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δ-Opioid receptor: the third extracellular loop determines naltrindole selectivity

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Abstract

Human δ/μ-opioid receptor chimeras were constructed to determine the role of the second and third extracellular loops in alkaloid ligand selectivity. Exchanging the third extracellular loop of the δ-opioid receptor with that of the μ-opioid receptor dramatically decreased the affinity of naltrindole, but not that of morphine. The results suggest that different domains of the opioid receptor are involved in the binding of naltrindole and morphine.

Keywords: Opioid receptor, human; Opiate; Binding site

The cDNAs encoding the major opioid receptor types (μ, δ and κ) have been cloned and belong to the G-protein-coupled receptor superfamily. The most striking feature of the opioid receptor subfamily is the extremely high homology in the putative seven transmembrane and intracellular loop domains where the ligand binding and the G-protein coupling occur in other cationic amine neurotransmitter receptors. The high degree of conservation within these regions of the opioid receptors makes it difficult to explain the selectivity of the opioid receptor ligands.

Construction of chimeric receptors is a powerful tool for mapping the regions involved in drug selectivity. Recently, several chimeras have been prepared between the opioid receptor types (Metzger and Ferguson, 1995; Knapp et al., 1995). In the present study, we constructed chimeras by exchanging the second and the third extracellular loops between the human μ-opioid (Wang et al., 1994) and δ-opioid (Knapp et al., 1994) receptors to determine their role in ligand recognition. The chimeras were spliced together using a modified polymerase chain reaction (PCR) overlap extension method using the human μ- and δ-opioid receptor clones as templates. The beginning and the end of the putative extracellular loops were determined by hydropathy analysis and sequence alignment.

The second loop chimera: δ(1–186)-μ(208–234)-δ(213–372) was synthesized in two consecutive PCR reactions using the primers (the μ-opioid receptor sequences are in bold): (1) TCA TGG TCA TGG CTA **CAA CAA AAT ACA GG**; (2) GAA AAC ACA GAT CTT CAC GAG GTT T; and (3) TGC AAG GCT GTG CC TCC AT. In the second PCR, we used the product of the first PCR reaction as the upper primer under low-stringency conditions (annealing temperature 50°C) in the first five cycles, then under high-stringency (annealing temperature 63°C) conditions.

The third loop chimera: δ(1–282)-μ(304–320)-δ(301–372) was assembled in three consecutive PCR reactions using the primers: (4) **GTT TCT TGG CAC TTC** TGC ATC GCG CTG GG; (5) CGG GTC TGG GGT CGT CGA AGT CGG C; (6) CAG CTG GTA CTG GGA CAC GGT GAC CAA GAT; and (7) **TGG TAA CCA AGG CTT TG**A CGA TGA CGA AGA T. The last PCR followed a two-step process using the products from the previous reactions as primers under low-stringency conditions (annealing temperature 50°C) in the first five cycles,

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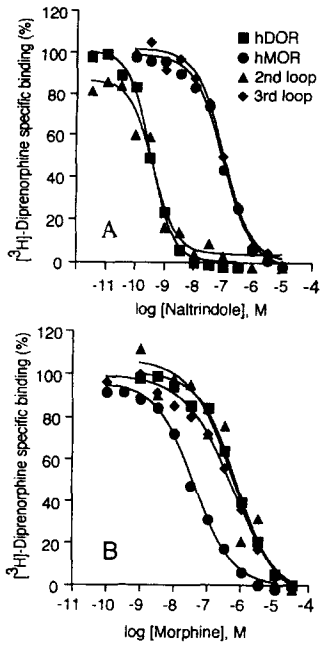

Fig. 1. The inhibition of specific [^{3}H]diprenorphine binding to COS-7 cell membranes transiently transfected with the wild-type μ-opioid (●), δ-opioid (■) receptors and the second (▲) and third (◆) loop chimeric constructs. The cell membranes were incubated with [^{3}H]diprenorphine (0.4 nM, 25°C, 3 h) in the presence of increasing concentrations of (A) naltrindole and (B) morphine. The values are the mean of two determinations carried out in duplicates.

then primers 5 and 6 under high-stringency conditions (annealing temperature 65°C) to exponentially amplify the product from the first five cycles.

Each PCR fragment carried unique restriction sites on each end and was inserted into the wild-type δ-opioid receptor sequence in the pcDNA3 vector to obtain the chimeras. The sequence of the chimeric constructs was verified by the dideoxy chain termination method. African green monkey kidney (COS-7) cells were transiently transfected with the chimeric and the wild-type receptors using the *O*-(diethylaminoethyl)-dextran transfection method as previously described (Knapp et al., 1994). Cell membranes were prepared 48–72 h after the transfection and the chimeric and wild-type receptors were characterized by [^{3}H]diprenorphine binding. Saturation experiments with [^{3}H]diprenorphine (0.1–5 nM) show that the affinity of the nonselective opioid receptor ligand is not affected by the exchange of the extracellular loops ($K_D \sim 0.5$ nM), indicating that there are no major changes in the secondary structure of the chimeric proteins. The inhibition of specific [^{3}H]diprenorphine binding (0.4 nM, 3 h incubation at 25°C) by morphine and naltrindole was used to further characterize the ligand selectivity of the chimeras.

The affinity of the selective δ-opioid receptor antagonist naltrindole was dramatically decreased (300-fold) by the replacement of the third extracellular loop of the δ-opioid receptor (Fig. 1). It has been shown previously that the exchange of the C-terminus (from transmembrane V–VII) between opioid receptor types affects the affinity of antagonists (Fukuda et al., 1995; Watson et al., 1995). In the present study, we show the importance of the third extracellular loop, without the transmembrane regions, in determining the affinity of naltrindole. Interestingly, the affinity of the opiate agonist morphine was not altered by the exchange of the extracellular loops. Together with the results from other chimeric studies (Onogi et al., 1995; Fukuda et al., 1995), our study suggests that the binding determinants for morphine reside elsewhere, presumably in the transmembrane regions. The second loop chimera fully retained its δ-opioid receptor characteristics, showing that this region is not involved in the selectivity or binding of naltrindole (Fig. 1).

The major finding of this study is that the replacement of the third extracellular loop of the human δ-opioid receptor with that of the μ-opioid receptor dramatically decreases the affinity of naltrindole while having no effect on the affinity of morphine.

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References

Fukuda, K., S. Kato and K. Mori, 1995, Location of regions of the opioid receptor involved in selective agonist binding, J. Biol. Chem. 270, 6702.

Knapp, R.J., E. Malatynska, L. Fang, X. Li, E. Babin, M. Nguyen, G. Santoro, E.V. Varga, V.J. Hruby, W.R. Roeske and H.I. Yamamura, 1994, Identification of a human δ-opioid receptor: cloning and expression, Life Sci. 54, 463.

Knapp, R.J., E. Malatynska, N. Collins, L. Fang, J.Y. Wang, V.J. Hruby, W.R. Roeske and H.I. Yamamura, 1995, Molecular biology and pharmacology of cloned opioid receptors, FASEB J. 9, 516.

Metzger, T.G. and D.M. Ferguson, 1995, On the role of the extracellular loops of opioid receptors in conferring ligand selectivity, FEBS Lett. 375, 1.

Onogi, T., M. Minami, Y. Katao, T. Nakagawa, Y. Aoki, T. Toya, S. Katsumata and M. Satoh, 1995, DAMGO, a μ-opioid receptor selective agonist distinguishes between μ and δ-opioid receptors around their first extracellular loops, FEBS Lett. 357, 93.

Wang, J.-B., P.S. Johnson, A.M. Persico, A.L. Hawkins, C.A. Griffin and G.R. Uhl, 1994, Human μ-opiate receptor. cDNA and genomic clones, pharmacologic characterization and chromosomal assignment, FEBS Lett. 338, 217.

Watson, B., F. Meng, R.C. Thompson and H. Akil, 1995, Structural studies of the μ-opioid receptor using chimeric constructs, Analgesia 1, 825.