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Short communication

Thermodynamics of ligand binding to the cloned δ-opioid receptor

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Abstract

The goal of this study was to determine the relative contribution of entropy and enthalpy to the free energies of binding to recombinant mouse δ-opioid receptors for the peptide agonist, DPDPE ([D-Pen^2,D-Pen^5]enkephalin), the peptide antagonist, TIPP(ψ) (Tyr-Tic ψ[CH_2NH]Phe-Phe-OH), the nonpeptide agonist, SNC80 ((+)-4-[(α *R*)-α-((2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide), and the nonpeptide antagonist, naltrindole. Competitive binding studies were carried out using [^{3}H]naltrindole at 0°C, 12°C, 25°C and 37°C, the affinities calculated and van't Hoff plots constructed for each ligand. The temperature dependence of binding and van't Hoff plots indicated that the entropy contribution is the major component of the free energy, for all four ligands, independent of its activity or chemical nature.

Keywords: δ-Opioid receptor; Ligand binding thermodynamics; Pharmacophore; Model

1. Introduction

The temperature dependence of the affinity of ligands for their binding sites can provide valuable information about the nature of the ligand-receptor interactions since, when analyzed using the laws of thermodynamics, it allows the separate determination of enthalpies and entropies of binding (for review, see Raffa and Porreca, 1989). Early studies of β-adrenoceptors suggested that agonists and antagonists may demonstrate different thermodynamic behavior, with antagonist binding being entropy-driven while agonist binding was enthalpy-driven, presumably as part of the signal transduction process (Weiland et al., 1979, 1980). This generalization has not held true for other G-protein-coupled receptors, including opioid receptors.

Early thermodynamics studies of binding to opioid receptors used nonselective ligands and therefore any conclusions drawn would be an average effect on all receptors (Hitzemann et al., 1985). A later study, using more selective radioligands, found an anomalous break in the van't Hoff plots for the two peptide ligands studied, not found for a nonpeptide ligand (Borea et al., 1988). Recent thermodynamics studies of the δ-opioid receptor found qualitatively different results if the dissociation constants were determined in an isolated tissue preparation (Raffa et al., 1992, 1993) or in receptor binding studies (Wild et al., 1994).

The goal of the current study was to further clarify two important unresolved questions about the thermodynamics of ligand binding to the δ-opioid receptor: (i) are there different modes of binding for agonists and antagonists? and (ii) is there a difference between peptide and nonpeptide ligands? To this end, the δ-selective peptide agonist, DPDPE ([D-Pen^2,D-Pen^5]enkephalin), peptide antagonist TIPP(ψ) (Tyr-Tic ψ[CH_2NH]Phe-Phe-OH), nonpeptide agonist, SNC80 ((+)-4-[(α *R*)-α-((2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide) and nonpeptide antagonist, naltrindole, were chosen for study. Competitive binding of these ligands to the cloned δ-opioid receptor (DOR-1) from mouse brain was measured at four different temperatures, 0°C, 12°C, 25°C and 37°C using [^{3}H]naltrindole as the radioligand. The results obtained clearly showed that binding of all four ligands to the δ-opioid receptor is driven by an increase in entropy.

2. Materials and methods

2.1. Materials

The recombinant mouse δ-opioid receptor was purchased from BioSignal (Montreal, Quebec, Canada),

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[^{3}H]Naltrindole from Dupont NEN (Boston, MA, USA), and naltrindole from Research Biochemicals International (Natick, MA, USA). DPDPE and TIPP(ψ) were kindly provided by the National Institute on Drug Abuse and SNC80 by the laboratory of Dr. K.C. Rice at the National Institutes of Health (Bethesda, MD, USA).

2.2. Receptor binding assays

The assay protocol developed by BioSignal for their recombinant receptors was modified for this study. Membranes in assay buffer (50 mM Tris-HCl (pH 7.4 at incubation temperature), 10 mM $MgCl_2$, 1 mM EDTA, 0.1% bovine serum albumin) were incubated with 0.3 nM [^{3}H]naltrindole and 8 concentrations of unlabeled ligand (10^{-11}–10^{-5} M) in a total volume of 540 μl in 96-well incubation plates. Nonspecific binding was determined in the presence of 10 μM naltrindole. The time to reach a steady state, determined at each assay temperature, was 30, 60, 90 and 120 min for 37°C, 25°C, 12°C and 0°C, respectively. The incubations were stopped by filtration (FilterMate cell harvester, Packard Instruments, Meriden, CT, USA) over GF/B filters presoaked in 0.3% polyethyleneimine and washed 4 times with 500 μl ice-cold wash buffer (50 mM Tris-HCl, pH 7.4 at 0°C). The radioactivity retained on the filters was determined by liquid scintillation counting (MicroScint 0, Packard Instruments).

2.3. Data analysis

The receptor binding data were analyzed by a modification (Toll et al., 1984) of the program LIGAND (Munson and Rodbard, 1980).

2.4. Calculation of thermodynamic parameters

The change in the standard Gibbs free energy (ΔG°) was calculated using Eq. (1):

$$\Delta G^\circ = -RT \ln(1/K_i) \quad (1)$$

where R is the ideal gas constant (1.98 cal/mol K), T is the temperature in Kelvin units, and K_i (M) is the calculated equilibrium dissociation constant for a compound at temperature T. Since

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (2)$$

combining Eqs. (1) and (2), we obtain the integrated van't Hoff equation:

$$-\ln(1/K_i) = \Delta H^\circ/RT - \Delta S^\circ/R \quad (3)$$

Therefore, plotting $[-\ln(1/K_i)]$ versus $[1/T]$, the change in enthalpy of binding (ΔH°) can be obtained from the slope ($\Delta H^\circ/R$), while the entropy change (ΔS°) can be obtained from its y-intercept ($\Delta S^\circ/R$).

3. Results

Competitive binding assays were carried out at 0°C, 12°C, 25°C and 37°C until a steady state was reached. In order to assure that only temperature effects were being observed, care was taken to adjust the pH of the incubation buffer (containing Tris-HCl) to 7.4 at each incubation temperature. Nonspecific binding, in the presence of 10 μM naltrindole, accounted for ≤ 10% of the total binding at each temperature.

The K_i values for each ligand at each temperature are shown in Table 1. In general, there was less than a 2-fold change in affinity over the temperature range studied for any of the ligands. The affinities (ln $1/K_i$ in M) were plotted against temperature ($1/T$ in K) (van't Hoff plot; Fig. 1). The enthalpy change (ΔH°) was calculated from the slope and the entropy change (ΔS°) from the y-intercept of the van't Hoff plot as described above. The calculated enthalpy does not change with temperature, resulting in linear van't Hoff plots (Fig. 1).

The binding of the two peptide ligands and the nonpeptide agonist SNC80 was exothermic ($-\Delta H^\circ$) while that of naltrindole, the nonpeptide antagonist, was nearly isoenthalpic ($\Delta H^\circ \sim 0$). The entropy contribution to the free energy ($-T\Delta S^\circ$) was calculated at 37°C and compared to the enthalpic contribution (ΔH°) (Table 1). In each case, the entropic contribution to the free energy of binding was significantly greater than the enthalpic component, and

Table 1
Thermodynamics of binding to the δ-opioid receptor

	K_i (nM) ± S.E. [a]				ΔS° [b]	ΔH° [c]	$-T\Delta S^\circ$ [d]
	0°C	12°C	25°C	37°C	(cal/mol K)	(kcal/mol)	(kcal/mol)
Naltrindole	1.5 ± 0.2	0.9 ± 0.1	0.8 ± 0.1	1.5 ± 0.1	42.3	0.39	−13.1
DPDPE	440 ± 70	410 ± 60	540 ± 60	600 ± 70	23.3	−1.61	−7.23
SNC80	45 ± 7	52 ± 8	61 ± 7	78 ± 9	24.8	−2.42	−7.69
TIPP(ψ)	18 ± 3	21 ± 3	18 ± 2	47 ± 5	22.4	−3.64	−6.94

[a] Inhibition of [^{3}H]naltrindole binding (0.3 nM) to recombinant mouse δ-opioid receptors. Values are mean ± S.E. as calculated by LIGAND (n = 3–7 assays per compound per temperature). [b] Entropy change (ΔS°) calculated from the y-intercept and [c] enthalpy change (ΔH°) from the slope of the van't Hoff plot (Fig. 1) as described in Section 2 (Eq. (3)). [d] The entropic contribution to free energy ($-T\Delta S^\circ$) was calculated at 37°C (310 K).

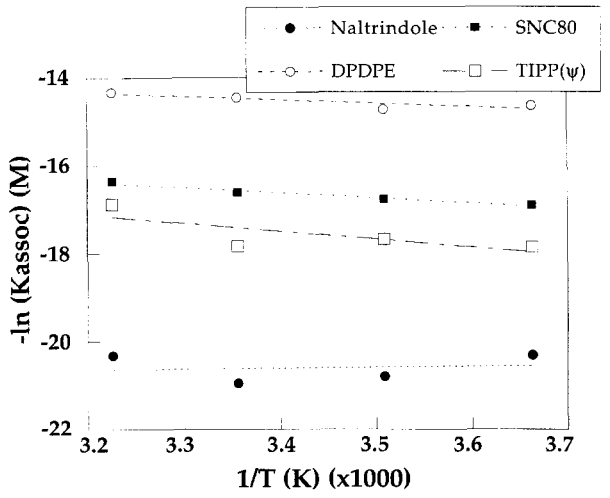

Fig. 1. Van't Hoff plots of ln K_i vs. $1/T$ for each ligand constructed from their binding affinities determined at each assay temperature as shown in Table 1. The $\Delta H°$ and $\Delta S°$ were calculated from the slope and y-intercept, respectively, of these linear plots as described in Eq. (3).

hence the binding of all of the ligands studied is entropy-driven.

4. Discussion

A thermodynamic process will spontaneously occur if the Gibbs free energy ($\Delta G°$) is negative. Therefore, negative changes in enthalpy ($\Delta H°$), i.e. an exothermic process, and positive changes in entropy ($\Delta S°$) (see Eq. (2)), favor the formation of the ligand-receptor complex.

In order to use thermodynamic parameters to try to distinguish the modes of binding of agonists and antagonists at a particular receptor, four conditions should be met in the experimental design (Raffa and Porreca, 1989): the binding reaction must reach equilibrium at each temperature studied; binding to a single receptor class should be measured; multiple temperatures should be used; and the experimental conditions should be such that the receptor is in a pharmacologically relevant affinity state. We have attempted to meet these criteria by carrying out experiments at four temperatures, at each of which the time to reach equilibrium was experimentally determined. We have used a source of cloned δ-opioid receptor, assuring the presence of only one receptor type in our binding system and the assay condition, containing Mg^{2+}, but devoid of sodium and GTP, promotes the formation of a high agonist-affinity state of the receptor (Rodriguez et al., 1992; Gilman, 1987).

In the present study, as shown in Table 1, for naltrindole, TIPP(ψ), SNC80 and DPDPE, the entropic ($-T\Delta S$) contribution dominates the enthalpic ($\Delta H°$) contribution to the free energy of complex formation and therefore the binding of all of the ligands studied is entropy-driven. For all but naltrindole, their interaction with the δ-opioid receptor is an exothermic process. In addition there is no distinction between agonists and antagonists nor between peptides and nonpeptides in the relative importance of their enthalpic or entropic components to the free energy of binding.

Meaningful comparisons of the results from the current study with previous results are hampered by the fact that in only a few of the previous studies reported were all four of the above experimental design conditions (Raffa and Porreca, 1989) satisfied.

For example, several earlier studies appear to be consistent with the findings reported here but have complications that render these comparisons of limited meaning. In one these studies, Hitzemann et al. (1985) showed that the binding of both [^{3}H]etorphine (agonist) and [^{3}H]diprenorphine (antagonist) was entropy-driven, when assayed in the absence of NaCl, with etorphine binding endothermic and diprenorphine binding exothermic. In another earlier finding that is consistent with the current results, Borea et al. (1988) found that binding of [^{3}H]Tyr-D-Ala-Gly-Phe-Met-Gly-OH (DAMGO), [^{3}H]Tyr-D-Ala-Gly-Phe-D-Leu-NH_2 (DADLE) and [^{3}H](±)-ethylketocyclazocine (EKC) was entropy-driven and endothermic between 0°C and 30°C. However, since the ligands used in both of these studies are nonselective and the assays were done in a multiple receptor system, they do not represent a direct confirmation of our findings.

By contrast, in another study (Wild et al., 1994), results were obtained that are consistent with the thermodynamic behavior reported here and that do represent a meaningful comparison and validation of both results. In that study, [^{3}H]naltrindole binding to the δ-opioid receptor from mouse brain and NG108-15 cells (from which the DOR-1 used in the present study was originally cloned (Evans et al., 1992)) was found to be endothermic and entropy-driven. However, in those same studies (Wild et al., 1994), the temperature dependence of naltrindole binding in the mouse spinal cord was also determined and the binding was found to be enthalpy-driven with a negligible contribution of entropy. It was proposed that this difference was due to the presence of different δ-opioid receptor subtypes in spinal cord than in brain and NG108-15 cells.

The results reported for naltrindole in spinal cord are similar to recent studies of the thermodynamics of the dissociation constant for naloxone and DPDPE from δ-opioid receptors in the mouse vas deferens (Raffa et al., 1992, 1993). Using this functional assay instead of radioligand binding, both agonist and antagonist binding was found to be enthalpy-driven, with naloxone having an unfavorable entropic contribution. It is possible that the subtype present in the mouse vas deferens and the spinal cord receptor is the same, while that in the brain, NG108-15 cells and the DOR-1 receptor used in the present study represent a second δ-opioid receptor subtype.

Another earlier study presents results in contrast to

those reported here. In the current studies, the calculated enthalpy does not change with temperature, resulting in linear van't Hoff plots (Fig. 1). This finding is in contrast to the results of Borea et al. (1988) in which a sharp break in the van't Hoff plot was found for the binding of the peptide agonist [^{3}H]DADLE to guinea pig brain membranes. However, DADLE is not a selective ligand, having significant affinity to μ and δ receptors, and the guinea pig membrane is a multireceptor system, both of which could cause the observed non-linear effect. In fact, a later study (Wild et al., 1994) in which both of these ambiguities were eliminated, by using the δ-opioid receptor from NG108-15 cells and the δ-selective ligand, [^{3}H]naltrindole, showed a linear van't Hoff plot over the same temperature range.

The dominance of the entropic contribution to the binding free energy of the peptide and nonpeptide agonists and antagonists to the cloned δ-opioid receptor suggests that a major component of this binding energy comes from the loss of waters of solvation of the ligand and/or receptor during the formation of the receptor ligand complex. Direct interactons between receptor and ligand in these complexes can be both electrostatic (Testa et al., 1987) and hydrophobic (Hitzemann, 1988) in origin. We have proposed that each of these types of interactions is important for binding of ligands to the δ-opioid receptor (Agarwal et al., 1995; Huang et al., 1996).

In previous studies of the properties of the high and low affinity ligands of the δ-opioid receptor (Agarwal et al., 1995; Huang et al., 1996), we have developed a three-dimensional (3-D) pharmacophore for recognition of the δ-opioid receptor that is largely hydrophobic, except for the single electrostatic interaction between the protonated amine common to all active opioids and a candidate anionic receptor residue. Specifically, this pharmacophore proposes that three moieties in a specific geometric arrangement are the minimum requirements for high affinity binding: the protonated amine nitrogen, an aromatic ring and at least one of two hydrophobic moieties. The hydrophobic moieties are proposed to interact with one small and one extended lipophilic region within the binding site (Agarwal et al., 1995; Huang et al., 1996).

In a complimentary study, we have recently reported the construction of a 3-D model of the transmembrane region of the δ-opioid receptor and identification of a candidate ligand binding site (Alcorta and Loew, 1996). This binding site was shown to accommodate all four ligands studied in similar, but not identical, regions in a manner in which the ligand moieties proposed as determinants of recognition interact with complimentary amino acid residues in the binding site. It is very plausible that variable extent of dehydration of the binding site and the ligands during the binding process could be a contributing factor to the ligand binding affinity, consistent with our finding of an entropy-driven binding.

The results of the thermodynamic analysis of the binding interaction provide a cross-validation of the pharmacophore for recognition, the 3-D model of the transmembrane region of the δ-opioid receptor and the candidate binding site. Further refinement of the 3-D model and of the recognition pharmacophore is vital to the computer-aided drug design process. Future studies of the thermodynamics of binding to other opioid receptor subtypes should aid in the development of 3-D models and recognition pharmacophores for those receptors, leading to the design of new opioid therapeutic agents.

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