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Pharmacological Characterization of the Cloned κ-, δ-, and μ-Opioid Receptors

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SUMMARY

Opioid drugs, such as morphine, and the endogenous opioid peptides, namely the enkephalins, endorphins, and dynorphins, exert a wide spectrum of physiological and behavioral effects, including effects on pain perception, mood, motor control, and autonomic functions. These effects are mediated via membrane-bound receptors, of which the best characterized are the κ, δ, and μ receptors. The existence of these distinct types of opioid receptors has recently been confirmed by molecular cloning. In the present study, we have examined the pharmacological profiles of the cloned κ, δ, and μ receptors using a battery of widely employed opioid agents. Our results suggest that the cloned κ and μ receptors have pharmacological characteristics similar to those of the endogenously expressed κ_1 and μ receptors, respectively. The cloned δ receptor displays a pharmacological profile consistent with that of a δ_2 receptor. Opioid agents with abuse potential possess high affinities for the μ receptor. The availability of the cloned receptors will facilitate the identification and development of more specific and selective compounds with greater therapeutic potential and fewer undesirable side effects.

The myriad of biological effects of the opioid drugs, such as morphine, and of the endogenous opioid peptides, namely the endorphins, enkephalins, and dynorphins, are mediated via a family of membrane-bound receptors. These receptors, known as μ, κ, and δ, have been extensively characterized in various tissues and cell lines (1), but the lack of highly selective ligands for each receptor and the paucity of tissues that express individual opioid receptors have hindered their further characterization. Recently, several groups have reported the molecular cloning of the κ, δ, and μ receptors (2–5). The availability of the cloned receptors now allows for studies of individual opioid receptor types with regard to pharmacological profile, structure-function analysis, cellular effector coupling, anatomical distribution, and regulation of expression.

Opioids are used clinically in the management of pain, but their use is limited by a constellation of undesirable side effects, including respiratory depression, miosis, decreased gastrointestinal motility, sedation, nausea, and vomiting (6). A concern regarding the use of opioids in the treatment of chronic pain is their potential for dependence and abuse. Studies suggest that the clinical effects of opioids are mediated via a variety of receptors and that the therapeutic effects versus the undesirable side effects of opioids may be mediated by different receptor (sub)types (6, 7). Therefore, the therapeutic versus side effects could be separable with the use of more selective agents for receptor subtypes. In the present study, we have characterized the pharmacological properties of the cloned κ-, δ-, and μ-opioid receptors and characterized the receptor selectivity of widely used opioid ligands.

Materials and Methods

Isolation of stable transformants. PC-12 cells were grown in RPMI medium with 10% horse serum and 5% bovine serum, in 5% CO_2 at 37°, to 50% confluency. The cells were transfected, by the lipofection method (8), with 7 μg of the 1.2-kilobase *PstI* fragment of the mouse κ receptor cDNA cloned into the cytomegalovirus promoter-based expression vector pCMV-6c, as described previously (4). The cells were selected and maintained in a similar medium containing 200 μg/ml G418. The generation of the CHO-DG44 cell line stably expressing the mouse δ receptor was accomplished as described previously (9). Briefly, a 1.3-kilobase *EcoRI-SacI* fragment of the mouse δ-opioid receptor cDNA was inserted into the expression vector pCMV-6c and co-transfected with pSV2neo into CHO cells, and stable transfectants were selected and grown as described previously (4, 9). The rat μ

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ABBREVIATIONS: CHO, Chinese hamster ovary; BNTX, 7-benzylidenenaltrexone; DADL, [D-Ala^2,D-Leu^5]enkephalin; DAMGO, [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin; DPDPE, [D-penicillamine^2,D-penicillamine^5]enkephalin; DSLET, [D-Ser^2,D-Leu^5]enkephalyl-Thr; NTB, benzofuran analog of naltrindole; PLO17, Tyr-Pro-MePhe-D-Pro-NH_2; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid.

receptor was expressed transiently in COS-7 cells, as described previously (5, 10).

Binding studies. Receptor binding assays were performed using membranes from either PC12 cells stably expressing the cloned mouse κ receptor, CHO-DG44 cells stably expressing the mouse δ receptor, or COS-7 cells transiently expressing the rat μ receptor, 48–72 hr after transfection, as described previously (4, 10). For radioligand binding assays, cells were harvested in 50 mM Tris·HCl, pH 7.8, containing 1 mM EGTA, 5 mM $MgCl_2$, 10 μg/ml leupeptin, 10 μg/ml pepstatin, 200 μg/ml bacitracin, and 0.5 μg/ml aprotinin (buffer 1) and were centrifuged at 24,000 × *g* for 7 min at 4°. The pellet was homogenized in buffer 1 using a Polytron (setting 2.5, 30 sec; Brinkmann). The homogenate was then centrifuged at 48,000 × *g* for 20 min at 4°. The pellet was homogenized in buffer 1 and this membrane preparation was used for the radioligand binding studies. For inhibition studies, cell membranes (10–20 μg of protein) were incubated for 40 min at 25° with [^{3}H]U-69,593 (2 nM; specific activity, 47.4 Ci/mmol), [^{3}H]naltrindole (1 nM; specific activity, 31.2 Ci/mmol), or [^{3}H]DAMGO (1 nM; specific activity, 55 Ci/mmol) (NEN-DuPont, Wilmington, DE), in a final volume of 200 μl, in the presence or absence of competing agents. For saturation experiments, cell membranes were incubated with increasing concentrations of the radioligands. Nonspecific binding was defined as the radioactivity remaining bound in the presence of 10 μM naloxone for all radioligands. The binding reaction was terminated by the addition of ice-cold 50 mM Tris·HCl buffer, pH 7.8, and rapid filtration over Whatman GF/B glass fiber filters that had been pretreated with 0.5% polyethyleneimine/0.1% bovine serum albumin for at least 1 hr. The filters were then washed with 12 ml of ice-cold Tris·HCl buffer and the bound radioactivity was counted in a scintillation counter. Total binding and nonspecific binding were typically 800 cpm and 100 cpm for [^{3}H]U-69,593, 500 cpm and 50 cpm for [^{3}H]DAMGO, and 1000 cpm and 300 cpm for [^{3}H]naltrindole, respectively. Data from radioligand binding studies were used to generate inhibition curves. IC_{50} values were obtained from curve-fitting performed by the mathematical modeling program FITCOMP (11) and saturation data were analyzed using FITSAT (12), available on the National Institutes of Health-sponsored PROPHET system. The inhibitory binding constants (K_i) were calculated from the IC_{50} values using the Cheng-Prusoff equation (13).

Results

To pharmacologically characterize the cloned κ, δ, and μ receptors, we expressed the cDNAs encoding these receptors either stably in PC12 (κ) or CHO-DG44 (δ) cells or transiently in COS-7 cells (μ). The κ-, δ-, and μ-opioid receptors were labeled with the selective opioid radioligands [^{3}H]U-69,593, [^{3}H]naltrindole, and [^{3}H]DAMGO, respectively. The binding of these radioligands was saturable and of high affinity (Fig. 1). Analysis of the saturation experiments demonstrated that [^{3}H]U-69,593 bound to the cloned κ receptor with a K_d of 2.8 nM and a B_{max} of 3346 fmol/mg of protein. Similarly, [^{3}H]naltrindole bound to the cloned δ receptor with high affinity, with a K_d of 0.18 nM and a B_{max} of 1251 fmol/mg of protein. The K_d for [^{3}H]DAMGO binding to the cloned μ receptor was 0.57 nM and the B_{max} was 444 fmol/mg of protein. All data were best fit by a single-site analysis. No specific radioligand binding was detectable in appropriate nontransfected control cells.

To identify the pharmacological specificities of the cloned κ-, δ-, and μ-opioid receptors, we tested a battery of opioid ligands for their abilities to inhibit radioligand binding (Table 1). These include both peptide and nonpeptide compounds previously characterized as selective and nonselective agents for opioid receptors (14–18). The endogenous opioid peptide dynorphin A(1–17) was found to be selective for the κ receptor, whereas β-endorphin, Leu-enkephalin, and Met-enkephalin were selective for the μ and δ receptors, because they either did not bind to the κ receptor (Leu- and Met-enkephalin) or bound with low potency (β-endorphin). Des-Tyr^1-β-endorphin did not bind to any of the opioid receptors. The binding to each receptor was stereoselective, being inhibited by (−)-naloxone and levorphanol but not by their respective isomers (+)-naloxone and dextrorphan. Other relatively nonselective compounds tested were (±)-bremazocine, ethylketocyclazocine, etorphine, pentazocine, and diprenorphine. Each of these compounds was relatively nonselective for μ versus κ receptors and displayed higher affinities for these receptors than for the δ receptor. Analogous results were found with β-funaltrexamine and β-chlornaltrexamine, although the values given are not true K_i values due to the covalent nature of these ligands. Furthermore, naltrexone, nalbuphine, and nalorphine were also relatively selective for κ and μ receptors, binding to the δ receptor only at much higher concentrations.

We also tested a variety of compounds that have been previously characterized as μ selective (14–18), including both peptide and nonpeptide agonists and antagonists. As expected, most of these compounds bound to the cloned μ receptor with K_i values in the low nanomolar range (Table 1). Exceptions include morphine, codeine, morphiceptin, and PLO17, which bound with affinities in the 10–100 nM range. The majority of the ligands tested were selective for the μ receptor and did not bind to the κ or δ receptors. Of the ligands that showed cross-reactivity, fentanyl bound to the μ receptor with high selectivity but its derivatives lofentanil and sufentanil were less selective, interacting with both δ and κ receptors, albeit with lower affinity than with the μ receptor. Similar cross-reactivity was found with the compound naloxonazine, which has been suggested to discriminate between subtypes of μ receptors, having high affinity for the μ_1 receptor (19).

Results with the κ-selective ligands U-50,488, U-69,593, ICI 204488, and spiradoline (Table 1) confirmed previous results showing their κ selectivity (14–18). The κ antagonist norbinaltorphimine was also selective for the κ receptor, but less so than the agonists tested.

Various peptide and nonpeptide agonists and antagonists at the δ receptor (14–18) were tested and results confirmed the δ selectivity of these compounds (Table 1). Thus, the peptide agonists DPDPE and D-Ala^2-deltorphin II were highly selective for the δ receptor, whereas DSLET and DADL were less so. The recently developed nonpeptide agonists BW 3734 (20) and 7-spiroindinooxymorphone (21) were also examined, with the former being found to be highly δ selective. We also tested compounds that have been proposed to distinguish between δ_1 and δ_2 receptor subtypes (22–24). These agents bound to the cloned δ receptor with differing affinities. Thus, the agonists DSLET and D-Ala^2-deltorphin II, which have been proposed as δ_2 ligands, were more potent than DPDPE, which is δ_1 selective. Furthermore, the antagonists naltrindole and NTB were more potent than BNTX at the cloned δ receptor. These results suggest that the pharmacological profile of the cloned δ-opioid receptor matches that of the δ_2 receptor subtype.

To determine whether the pharmacological profiles of the cloned opioid receptors were similar to those reported previously for receptors expressed *in vivo* in biological tissues containing multiple opioid receptor subtypes, we performed correlational analyses comparing the K_i values obtained in this study

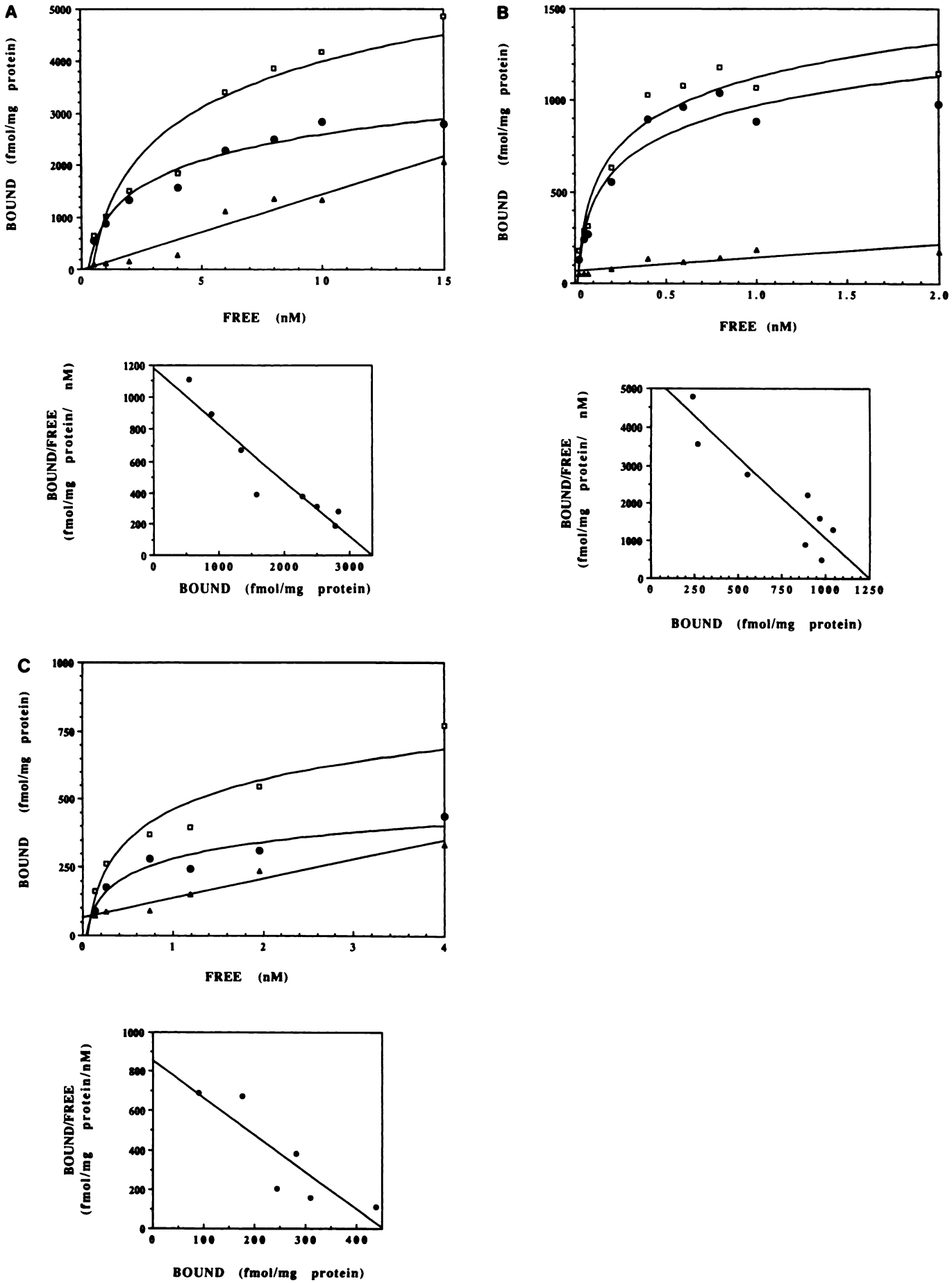

Fig. 1. Saturable binding of [^{3}H]U-69,593, [^{3}H]naltrindole, and [^{3}H]DAMGO to the cloned κ-, δ-, and μ-opioid receptors. Membranes from PC12 cells stably expressing the cloned κ receptor (A), CHO-DG44 cells stably expressing the cloned δ receptor (B), or COS-7 cells transiently expressing the

with those reported in the literature (15–18). Compounds for which literature values were not available or which did not bind to a given receptor were not included in the analysis. The correlation coefficients obtained for both the κ (Fig. 2A) and μ (Fig. 2B) receptors were very high, with r values of 0.879 (n = 25) and 0.954 (n = 16), respectively. In contrast, the correlation for the δ receptor was low (data not shown), with an r value of 0.185 (n = 17), indicating that the cloned δ receptor differs pharmacologically from those characterized in diverse tissues.

TABLE 1

Binding potencies (K_i) of ligands for the cloned κ-, δ-, and μ-opioid receptors

	κ Receptor, [^{3}H]U-69,593	δ Receptor, [^{3}H]naltrindole	μ Receptor, [^{3}H]DAMGO
		nM	
Nonselective compounds			
Dynorphin A	0.5	>1000	32
Leu-enkephalin	>1000	4.0	3.4
Met-enkephalin	>1000	1.7	0.65
β-Endorphin	52	1.0	1.0
Des-Tyr^1-β-endorphin	>1000	>1000	>1000
(−)-Naloxone	2.3	17	0.93
(+)-Naloxone	>1000	>1000	>1000
Levorphanol	6.5	5.0	0.086
Dextrorphan	>1000	>1000	>1000
(±)-Bremazocine	0.089	2.3	0.75
Ethylketocyclazocine	0.40	101	3.1
Etorphine	0.13	1.4	0.23
Pentazocine	7.2	31	5.7
Diprenorphine	0.017	0.23	0.072
β-CNA[a]	0.083	115	0.90
β-FNA	2.8	48	0.33
Naltrexone	3.9	149	1.0
Nalbuphine	3.9	>1000	11
Nalorphine	1.1	148	0.97
μ-Selective compounds			
CTOP	>1000	>1000	0.18
Dermorphin	>1000	>1000	0.33
Methadone	>1000	>1000	0.72
DAMGO	>1000	>1000	2.0
PLO17	>1000	>1000	30
Morphiceptin	>1000	>1000	56
Codeine	>1000	>1000	79
Fentanyl	255	>1000	0.39
Sufentanil	75	50	0.15
Lofentanil	5.9	5.5	0.68
Naloxonazine	11	8.6	0.054
Morphine	538	>1000	14
κ-Selective compounds			
Norbinaltorphimine	0.027	65	2.2
Spiradoline	0.036	>1000	21
U-50,488	0.12	>1000	>1000
U-69,593	0.59	>1000	>1000
ICI 204,488	0.71	>1000	>1000
δ-Selective compounds			
DPDPE	>1000	14	>1000
D-Ala^2-deltorphin II	>1000	3.3	>1000
DSLET	>1000	4.8	39
BW 3734	17	0.013	26
DADL	>1000	0.74	16
SIOM	>1000	1.7	33
Naltrindole	66	0.02	64
NTB	13	0.013	12
BNTX	55	0.66	18

[a] β-CNA, β-chloronaltrexamine; β-FNA, β-funaltrexamine; CTOP, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2; SIOM, 7-spiroindinooxymorphone.

Discussion

The pharmacological characteristics of the recently cloned subtypes of opioid receptors have been simultaneously characterized. The results indicate that the cloned κ receptor corresponds pharmacologically to the κ_1 receptor previously characterized in heterogeneous tissues (25). Furthermore, the high

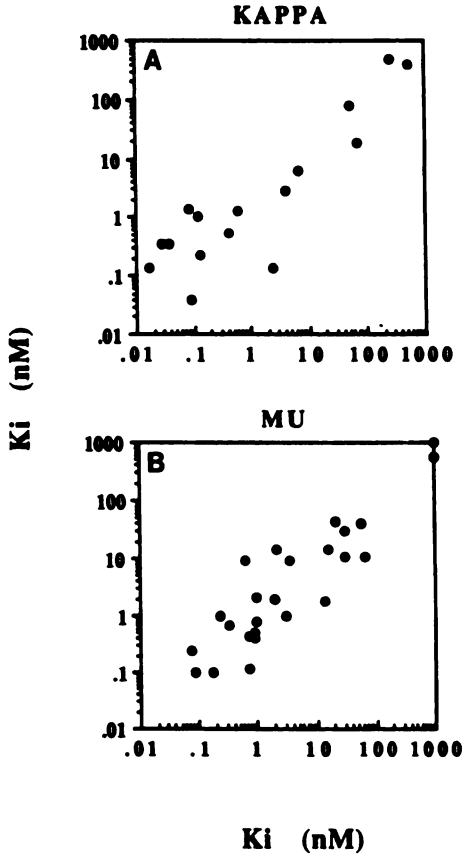

Fig. 2. Correlation of the potencies of opioid ligands to inhibit radioligand binding to the cloned κ-, δ-, and μ-opioid receptors with their potencies for opioid receptors characterized in heterogeneous tissues. Correlation analyses were performed by plotting the logarithm of the affinities of opioid ligands for the cloned κ (A) and cloned μ (B) receptors versus the logarithm of the potencies of these compounds to inhibit subtype-selective radioligand binding to these opioid receptor types in heterogeneous tissues. The affinities of ligands for the κ and μ receptors were highly correlated with literature values, with r values of 0.954 and 0.879, respectively. The correlation of potencies at the δ receptor was much poorer (r = 0.185) (not plotted).

cloned μ receptor (C) were incubated for 40 min at 25° with increasing concentrations of [^{3}H]U-69,593, [^{3}H]naltrindole, or [^{3}H]DAMGO, respectively, in the presence (△) or absence (□) of 10 μM naloxone to determine specific binding (●). *Upper*, saturation isotherms of representative experiments; *lower*, linearization of the saturation isotherm data. Analysis of the saturable binding to the κ receptor revealed that [^{3}H]U-69,593 bound to a single site with a K_d of 2.8 nM and a B_{max} of 3346 fmol/mg of protein. Analysis of the saturable binding to the δ receptor revealed that [^{3}H]naltrindole bound to a single site with a K_d of 0.18 nM and a B_{max} of 1251 fmol/mg of protein. Analysis of the saturable binding to the μ receptor revealed that [^{3}H]DAMGO bound to a single site with a K_d of 0.57 nM and a B_{max} of 444 fmol/mg of protein. All analyses were best fit by a single site, with improvements in F values of 0.411801 and 1.65416 at significance levels of 0.69 and 0.3 for the κ and μ receptors, respectively. No improvement in F value was found in analyses of δ receptor binding. Experiments were conducted in triplicate and the results of two or three independent experiments were similar.

affinity of the cloned μ receptor for naloxonazine, a compound possessing subtype selectivity (19), suggests that the cloned μ receptor corresponds to the endogenously expressed μ_1 receptor subtype. In contrast, the pharmacological profile of the cloned δ-opioid receptor differs from that of δ-opioid receptors previously characterized in diverse tissues. The existence of subtypes of δ receptors has been suggested based on behavioral data for compounds such as DPDPE and BNTX, which interact with δ_1 receptors, and DSLET, D-Ala^2-deltorphin II, and NTB, which interact with δ_2 receptors (22–24). The demonstration of the existence of δ receptor subtypes based on results of radioligand binding studies has been more ambiguous, perhaps due to the lack of sufficiently selective radioligands. The higher potencies of these latter compounds at the cloned δ receptor suggest that it may correspond to the δ_2 receptor.

Interestingly, the endogenous opioid peptides β-endorphin, Leu-enkephalin, and Met-enkephalin were selective for the μ and δ receptors versus the κ receptor. In fact, the K_i values for these peptides were comparable at the μ and δ receptors. Because the potencies of the enkephalins to bind to the μ and δ receptors are within physiological concentrations, these peptides may be endogenous ligands for both of these receptor subtypes.

Our results indicate that opioid agents with abuse liabilities possess high affinities for the μ receptor. Thus, the addictive compounds morphine, fentanyl, and methadone have high affinities for the cloned μ receptor but little or no affinity for the δ or κ receptors. Furthermore, etorphine, sufentanil, levorphanol, nalbuphine, and codeine, which have been shown to possess abuse liability (6), have in common relatively high affinity for the μ receptor. Development of analgesic agents that are κ- or δ-selective may obviate this limitation of μ-selective analgesics.

The ability to individually study the pharmacological properties of the cloned opioid receptor subtypes will allow for identification of structural features of ligands that permit selective interactions. Identification of the pharmacological interactions of drugs with the individual opioid receptors could lead to the identification of therapeutic agents less burdened with the potential to produce undesirable side effects.

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References

1. Herz, A., ed. *Opioids I*. Springer-Verlag, New York (1993).
2. Evans, C., D. Keith, H. Morrison, K. Magendzo, and R. Edwards. Cloning of a *delta* opioid receptor by functional expression. *Science (Washington D. C.)* **258**:1952–1955 (1992).
3. Kieffer, B. L., K. Befort, C. Gaveriaux-Ruff, and C. G. Hirth. The δ-opioid receptor: isolation of a cDNA by expression cloning and pharmacological characterization. *Proc. Natl. Acad. Sci. USA* **89**:12048–12052 (1992).
4. Yasuda, K., K. Raynor, H. Kong, C. Breder, J. Takeda, T. Reisine, and G. I. Bell. Cloning and functional comparison of κ and δ opioid receptors from mouse brain. *Proc. Natl. Acad. Sci. USA* **90**:6736–6740 (1993).
5. Chen, Y., A. Mestek, J. Liu, J. A. Hurley, and L. Yu. Molecular cloning and functional expression of a μ-opioid receptor from rat brain. *Mol. Pharmacol.* **44**:8–12 (1993).
6. Jaffe, J. M., and W. R. Martin. Opioid analgesics and antagonists, in *The Pharmacological Basis of Therapeutics* (A. Gilman, J. Rall, A. Nies, and P. Taylor, eds.), Ed. 8. Pergamon Press, New York, 485–573 (1990).
7. Pasternak, G. W. Pharmacological mechanism of opioid analgesia. *Clin. Neuropharmacol.* **16**:1–18 (1993).
8. Muller, S. R., P. D. Sullivan, D. O. Clegg, and S. L. Feinstein. Efficient transfection and expression of heterologous genes in PC12 cells. *DNA Cell Biol.* **9**:221–229 (1990).
9. Rens-Domiano, S., S. Law, Y. Yamada, S. Seino, G. I. Bell, and T. Reisine. Pharmacological properties of two cloned somatostatin receptors. *Mol. Pharmacol.* **42**:28–34 (1992).
10. Kong, H., K. Raynor, K. Yasuda, S. Moe, P. S. Portoghese, G. I. Bell, and T. Reisine. A single residue, aspartic acid 95, in the δ opioid receptor specifies selective high affinity agonist binding. *J. Biol. Chem.* **268**:23055–23058 (1993).
11. Perry, H., and P. McGonigle. FITCOMP, a program to fit 1-, 2-, and 3-site models to competition binding data, in *PROPHET Public Procedures Notebook*. Bolt, Berabek, and Newman, Inc., Cambridge, MA, 187–192 (1988).
12. McGonigle, P., C. Kanarick, and H. Perry. FITSAT, in *PROPHET Public Procedures Notebook*. Bolt, Berabek, and Newman, Inc., Cambridge, MA, 215–218 (1988).
13. Cheng, Y. C., and W. H. Prusoff. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **22**:3099–3102 (1973).
14. Lutz, R. A., and H. P. Pfister. Opioid receptors and their pharmacological profiles. *J. Recept. Res.* **12**:267–286 (1992).
15. Goldstein, A., and A. Naidu. Multiple opioid receptors: ligand selectivity profiles and binding site signatures. *Mol. Pharmacol.* **36**:265–272 (1989).
16. Schiller, P. W. Development of receptor selective opioid peptide analogs as pharmacologic tools and potential drugs, in *Opioids I, Handbook of Experimental Therapeutics* (A. Herz, ed.), Vol. 104/I. Springer-Verlag, New York, 681–710 (1993).
17. Portoghese, P. S. Selective nonpeptide opioid antagonists, in *Opioids I, Handbook of Experimental Therapeutics* (A. Herz, ed.), Vol. 104/I. Springer-Verlag, New York, 276–296 (1993).
18. Corbett, A. D., S. J. Paterson, and H. W. Kosterlitz. Selectivity of ligands for opioid receptors, in *Opioids I, Handbook of Experimental Therapeutics* (A. Herz, ed.), Vol. 104/I. Springer-Verlag, New York, 645–673 (1993).
19. Pasternak, G., and P. Wood. Multiple *mu* opiate receptors. *Life Sci.* **38**:1889–1898 (1986).
20. Chang, K.-J., G. Rigdon, J. Howard, and R. McNutt. A novel, potent and selective nonpeptidic delta-opioid receptor agonist BW373U86. *J. Pharmacol. Exp. Ther.* **267**:852–857 (1993).
21. Portoghese, P., S. Moe, and A. Takemori. A selective *delta*$_1$ opioid receptor agonist derived from oxymorphone: evidence for separate recognition sites for *delta*$_1$ opioid receptor agonists and antagonists. *J. Med. Chem.* **36**:2572–2574 (1993).
22. Sofuoglu, M., P. Portoghese, and A. Takemori. Differential antagonism of *delta* opioid agonists by naltrindole and its benzofuran analog (NTB) in mice: evidence for *delta* opioid receptor subtypes. *J. Pharmacol. Exp. Ther.* **257**:676–680 (1991).
23. Portoghese, P., M. Sultana, H. Nagase, and A. Takemori. A highly selective *delta*-1 opioid receptor antagonist: 7-benzylidenenaltrexone. *Eur. J. Pharmacol.* **218**:195–196 (1992).
24. Sofuoglu, M., P. Portoghese, and A. Takemori. Cross-tolerance studies in the spinal cord of β-FNA-treated mice provides further evidence for *delta* opioid receptor subtypes. *Life Sci.* **49**:PL-153–PL-156 (1991).
25. Clark, J. A., L. Lui, M. Price, B. Hersh, M. Edelson, and G. W. Pasternak. *Kappa* opiate receptor multiplicity: evidence for two U-50,488-sensitive *kappa*$_1$ subtypes and a novel *kappa*$_3$ subtype. *J. Pharmacol. Exp. Ther.* **251**:461–468 (1989).

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