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Molecular modeling study of the differential ligand–receptor interaction at the μ, δ and κ opioid receptors

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Summary

3D models of the opioid receptors μ, δ and κ were constructed using BUNDLE, an in-house program to build de novo models of G-protein coupled receptors at the atomic level. Once the three opioid receptors were constructed and before any energy refinement, models were assessed for their compatibility with the results available from point-site mutations carried out on these receptors. In a subsequent step, three selective antagonists to each of three receptors (naltrindole, naltrexone and nor-binaltorphamine) were docked onto each of the three receptors and subsequently energy minimized. The nine resulting complexes were checked for their ability to explain known results of structure-activity studies. Once the models were validated, analysis of the distances between different residues of the receptors and the ligands were computed. This analysis permitted us to identify key residues tentatively involved in direct interaction with the ligand.

Introduction

Morphine, the active ingredient of opium, came into use for minor surgical procedures about 150 years ago. Despite its beneficial therapeutic use as potent analgesic, the compound exhibits side effects, including respiratory depression, modulation of gastrointestinal motility and physical dependence. Over the years numerous analogs in many diverse chemical families have been synthesized and evaluated. This synthetic effort was aimed at finding new opioid analgesics without the adverse side effects of morphine [1–3]. Although much knowledge has been gathered in the past, all opiates disclosed until now exhibit similar deleterious effects to morphine and consequently the quest for an opioid analgesic without side effects continues. On the other hand, this continued effort of generating novel compounds, gives us the opportunity to access a long list of analogs suitable for carrying out structure-activity relationships studies. Work carried out in this direction in previous years has generated hypotheses concerning the stereochemical requirements of ligands at the opioid receptors [1,4–7]. In particular, Schwyzer's 'message-address' concept was demonstrated to be very useful in rationalizing the molecular basis of opioid ligand–receptor interactions [8].

Pharmacological studies suggest that the analgesic properties of opiates are mediated through at least three different receptors, known as μ, δ and κ [9]. These receptors were successfully cloned and expressed a few years ago [10–15]. Furthermore, site directed mutagenesis studies have provided knowledge of the importance of some residues for receptor functionality [16–24]. However, only knowledge of the 3D structure of these receptors can help to predict the role of different residues on binding, as well as help us understand different ligand binding modes and selectivity, providing insights into the design of new opiates with various degrees of receptor selectivity.

Opioid receptors belong to the superfamily of integral proteins known as G-protein coupled receptors (GPCRs). They consist of a single polypep-

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tide chain containing seven transmembrane domains (TM1–TM7) looping back and forth across the lipid membrane. These hydrophobic domains exhibit helical secondary structures and pack to form a seven helix bundle with a hydrophobic exterior facing the lipid membrane and a hydrophilic interior, where the ligands bind. At present there are considerable technical difficulties, including purification and isolation in large enough quantities, that hinder the preparation of crystals of these proteins suitable for X-ray diffraction studies. Rhodopsin is the only GPCR to have been structurally characterized to date by electron microscopy analysis of 2D crystals. Unfortunately, the resolution of the available structures is too low (9 and 7 Å, respectively) to furnish detailed structural information [25–27].

Molecular modeling can be used to provide atomic resolution models of these receptors using all available experimental information. Models of the μ, δ and κ opioid receptors using bacteriorhodopsin as template [28–30], as well as using de novo methodologies [31,32] have been published in recent years. Although these models provided useful insights into the structure of the receptors they have inherent drawbacks associated with the different helical packing exhibited by rhodopsin and bacteriorhodopsin. Besides, those models that were constructed by homology modeling using bacteriorhodopsin as template have drawbacks associated with the low sequence identity between the template and the protein modeled. We have recently proposed a general procedure for building GPCRs de novo [33,34] using information deduced from the rhodopsin 2D electron density map. In the present study, we apply this methodology for the construction of the three known opioid receptors. In addition, three selective 4,5-epoxymorphinan antagonists: naltrexone, naltrindole and nor-binaltorphamine (nor-BNI) were docked onto the three constructed receptors. These ligands were selected because they share a certain structural similarity and at the same time they cover different opiate binding profiles. It needs to be stressed that, due to the limited amount of information available for a precise construction of these receptors, models reported in the present work need to be considered as semi-quantitative. Despite this limitation, they provide new insights into the requirements for opioid ligand selectivity and represent a useful tool to design new experiments.

Methods

Choice of the putative transmembrane regions of the opioid receptors

The receptor models reported in this work were constructed using rat μ, δ and mouse κ opioid sequences. This selection was guided by the amount of information available on them, since these receptors have been the most commonly used in mutagenesis experiments. Each of the three opioid receptors shares a sequence identity higher than 90% between different species, whereas different receptor types share an overall sequence identity higher than 60% with each other within the same species. This sequence identity can increase up to 75% when only transmembrane regions are compared in the alignment. Interestingly, TM2 and TM3 exhibit a sequence identity higher than the average value, whereas TM4 and TM1 exhibit lower sequence identity than the average value [35]. Amino acid sequences of the μ, δ and κ opioid receptors were taken from the SWISS-PROT protein sequence data bank [36]. The putative transmembrane segments of the three opioid receptor types were assessed using the profile fed neural network system (PHDhtm) from the EMBL in Heidelberg [37]. Figure 1 shows the sequences of the three receptors with the seven putative transmembrane regions highlighted, where numbering refers to the delta receptor.

Construction of an initial structure for the opioid receptors

Each of the transmembrane domains in every receptor was forced to adopt an ideal α-helix ($\phi = -57°, \Psi = -47°$), except for prolines ($\phi = -65°$), and with the side chains kept in an extended conformation. Atomic coordinates were computed using the AMBER4.0 program [38]. Helices were automatically arranged to generate a bundle, using the in-house program BUNDLE [33,34]. Specifically, the program treats every helix as a rigid body. The atomic coordinates of a helix are first transformed to a local, right-handed orthonormal coordinate system, centered at the mean position of the helix and with one of the coordinate axis being the helical one. The main steps performed by the program on every helix to construct the final model are: (i) rotation of the helix around one of the non-helical axes in order to generate an adequate antiparallel orientation in the bundle; (ii) computation of its hydrophobic moment according to the method described by Eisenberg and co-workers [39]; (iii) rotation of the helix

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                1                                                  50
oprd_rat        MEPVPSARAE LQFSLLANVS DTFPSAFPSA SANASGSPGA RSASSLALAI
oprm_rat        .DPLAQASCS PAPGSWLNLS HVDGNQSDPC GLNRTGLGGN DSSPSMVTAI
oprk_mouse      FRGDPGPTCS PSACLLPNSS SWFPNWAESD SNGSVGSEDQ QLESSPAIPV

                51            I                          II    100
oprd_rat        AITALYSAVC AVGLLGNVLV MFGIVRYTKL KTATNIYIFN LALADALATS
oprm_rat        TIMALYSIVC VVGLFGNFLV MYVIVRYTKM KTATNIYIFN LALADALATS
oprk_mouse      IITAVYSVVF VVGLVGNSLV MFVIIRYTKM KTATNIYIFN LALADALVTT

                101                          III                150
oprd_rat        TLPFQSAKYL METWPFGELL CKAVLSIDYY NMFTSIFTLT MMSVDRYIAV
oprm_rat        TLPFQSVNYL MGTWPFGTIL CKIVISIDYY NMFTSIFTLC TMSVDRYIAV
oprk_mouse      TMPFQSAVYL MNSWPFGDVL CKIVISIDYY NMFTSIFTLT MMSVDRYIAV

                151                         IV                 200
oprd_rat        CHPVKALDFR TPAKAKLINI CIWVLASGVG VPIMVMAVTQ PRDGAVVCTL
oprm_rat        CHPVKALDFR TPRNAKIVNV CNWILSSAIG LPVMFMATTK YRQGSIDCTL
oprk_mouse      CHPVKALDFR TPLKAKIINI CIWLLASSVG ISAIVLGGTK VREDviECSL

                201                        V                  250
oprd_rat        QFPSPSWYWD TVTKICVFLF AFVVPILIIT VCYGLMLLRL RSVRLLSGSK
oprm_rat        TFSHPTWYWE NLLKICVFIF AFIMPVLIIT VCYGLMILRL KSVRMLSGSK
oprk_mouse      QFPDDEYWWD LFMKICVFVF AFVIPVLIII VCYTLMILRL KSVRLLSGSR

                251                       VI                  300
oprd_rat        EKDRSLRRIT RMVLVVVGAF VVCWAPIHIF VIVWTLVDIN RRDPLVVAAL
oprm_rat        EKDRNLRRIT RMVLVVVAVF IVCWTPIHIY VIIKALITI. PETTFQTVSW
oprk_mouse      EKDRNLRRIT KLVLVVVAVF IICWTPIHIF ILVEALGSTS HSTAAL.SSY

                301      VII                                       350
oprd_rat        HLCIALGYAN SSLNPVLYAF LDENFKRCFR QLCRAPCGGQ EPGSLRRPRQ
oprm_rat        HFCIALGYTN SCLNPVLYAF LDENFKRCFR EFCIPTSSTI EQQNSTRVRQ
oprk_mouse      YFCIALGYTN SSLNPVLYAF LDENFKRCFR DFCF...... .PIKMRMERQ

                351                  372
oprd_rat        ATARERVTAC TPSDGPGGGA AA
oprm_rat        NTREHPSTAN T......... ..
oprk_mouse      STNRVRNTVQ DPASMRDVG. ..
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Figure 1. Sequence alignment of the rat δ, μ and mouse κ opioid receptors with the putative seven transmembrane regions highlighted. Sequence numbering refers to the δ receptor. Intrinsic numbers of the μ and κ receptors require the addition of 19 and 10 residues, respectively.

around its helical axis to exhibit its hydrophobic moment pointing outwards of the helix bundle and in the direction of the bisector of the angle defined between the centers of the previous helix, the helix being computed, and the following one; (iv) tilting of the helix according to the available information deduced from the projection map of rhodopsin; and (v) translation of the center of the helix according to the rhodopsin electron density map.

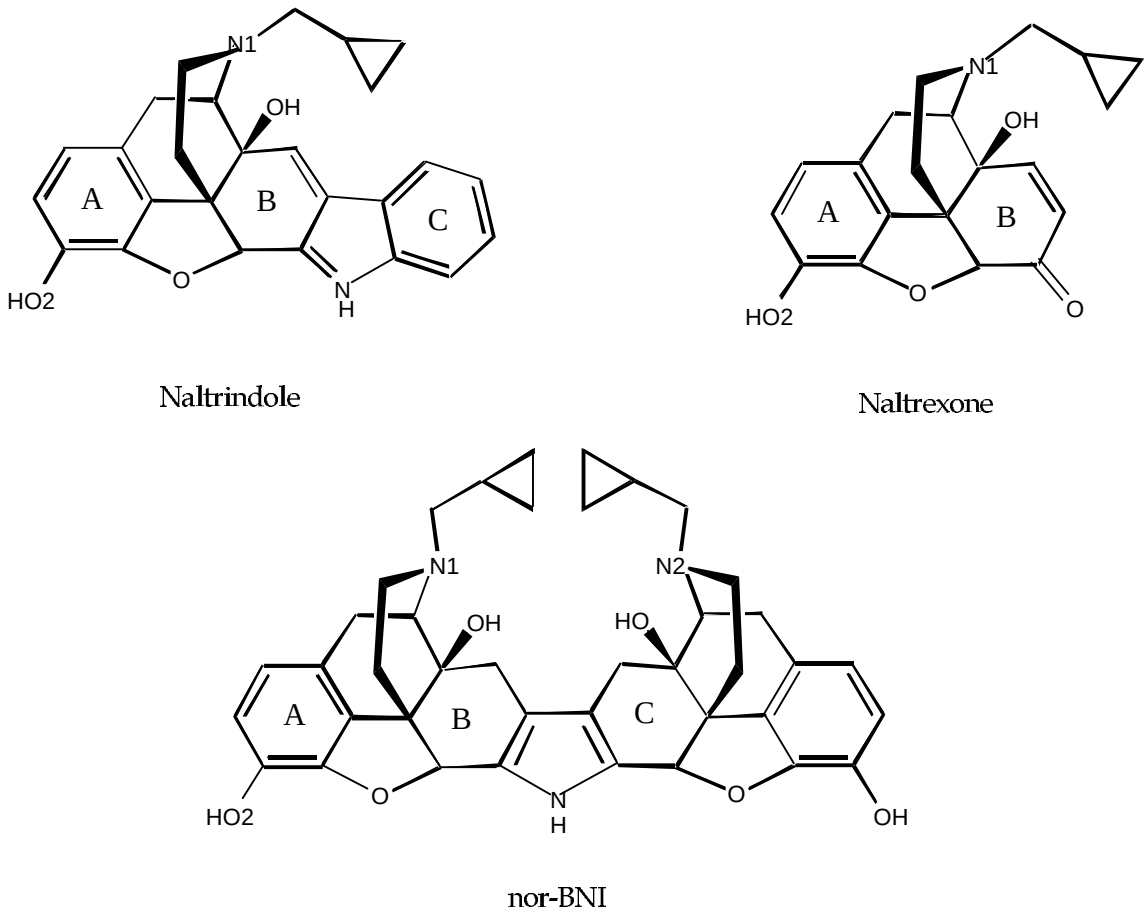

Figure 2. Chemical structures of naltrindole, naltrexone and nor-BNI. A, B and C designate different key chemical moieties for each ligand involved in ligand–receptor interactions.

Selected ligands and construction of the starting structure of the complexes

Molecular mechanics calculations of the ligands and of the complexes were carried out using the parm91 parametrization [40] of the AMBER force field. Structures of the three selected ligands (naltrexone, naltrindole and nor-BNI) were constructed with the PREP module of AMBER 4.0 [38] guided by the structures of similar ligands taken from the Cambridge data bank. Consistently with the parm91 parametrization of the AMBER force field, atomic partial charges for the antagonists were generated by fitting the molecular electrostatic potential computed with a STO-3G basis set using the GAUSSIAN94 suite of programs [41]. Figure 2 shows the chemical structures of the three ligands selected for the present study. Ligands were energy minimized and subsequently, manually docked inside the helix bundle of each of the three opioid receptors.

Complex refinement

Structures of the nine complexes after docking naltrindole, naltrexone and nor-BNI to the δ, μ and κ receptors were refined by energy minimization. The procedure was carried out in several steps using a dielectric constant of 4*r*. First, the opioid complexes were subjected to 1 500 cycles of steepest descent, keeping all the backbone and ligand atoms frozen in their initial positions to eliminate the worst steric interactions between atoms of the side chains. Under the same conditions, complexes were subsequently minimized using a conjugate gradient method to reach a convergence on the rmsd of the gradient when less than 0.001 Å. Subsequent steps consisted of smoothly lifting the constraints on the structure. In every step

minimization was performed with 500 cycles of steepest descent followed by a conjugate gradient algorithm until convergence was achieved when the rmsd of the gradient was less than 0.001 Å. In the first step backbone constraints were lifted although new constraints were imposed on helix lengths and on the distances between neighboring helices. In a subsequent step, constraints on the ligand were lifted and those on the distance between neighbor helices reduced to one half. Next, constraints on the distances between helices were lifted and constraints on the lengths reduced to one half. In the final step constraints on the lengths of the helices were lifted and the structures of the complexes were completely relaxed.

Results and discussion

Three-dimensional models of the three opioid receptors were constructed using BUNDLE [34], as explained in the Methods section. There are two sources of information to assess the reliability of the models. First, the results available from site-directed mutagenesis studies, deletions and chimeric opioid receptors. Second, the available structure-activity relationships studies on opiates. So, before doing any refining, the receptors were inspected to check if residues reported by site-directed mutagenesis experiments to affect the pharmacological profile of different opioid ligands, were oriented towards the interior of the bundle. However, it should be borne in mind that it is difficult to assign the results of many mutagenesis studies to direct effects on the interaction with ligands. In fact, mutations can alter the three-dimensional structure of the receptor, modifying the binding profile of the ligands. Information about point mutations of residues located on the transmembrane domains of the receptors were gathered from the on-line 'CPCR–family A–point mutation database' (GRAP) [42]. These residues include (numbering refers to each receptor, respectively): Asp^{95} in TM2; Ala^{123} and Leu^{125} in TM3 at the δ receptor. Asp^{114}, Val^{126}, Asn^{127} in TM2; Thr^{137}, Ile^{138}, Ile^{142}, Ile^{144} and Asp^{147} in TM3 and His^{297} in TM6 at the μ receptor. Finally, Asp^{105} in TM2; Asp138 in TM3 and Glu^{297} in TM6 at the κ receptor. Additional mutations were taken from the literature. These include Asp^{128} and Tyr^{129} in TM3 [43], Ser^{177} in TM4 [35] and Trp^{274} in TM6 [43] at the δ receptor, Ser^{196} in TM4 [35] at the μ receptor. Most of the mutated residues in our models face the interior of the receptor, except Ala^{123} and Trp^{274} in δ, and Ile^{138} and Ile^{142} in μ. These residues could be implicated in the structural stability of the receptor more than in a direct interaction with the ligands.

In order to rationalize ligand–receptor interactions on opiates, three selective opioid antagonists to each of the three receptors: naltrindole, naltrexone and nor-BNI were docked into the three unrefined receptor models. These 4,5-epoxymorphinans were selected because they are rigid molecules that cover the different pharmacological profiles exhibited by opiates with a minimal structural diversity. In these conditions, the role of the extracellular loops as part of the binding site is minimized, in contrast to the binding characteristics of peptide ligands as suggested by site-directed mutagenesis studies on opioids [16–24] and other receptors [44]. Furthermore, since they present common structural elements they may bind to the receptors in a similar fashion [29], being good models to investigate the message-address concept [8]. Furthermore, ligands were chosen to be antagonists, avoiding problems associated with receptor activation. Consequently, this study was aimed at providing insights into the molecular features that furnish selectivity among ligands sharing structural similarities.

The energy minimized structures of the ligands were manually docked into the seven helix bundle pockets of the δ, μ and κ receptors, respectively, guided by the results of mutagenesis studies on these receptors and previous structure–activity relationships studies. In what follows numbering of receptor residues refers to the δ receptor as displayed in Figure 1. Residue Asp^{128} is one of the reference points for docking the ligands. This residue is highly conserved in all the cationic neurotransmitter receptors [35, 45] and it has been demonstrated to be involved in ligand–receptor interactions in the case of the μ-opioid [18], m1 muscarinic [47], and β [48] and α_{2A} adrenergic receptors [49] by mutagenesis experiments. Besides the key role of Asp^{128} there is also information about the orientation that ligands may adopt when bound to their receptors. Indeed, pharmacological studies on chimeric receptors [21] suggest that binding of two μ-selective alkaloids, morphine and codeine, as well as that of the δ-selective cyclic peptide DPDPE takes place on transmembrane segments TM5-TM7. This information can be contrasted with the results accumulated on structure–activity studies of opiates, suitably described by means of the 'message-address' concept of the ligand–receptor interactions [8]. According to this hypothesis, all opiates exhibit some universal structural features, common to the ligands,

independently of their selectivity towards the different opioid receptors. These features constitute the message portion of the molecule and includes the amine nitrogen plus the phenoxyl group, and the N-terminal group and the tyrosine side chain in the case of opioid peptides. On the other hand, the address portion of the molecules carry some structural features that confer selectivity on the different ligands. In the case of the μ receptor, a polar interaction has been claimed to be responsible for ligand selectivity [4], whereas in the case of the δ receptor a non-specific hydrophobic moiety has been implicated in selectivity [7]. In the case of the κ receptor, naltrexone-derived bivalent ligands demonstrated that the second amine nitrogen functions as the address moiety of these ligands [49].

Assuming that the three antagonists selected for the present study bind to the receptors in the same fashion, these results suggest: (i) chemical groups involved in the message portion of the opioid ligands need to be accommodated in a region of the receptor that is common to all three opioid types; (ii) the address portion requires to be accommodated in a region where residues are not conserved in order to explain ligand selectivity. These constraints can be fulfilled if the amine nitrogen is placed in the neighborhood of the conserved Asp^{128} of TM3 and, simultaneously, the phenoxyl group accommodated in the region between TM3 and TM5, where three conserved residues: Tyr^{129}, Lys^{214} and Phe^{218} can provide a good interaction with this moiety. Moreover, when the message-constituting groups are placed in this way, ligands exhibit their address moieties facing a receptor region where several non-conserved residues of TM6 and TM7 lie close to the ligand, providing possible explanations for ligand selectivity.

The initial structures of the nine ligand–receptor complexes were energy minimized using the protocol described in the Methods section. After minimization, the starting orientation of the ligands changed slightly. The distance between the amine nitrogen and Asp^{128} increased in all the complexes and molecules rotated around an axis defined between the amine nitrogen and the furan oxygen common to all the ligands, in such a way that the phenoxyl group ended being more deeply buried inside the receptors. The refined structures were subsequently used to identify conserved and non-conserved residues surrounding the ligands. Analysis of the nine ligand–receptor complexes permitted the identification of 10 residues lying in close proximity to the ligands, presumably involved in ligand–receptor interactions: Asp^{128} and Tyr^{129} in TM3; Lys^{214} and Phe^{218} in TM5; Val^{281} (Ile in κ receptor), Thr^{285} (Ala in both μ and κ receptors), His^{278} and Trp^{284} (Lys and Glu in μ and κ receptors, respectively) in TM6; Leu^{300} (Trp and Tyr in μ and κ receptors, respectively) and Val^{296} (Gln and Leu in μ and κ receptors, respectively) in TM7.

As an illustration, Figures 3–5 depict pictorially the δ-naltrindole, μ-naltrexone and κ-nor-BNI complexes, respectively. Distances between the selected opioid receptor side chains and the ligands of the nine complexes after minimization are summarized in Table 1. Distances were computed from the center of the phenyl ring for Phe^{218}, Tyr^{129}, Tyr^{300}, Trp^{300} and His^{278}; from atom Cγ for Asp^{128}, Leu^{300} , Glu^{284} and Thr^{285}; atom Cβ for Val^{281}, Ile^{281}, Val^{296}, Ala^{285} and Leu^{296}; atom Nζ for Lys^{214} and Lys^{284}; atom $N\varepsilon_2$ for Gln^{296} and atom Nε for Trp^{284} to atoms on the ligands or pseudo-atoms representing the ring centers of the different moieties as shown in Figure 2. Analysis of the results displayed in Table 1 provides qualitative explanations of ligand affinities and selectivities for the different receptors on the basis of the number and type of polar and hydrophobic interactions between them and the receptors. However, due to a lack of experimental information on these receptors, both structural and in regard to the precise assignment of the different protein domains, distances measured on the nine ligand–receptor complexes need to be interpreted within a certain tolerance estimated to be around ±3 Å [50].

Let us first analyze ligand–receptor interactions at the message portion of the ligands. Table 1 shows that the distance between the amine nitrogen (N1) and Asp^{128} on TM3 ranges from 3.5–6.3 Å in the complexes. Except for the case of the nor-BNI-κ complex, this distance is rather long for a hydrogen bond interaction. However, such a long distance may justify the small effect observed when Asp^{128} is mutated to Ala or Asn on the pharmacological profile of naltrindole and naloxone, a molecule similar to naltrexone, both in δ and μ receptors [42]. Other residues putatively involved in the interaction with the receptor at the message region are Phe^{218} and Tyr^{129}. These two amino acids exhibit a similar distance to ring A in most of the complexes, and can be considered to be involved in either π–π interactions with the ligand or, more generally speaking, in a less specific hydrophobic interaction. Indeed, when Tyr^{129} is mutated to Phe^{129} in the δ receptor there is no alteration of the pharmacological profile of naltrindole and naloxone, whereas when it is mutated to Ala, the binding affinity

Table 1. Values of distances (in Å) between the selected opioid receptor side chains and the ligands in the minimized models

δ, μ, κ	Moiety	Naltrindole			Naltrexone			nor-BNI		
		δ	μ	κ	δ	μ	κ	δ	μ	κ
Asp^{128}	N1	5.1	4.1	5.0	5.9	3.9	6.3	4.9	5.3	3.5
Lys^{214}	O2	2.9	3.1	16.5	3.6	7.8	15.9	6.1	8.9	14.2
Phe^{218}	A	4.1	5.8	11.3	5.5	4.5	10.7	4.7	5.4	8.9
Tyr^{129}	A	4.9	8.0	8.1	4.9	4.6	7.8	5.5	5.2	5.3
Val^{281}(Val, Ile)	B	9.3	6.0	6.3	6.4	5.8	5.8	7.2	6.8	5.3
	C	5.8	5.9	4.9	–	–	–	6.2	7.6	5.6
Thr^{285}(Ala, Ala)	B	10.0	7.1	8.6	9.7	7.4	8.2	8.5	8.0	7.8
	C	5.8	5.0	10.2	–	–	–	5.1	5.8	5.0
Leu^{300}(Trp, Tyr)	B	7.5	6.5	6.5	5.7	5.8	6.0	10.1	5.8	6.9
	C	6.4	7.5	9.2	–	–	–	9.8	6.8	6.6
Val^{296}(Gln,Leu)	B	13.8	5.8	15.4	12.8	10.8	9.9	10.0	11.5	13.0
	C	11.3	3.4	17.1	–	–	–	7.4	9.0	11.4
His^{278}	B	6.1	6.9	7.7	6.0	7.1	10.8	10.2	7.4	8.3
	C	5.8	10.8	9.1	–	–	–	11.4	10.2	10.4
Trp^{284} (Lys, Glu)	N1	18.5	9.9	9.6	17.2	11.4	10.4	16.2	10.0	10.0
	N2	–	–	–	–	–	–	6.7	8.3	3.8

of naloxone is considerably affected [43]. However, it has been shown that mutation of Phe^{218} to Ala in the δ receptor does not change the pharmacological profile of naltrindole or naloxone [43], suggesting that Tyr^{129} is more directly involved in a more specific interaction with ring A of opiates. Furthermore, not only the presence of the aromatic ring of Tyr^{129}, but its spatial arrangement may also be important for ligand–receptor interactions. Thus, in a mutational study involving the conserved Ser^{177} on TM4, the mutation S177L in δ and μ receptors turned classical antagonists into agonists [36]. Although in our models ligands do not interact directly with Ser^{177}, this residue exhibits a hydrogen bond with the conserved Tyr^{129} of TM3 in all the models, fixing its side chain conformation. Substitution of serine for the more voluminous Leu may force to a reorientation of the Tyr^{129} side chain that could lead to different activation modes. Present models also suggest that the conserved residue Lys^{214} interacts with the hydroxyl group of, the phenoxyl moiety. This is particularly clear in the δ and μ complexes with naltrindole and naltrexone (Figures 3 and 4). This structural feature could explain the favorable effect on the affinity exhibited by different opiates of the hydroxyl moiety on ring A [1]. In the case of the κ receptor, Lys^{214} and Phe^{129} appear to be a little removed from the interaction with the ligands (Figure 5), although this might be considered an artifact of the model due to the orientation of the helix and caused by an inaccurate transmembrane sequence assignment.

Let us now analyze the interaction of the address portion of the ligands considered to provide explanations of the observed ligand selectivity towards the different receptors. The high affinity of naltrindole at the δ receptor ($K_i = 0.10$ nM) [51] can be explained by the good complementarity between the hydrophobic indole moiety of the ligand and the hydrophobic receptor pocket located between TM6 and TM7. Figure 3 shows good interaction contacts between the ligand and His^{278}, Val^{281}, Thr^{285}, Leu^{309} or Ile^{174} (not shown). In contrast, naltrexone does not have a large hydrophobic moiety, providing a possible explanation for the 100 times lower affinity with respect to naltrindole, observed for the δ-receptor ($K_i = 10.7$ nM) [51]. With regard to the nor-BNI no K_i value has yet been reported for the δ-receptor, however, the ligand has been described as κ-selective [52]. Accordingly, one cannot expect a ligand–hydrophobic pocket fit as good as is observed in the naltrindole– δ-receptor complex. Indeed, distances between the ligand and the side chains (Table 1) are longer than in the naltrindole complex, suggesting an unsatisfactory ligand–receptor interaction.

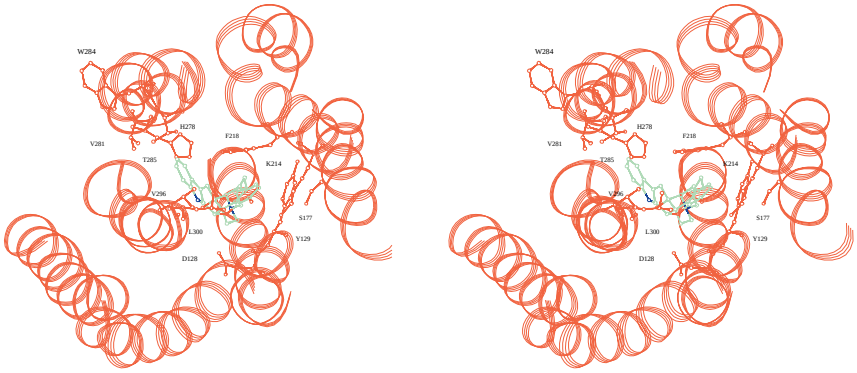

Figure 3. 3D structure of the refined model of the naltrindole–δ-opioid complex. Relevant amino acid residues involved in the interaction with the ligand are displayed.

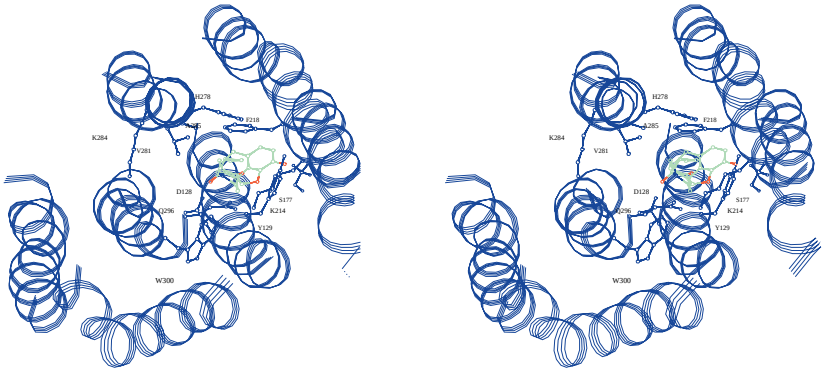

Figure 4. 3D structure of the refined model of the naltrexone–μ-opioid complex. Relevant amino acid residues involved in the interaction with the ligand are displayed.

The μ-receptor exhibits a non-conserved residue Gln^{296} that corresponds to a Val and a Leu in delta and kappa, respectively. This residue can provide a polar ligand–receptor interaction, which may explain the higher affinity exhibited by naltrexone for this receptor (K_i = 0.27 nM) [51], due to an interaction of Gln^{296} with the carbonyl oxygen of ring B, as can be seen in Figure 4. Naltrindole exhibits a hydrogen donor in an equivalent position to naltrexone carbonyl oxygen, suitable for interaction with the glutamine residue. However, the bulky hydrophobic indole moiety may prevent this interaction, providing an explanation for the 100-fold decrease in affinity observed by naltrindole in regard to naltrexone at the μ-receptor (K_i = 33.9 nM) [51]. No affinity data are available in the case of nor-BNI, but from inspection of the distances between the ligand and receptor and the nature of the intermolecular interactions, we conclude that the ligand should exhibit an affinity for this receptor similar to that of naltrindole.

Finally, with regard to the κ receptor, the higher affinity exhibited by nor-BNI can be explained, as was suggested earlier [53], on the basis of the strong interaction between the non-conserved residue Glu^{284} (a Trp in δ and a Lys in μ) with the second charged nitrogen (N2) not involved in an interaction with Asp^{128}. Figure 5 shows the nor-BNI–κ-opioid complex. The interaction between this residue and the N2 atom of nor-BNI maintains the ligand in a position in which a stronger interaction can be established between Asp^{128} and the other charged nitrogen (N1) of the ligand. This interaction cannot be conferred by the other two ligands, and this is thus a plausible reason for the selectivity exhibited by nor-BNI towards this receptor. Naltrexone exhibits distances to the conserved residues of this pocket similar to those measured on the μ-receptor, which means that its affinity for the κ-receptor is high (K_i = 0.93 nM). In contrast, naltrindole exhibits a lower affinity (K_i = 19.6 nM), which can be explained on the basis of a

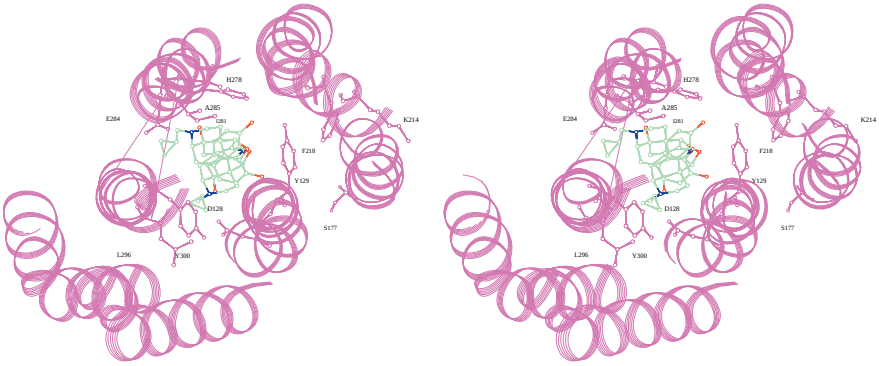

Figure 5. 3D structure of the refined model of the nor-BNI–κ-opioid complex. Relevant amino acid residues involved in the interaction with the ligand are displayed.

poor ligand–receptor interaction at the address pocket of the receptor.

The present modeling studies provide new insights into the interaction of three antagonists and the three opioid receptor types and supports the message–address concept as a basis for explaining ligand–receptor interactions in opiates. The message–address concept was developed exclusively on the basis of ligand features and was originally proposed to explain the interaction of bioactive peptides with their receptors [54]. Accordingly, ligands exhibit two clearly differentiated receptor recognition elements structurally joined by a spacer. One of them is common to all ligands of a target receptor (message), whereas the second one (address), in addition to being involved in binding, also determines ligand selectivity. Structure–activity relationship studies on opiates soon suggested that these ligands fit this pattern well [8], and this model of ligand–receptor interaction has been widely used since then.

On the other hand, with regard to the features of the receptors, consistent with the information deduced from rhodopsin, TM3 is less exposed to the lipid bilayer than the other transmembrane regions, dividing the binding site into two separated regions. Furthermore, mutagenesis studies reveal that both TM3 and TM5–TM7 are involved in ligand binding. TM3 contains an Asp^{128} residue that is conserved among several amine receptors and this is suggested to be responsible for the amine–receptor interaction. Interestingly, if the amine nitrogen is situated at a short distance from Asp^{128}, the message and the address ligand segments occupy the two respective receptor pockets. Residues in the pocket formed between TM3 and TM5 are conserved in the three receptors and allow an explanation to be given of the nature of the interactions of the message portion of the ligands. On the other hand, the pocket involving TM6 and TM7 contains non-conserved. residues on the three opioid receptor types and can provide explanations for the observed selectivity of the ligands. The message–address concept was also invoked by Metzger et al. [28] in using their models to explain the binding characteristics of different opiate ligands at their receptors. They proposed that the cationic amine of the tyramine moiety of the message portion of opiates interacts with the conserved Asp^{128}, the aromatic portion being implicated in a π–π interaction with different residues, such as Tyr^{129}, Phe^{218}, Trp^{284} and His^{278}. The first two of these residues are also implicated in present models, whereas the last two participate in the interaction with the address portion of the ligands. These differences are probably an artifact of the models constructed in Reference 28, since the authors used bacteriorhodopsin as a template to build the opioid receptors. Bacteriorhodopsin has only one binding pocket and consequently the authors could not differentiate two regions that, according to the present results, can be clearly associated with the message and address portions of the ligands. Regarding the address portion, the authors pointed out, as we do in the present models, the role of Glu^{284} in the selectivity of the κ receptor. Other models published in the literature also propose that Asp^{128} is implicated in the interaction with the cationic amine moiety [29, 30].

In summary, the models suggested here make predictions about key residues involved in ligand–receptor interactions. The present work suggests several residues that have not yet been studied by mutagenesis, which are thought to be involved in ligand–receptor interactions. These include Tyr^{129} in TM3 (in other receptors than δ), Lys^{214} and Phe^{218} (in other

receptors than δ) in TM5, Val^{281} (Ile in the κ receptor) Thr^{285} (Ala in both μ and κ receptors), His^{278} (in other receptors than μ) and Trp^{284} (Lys and Glu in μ and κ receptors, respectively) in TM6, Leu^{300} (Trp and Tyr in μ and κ receptors, respectively) and Val^{296} (Gln and Leu in μ and κ receptors, respectively) in TM7. Confirmation of these results by site-directed mutagenesis studies would further validate the models and would reveal whether they are accurate enough to be used for de novo ligand design.

Conclusions

In the present study we have applied a general strategy to construct the three opioid receptor types, μ, δ and κ. Before any refinement, models were checked for their compatibility with the information available from site-directed mutagenesis studies on these receptors. The orientation of most of the residues, with the exception of Ala^{123} in δ, and Ile^{138} and Ile^{142} in μ, were adequately predicted. Models were used to dock three selective antagonists onto the three receptor models. Docking was carried out manually and guided by positioning the ligands in such a way that the conserved aspartic residue of TM3 was close to the amine nitrogen of the ligands and so that the phenoxyl group was placed in a region between TM3 and TM5 common to the three receptors. Specifically, the aromatic ring was placed in a pocket formed by the conserved Tyr^{129} of TM3 and Phe^{218} of TMS, whereas the hydroxyl group was accommodated close to the conserved Lys^{214} of TM5. In this orientation the ligands extended their address portion over the TM6 and TM7 regions. These helices exhibit residues that can explain the observed selectivity of the three antagonists.

The models satisfactorily explain the structure–activity relationships observed in opiates. Furthermore, these models can be used to guide interesting point mutations of residues that are in direct interaction with the ligands. Studies in this direction would further validate the present models, which could subsequently be used for de novo ligand design.

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