and Met-enkephalins are relatively selective δ-agonists with some cross-reactivity to μ-receptors, weak activity at ε-receptors, and essentially no activity at κ-receptors.

REFERENCES

1. Chang, K.-J., and P. Cuatrecasas. Heterogeneity and properties of opiate receptors. *Fed. Proc.* **40**:2729–2734 (1981).
2. Chang, K.-J., and P. Cuatrecasas. Multiple opiate receptors: enkephalins and morphine bind to receptors of different specificity. *J. Biol. Chem.* **254**:2610–2618 (1979).
3. Chang, K.-J., B. R. Cooper, E. Hazum, and P. Cuatrecasas. Multiple opiate receptors: different regional distribution in the brain and differential binding of opiates and opioid peptides. *Mol. Pharmacol.* **16**:91–104 (1979).
4. Lord, J. A. H., A. A. Waterfield, J. Hughes, and H. W. Kosterlitz. Endogenous opioid peptides: multiple agonists and receptors. *Nature* **267**:495–499 (1977).
5. Kosterlitz, H. W., S. J. Paterson, and L. E. Robson. Characterization of the κ-subtype of the opiate receptor in the guinea pig brain. *Br. J. Pharmacol.* **73**:939–949 (1981).
6. James, I. F., C. Chavkin, and A. Goldstein. Preparation of brain membranes containing a single type of opioid receptor highly selective for dynorphin. *Proc. Natl. Acad. Sci. U.S.A.* **79**:7570–7574 (1982).
7. Porthe, G., A. Valette, and J. Cros. Kappa opiate binding sites in human placenta. *Biochem. Biophys. Res. Commun.* **101**:1–6 (1981).
8. Goodman, R. R., S. H. Snyder, M. J. Kuhar, and W. S. Young. Differentiation of delta and mu opiate receptor localizations by light microscopic autoradiography. *Proc. Natl. Acad. Sci. U.S.A.* **77**:6239–6243 (1980).
9. Goodman, R. R., and S. H. Snyder. κ-Opiate receptors localized by autoradiography to deep layers of cerebral cortex: relation to sedative effects. *Proc. Natl. Acad. Sci. U.S.A.* **79**:5703–5707 (1982).
10. Herkenham, M., and C. B. Pert. *In vitro* autoradiography of opiate receptors in rat brain suggests loci of "opiatergic" pathways. *Proc. Natl. Acad. Sci. U.S.A.* **77**:5532–5536 (1980).
11. Chang, K.-J., A. Killian, E. Hazum, P. Cuatrecasas, and J.-K. Chang. Morphiceptin: a potent and specific agonist for morphine (μ) receptors. *Science* **212**:75–77 (1981).
12. Chang, K.-J., E. T. Wei, A. Killian, and J.-K. Chang. Potent morphiceptin analogs: structure activity relationships and morphine-like activities. *J. Pharmacol. Exp. Ther.* **227**:403–408 (1983).
13. Lahti, R. A., P. F. VonVoigtlander, and C. Barsuhn. Properties of a selective kappa agonist U-50, 488H. *Life Sci.* **31**:2257–2260 (1982).
14. Mosberg, H. I., R. Hurst, V. J. Hruby, K. Gee, H. I. Yamamura, J. J. Galligan, and T. F. Burks. Bis-penicillamine enkephalins possess highly improved specificity toward δ-opioid receptors. *Proc. Natl. Acad. Sci. U.S.A.* **80**:5871–5874 (1983).
15. Zajac, J.-M., G. Gacel, F. Petit, P. Dodey, P. Rossignol, and B. P. Roques. Deltakephalin, Tyr-D-Thr-Gly-Phe-Leu-Thr: a new highly potent and fully specific agonist for opiate δ-receptors. *Biochem. Biophys. Res. Commun.* **111**:390–397 (1983).
16. Portoghese, P. S., D. L. Larson, L. M. Sayre, D. S. Fries, and A. E. Takemoni. A novel opioid receptor site directed alkylating agent with irreversible narcotic antagonistic and reversible agonistic activities. *J. Med. Chem* **23**:233–234 (1980).
17. Schulz, R., E. Faase, M. Wüster, and A. Herz. Selective receptors for β-endorphin on the rat vas deferens. *Life Sci.* **24**:843–850 (1979).
18. Schulz, R., M. Wüster, and A. Herz. Pharmacological characterization of the ε-opiate receptor. *J. Pharmacol. Exp. Ther.* **216**:604–606 (1981).
19. Huidobro-Toro, J. P., E. M. Caturay, N. Ling, N. M. Lee, H. H. Loh, and E. L. Way. Studies on the structural prerequisites for the activation of the β-endorphin receptor on the rat vas deferens. *J. Pharmacol. Exp. Ther.* **222**:262–269 (1982).
20. Huidobro, F., J. P. Huidobro-Toro, and H. Miranda. Interactions between morphine and the opioid-like peptides in the rat vas deferens. *Br. J. Pharmacol.* **70**:519–525 (1980).
21. Gillan, M. G. C., H. W. Kosterlitz, and J. Magnan. Unexpected antagonism in the rat vas deferens by benzomorphans which are agonists in other pharmacological tests. *Br. J. Pharmacol.* **72**:13–15 (1981).
22. Law, P.-Y., H. H. Loh, and C. H. Li. Properties and localization of β-endorphin receptor in rat brain. *Proc. Natl. Acad. Sci. U.S.A.* **76**:5455–5459 (1979).
23. Chang, K.-J., E. Hazum, and P. Cuatrecasas. Novel opiate binding sites selective for benzomorphan drugs. *Proc. Natl. Acad. Sci. U.S.A.* **78**:4141–4145 (1981).
24. Corbett, A. D., S. J. Paterson, A. T. McKnight, J. Magnan, and Kosterlitz. Dynorphin$_{1-8}$ and dynorphin$_{1-9}$ are ligands for the κ-subtype of opiate receptor. *Nature* **299**:79–81 (1982).
25. Chang, K.-J., and J.-K. Chang. The role of amino-terminal sequence of β-endorphin and dynorphin in the determination of opiate receptor subtype selectivity. *Life Sci.* (Suppl. 1) **33**:267–270 (1983).
26. Oka, T., K. Negishi, M. Suda, T. Matsumiya, T. Inazu, and M. Ueki. Rabbit vas deferens: a specific bioassay for opioid κ-receptor agonists. *Eur. J. Pharmacol.* **73**:235–236 (1980).
27. Chang, K.-J., and P. Cuatrecasas. Characterization of novel opiate binding sites selective for benzomorphan drugs, in *Advances in Endogenous and Exogenous Opioids* (H. Tokage and E. J. Simon, eds.). Elsevier Biomedical Press, New York, 5–8 (1981).
28. Cheng, Y.-L., and W. H. Prusoff. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **22**:3099–3102 (1973).
29. McKnight, A. T., A. D. Corbett, and H. W. Kosterlitz. Increase in potencies of opioid peptides after peptidase inhibition. *Eur. J. Pharmacol.* **86**:393–402 (1983).
30. Attali, B., C. Gouarderes, H. Mazarguil, Y. Adigies, and J. Cros. Evidence for multiple "kappa" binding sites by use of opioid peptides in the guinea-pig lumbo-sacral spinal cord. *Neuropeptides* **3**:53–64 (1982).
31. Pfeiffer, A., A. Pasi, P. Mehraein, and A. Herz. A subclassification of κ-sites in human brain by use of dynorphin-17. *Neuropeptides* **2**:89–97 (1981).
32. Osborne, H. H., and A. Herz. Opiate binding sites in bovine retina: evidence for benzomorphan selective binding sites. *Eur. J. Pharmacol.* **86**:373–378 (1983).
33. Castanas, E., P. Giraud, Y. Audigier, R. Drissi, F. Boudouresque, B. Conte-Devolx, and C. Oliver. Adrenal medullary opiate receptors: pharmacological characterization in bovine adrenal medulla and a human pheochromocytoma. *Mol. Pharmacol.* **25**:38–45 (1984).
34. Valdes, F., B. Crain, K. Chang, and J. McNamara. Radiohistochemical localization of opiate receptors in rat brain: the benzomorphan binding sites, *Abstr. Soc. Neurosci.* **8**:647 (1982).
35. Pert, C. B., and S. H. Snyder. Opiate receptors binding of agonists and antagonists affected differently by sodium. *Mol. Pharmacol.* **10**:868–879 (1974).
36. Blume, A. J. Opiate binding to membrane preparations of neuroblastoma × glioma hybrid cells NG108-15: effect of ions and nucleotides. *Life Sci.* **22**:1843–1852 (1978).
37. Childers, S. R., and S. H. Snyder. Differential regulation of quanine nucleotide of opiate agonist and antagonist receptor interactions. *J. Neurochem.* **34**:583–593 (1980).
38. Blume, A. J., D. Lichtstein, and G. Boone. Coupling of opiate receptors to adenylate cyclase: requirement of Na^+ and GTP. *Proc. Natl. Acad. Sci. U.S.A.* **76**:5626–5630 (1979).
39. Hammonds, Jr., R. G., P. Nicolas, and C. H. Li. β-Endorphin-(1–27) is an antagonist of β-endorphin analgesia. *Proc. Natl. Acad. Sci. U.S.A.* **81**:1389–1390 (1984).

Send reprint requests to: Dr. K.-J. Chang, Department of Molecular Biology, The Wellcome Research Laboratories, Research Triangle Park, NC 27709.