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Research Article

THEORETICAL MODELING AND DOCKING STUDIES OF SILKWORM OCTOPAMINE RECEPTORS

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ABSTRACT

Objective: The octopamine receptor which belongs to the rhodopsin receptor family ie., class A of G protein-coupled receptors serves as neurohormone, neurotransmitter and neuromodulator. So far the information on the three dimensional structure for octopamine receptor is not available for any organism including silkworm. Here we modelled the octopamine receptor and analyzed the binding site of known aagonist and screened the novel candidates using the pharmacophore method.

Methods: The octopamine receptor was docked with the known agonists such as clondine, naphazoline etc and their interaction sites were predicted using the Autodock program. The pharmacophore models were derived and filtered using Lipinski's rule of five criteria.

Results: The three-dimensional structure of this protein was modelled with the template 2rh1A using the MODELLER program and validated using the SAVES server which is reliable and found the compounds which shares the same binding site of known agonist using pharmacophore method.

Conclusion: The results on the structure of the receptor and their binding site interaction residues will pave the way for locating various drug targets of transmembrane protein.

Keywords: Octopamine receptor, Comparative modeling, Docking, Pharmacophores.

INTRODUCTION

The octopamine receptor belongs to the rhodopsin receptor family ie. class A of G protein-coupled receptors that are a largest superfamily of integral membrane proteins. In insects, the first octopamine receptor was isolated from the fruit fly *Drosophila melanogaster* [1]. All GPCR proteins are integral membrane proteins and characterized by three extracellular N-terminal and three intracellular C-terminal, seven transmembrane alpha helices. The octopamine receptor interacts with octopamine that elevates the release of cAMP and intracellular ca2+ concentration via the action of G proteins and their effectors [2]. It serves as neurohormone, neurotransmitter and neuromodulator involved in many processes including the endocrine gland activity, mobilization of carbohydrates and lipids, sensory inputs, motor pattern etc [3].

In the inactive state of the rhodopsin and β2-adrenergic receptors, it has been shown that the third transmembrane arginine residues is caged by the salt bridges formed between Asp and Glu of third and at the bottom of sixth transmembrane region [4-6]. The structural studies of alpha 1-adrenergic receptor have revealed that critically important extracellular loop residues involved in subtype-selective antagonist binding sites [7-9]. In 6th transmembrane region, phenylalanine is entirely conserved and recognized as being important for agonist activation of the rhodopsin receptor [10]. In *Periplaneta americana,* docking studies of octopamine agonist with the octopamine receptor has revealed the important interaction residues of the agonist binding site [11]. Despite the great deal of interest, the structure of the octopamine receptor protein has not been determined experimentally because of the difficulty in expression, purificaton and crystallization of the membrane proteins. So far three-dimensional structure of the four GPCR proteins such as rhodopsin, the β1-adrenergic receptor, the β2-adrenergic receptor, and the A2A adenosine receptor has been determined experimentally.

In the present study, we characterized the octopamine receptor by modelling the three dimensional structure of octopamine receptor which circumvented the absence of a structure of silkworm octopamine receptor and analyzed the binding site of the known agonists of the octopamine receptor. These interaction studies will be help to decipher the structural features and elucidate many promising molecule candidates. The novel agonists which activate the octopamine receptor were screened using the pharmacophore method.

MATERIALS AND METHODS

The silkworm, *Bombyx mori* octopamine receptor (Q08JR9_BOMMO) was retrieved from the SWISSPROT, a public domain database [12]. The physico-chemical properties of the octopamine receptor sequence were computed by the Expasy's ProtParam server such as theoretical isoelectric point (pI), molecular weight, extinction coefficient, aliphatic index, total number of positive and negative residues, instability index and grand average hydropathy (GRAVY) [13]. The transmembrane regions of the octopamine receptor was found out using the Transmembrane Hidden Markov Model (TMHMM) Program, that predicts the transmembrane regions of the sequence using of not needed Hidden Markov Model [14]. The secondary structures of the protein were determined using the Self Optimized Prediction from Multiple Alignment (SOPMA) server which uses the nearest-neighbor approach for the prediction of secondary structure [15] and the octopamine receptor functional domains were predicted using the Scanprosite program [16].

Molecular Modeling and Analysis

The homology sequences of the octopamine receptor was found and selected using the Basic Local Alignment Search Tool (BLAST) program which was performed against Brookhaven Protein Data Bank to find the suitable templates for homology modeling [17]. According to the selected template, the modeling of the three-dimensional structure of octopamine receptor was performed using the MODELLER program [18]. The constructed 3D models were energy minimized in GROMOS96 force field using steepest descent minimization algorithms [19]. The validation for structure models was performed by using SAVES metaserver [20], which comprises of several tools for protein structure verification such as Procheck [21], Whatcheck [22], Errat [23] etc for analyzing stereochemical quality and validating protein structures using the Ramachandran Plot [24]. The PyMOL program was employed for interactive visualization and analysis of molecular structures [25].

Docking and Pharmacophore Analysis

The active binding site of this modelled octopamine receptor protein was identified using the Q-Sitefinder Program that

predicts ligand-binding site pockets [26]. The binding sites of the known agonists of the octopamine receptor such as clonidine, naphazoline, octopamine, phentolamine, phenylethanolamine, synephrine, tolazoline etc were analyzed. The spatial arrangement of agonists with an octopamine receptor was detected using the PharmaGist webserver for pharmacophore detection [27]. The agonists that are known to bind the octopamine receptor were used as input structure and then the pharmacophores were computed. These pharmacophores were searched against the ZINC database using the ZINCPharmer software [28]. The compounds were retrieved and considered only if it possesses the drug like properties using FAF drugs-ADME/Tox filtering [29]. The virtual screening of the screened compounds and the receptor ligand interactions studies was carried out using the standard docking procedure of Autovina [30] and Autodock Program [31]. The docking energy, Kcal/mol and the inhibition constant, Ki was obtained for the docked molecules. The interaction studies such as hydrogen bonding /π-π interactions, RMSD calculations were carried out between the agonist and the receptor protein.

RESULTS

The physico-chemical properties of the octopamine receptor protein with length of 507 amino acids were computed by Expasy Protparam tool which is presented in Table 1. The receptor protein is basic in nature and unstable membrane protein, which is revealed from the PI and the instability index value. The aliphatic index was 85.76 which indicate that the protein may be stable for a wide range of temperatures. The percentage of alpha helix, random coil, extended strand, beta turn is 33.93, 43.98, 18.15 and 3.94 respectively. The octopamine protein was characterized by seven transmembrane helices and these regions are 25-47, 60-82, 97-119, 140-162, 193-215, 394-416 respectively of which were predicted using the THMMM program. The thermostability of protein was determined using the disulphide bridges, between Cys96 of Extracellular region 1 and Cys186 of Extracellular region 2 which was predicted as disulphide bridges and the functional domain G_Protein_ReCep_F1 of this receptor was identified using Scanprosite. The secondary structural features of the protein were predicted using the SOPMA program and it infers that the random coil predominates with alpha helix followed by extended strand and beta turn respectively.

The three-dimensional structure of this protein was modelled using the MODELLER program with the template 2rh1A that has 32% identity was detected using the BLAST Program. The reliable model has predicted the correct fold, which has probability more than 95%, and it was shown by model score of 0.98 which was more than the cut off value of 0.7 and the modelled three-dimensional structure was validated using the SAVES server and Ramachandran plot was mapped and it was found to be reliable. The percentage of residue lying in the most favoured, additionally allowed, generously allowed and disallowed regions were 93.9%, 6.1%, 0% and 0% respectively which was found using the Ramachandran plot analysis. The procheck G-factor for dihedrals and overall PG-factor was 0.09 and 0.04, respectively. In the ERRAT graphs, the overall quality factor was 71.529 and indicates reasonably good model. This modelled structure was validated by the WHATCHECK program in which the z-scores of bond lengths, bond angles, improper dihedral distribution, inside/outside distribution, omega angle restraints, and side chain planarity are 0.986, 1.260, 0.971, 1.214, 0.674 and 0.332 respectively. The refined model structure was acceptable because all the scores are positive; positive is better than average. The possible binding sites of octopamine receptor proteins were searched using Q-Sitefinder program. It was found that the binding sites of the receptor has residues such as Asp93, Val94, Cys96, Ser97, Leu100, Leu159, Val160, Gly161, Asp164, Pro180, Pro181, Gln183, Trp184, Thr185, Cys186, Glu187, Leu188 and this region was considered as most favorable binding site of protein for docking.

DISCUSSION

The proposed pharmacophore study was also carried out in the previous studies [32- 34]. Efforts were made to study the docking of the known ligands such as clonidine, naphazoline, octopamine, phentolamine, phenylethanolamine, synephrine, tolazoline inside the binding pocket of the Octopamine receptor was performed using the Autodock program and found that the agonists are interacting with the residues of the octopamine receptor protein. The features of octopamine receptor agonists are shown in different color such as blue, grey, yellow and green which represents aromatic, hydrophobic, donors and acceptors respectively (Figure 1). Among amine receptors, aspartate, serine and phenylalanine residues are conserved in TM3, TM5, and TM6 respectively. octopamine receptor has highly conserved residues in between the two serine residues of the 5th transmembrane region.

The pharmacophore detection of the agonists which are known to bind to the receptor was carried out using PharmaGist web server shown in Table 2. By using these pharmacophores ZINC druggable database was searched and found that the 666 compounds satisfied the ADMET properties using ZINCPharmer. These compounds were prepared as ligands and virtual screening of these compounds was carried out.

Molecular docking studies revealed that the ZINC00004785, ZINC03604145, ZINC00119985 compounds were found docked into the same binding site of the known agonists with better binding energy of -7.8 which shows good binding interactions. The study revealed that the compounds could potentially form hydrogen bonds with the octopamine receptor Asp93, Gln183, Glu187, Asp164 residues.

The ZINC03604145 compound form interaction with Gln183 residue with the distance of 1.9 and Asp164 residue with the distance of 2.5 and 2. The residues Asp164 and Leu159 form hydrogen bonds with ZINC00004785 compound with the distance of 3.2, 3.5 and 3.3 respectively.

The ZINC00119985 compound interacts with Asp93 residues with distance of 2.6 and Glu187 with the distance of 2.9, 2.5 respectively (Figure 2). It was also shown that these compounds have the interaction with the residues such as Asp167, Thr189, Thr423 and Ser428 of octopamine receptor.

CONCLUSION

These studies shows that these compounds have agonist activity on octopamine receptor and this molecular docking analysis with pharmacophore method used for identification of novel candidates for activation of octopamine receptor for better productivity.

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Table 1: Physico-chemical properties of octopamine receptor protein

Accession No.	Length	M.wt	pI	-R	+R	EC	II	AI	GRAVY
Q08JR9	507	56899.6	9.47	44	66	89350 88350	51.52 unstable	85.76	-0.085

Table 2: The detected features of octopamine receptor agonists

Molecule	Atoms	Features	Spatial Features	Aromatic	Hydrophobic	Donors	Acceptors
Clonidine	23	5	5	1	0	2	1
naphazoline	30	4	4	2	0	1	1
Ocopamine	22	7	4	1	0	3	3
Phentolamine	40	8	7	2	2	2	2
Phenylethanol amine	21	5	3	1	0	2	2
synephrine	25	8	5	1	1	3	3
tolazoline	24	3	3	1	0	3	1

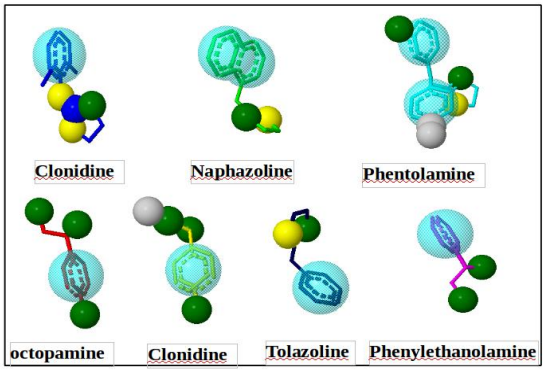

Fig. 1: The Octopamine receptor agonists.

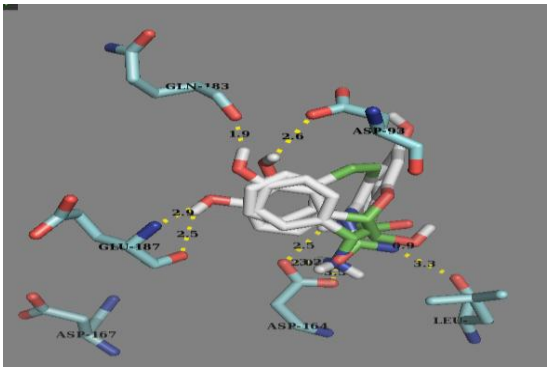

Fig. 2: Interaction of Compounds with the octopamine receptor.

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