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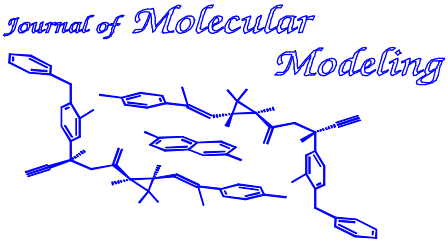

Computer-Aided Receptor Modelling of Human Opioid Receptors: (*Mu, Kappa* & *Delta*).

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Abstract

Opioid receptors (OPRs) are important agents in the centeral nervous system (CNS) function. These receptors belong to "G-Protein Coupled Receptors (GPCRs)" which have structural similarity with the *BACTERIORHODOPSIN* (bR). Because of receptor location in the membrane, three dimensional (3D) structure of GPCRs are unknown. The Computer-Aided Receptor Modelling on the basis of amino acid sequence, accompanied by the experimental results is a useful method to understanding the structure and mechanism of these receptors.

In this study we tried to modell three types of Human Opioid Receptors; *Mu*, *Kappa* and *Delta*. We applied several methods to predict secondary structure (such as Hydropathicity Plot) of opioid receptors and also determined the possible regions of transmembrane helices (TMHs). Results were confirmed by inclusion of other human GPCRs sequence in multiple alignment methods. Then similarity between these receptors and bR were calculated on the basis of parameters such as Mutation Matrix and Secondary Structure Scale. After calculation and refinment of geometric coordinates of atoms located in helices by computerized mutation method (on the basis of 3D structure of bR, as a template) these data were corrected and optimized using Molecular Mechanics Calculations (AMBER Force Field). We used Morphin, Naloxone, Ethylketazocine (EKC) and SKF-10047 as general/specific ligand for these receptors. We optimized conformation of ligands by Quantum Mechanical Semiemprical Calculations (MOPAC). In final step we tried to dock ligands into the receptor cavity with attention to Mutagenesis Data and Structure-Activity Relationships (SAR) information.

Our results show that in Delat receptors 'ASP-96' in TMH-II is important to binding of agonists and antagonists. In *Mu* receptors charged amino acid residues in TMH-II (ASP-116), TMH-III (ASP-149) and TMH-VI (HIS-299) interact with agonists. In Kappa receptors TMH-VI (GLU-297) and TMH-II (ASP-106) play a major role in interaction with antagonists. All of the mentioned residues are located in or near the inner cavity of receptors. With attention to results we suggest that other sites of receptors (such as loops and terminals) may be interact with ligands.

Keywords: Opioid Receptors, Morphine, Modelling, G-Protein Coupled Receptors, Bacteriorhodopsin.

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Introduction

Opioid receptors are most identified with the analgesic properties of opiate drugs [1]. Their agonists can modify pain in virtually every test of spinal and supraspinal analgesia and they perominently implicated in mechanism of opiate-induced reward and reinforcement [2]. Opioid drugs are the principle agents used for treating sever pain. Their value is reflected by the effort expended on producing new compounds and understanding their mechanism of action and effects.

The cloning of cDNAs encoding a number of OPRs [3-5] has demonstrate that the three most prevalent OPR subtype *Kappa, Mu* and *Delta*, all belong to the family of Rhodopsin-like receptors within the superfamily of GPCRs [6]. A number of observation suggests that all GPCRs evolved from a common ancestor.

Because of location GPCRs in membrane, The 3D structure of these receptors are unknown. There is now enough evidence to generate reasonable 3D models of GPCRs using "*Computer-Aided Molecular/Receptor Modelling*". But 3D interpretation is still very speculative, so the projection map can not be used directly as a modelling template. However, it clearly exhibits a 7-helix bundle, and a number of further criteria [7] suggests that the GPCRs are structurally anlogous to *BACTERIORHODOPSIN* although sequence homology is not detectable [8]. Despite of sequence homology with GPCRs, the parallel between the overall 3D structural patterns is striking. The 3D structure of helices in bR was revealed by electron cryomicroscopy [9].

Attemps to build a GPCR model vary in their degree of adherence to the bR structure. In several models the overall topology, i.e. the 7-helix bundle, was incorporated, and this information was supplemented by general structural features of membrane proteins and by experimental data [10-11]. On the other hand, these models are based on the assumption of structural analogy and, hence adhere more closely to the bR structure [12-13].

Beacause of human OPRs are the ultimate targets of therapeutic opiods drugs, it is particularly important to have models of these receptors. We report here our investigation of human OPRs primary sequence homology and alignment, prediction of secondary structure and the construction of 3D models for *Mu, Kappa* and *Delta* human opioid receptors with their general ligands using bR as a template.

Methods

As a first step in the construction of the GPCR 3D models, exhausive primary sequence comparison and hydropathicity analysis were required. The following GPCR sequences were analyzed: Human *Mu* (*OPRM_HUMAN*), *Kappa* (*OPRK_HUMAN*) and *Delta* (*OPRD_HUMAN*).

The alignment was performed with the method of Needleman-Wunsch [14] and Lipman-Pearson [15] using the Dayhoff Similarity Table [16] for amino acids as implemented in the HUSAR [17] and MULTALIN [18] softwares. To obtain an optimal alignment, we used several Gap Penalty and finally the comparison was refined manually (Fig. 1).

The prediction of secondary structure of OPRs was performed with the several methods:

- Kyte-Doolittle Parameter Set [19].
- Goldman-Engelman-Steitz Parameter Set [20].
- Garnier Scale (GOR Method) [21].
- Manual Refinment (GPCRs Overall Topology).

The refined model of bR was obtained from Brookhaven Protein Databank (entry *1BRD*) and the primary structure of bR and human OPRs (*Mu, Kappa* and *Delta*) were obtained from Swiss-Prot databank (entry *P02945, P35372, P41145* and *P41143*, respectively).

Due to the conformational flexability of the extra- and intracellular loop region, we have only attempt to model the transmembrane helices (TMHs) of the OPRs. In order to obtain a Homology-Based Model of the TMHs of the OPRs the following protocol was followed:

i) The sequences was aligned with that of bR as described above. (Fig. 2).
ii) The backbone of bR (1BRD) was used as a template for the positioning of the TMHs of the OPRs.
iii) The side chains were adjusted to adopt likely positions.

The receptors were optimized by the AMBER, Ver.4.1 [22] force fields using molecular mechanics calculations with the „Kollman All Atoms“ parameter set (unconstrained pathway) in the following way:

Step I) The single helices were minimized for 1000 steps using the conjugate gradient minimizer.
Step II) The transmembrane part the receptor models was constructed and again minimized for 2000 steps.

A nonbonded cutoff of 8 Å was used. To account to some extent for the membrane environment, a distance-dependent dielectric constant of 5 and 1-4 non bonded interactions of 0.5 were chosen. Conjugate gradient minimization used until the RMS energy gradient was acheived a value below 0.1 Kcal/mol·Å^2. The N-terminus was capped with an Acetamido group, and C-terminus with a Carboxamido group.

We used some compounds as a general/selective or specific ligands:

- For Mu : Morphine as a agonist and Naloxone as a antagonist (Fig. 3 and 4, respectively).

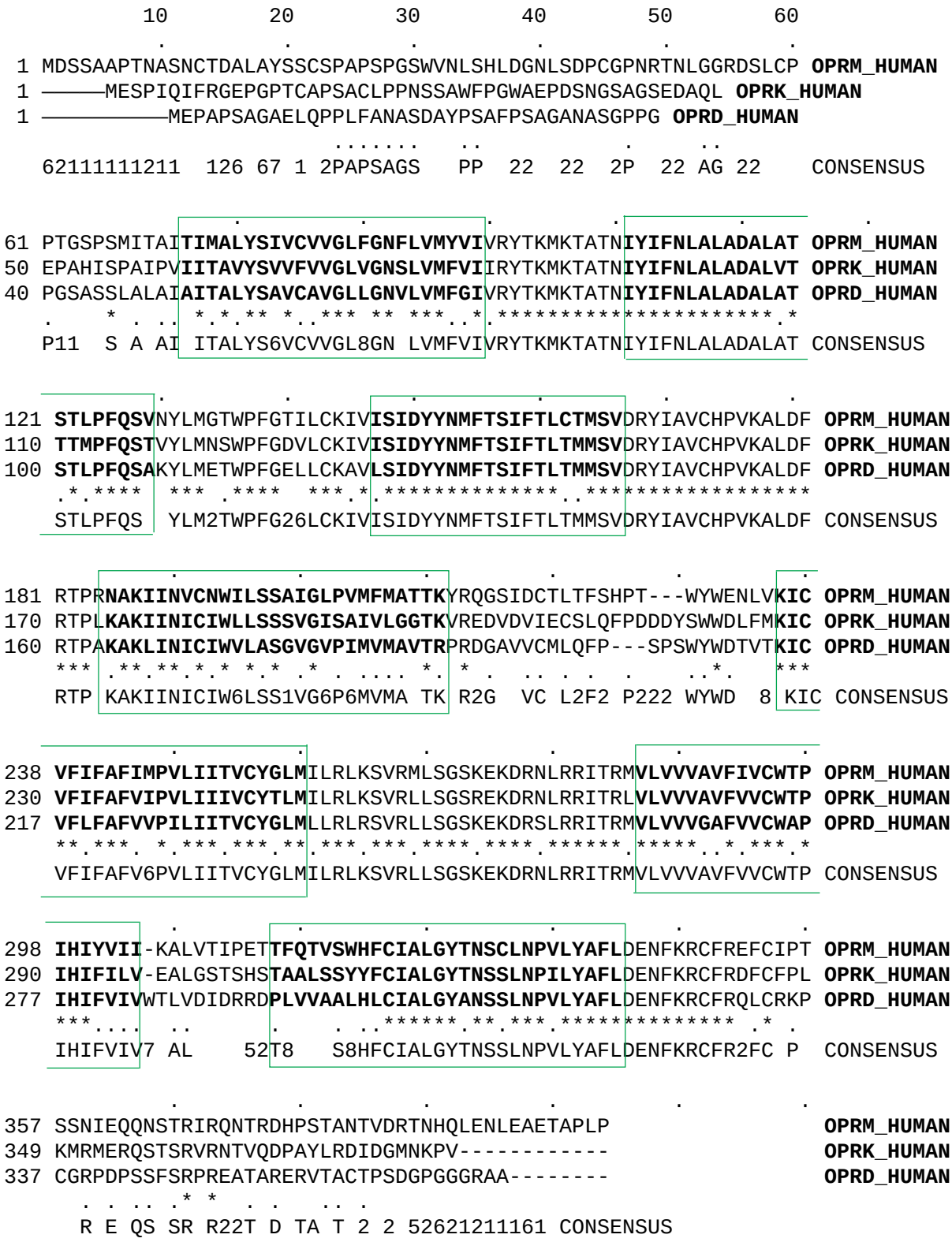

Figure 1. *The Multiple Alignment of Human Opioid Receptors. Blocks Showed Our Predicted Trans Membrane Helices Region.*

Figure 2 (next page). *The Alignments of Helices Between Bacteriorhodopsin (*BACR_HALHA*) and Human Opioid Receptors.*

- **HELIX I**:

BACR_HALHA_I	21	PEWIWLALGTALMGLGTLYFLVKGM	45
OPRM_HUMAN_I	81	ITIMALYSIVCVVGLFGNFLVMYVI	105
OPRK_HUMAN_I	60	VIITAVYSVVFVVGLVGNSLVMFVI	84
OPRD_HUMAN_I	50	IAITALYSAVCAVGLLGNVLVMFGI	74

- **HELIX II**:

BACR_HALHA_II	51	DAKKFYAITTLVPAIAFTMYLSMLL	69
OPRM_HUMAN_II	106	.NIYIFNLALADALATSTL.....	123
OPRK_HUMAN_II	96	..IYIFNLALADALVTTTMPFQST.	117
OPRD_HUMAN_II	85	.NIYIFNLALADALATSTL.....	102

- **HELIX III**:

BACR_HALHA_III	87	EQNPIYWARYADWLFTTPLLLL	108
OPRM_HUMAN_III	146	.ISIDYYNMFTSIFTLCTMSV.	165
OPRK_HUMAN_III	133	IVISIDYYNMFTSIFTLTMMSV	154
OPRD_HUMAN_III	125	.LSIDYYNMFTSIFTLTMMSV.	144

- **HELIX IV**:

BACR_HALHA_IV	119	GTILALVGADGIMIGTGLVGAL	140
OPRM_HUMAN_IV	196	..LSSAIGLPVMFMATTK....	211
OPRK_HUMAN_IV	174	KAKIINICIWLLSSSVGISAIV	195
OPRD_HUMAN_IV	175	.....LASGVGVPIMVMAVTR.	190

- **HELIX V**:

BACR_HALHA_V	149	VWWAISTAAMLYILYVLFFGFT	170
OPRM_HUMAN_V	235	.KICVFIFAFIMPVLIITVCYG	256
OPRK_HUMAN_V	227	.KICVFIFAFVIPVLIIIVCYT	247
OPRD_HUMAN_V	215	.KICVFLFAFVVPILIITVCYG	235

- **HELIX VI**:

BACR_HALHA_VI	179	EVASTFKVLRNVTVVLWSAYPVVWLI	204
OPRM_HUMAN_VI	283	MVLVVVAVFIVCWTPIHIYVIIK...	305
OPRK_HUMAN_VI	276	VLVVVAVFVVCWTPIHIFILVEAL..	299
OPRD_HUMAN_VI	262	.MVLVVVGAFVVCWAPIHIFVIVW..	284

- **HELIX VII**:

BACR_HALHA_VII	215	NIETLLFMVLDVSAKVGFGLILLR	238
OPRM_HUMAN_VII	314	.TFQTVSWHFCIALGYTN.....	330
OPRK_HUMAN_VII	312	YYFCIALGYTNSSLNPILYAFL..	333
OPRD_HUMAN_VII	294	...PLVVAALHLCIALGYAN....	

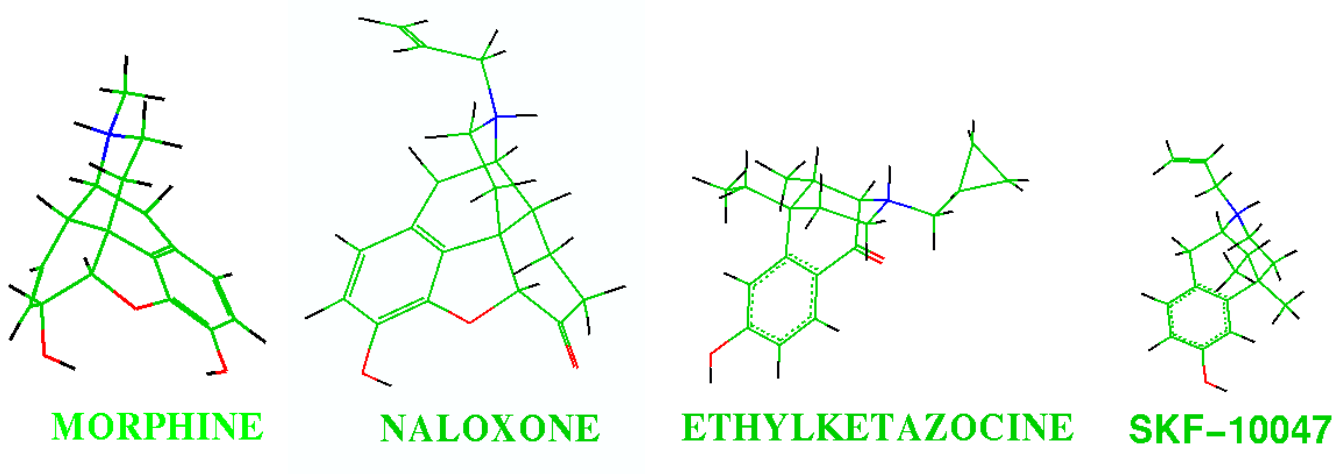

Figure 3 - 6. *The Structure of General / Specific Ligands for Opioid Receptors.*

- For Kappa: Ethylketazocine (EKC) as a agonist and SKF-10047 as a antagonist (Fig. 5 and 6, respectively).
- For Delta: SKF-10047 as a antagonist.

A systematic search for obtaining best conformation of ligands was performed by the INSIGHT II package and finally geometry and charges distribution of suitable conformation of ligands was calculated by the MOPAC package [23], using PM3 and AM1 [24] hamiltonian. (Figure 3 – 6)

Data about probable binding sites and important residues involved in interaction of ligand-receptor obtained from mutagenesis experiments. With attention to these data and structure-activity relationships studies of ligands, the selective and/or specific ligands were manually and rigidly docked into their putative binding sites. The docking procedure was repeated several times with different initial orientations of the side chains and of the ligand in order to obtain the best possible inteaction complexes. Charges for the ligands were imported from the MOPAC output files.

The drug-receptor complexes were optimized by molecular mechanics calculation (AMBER force fields, 4000 Steps, Conjugate Gradient, Cutoff = 8 Å and Gradient less than 0.1 Kcal/mol·Å^2). Final geometry was acheived for ligands and receptors (Figures 7, 8, 9 and 10)

The primary interactive modelling, display and file generation was acheived with molecular modelling package; WhatIf, Ver.3.0 [25] and finally display, systematic search of ligands conformation and file generation was acheived with molecular modelling package INSIGHT II, Ver.2.9/3.1 [26]. All calculations were performed on Silicon Graphics and SP2 computers.

Results and Discussion

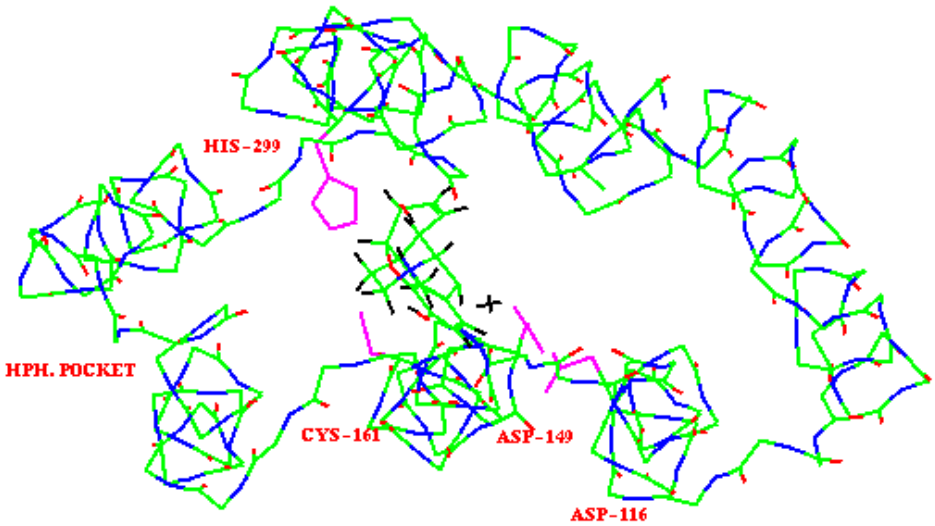

Figure 7. *Three-Dimensional Views of Ligands-Opioid Receptors Complexes. Backbone of human Mu receptor, active site and morphine*

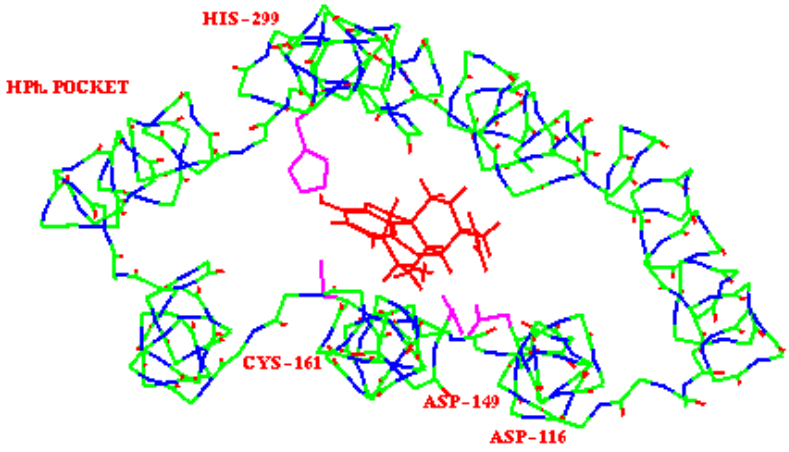

Figure 8. *Three-Dimensional Views of Ligands-Opioid Receptors Complexes. Backbone of human, Mu receptor active site and naxolone.*

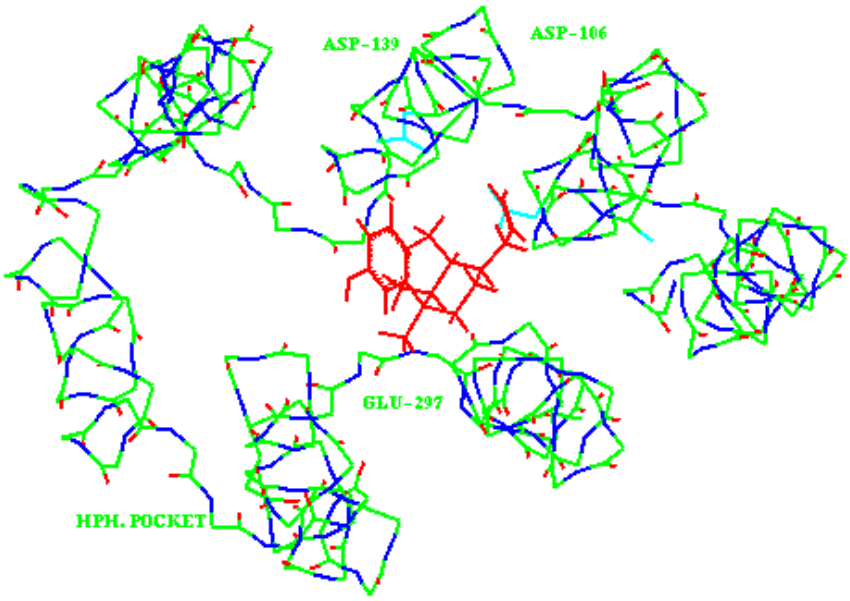

Figure 9. *Backbone of human Kappa receptor, active site and ethylketazocine.*

We briefly explain the results obtained through this study:

1.) In TMH-I, the motif GXXGN occures in OPRs, rather than the GN motif present in biogenetic amine. In all sequences the last five C-terminal residues of this helix are frequently occupied by basic residues, indicating the end of trans membrane domain. Such basic residues may serve as "Membrane Anchors".

2.) In TMH-II, the residue preceding the conserved Leu in the LXXXD motif is a conserved Serine for the biogeneic amine receptors, but is an Asn in the OPRs. The PRO is consistently spaced by seven residues from the 'ASP' in the OPRs (eight residues in biogenic amine receptors).

3.) The conserved 'ASP' in TMH-II was shown to be a Sodium-Dependent Allosteric Regulatory Site in the OPRs.

4.) The DRY motif, characteristic of the third transmembrane domain in GPCRs, is supposedly important for coupling of the OPRs to G-proteins unit and not for ligand binding.

5.) In *Kappa* receptor one or more of the first six positions in the N-terminal sequence of TMH-IV are generally occupied by LYS or ARG residues. Again these residues could well serve as "Membrane Anchor". This condition has not been found in *Mu* and *Delta* receptors.

6.) The CXXP motif and WXP motif in TMH-VI has been found in all of the OPRs.

7.) Results showed that the hydrophobic side of each helix was facing the lipid face and the hydrophilic side of each helix was facing either another helix or the pore formed by the putative bundle.

8.) The assembly of helices maintained a clockwise order, when seen from the intracellular side. Non of the helices were intersecting.

9.) Five potential glycosylation sites are present on the extracellular N-terminal amino acid sequence.

10.) The *Mu* receptor has about 60% amino acid identity to the *Kappa* and 65% to *Delta* receptors.

11.) The *Kappa* receptor has about 59% amino acid identity to the *Delta* and 61% to *Mu* receptors.

12.) 'ASP' in TMH-II and TMH-III,is generally seen as the main anchoring point for agonist and antagonist binding. Residues in other TMH domain (TMH-V) appear to be involved in determining the selectivity of these receptors for their agonists, e.g. Serine interacts with the agonist hydroxyl moiety. Our finding suggest that the induction of a conformational change in 'ASP' by an agonist could be a genertal and crucial step in OPRs stimulation. On the other hand conformational changes in intracellular loops to rearrangment of the seven-helix bundle, the so called „ARG Switch" [27], or ligand-mediated "Proton Transfer" mechanims [28].

13.) Docking showed that the amino group of Morphine to be within 3 to 4 Å of the 'ASP' residue (in TMH-III) and the aromatic ring to be in close (less than 5 Å) proximity of TRP residues of the TMH-IV and TMH-VI.

14.) It appears that the ligand is in contact with only four of the TMH-III, TMH-IV, TMH-V and TMH-VI, suggesting that these helices are responsible for binding selectivity. Probabely 'HIS' in TMH-VI is important in interaction with ligands.

15.) Our results showed that following residue are important in the drug-receptor interactions:

- In **Mu** Receptor:
  - 'ASP-116' in TMH-II.
  - 'ASP-149' in TMH-III.
  - Hydrophobic residues in TMH-VII, TMH-VI and TMH-I as a hydrophobic pocket.
  - 'HIS-299' in TMH-VI.
  - 'CYS-161' in TMH-IV.
- In **Kappa** Receptor:
  - 'ASP-106' in TMH-II.
  - 'ASP-139' in TMH-III.
  - Hydrophobic residues in TMH-VII, TMH-VI and TMH-I as a hydrophobic pocket.
  - 'GLU-297' in TMH-VI.
- In **Delta** Receptor:
  - 'ASP-96' in TMH-II.
  - 'ASP-129' in TMH-III.
  - Hydrophobic residues in TMH-VII, TMH-VI and TMH-I as a hydrophobic pocket.

Conclusion

In the present study we have combined results from site-specific mutagenesis studies on the OPRs with findings from different molecular modelling approaches such as conformational analysis, pharmacophore fitting and receptor docking studies. From our findings the followin conclusions emerge:

- The different conformations of 'ASP' in TMH-III and TMH-II observed in our modelling studies upon agonists or antagonists binding indicate that these Aspartic Acids may play a key role in receptor simulation. Upon binding of agonists one of Aspartic Acids changes its conformation and points in the direction of TMH-V, which contains residues responsible for the observed selectivity, i.e. LYS. In this way Aspartic Acids in TMH-III and TMH-II is assigned a crucial function in triggering GPCRs stimulation.
- There still remain much work to be done on the characteization of OPRs ligand recognition domaine, loop building (important for peptide ligands), G-protein coupling mechanisms, and receptor correlates for opioid tolerance and dependence.
- Modelling of GPCRs has been become an important tool in understanding drug-receptor interactions and in the development of new ligands for these receptors.

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