American Journal of Biochemistry and Biotechnology 3 (4): 216-224, 2007
ISSN 1553-3468

In silico Modeling of α1A-Adrenoceptor: Interaction of its Normal and Mutated Active Sites with Noradrenaline as well as its Agonist and Antagonist

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Abstract: Noradrenaline, like most other neurotransmitters, acts through various adrenoceptor subtypes. The structure and active site of adrenoceptors for the binding of noradrenaline were unknown, however, such information are crucial for understanding the molecular mechanism of action of neurotransmitters, including noradrenaline, in health and disease as well as for drug designing. In this *in silico* study, we modeled the α1A-adrenoceptor; a G protein coupled receptor and defined its active site. Further, molecular docking and interaction of noradrenaline and its agonist as well as antagonist with the so defined active site of the receptor was studied before and after *in silico* site directed mutation of several amino acid residues forming the active site. Our results indicate that the ARG166 is the most crucial residue for binding of noradrenaline and methoxamine to α1A-adrenoceptor and ILE178 is the most important residue for binding of prazosin to it. Thus, the observations provide new insights into the structure function relationship of α1A-adrenoceptor. A significant finding of this study is that the same residue of the active site may not be necessary for binding of a receptor with its natural ligand and its pharmacologically active known agonist and antagonist.

Key words: α1A-adrenoceptor, *In silico* mutagenesis, Methoxamine, Molecular docking, Noradrenaline, Prazosin, WB4101

INTRODUCTION

Rapid eye movement (REM) sleep is an integral component of sleep-waking rhythm, is present in mammals including humans and throughout ones life span, although its quantum varies through age and in different species[1]. Its importance may be gauged by the fact that REM sleep loss tends to be compensated by its rebound increase and if the deprivation is prolonged, several patho-physio-psycho-behavioral disorders set in[2-9]. As a mechanism of action it has been shown that cessation of activity of the noradrenaline (NA) containing neurons in the locus coeruleus is a prerequisite for REM sleep generation[10] and upon REM sleep deprivation they do not cease activity[11] resulting in increased levels of NA in the brain[12-14]. This increased NA is the primary cause for REM sleep deprivation associated changes including increased Na-K ATPase activity[15] neuronal cytomorphometry[16] and apoptosis[17] and all these changes were mediated by NA acting upon α1-adrenoceptor. Additionally we showed that at least the increase in Na-K ATPase activity was mediated by α1A-adrenoceptor[18]. For further understanding of the mechanism of action at the molecular level, the next step was to study the interactions between NA and α1A-adrenoceptor, however, although the structure of NA was known, the structure and active site of the α1A-adrenoceptor was unknown. Hence, we took the help of bioinformatics to first model the α1A-adrenoceptor *in silico* and deposited it in the PDB as 2F75. Thereafter, in this study we modeled the active site of the α1A-adrenoceptor and carried out *in silico* mutation of different amino acids within the modeled active site to determine the component of the adrenoceptor that is essential for binding with NA. In addition to NA, we also studied the interactions of the so modeled active site of the receptor with methoxamine, prazosin and WB4101 the known pharmacologically active agonist and antagonist of α1A-adrenoceptor.

MATERIALS AND METHODS

Homology Modeling: The sequence of rat α1A-adrenoceptor was obtained from the Swiss-Prot Database[19]; the protein contains 466 amino acid (Accession number: P43140). BLAST[20] search among the proteins of known 3D structure revealed that the bovine rhodopsin showed the highest (21%) sequence

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identity score with α1A-adrenoceptor; the sequence similarity was 31%. 3D-JURY[21] also picked bovine rhodopsin 1GZM at 2.65 Å as the template. Hence, in this study X-ray structure of bovine rhodopsin from Bos taurus (PDB code: 1l9h)[22] was selected as a template and based on that the 3D structure of α1A-adrenoceptor was predicted. However, the initial N-terminal residues 1-35 and final C-terminal residues 342-466 were not modeled because these were absent in the template. The 3DJIGSAW, automated homology modeling tool[23], was used to model the α1A-adrenoceptor framework for residues 36-341. STRIDE[24], which uses hydrogen bond energy and main chain dihedral angles to recognize helix, coils and strands was used to predict the secondary structure of the so modeled α1A-adrenoceptor. Hydropathy of α1A-adrenoceptor was analyzed according to the algorithm of Kyte and Doolittle[25]. The weighted root mean square deviation (RMSD) of the modeled protein was calculated using combinatorial extension (CE) algorithm[26]. Solvent accessibility was measured using the program GEPOL[27]. The computed structure of the α1A-adrenoceptor obtained was refined by energy minimization with CHARMM force field until the energy showed stability in sequential repetition[28]. The stability of the so predicted theoretical model was evaluated sterically with procheck[29].

Gold docking: The chemical structures of α1A-adrenoceptor agonists and antagonists were extracted from pubchem database (http://pubchem.ncbi.nlm.nih.gov/). Structures of all the five ligands (adrenaline, NA, methoxamine, prazosin and WB4101) were retrieved into two-dimensional MDL/SDF format. Three - dimensional coordinates were generated using the CORINA program[30]. The chemical structures of NA and the known pharmacological agonist (methoxamine) and antagonist (prazosin and WB4101) of α1A-adrenoceptor are shown in Fig. 1. Docking was performed using GOLD Software (Genetic Optimization Ligand Docking). GOLD uses a genetic algorithm to explore the full range of ligand conformational flexibility with partial flexibility of the protein[31]. Docking procedure consisted of three interrelated components; a) identification of binding site b) a search algorithm to effectively sample the search space (the set of possible ligand positions and conformations on the protein surface) and c) a scoring function. The GOLD fitness function consisted of four components: a) protein-ligand hydrogen bond energy (external H-bond); b) protein-ligand van der Waals (vdw) energy (external vdw); c) ligand internal vdw energy (internal vdw); d) ligand torsional strain energy (internal torsion). Standard default settings, consisting of population size-100, number of islands-5, selection pressure-1.1, niche size-2, migrate-10, cross over-95, number of operations-100,000, number of dockings-10, were adopted for GOLD docking. For each ligand-α1A-adrenoceptor binding, 10 docking conformations (poses) were tested and the best GOLD score was selected for studies. The consensus scoring function XSCORE was used to estimate the binding affinity of the α1A-adrenoceptor with its ligands NA, its agonist methoxamine as well as antagonist prazosin and WB4101[32]. SILVER was used to predict the interactions of α1A-adrenoceptor and ligand complex[31].

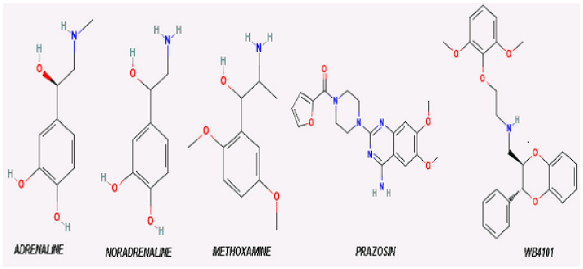

Fig. 1: Chemical structures of α1A-adrenoceptor ligands

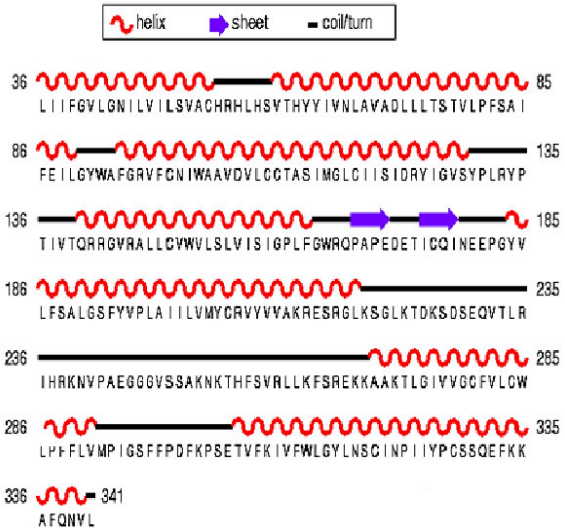

Fig. 2: Secondary structure assignment of our modeled α1A-adrenoceptor

***In silico* Site-directed Mutagenesis:** *In silico* site-directed mutagenesis has been widely used to identify the critical residue(s) for binding of a ligand; NA with α1A-adrenoceptor, in this case. The following *in silico* mutations were performed using Swiss-Pdb Viewer Software[33] and minimized the structure with

CHARMM force field[28]: a) polar amino acid(s) was replaced with polar amino acid(s); b) non-polar group replaced with non-polar group; c) polar group was replaced with non-polar amino acid(s); d) non-polar group replaced with polar amino acid(s); either one amino acid was replaced at a time or a set of amino acids were mutated simultaneously.

RESULTS AND DISCUSSION

Procheck evaluation: The sequence information of rat α1A-adrenoceptor was described in the section materials and methods. Secondary structure assignment using STRIDE provided the physical features of the modeled structure (protein), such as helix, coil as well as extended strands (Fig. 2). We found a proline residue, known to introduce kink, in the middle of the 5th, 6th and 7th transmembrane helices that might alter the helicity of the bundle. To avoid error we used appropriate parameters[34,35] so that helix bundle geometry was properly represented. The output of hydropathy analysis of α1A-adrenoceptor was comparable to the results of secondary structure assignment. The 3D structural model of α1A-adrenoceptor was obtained as described in the materials and methods section. The stereo-chemical quality of the modeled α1A-adrenoceptor was estimated by Procheck. The phi/psi angles of 81.7% residues fell in the most favored regions, 13.2% residues lied in the additional allowed regions and 2.9% fell in the generously allowed regions; only 2.2% of residues lied in the disallowed conformations (Fig. 3). Thus, statistical analysis suggests that the backbone conformation of our predicted model of α1A-adrenoceptor was almost as good as that of the template; the 3D conformation of the predicted model of α1A-adrenoceptor has been shown in Fig. 4. The weighted RMSD of C^{α} trace between the template (1L9H) and final refined model of α1A-adrenoceptor was 1.5Å with a significant Z-score of 6.5.

Protein model deposition: The atomic coordinates of the theoretical model of α1A-adrenoceptor have been deposited with RCSB Protein Data Bank[36], which can be accessed with the code 2F75.

Gold docking: One of the most appropriate methods to predict structural features of active site of a molecule is through docking studies with selected ligands. Therefore, once a theoretical model of the α1A-adrenoceptor was obtained, its active site was predicted and characterized *in silico* by docking pharmacologically confirmed analogues/agonists (adrenaline, noradrenaline and methoxamine) and antagonists (Prazosin and WB4101) with the receptor. For such prediction we considered a cavity size of 10 Å around SER188, because it (SER188) has been reported

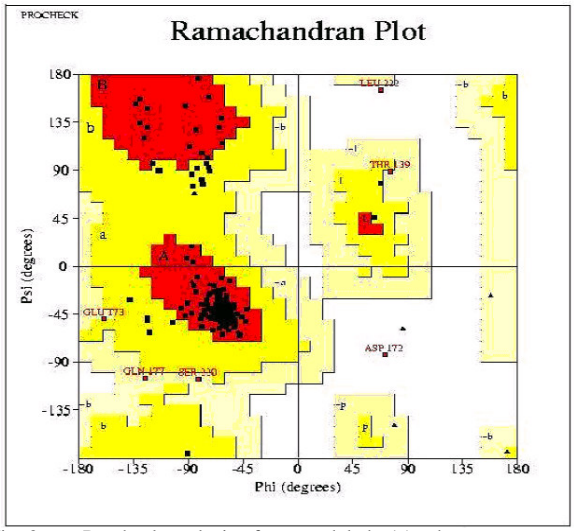

Fig. 3: Procheck analysis of our modeled α1A-adrenoceptor

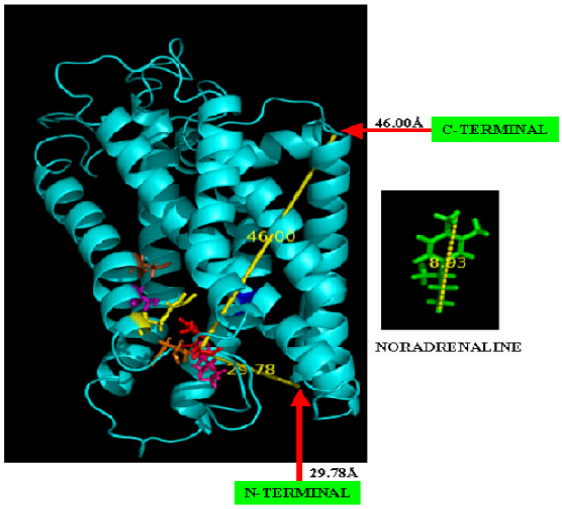

Fig. 4: Ribbon representations of our modeled α1A-adrenoceptor. Important residues are shown in sticks. The distance from the active site (ARG 166) to N-(29.78 Å) and C-(46.00 Å) terminals of the modeled receptor, as calculated by pymol, are shown. The inset shows the length (8.93 Å) of noradrenaline with its structure. Color code: ASP106-blue; ARG166-red; ILE178-pink; ASN179-orange; TYR184-yellow; SER188-violet and SER192-brown

to be the key residue for binding with adrenaline, a methylated product of NA that also binds to α1A-adrenoceptor with comparable affinity[37]. Such interactions enabled us to predict the following interactive residues at the active site in our modeled α1A- adrenoceptor, ASP106, ARG166, ILE178, ASN179, TRY184, SER188 and SER192 and the details are explained below.

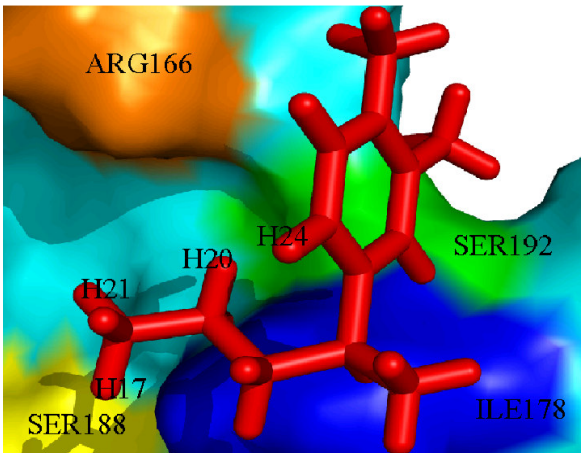

Fig. 5a: Hydrogen bond interactions between α1A-adrenoceptor and adrenaline. α1A-adrenoceptor is shown in cyan color (surface). Color code: ARG166-orange, ILE178-blue, SER188-yellow, SER192-green and adrenaline-red

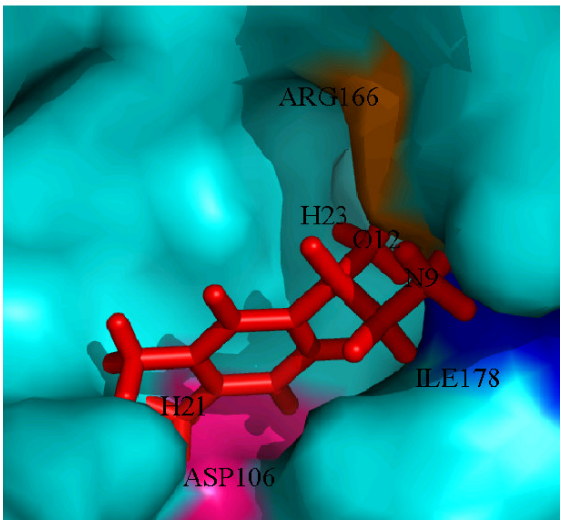

Fig. 5b: Hydrogen bond interactions between α1A-adrenoceptor and noradrenaline. α1A-adrenoceptor is shown in cyan color (surface). Color code: ARG166-orange, ILE178-blue, ASP106-rose, SER192-green and noradrenaline-red

Interactions with adrenaline: The following 4 hydrogen bond interactions were observed between adrenaline and α1A-adrenoceptor (Fig. 5a) (Table 1). (1) Non-polar residues: The NH2 atom of ARG166 of the active site of the receptor forms hydrogen bond with H17 of adrenaline. The O atom of ILE178 of the active site of the receptor forms hydrogen bond with H21 of adrenaline. (2) Polar residue: O atom of SER188, of the predicted active site forms hydrogen bond with H20 of adrenaline and N atom of SER192 of the active site of the receptor forms hydrogen bond with H24 of adrenaline.

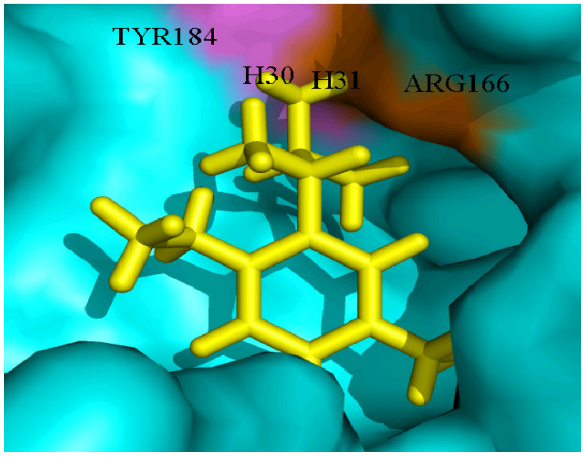

Fig. 5c: Hydrogen bond interactions between α1A-adrenoceptor and methoxamine. α1A-adrenoceptor is shown in cyan color (surface). Color code: ARG166-orange, YR184-rose and methoxamine-yellow

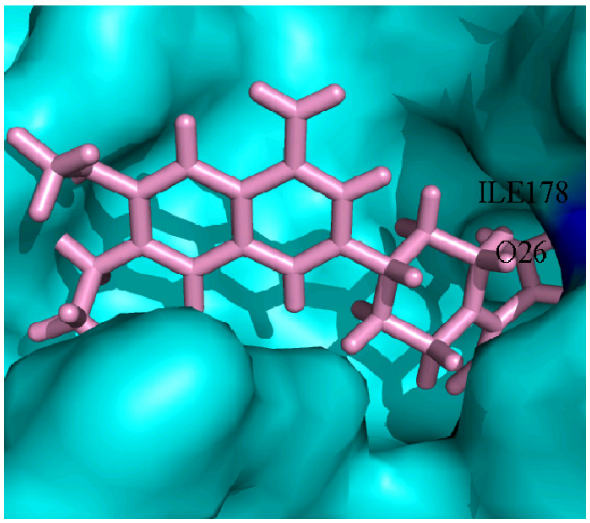

Fig. 5d: Hydrogen bond interactions between α1A-adrenoceptor and prazosin. α1A- adrenoceptor is shown in cyan color (surface). Color code: ILE178-blue and prazosin-rose

Interactions with noradrenaline: There are the following 6 hydrogen bond interactions between NA and α1A-adrenoceptor (Fig. 5b) (Table 1). (1) Non-polar residues: The NH1 and O atom of ARG166 of the active site of the receptor forms hydrogen bond with O12 and N9 of NA. The NH2 atom of ARG166 of the active site of the receptor, hydrogen bonds with H23 of NA. The O atom of ILE178 of the active site of the receptor form hydrogen bonds with H23 of NA. (2) Polar residue: O atom of ASP106, with the predicted active site forms hydrogen bond with H21 of NA.

Table 1: Hydrogen bond interactions between α1A -adrenoceptor with adrenaline, NA and known NA-agonist, methoxamine and NA-antagonist, prazosin and WB4101

Ligands	AA Residues	AA Residue Number	AA Residue Atom Name	Ligand Atom Name	Distance Å
AD	ARG	166	NH2	H17	2.14
	ILE	178	O	H21	2.20
	SER	188	O	H20	2.32
	SER	192	N	H24	2.36
NA	ASP	106	O	H21	2.35
	ARG	166	NH1	O12	2.36
	ARG	166	O	N9	2.14
	ARG	166	NH2	H23	1.78
	ILE	178	O	H23	2.45
	ILE	178	O	O12	2.16
METH	ARG	166	NH2	H30	1.65
	ARG	166	NH1	H30	2.11
	TYR	184	O	H31	1.89
PRZ	ILE	178	O	O26	2.19
WB4101	ARG	166	NH2	H58	2.35
	ILE	178	O	O27	1.34
	ILE	178	O	N26	2.25
	ILE	178	O	H58	2.36
	ASN	179	N	O29	2.65

Table 2: Comparison of the experimental binding affinities* and the calculated scores using GOLD score and XSCORE for α1A-adrenoceptor

Ligands	M.wt	M. formula	H bond donor count	H bond acceptor count	X logP	GOLD score	XSCORE K.cal/ mol	*Exp energy (pKi value)
AD	183.20	$C_9H_{13}NO_3$	4	4	0.26	39.24	-6.35	6.3
NA	169.18	$C_8H_{11}NO_3$	4	4	43.6	38.39	-6.01	6.2
METH	211.26	$C_{11}H_{17}NO_3$	2	4	1.5	43.18	-7.14	7.43
PRZ	375.86	$C_{21}H_{23}CFNO_2$	1	4	2.86	43.53	-8.75	8.5
WB4101	345.39	$C_{19}H_{17}NO_3$	1	6	-0.28	41.85	-7.05	8.8

Abb: AD- adrenaline, NA- noradrenaline, METH- methoxamine and PRZ- prazosin.
[*Experimental energy taken from GPCR Database (www.iuphar-db.org/ <http://www.iuphar-db.org/>)]

Table 3: First, second and third best conformations of GOLD docking using GOLD score and XSCORE for α1A-adrenoceptor

Ligands	Gold score			Xscore (K.cal/mol)		
	First	Second	Third	First	Second	Third
AD	39.24	38.83	37.78	-6.35	-6.24	-6.15
NA	38.39	37.45	37.18	-6.01	-5.88	-5.40
METH	43.18	42.69	39.68	-7.14	-8.10	-6.77
PRZ	43.53	40.65	39.50	-8.75	-7.98	-6.55
WB4101	41.85	38.26	38.21	-7.05	-6.05	-6.00

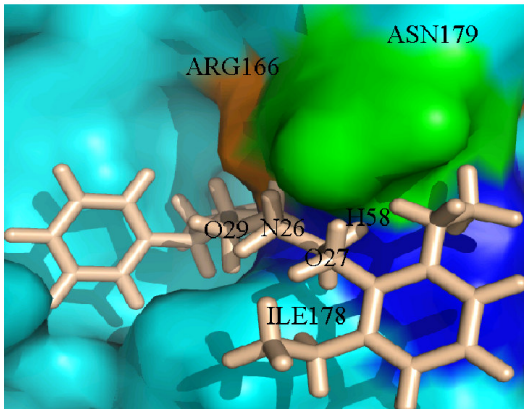

Fig. 5e: Hydrogen bond interactions between α1A-adrenoceptor and WB4101. α1A-adrenoceptor is shown in cyan color (surface). Color code: ARG166-orange, ILE178-blue, ASN179-green and WB4101-sandal

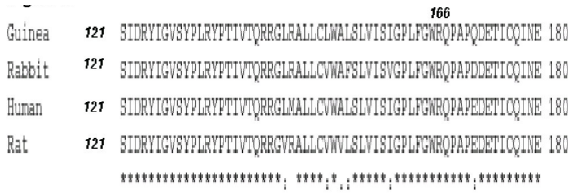

Fig. 6: Sequence of α1A-Adrenoceptor from four mammalian species, as obtained from Swiss Prot database, was aligned by CLUSTAL W. The critical residue R166 of α1A-Adrenoceptor, as observed in this study, is conserved in all the species

Interactions with methoxamine: An examination of the binding interaction of the agonist methoxamine with predicted active site showed that there are 3 hydrogen bonds. (1) Non-polar residues: The NH1 and NH2 atom of ARG166 make hydrogen bonds that tether H30 of the methoxamine. (2) Polar residue: O atom of TYR184

of the active site of the receptor forms hydrogen bond with H31 of NA (Fig. 5c) (Table 1).

Interactions with prazosin: There was only one interaction between non-polar residue O atom of ILE178 that forms hydrogen bond with O26 of prazosin (Fig. 5d) (Table 1).

Interactions with WB4101: There are 6 hydrogen bond interactions between WB4101 and α1A-adrenoceptor. (1) Non-polar residues: The O atom of ILE178 forms hydrogen bond with O27, N26 and H58 of WB4101. The NH2 atom of ARG166 makes hydrogen bonds that tether H58 of the WB4101; and (2) Polar residue: N of ASP179 of the active site of the α1A-adrenoceptor makes hydrogen bond with 029 of WB4101 (Fig. 5e) (Table 1).

Validation of docking: XSCORE was used to estimate the binding affinity of the receptor-ligand complex of α1A-adrenoceptor (Table 2). This scoring function included terms accounting for van der Waals interaction, hydrogen bonding, deformation penalty and hydrophobic effect. The XSCORE performs better in identifying the correct bound conformations used for docking analysis. We evaluated top three docking conformations using GOLD score and XSCORE for all the ligands with the modeled structure (Table 3), however, as a normal practice, we have taken the best gold score for further studies.

***In silico* mutagenesis:** The hydrogen bond residues mentioned above were present in the cavity that formed active site of the α1A-adrenoceptor. Hence, to further explore that which of those residues was critical for binding of NA and its agonist/antagonist, we performed *in silico* mutation of the active site residues and then studied the binding affinities of the mutated active site with NA and its agonist as well as antagonist mentioned above (Table 4).

In an earlier *in situ* mutation study[37], SER 188 and SER192 of α1A-adrenoceptor were mutated and their interactions with adrenaline, a methylated product of NA, was studied. Such study identified SER188 as the key residue for binding with adrenaline. To start with we evaluated whether the same residue of the receptor was playing a crucial role for binding with NA and known specific pharmacological agonist methoxamine and known specific antagonist prazosin as well as WB4101, we mutated either SER188 with ALA 188 or, SER192 with ALA192 or both simultaneously (SER188 and SER192 with ALA188 and ALA192). It was observed that there was no change in the cavity and binding affinity of NA, methoxamine and prazosin.

Thereafter, we replaced all four polar amino acid residues of α1A-adrenoceptor (ASP106, ARG166, ASN179, TYR184) with four non-polar amino acid residues (VAL106, ALA166, LEU179, GLY184); all simultaneously or one at a time. Solvent Accessibility Surface area (SAS) was calculated for natural α1A-adrenoceptor (without mutation) and that after each of such *in silico* mutation mentioned above (Table 5). The results showed that the size of the cavity and binding affinity with NA, methoxamine and prazosin were significantly decreased. Subsequently, we studied the effects of replacing one amino acid at a time of the active site of the modeled receptor on SAS and binding affinities with NA, methoxamine, prazosin and WB4101. Replacement of polar residue ASP106 with a non-polar residue VAL106 did not significantly change the cavity size and binding affinity of α1A-adrenoceptor with NA, methoxamine and prazosin. Similarly, upon replacement of the polar residue ASN179 with a non-polar residue LEU179, there was no change in the cavity size and binding affinity of NA, methoxamine and prazosin with α1A-adrenoceptor.

Substitution of the non-polar residue ILE178 with a polar residue THR178 increased the cavity size but there was no change in the binding affinity of NA and methoxamine, however, the prazosin binding affinity with α1A-adrenoceptor was decreased. Mutation of the polar residue ARG166 with a non-polar residue ALA166 increased the cavity size and the binding affinities of NA, methoxamine and prazosin were significantly decreased with α1A-adrenoceptor. Substitution of the polar residue TYR184 with a non-polar residue GLY184 increased the cavity size but the binding affinities of NA and methoxamine were not significantly altered, however, the prazosin binding affinity with the receptor was decreased. These data suggest that ARG166 is the most crucial residue for binding of NA and methoxamine to α1A-adrenoceptor; however, not for prazosin because ARG166 is not its interacting residue. Our prediction may be supported by the fact that the ARG166 is conserved in all the four mammalian species α1A-adrenoceptor whose sequence we compared (Fig. 6). The sequence alignment was derived using CLUSTAL W package[38]. Further, ILE178 is the most important residue for binding of prazosin to α1A-adrenoceptor. However, at present we cannot comment on why in all the mutations the binding affinities of WB4101 to α1A-adrenoceptor increased. Finally, a word of caution that our model does not account for the portions of the sequence of the α1A-adrenoceptor, which was not present in the template bovine rhodopsin. Nevertheless, as an indirect evidence, we calculated the distance from the active site (ARG166) to N- (29.78 Å) and C- (46.00 Å) terminals of the modeled receptor and the length of the NA

Table 4: *In silico* mutation of α1A-adrenoceptor and their interactions with NA, its agonist methoxamine and antagonist prazosin and WB4101. GOLD score and XSCORE were used to calculate the binding affinities of normal and mutated α1A adrenoceptor

Ligands		NA		METH		PRZ		WB4101	
		Gold score	X score K.cal/mol	Gold score	X score K.cal/mol	Gold score	X score K.cal/mol	Gold score	X score K.cal/mol
	Substitution								
Normal		38.39	-6.01	43.18	-7.14	43.53	-8.75	41.85	-7.05
Polar To Non-Polar	S188A	38.87	-6.34	41.99	-7.12	44.34	-8.85	59.09	-9.28
	S192A	39.34	-6.41	43.43	-7.05	45.06	-8.77	58.91	-9.45
	S188A/S192A	39.98	-6.28	42.63	-7.13	41.57	-8.82	57.07	-9.14
	D106V	39.1	-6.4	43.05	-6.98	44.72	-8.71	55.12	-9.09
	R166A	32.05	-6.13	30.82	-6.6	37.3	-6.67	45.75	-7.33
	N179L	37.41	-6.12	40.55	-7.1	43.17	-7.86	66.76	-8.49
	Y184G	38.95	-6.4	41.82	-7.09	23.73	-8.29	48.02	-8.87
	ALL REPLACED	36.23	-6.05	38.78	-6.31	39.97	-6.54	43.42	-8.65
Non-Polar To Polar	I178T	39.41	-6.39	42.12	-7.13	35.89	-8.33	41.19	-8.96

Mutation has been shown under substitution as follows: SER188 to ALA 188=S188A; SER192 to ALA192=S192A; SER188 to ALA188/SER192 to ALA192= S188A/S192A; ASP 106 to VAL106=D106V; ARG166 to ALA166=R166A; ASN179 to LEU179=N179L; TYR184 to GLY184=Y184G; ILE178 to THR178=I178T

Table 5: Solvent accessibility surface area (SAS) for normal and mutated α1A-adrenoceptor

Residue		Total SAS (A)	Per Residue SAS (Normal) (A°)	Per Residue SAS (Mutation) (A°)	Percent Change
Normal protein		15811.04			
	(Polar to Non-polar) S188A	15810.9	14.21	14.8	4.15
	(Polar to Non-polar) S192A	15812.5	17.18	17.07	0.64
	Polar to Non-Polar) D106V	15804.35	8.99	8.6	3.26
Mutation	(Polar to Non-Polar) R166A	15826.81	29.99	9.77	67.42
	(Polar to Non-Polar) N179L	15801.94	21.25	27.29	28.42
	(Polar to Non-Polar) Y184G	15847.79	1.52	2.97	95.3
	(Non-Polar to Polar) I178T	15825.87	49.48	38.06	23.08

Mutation has been shown under substitution as follows: SER188 to ALA 88=S188A; SER192 to ALA192=S192A; ASP 106 to VAL106=D106V; ARG166 to ALA166=R166A; ASN179 to LEU179=N179L; TYR184 to GLY184=Y184G; ILE178 to THR178=I178T

molecule (8.93 Å) in its full extent, as shown in the Fig. 4. Since to our knowledge there is no biochemical data available to confirm if the non-modeled portion of the receptor is not necessary for NA binding, based on the distance, it is expected that indeed those regions are unlikely to be directly participating for NA binding to α1A-adrenoceptor. Also, we are aware that while mutation, we have considered the polar and non-polar properties of the amino acids; their biological potency and whether they can really be synthesized are unknown hence, our prediction are subject to *in vivo* confirmation.

CONCLUSION

The structure of α1A-adrenoceptor was modeled *in silico* based on X-ray crystallography structure of bovine rhodopsin (1l9h) taken as a template. The strong correlation between the GOLD score and XSCORE values add confidence to the accuracy of the model. The validity of the refined modeled structure was evaluated by studying its molecular docking affinities with known pharmacological agonist and antagonists before and after *in silico* site-directed mutagenesis of the active site of the model. The findings provide new information about the intra- and inter-molecular interactions characterizing the α1A-adrenoceptor-NA complex. Our analysis provides strong evidence that ARG166 is the most crucial residue for binding of NA and methoxamine to α1A-adrenoceptor and ILE178 is the most important residue for binding of prazosin to α1A-adrenoceptor. These findings advance our knowledge on specific interactions on NA binding with α1A-adrenoceptor, which subject to confirmation *in vivo* may facilitate designing of related therapeutics.

ACKNOWLEDGEMENT

Thanks to Prof. Alok Bhattacharya, School of Life Sciences and School of Information Technology; Jawaharlal Nehru University; for his constructive criticism while preparing the manuscript.
Funding and fellowship to RV from UPOE, UGC and Department of Biotechnology, India are acknowledged.

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