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Homology modeling and docking studies of Human Neurokinin receptors NKR1, NKR2 and NKR3

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Abstract

In order to understand the mechanisms of ligand binding and the interaction between the ligand and the Human Neurokinin receptors NKR1, NKR2 and NKR3, three-dimensional (3D) models of the three receptors are generated based on the crystal structure of the bovine rhodopsin (PDB code 1u19A) by using Modeller . Accordingly 25 models are generated and the model having lowest modeler objective function was choosed for the further assessment. The models thus generated are assessed using ProSa II and Procheck. The results show that the models are reliable. With the generated models, a flexible docking study was performed. The docking results indicated the conserved residues involved in antagonist binding. The interactions proposed in this study are useful to understand the potential mechanisms of the respective antagonists used in the study.

Keywords: Homology Modeling, Docking, Neurokinin Receptors, Modeller, AutoDock, ProSa, Procheck.

Introduction

The neurokinin receptors (NKR) are peptide compounds of 350- 500 aminoacids. They belong to the family of G-protein coupled receptors (GPCR's [1]. Three NKR subtypes, NKR1, NKR2 and NKR3 have been identified by molecular cloning and sequence analysis. They are characterized by their endogenous ligands substance p, neurokinin A and neurokinin B. The endogenous ligands belong to a family of amidated neuropeptides tachykinins [2].

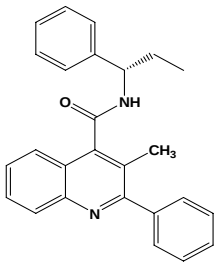
Some experimental work gives structural information of the NKR. Three neurokinin antagonist pharmacophore models accounting for hydrogen bonding groups in the 'head' and 'tail' of NK receptor ligands was developed by a procedure for treatment of hydrogen bonds during superimposition [3]. Elling *et al.* [4, 5] have

performed extensive mutagenesis work to identify zinc ion binding sites in the NK1R. Donnelly et al [6] have made a Asp79Asn + Asn303Asp NK2R double mutant and they conclude that there is a direct interaction between the side chains of the two residues, that places TM2 and TM7 in contact, and the relative orientation of the two helices can be deduced.

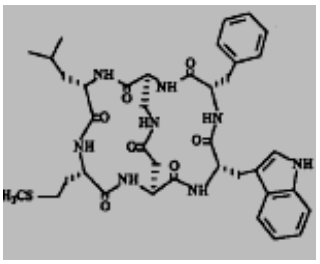
The NK1 receptor is implicated in depression, asthma, emesis, anxiety, and pain. A role for SP is proposed in the regulation of pain, asthma, psoriasis, inflamatory bowel disease and, in the CNS, emesis, migraine, schizophrenia, depression and anxiety. It was also shown that a series of N-acyl-L-typtophan benzyl esters are potent antagonists of the human NK-1 receptor [7]. Interaction of a potent representative of tryptophan series, N-acetyl-~-tryptophan-3,5- bistrifluoromethylbenzyl ester (L-32,138), with the human NK-1 receptor was well characterized.L-732,138 inhibits the binding of 1251-SP to the human NK-1 receptor stably expressed in CHO cells. L-732,138 has >1000-fold lower affinity for the homologous NK-2 and NK-3 receptors.

Neurokinin A, exerts prominent biological effects, preferentially mediated by activation of the tachykinin NK 2 receptor [8-11]. NK 2 receptors are widely distributed in the peripheral nervous system of mammals and are especially important in mediating smooth muscle contraction produced by endogenous tachykinins in the airways, intestine and genitourinary tract [12]. MEN 10,627 displays an affinity for NK 2 receptors at least 10-times higher than its monocyclic precursor [13]. The selectivity is clearly remarked by the in vitro antagonist activity, which has been determined in a number of different bioassays. The affinity of MEN 10,627 for NK 2 receptors is 10 to100fold higher than that of L 659,877 [14]. Because of its high affinity, MEN 10,627 appears as a very valuable tool for exploring the structural and functional relationships of the NK 2 receptor protein.

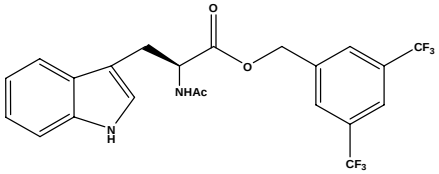
The physiological and pathophysiological roles of NK-3 receptors in mammalian CNS are unknown, although there is evidence from several studies in animal that activation of this tachykinin receptor modulates the release of a variety of neurotransmitters, including 5-HT (Stoessl *et al*.1990), acetylcholine [15], dopamine and vasopressin (Saigo *et al.,* 1993). Based on such information, in addition to results describing changes in the expression of the NK-3R and/or NKB and the effects of tachykinin ligands in vivo, it has been speculated that NK-3Rs may play a pathophysiological role in various diseases, including epilepsy anxiety and Parkinson's disease (Bannon *et al.,* 1995).SB-222200 would appear to be an appropriate tool compound with which to assess the role of NK-3Rs in animal models of CNS diseases. SB-222200 belongs to the recently described class of non peptide NK-3R antagonists, which are based on the 4- quinolinecarboxamide backbone [16]. Functional and binding studies indicate that SB-222200 is a high affinity antagonist for the hNK-3R.



Structure of SB-222200



Structure of MEN 10627



Structure of L-732, 138

With respect to the tachykinin receptors SB-222200 has about a 60-fold selectivity for the hNK-3R versus the hNK-2R and over 100,000-fold selectivity versus the hNK-1R.

Methods

Sequence alignment

Comparative sequence analysis of the Neurokinin receptors NKR1, NKR2 and NKR3 with bovine rhodopsin (PDB code 1ui9A) was performed by clustal W [17]. GPCR's TM domain show higher homology, than the extracellular (EC) or intracellular (IC) domains. The long IC4 loop of the three receptors were excluded from modeling, since it has no equivalent in the bovine rhodopsin sequence. Here we have to mention that the bovine rhodopsin crystal structure is in its inactive conformation, therefore we assumed that homology modeling of the antagonist binding site is reasonable.

The sequence alignment of the receptors NKR1, NKR2 (with out IC4 resulted in the identities of 25%, 22% and 22% respectively. The automatic sequence alignment was followed by manual adjustment, paying attention to the position of the conserved GPCR residues.

Homology Modeling

For modeling 3D structure receptors, the program MODELLER was used [18]. Modeller is an implementation of an automated approach to comparative modeling by satisfaction of spatial restraints [19,20].

Accordingly twenty five models are generated using the loop refinement method and the model having the lowest Modeller objective function was chosen for further validations.

Model Validation

PROCHECK and ProSa are used for the validation of the models generated. The main geometric parameters of the models were determined by PROCHECK [21]. We choose the first model for further investigations, which possesses on average the most favorable features.

In the last step of homology modeling the selected model was subjected to a series of tests for its internal consistency and reliability. Backbone conformation was evaluated by the inspection of the psi/phi Ramachandran plot obtained from PROCHECK analysis. The PROSA [22] test was applied to check for energy criteria in comparison with the potential of mean force derived from large set of known protein structures.

Docking

Molecular docking can fit molecules together in a favorable configuration to form a complex system. The structural information from the theoretically modeled complex may help us to clarify the binding mechanism of ligands to the receptor. The 3D structures of the ligands were generated using chemdraw program and the geometry was optimized using prodrg server.

Autodock 3.05 and Autodock tools were used to perform the automated molecular docking. All docking calculations were performed by the Lamarckian Genetic Algorithm. Running LGA with default parameters resulted in insufficient sampling efficacy, parameters of LGA were changed [23]. The number of generations, energy evaluations and the number of docking runs were set to 27, 000, 1,500,000, and 100 respectively. These changes contributed higher diversity of sampled configuration of genes and allowed us to achieve sufficient sampling of the conformational space available for ligands within the binding site.

Results

Homology modeling

The final alignment of the Neurokinin receptors NKR1, NKR2 and NKR3 to that of bovine rhodopsin was carefully checked in TM regions and we found that all the critical structural elements involved in the binding of their natural substrates are intact. Therefore, we conclude that this alignment can be used to construct reliable 3D models.

Validation of the homology models involved two independent tests. The first test was to compare the residue backbone conformations in our model with the preferred

values obtained from Protein Data Bank of known structures.

The results of PROCHECK analysis indicate that relatively low percentage of residues having phi/psi angles in the disallowed ranges, the quality of Ramchandran plots is acceptable for all models. The percentage of residues in the "core" region of modeled NK-1, NK-2, and NK-3 were found to be 84.3%, 83.9%, 81.5% respectively. The stereochemical quality of the models were found to be satisfactory. The Ramachandran plots of the modeled receptors were shown in the fig 1.

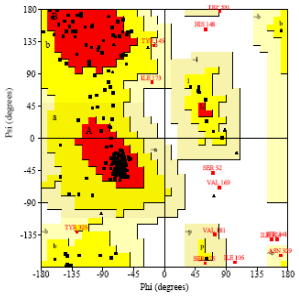


Figure 1a

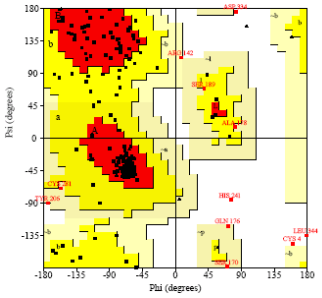


Figure 1b

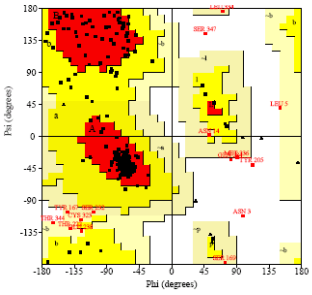
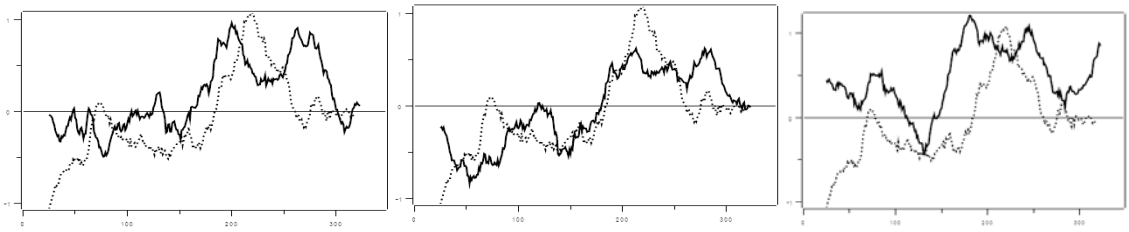


Figure 1c

Figure 1 : Ramchandran plot of the Human NKR-1, 2 and 3 Models. The most favored regions are coloured red, additional allowed, generously allowed and disallowed regions are indicated as yellow, light yellow and white fields, respectively

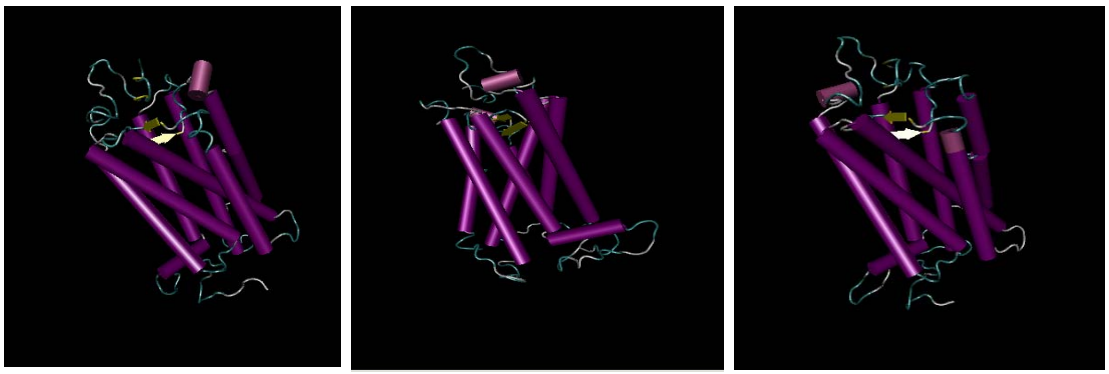
The second test was to apply energy criteria using PROSA. We investigated whether the interaction energy of each residue with the remainder of the protein is negative. PROSA energy plots for the models and bovine rhodopsin are shown in fig 2.



(a) (b) (c)

Figure 2 : Prosa plots calculated for human NKR1, 2 and 3 models (dotted line) and The template (bold line)

In summary, the quality of the receptor models has been checked using two different criteria. The results showed that the backbone conformations (PROCHEK), the residue interaction (PROSA) are well within the limits established for reliable structures. Passing all tests by our structure suggests that we obtained adequate model for the NKR receptors to characterize the binding site and to explore interactions formed with different antagonists. The models were shown in Fig. 3.



(a) (b) (c)

Figure 3 : Cartoon representation of the Predicted structure of Human NKR-1, 2 and 3 by Homology Modeling using MODELLER

Docking

With the protein held static throughout the simulation, the antagonist molecule is manipulated to fit inside the cavity, largely by applying a random displacement to each degrees of freedom, i.e., translation of its centre of gravity, orientation and rotation around each of its flexible internal dihedral angles. These manipulations resulted in new confirmations of the antagonist molecule whose energy is evaluated.

The docked complexes of receptor-ligand were selected according to the criteria of interacting energy combined with geometrical matching quality. Accordingly the binding confirmations were evaluated and ranked. The most probable residues involved in antagonist binding are determined using the evaluations of interaction energies and the results are shown in tables 1a, 1b and 1c.

Table 1a : The total interaction energy (E total), Vander Waals energy (E vdw) and electrostatic energy (E elc) of residues of NKR1 when docked with L-732,138.

Residues	E vdw	Ele (kcal/mol)	E total (kcal/mol)
TYR 216	-1.29	0.10	-1.19
ILE 135	-1.10	-0.05	-1.15
ALA 215	-1.32	0.26	-1.06
ILE 212	-0.66	0.10	-0.76
THR 222	-1.09	-0.19	-1.28
TRP 224	-1.41	-0.22	-1.63
ILE 135	-1.10	-0.05	-1.15
THR 217	-1.12	-0.12	-1.24
VAL 219	-0.51	-0.05	-0.56

Table 1b : The total interaction energy (E total), Vander Waals energy (E vdw) and electrostatic energy (E elc) of residues of NKR2 when docked with MEN-1026

Residues	E vdw	Ele (kcal/mol)	E total(kcal/mol)
SER 298	-1.03	-0.12	-1.15
MET 124	-1.05	0.59	-2.04
ARG 226	-0.21	-0.26	-0.47
TRP 294	-1.02	0.12	-1.14
TYR 93	-0.98	-0.08	-1.06
THR 179	-1.24	-0.11	-1.35
PRO 230	-1.02	-0.10	-1.12
LEU 295	- 1.00	-0.22	-1.22
MET 297	-0.59	-0.12	-0.71

Table 1c : The total interaction energy (E total), Vander Waals energy (E vdw) and electrostatic energy (E elc) of residues of NKR3 when docked with SB –222200

Residues	E vdw	Ele (kcal/mol)	E total (kcal/mol)
SER 206	-0.98	-0.12	-1.10
VAL 203	-1.09	0.15	-1.19
ILE 207	-1.21	-0.01	-1.22
PRO 259	-1.01	0.05	-1.06
ALA 211	-1.46	-0.09	-1.55
THR 200	-1.12	-0.31	-1.43
LEU 260	-1.17	-0.07	-1.54
LEU 232	-0.91	0.22	-0.69
ARG 230	-1.92	0.12	-1.80

Discussion

To determine the key residues that comprise the binding pocket of the model, the interaction energies of the substrates with each individual amino acid in the receptor were calculated. Significant binding- site residues in the models were identified by the total interaction energy between the substrates and each amino acid residues in the receptor. This identification, compared with a definition based on the distance from the substrate, can clearly show the relative significance for every residue.

The conserved histidine residues at positions 197 and 265 in transmembrane domains 5 and 6 of the human NK-1 receptor are important determinants of the binding of quinuclidine antagonists to the receptor. The indole substituent of the tryptophan antagonists may specifically interact with histidine 197 via either an amino-aromatic or an aromatic-aromatic interaction an aromatic group, have been observed in many protein structures.

The results indicated a remarkable consistent mode of antagonist- receptor interaction. The possible contributions to binding of the antagonist in the case of NKR1 suggested are to be ILE 212, VAL 219, TRY 216, ILE 135, ALA 215, ALA 258, THR 222, TRP 224, THR 217. Similarly the residues SER 298, MET 124, ARG 226, TRP 294, TYR 93, THR 179, PRO 230, LEU 295, MET 297 were found to be important anchoring residues for antagonist MEN 1026 and receptor NKR-2. In the same way the residues SER 206, VAL 203, ILE 207, PRO 259, ALA 211, THR 200, LEU 260, LEU 232, ARG 230, CYS 233 were found to be important for the binding of antagonist SB 222200 to the receptor NKR-3.

This implies that high affinity binding of nonpeptide antagonists appears to depend predominantly on elements in the fifth to seventh transmembrane segments whereas the peptide the peptide ligands appear to make a larger number of contacts within the helical bundle. Helix 7 is completely conserved in the tachykinin receptors apart from two positions near the exacellular environment.

The energy information of the receptor ligand interactions may guide the selection of candidate sites for further experimental studies of site-directed mutagenesis. Furthermore, these residues, as well as the others are suggested as candidates for further experimental studies of structure–function relationships.

In conclusion, homology modeling was done to construct the 3D models of the three neurokinin receptors. The models generated are assessed by PROCHECK and PROSA. The stable structure is further used to perform the docking to identify the role of several amino acids having an effect on agonist or antagonist binding. Three known, well characterized, selective and potent antagonists and natural ligands were used for docking. We found several potential aromatic interaction points in the receptors that took part in forming the liphophilic side of the antagonist binding cavity with other residues.

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