Molecular Docking-Based Study of Vasopressin Analogues Modified at Positions 2 and 3 with *N*-Methylphenylalanine: Influence on Receptor-Bound Conformations and Interactions with Vasopressin and Oxytocin Receptors

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In this study, four cyclic vasopressin (CYFQNCPRG-NH_2, AVP) analogues substituted at positions 2 and 3 with four combinations of enantiomers of *N*-methylphenylalanine have been investigated. Three-dimensional structures of analogues have been formerly determined using NMR spectroscopy in dimethyl sulfoxide. Three-dimensional models of the vasopressin and oxytocin receptors were constructed by combining the multiple sequence alignment and the RD crystal structure as a template. The analogues have been docked into the receptor using the AutoDock program. The relaxation of the receptor−ligand complexes using energy minimization, followed by the constrained simulated annealing protocols (CSA), has been performed. The receptor-bound conformations of the investigated analogues have been proposed. We concluded that the N-methylated residues at positions 2 and 3 act as a structural restraint, determining the conformation of analogues, their location inside the receptor cavity, and mutual arrangement of the aromatic side chains. The conserved polar residues constitute the handles keeping the biologically active analogues inside the binding cavity. The Arg^8-$D^{2.65}$ salt bridge might be responsible for analogue-selective binding in OTR and V1aR versus V2R, where the positively charged $K^{2.65}$ 100 is present at the equivalent position.

Introduction

Arginine vasopressin (CYFQNCPRG-NH_2, AVP) is an endogenous nonapeptide synthesized in the hypothalamic magnocellular neurons and stored in the posterior pituitary gland wherefrom it is released into the peripheral circulation under three main stimuli: hyperosmolality, hypovolaemia, and hypotension.[1] The main physiological roles of AVP are the regulation of water balance, the control of blood pressure, and the adrenocorticotropin hormone (ACTH) secretion mediated via three subtypes of vasopressin receptors: V2 (V2R), V1a (V1aR), and V1b (V1bR), respectively.[2−4] Moreover, AVP to some extent also exhibits the typical oxytocin ([I3,L8]AVP, OT) activities: the contractions of uterine smooth muscle and mammary myoepithelium via interaction with the oxytocin receptor (OTR).[2−5] Furthermore, AVP and OT act as neurotransmitters in the central nervous system playing a role in many reproductive, developmental, behavioral, and social functions.[6−9] Vasopressin and oxytocin receptors are included in the neurohypophyseal hormone receptors subgroup and belong the best studied class A G protein-coupled receptors (GPCRs).[10−13] GPCRs are the integral membrane proteins consisting of seven transmembrane helices (TM1−TM7), connected by alternating extracellular (EL) and intracellular (IL) loops with the extracellular N-terminus and the cytosolic C-terminus.[14−16] They occupy almost 3% of the human genome and are targets for more than 60% drugs on the market.[17−20] Neurohypophyseal hormone receptors are involved in a number of pathological conditions. Identification of the difference between their interactions with agonists, associated with the receptor activation and signal transduction and those with antagonist, resulting in a blockade of the receptors binding domain, is an important task in rational drug design.[17,20,21]

In this study the cyclic AVP analogues, substituted at positions 2 and 3 with the combinations of enantiomers of *N*-methylphenylalanine, have been investigated. The pharmacological properties of these analogues were tested previously in the uterotonic, pressor, and antidiuretic tests in vitro (see Table 1).[22] The modification of AVP molecule at positions 2 and 3 with *N*-methylphenylalanine results in a structural constraint, determining conformation of the macrocyclic ring and spatial orientation of the aromatic side chains at positions 2 and 3. Moreover, the lack of the phenol group in the aromatic side chain at position 2, crucial for transduction, results in loss of agonistic properties. It is known that a proper orientation of Tyr^2 side chain is necessary for agonistic activity.[23] The substitution of Tyr^2 with D-Tyr^2 produces an analogue with only partial agonist activity, whereas the deletion or O-alkylation of Tyr^2 hydroxyl group results in antagonistic properties.[23] With the intention of explaining the differences in biological activities of the investigated analogues, their three-dimensional structures have been formerly resolved using NMR spectroscopy in aqueous solution and dimethyl sulfoxide.[24,39] It has been shown that the geometry of a disulfide bridge does not influence on the activity of the analogues, and it has been concluded that the biological activity could rather depend on a mutual arrangement of the aromatic side chains at positions 2 and 3.[24,39] In this study the molecular docking has been used to determine

Table 1. Biological Activity of AVP Analogues Substituted at Positions 2 and 3 with Enantiomers of *N*-Methylphenylalanine toward the Respective Receptors[22]

	OTR	V1aR	V2R
[D-$MePhe^{2,3}$]AVP	6.4	0	0
[D-$MePhe^2$,$MePhe^3$]AVP	6.6	5.8	0
[$MePhe^2$,D-$MePhe^3$]AVP	0	0	0
[$MePhe^{2,3}$]AVP	0	0	0

[a] The pharmacological properties of analogues were tested in the uterotonic, pressor, and antidiuretic tests in vitro The activity values are given in the pA_2 (pA_2 values represent the negative base 10 logarithm of the average molar concentration of the antagonist that will reduce the appropriate biological response to 2*x* units of agonist to the level of *x* units).

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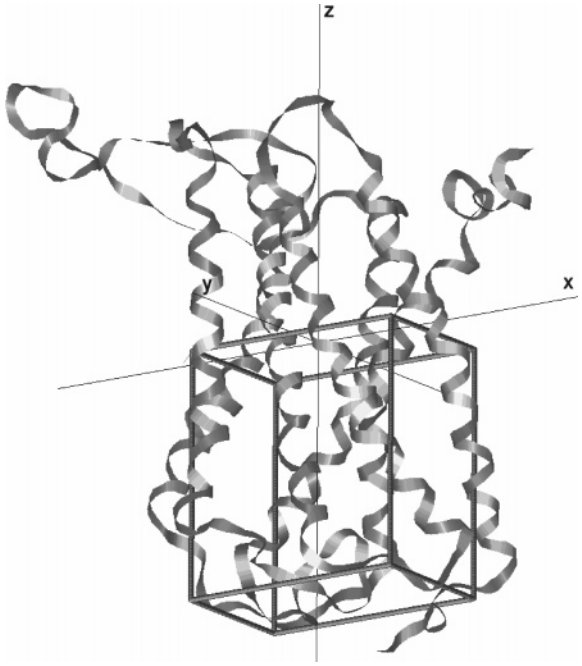

Figure 1. Representation of the box delimiting the ligands docking space. The approximate location and size of the ligand binding site has been defined before the docking.

receptor-bound conformations of the investigated analogues and to validate a thesis on a crucial role of the aromatic side chains orientations on the analogue activity. Molecular modeling approach may provide a "biologically active" conformation of the ligand and explain the receptor−ligand interactions at the atomic resolution.

Methods

Model Building and Molecular Docking. Nonstandard amino acid residues and other structure fragments were parametrized as recommended in the Amber 7.0 manual.[35−37] Specifically, the point atom charges were fitted by applying the Resp procedure to the electrostatic potential calculated in the 6-31G* basis set using the program Gamess.[36,38] The three-dimensional models of vasopressin analogues were built using the obtained NMR coordinates.[39] The three-dimensional models of the neurohypophyseal receptors (V2R, V1aR, and OTR) were constructed as described previously.[40−43] Briefly, the receptor models were built by applying multiple sequence alignment for bovine rhodopsin (RD), OTR, V1aR, and V2R and subsequently using the three-dimensional model of inactive RD as the template.[16,44] Computer mutations, insertions, and/or deletions ensuing from multiple sequence alignment were performed using standard Amber 7.0 tools and the Biopolymer module of Sybyl.[35,45] Subsequently, all receptor and ligand models were energy-minimized and relaxed using the Amber 7.0 force field.[35]

In the next step, all four analogues were docked to the OTR, V1aR, and V2R, using a modified genetic algorithm (GA) as implemented in the AutoDock program.[46,47] This docking procedure is a hybrid search technique that implements an adaptive global optimizer with local search. The global search method does not require gradient information in order to proceed, and it also uses fixed variances for the determination of the probabilistic way of a change of a particular state variable, such as the x-translation.[47] These variances are either doubled or halved during the search, depending on the number of consecutive successful (drop in energy) or failed moves.[48] To execute docking, the approximate location and size of the ligand binding site in the receptor central cavity was defined using AutoGrid [46] (module of the AutoDock program) in agreement with the experimental structure−activity data.[3,5,49−53] In Figure 1 the representation of the "box" delimiting the ligand binding site in the docking, is presented. The obtained complexes were previously minimized in Amber 7.0.[35] The low-energy complex for each receptor−ligand set was selected. In the next step the relaxation of the selected complexes, using a constrained simulated annealing protocols (CSA) in vacuo for 15 ps with positional constrains for C^{α} atoms in transmembrane domains, was performed. [54−56] This procedure allowed us to maintain the receptor three-dimensional structure within the 7TM domain in homology to RD. The selected lowest-energy models of complexes were used for characterization and discussion of the ligand conformation and the receptor−ligand interactions.

Results and Discussion

Residue Indexing and Abbreviations of the Complexes Names. The ligand residues are identified using three letter codes with the indices in superscript, e.g. Phe^{3}. The receptors residues are identified using one letter codes with the universal class A indices (Ballesteros−Weinstein numbering scheme[25]) placed as superscripts, followed by the absolute numbers, e.g. V1aR $Y^{6.51}300$, facilitating match between equivalent residues in different receptors, e.g. $W^{6.48}$(288, 304) in (OTR, V1aR), respectively. Briefly, in the Ballesteros−Weinstein scheme, the specific superscript N.XX defines a position relative to the most conserved residue in the TM helix 'N', which is assigned the number 'N'.50. Residues placed in loops are identified with one letter code, followed only by the residue absolute number, e.g. EL2 D186. Describing receptor−ligand interactions, the names of the ligands [D-$MePhe^{2,3}$]AVP, [D-$MePhe^{2}$,$MePhe^{3}$]-AVP, [$MePhe^{2}$,D-$MePhe^{3}$]AVP, and [$MePhe^{2,3}$]AVP will be abbreviated to DD, DL, LD, and LL, respectively.

Energy of Obtained Complexes and Location of the Docked Analogues. After the molecular docking, 360 relaxed receptor−ligand complexes have been obtained. Important factors validating the docking procedure are energy of the obtained complex and locations of docked analogue inside the receptor binding pocket. Both energy of a complex and location of a ligand is pertinent to the quality of fitting of the analogue in the receptor pocket, in relation to its biological activity.[40,42,43] For some receptor−ligand complexes the values of interaction energy following from docking are extremely (maximally) high, showing the difficulty with appropriate docking of this particular conformation of ligand. Indeed, if the AutoDock-calculated energy is in the order of 10^{12} kcal/mol (maximum), it simply means the impossibility of proper docking of the ligand. For complexes of all analogues with V2R as many as 40−50% complexes were those of maximum-energy, suggesting poor ligand fitting in the receptor binding cavity, in the agreement with experimental data (see Table 1). Among the remaining complexes, both with OTR and V1aR, poor "hits" do not exceed 20−30%. The difference in the number of improperly docked ligands is probably a result of a better sequence identity between OTR and V1aR than between any of the former and V2R.

The ligand locations in specific receptors are dissimilar and might partially explain the differences in their biological activities. It is known that the macrocyclic ring of AVP is docked inside the 7TM domain, while the three-residue tail, and especially Arg^{8}, protrudes outside the 7TM, toward the extracellular domain.[49−53] The described location is well correlated with the dual polarity of the receptor: hydrophobic cavity and hydrophilic extra- and intracellular domains. During the docking in AutoDock, where the ligand is instantly generated in the optimal (lowest-energy) location inside the binding cavity, nonphysiological ligand orientation, incompatible with the receptor pocket polarity, is permissible. In Table 2 the statistics of analogues orientation is given. The percentage shows the

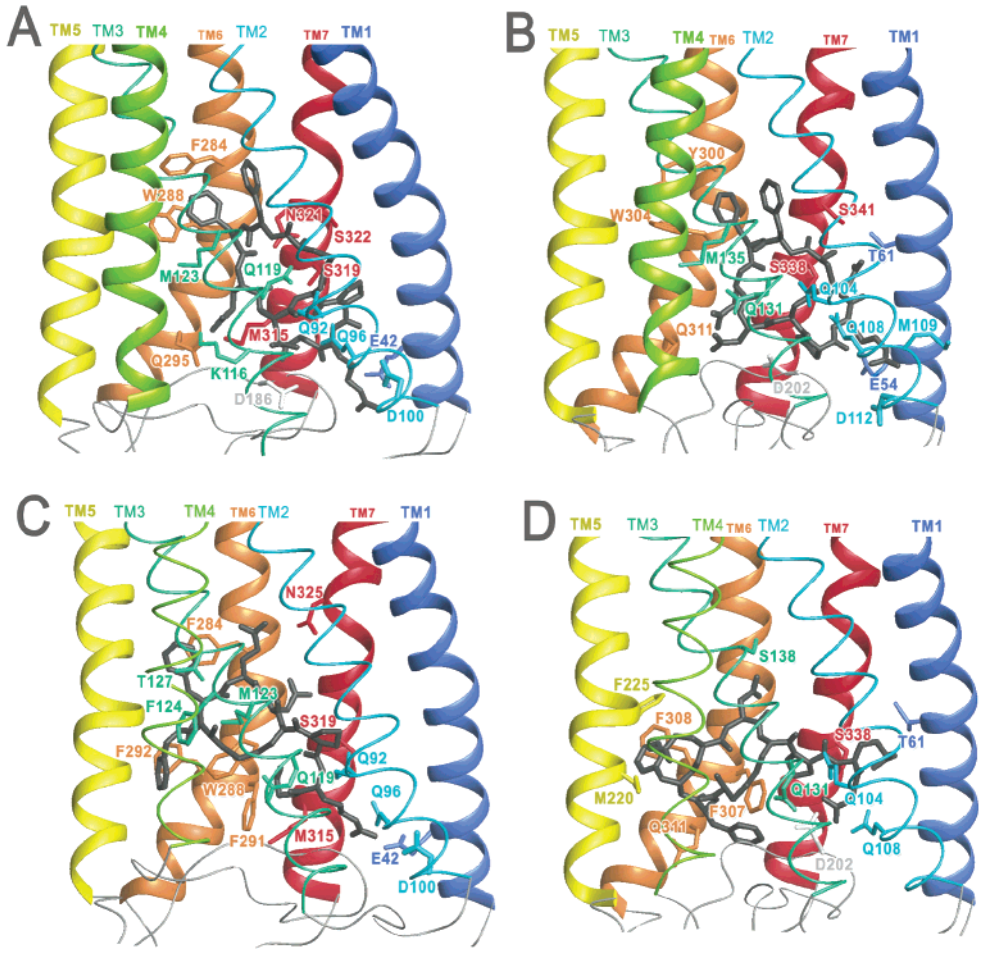

Figure 2. Representation of the DL and DD analogues binding pockets in OTR and V1aR. Panel A, OTR−DL; panel B, V1aR−DL; panel C, OTR−DD; panel D, V1aR−DD. Only receptor extracellular parts are shown. The TM helices are arranged counterclockwise from TM1 (blue) to TM7 (red); ligand is black. Several helices are drawn thinner for clarity. The binding amino acid residues are marked, and their side chains are exposed.

Table 2. The Percentage of the Ligand Orientations Having the Macrocyclic Ring Docked Inside the Binding Cavity and Arg[8] Protruding toward the Extracellular Side

	DL	DD	LD	LL
OTR	50	55	35	25
V1aR	40	25	35	35
V2R	30	30	30	30

proper ligand orientation compatible with the receptor polarity. In all complexes the percentage of proper ligand location is relatively low in agreement with the low biological activity of investigated analogues (see Table 2). Nevertheless, the detailed comparison of Tables 1 and 2 reveal that the differences in the experimentally determined receptor−ligand affinities (Table 1) are reflected in the differences of AutoDock-resulting locations of the analogues inside the receptor cavities (Table 2). Accordingly, the previously investigated strong OTR antagonists, atosiban and barusiban, were properly orientated inside the receptor binding cavity in the majority of complexes.[40,42,43] Moreover, the mutual arrangement of the aromatic side chains at positions 2 and 3 is probably the most important factor determining the binding of DD and DL *versus* LD and LL analogues in specific receptors. It is possible that their arrangement in LD and LL analogues makes difficult fitting the tocin ring of a in the binding cavity due to the steric hindrances.

Analysis of DL and DD Complexes with OTR and V1aR. For two analogues exhibiting biological activity, viz. ([D-MePhe[2,3]]AVP and [D-MePhe[2],MePhe[3]]AVP, see Table 1) four low-energy complexes: both DL and DD per OTR and both DL and DD per V1aR, were chosen for further detailed examination after CSA (see Methods). The receptors amino acid residues forming the putative ligand binding pockets were selected using the distance criterion. Thus, all receptor residues whose any atom was not farther than 3.5 Å from any atom of the ligand were selected. The OTR and V1aR residues proposed as interacting with the DL and DD are given in Table 3. The most important receptor−ligand interactions are presented in Figure 2.

The location of DL is vertical (parallel to the longer axis of the receptor) in both OTR and V1aR, with the aromatic rings immersed deeply into the hydrophobic bottom of the cavity and the Arg[8] guanidinium group exposed toward the hydrophilic extracellular side (see Figure 2, panels A, B). The location of DD analogue in OTR, although rather tilted than vertical, places the aromatic side chains still in a close contact with the hydrophobic bottom of the binding pocket with Arg[8] guanidinium group turned toward hydrophilic entrance of the binding pocket (see Figure 2, panel C). on the contrary to situation of the same analogue in V1aR, where the ligand position is more even more "horizontal" (i.e. perpendicular to the longer axis of the receptor). In the latter complex, the aromatic rings are situated approximately at the same depth as the Arg[8] guanidinium group, which interacts with the deeper part of the receptor than in the former three complexes (see Figure 2, panel D). It is noticeable that for the V1aR−DD complex "inap-

Table 3. List of the OTR and V1AR Residues Involved in the Interactions with the Ligands

TM 'N'domain	[D-MePhe^2,MePhe^3]AVP		[D-MePhe^{2,3}]AVP		universal numbering[25]
	OTR	V1aR	OTR	V1aR	
TM1	E42	E54	E42	−	1.35
	−	T61	−	T61	1.42
TM2	D85	D97	−	−	2.50
	V88	V100	V88	V100	2.53
	Q92	Q104	Q92	Q104	2.57
	V93	V105	V93	V105	2.58
	Q96	Q108	Q96	Q108	2.61
	−	M109	−	−	2.62
	D100	D112	D100	−	2.65
TM3	V115	V127	V115	V127	3.28
	K116	−	−	−	3.29
	Q119	Q131	Q119	Q131	3.32
	V120	−	V120	V132	3.33
	M123	M135	M123	M135	3.36
	−	−	F124	−	3.37
	S126	S138	S126	S138	3.39
	T127	−	T127	−	3.40
TM5	−	−	−	M220	5.42
	−	−	T205	T221	5.43
	−	−	−	F225	5.47
	−	−	V213	−	5.51
TM6	F284	Y300	F284	Y300	6.44
	C287	−	C287	−	6.47
	W288	W304	W288	W304	6.48
	F291	F307	F291	F307	6.51
	−	−	F292	F308	6.52
	Q295	Q311	Q295	Q311	6.55
TM7	I312	−	I312	−	7.36
	−	T333	−	−	7.38
	M315	A334	M315	A334	7.39
	A318	L335	A318	L335	7.41
	S319	S338	S319	S338	7.43
	N321	N340	N321	N340	7.45
	S322	S341	S322	S341	7.46
	N325	N344	N325	−	7.49
EL2	V184	−	V184	−	−
	F185	R201	F185	−	−
	D186	D202	−	D202	−
	W188	W204	W188	W204	−

propriate" locations of the docked ligand (see Table 2) indicate difficulty with the fitting ligand into the receptor cavity in the docking process, thus suggesting a weaker binding of DD in V1aR in analogy to the nonactive LL and LD analogues.

In Figure 3, the superimpositions of NMR-based conformations (with the highest statistical weight) of the DL and DD analogues,[39] and their receptor-bound conformations (from the lowest-energy complex) are given. The conformation of the tocin ring of DL and DD analogues did not change considerably during docking, and the RMSd measured on the C^{α} atoms of the tocin ring were as follows: 0.82 Å for DL docked in OTR; 0.50 Å for DL docked in V1aR; 0.72 Å for DD docked in OTR; 0.55 Å for DD docked in V1R. Conversely, the significant changes of the side chain conformation and the C-terminal three-residue tails as well as their location relative to macrocyclic ring can be observed in both DL and DD. In the receptor-bound conformation of DL, the aromatic side chains of the residues at positions 2 and 3 are situated on the edge of the ligand and may strongly interact with each other stronger than in the unbounded analogue. Thus, in both OTR−DL and V1aR−DL complexes, the dislocation of D-MePhe^3 aromatic side chain results in the increase of the intramolecular $\pi-\pi$ interaction in both OTR-bound and V1aR-bound conformations of DL (see Figure 3, panels A and B). However, this interaction is to a little extent weaker in the latter, where a small rotation of D-MePhe^3 aromatic ring is observed (Figure 3, panel B). Completely different phenomena can be observed in the receptor-bound conformations of DD analogue. Unexpectedly, the energetically favorable, almost parallel orientation of the

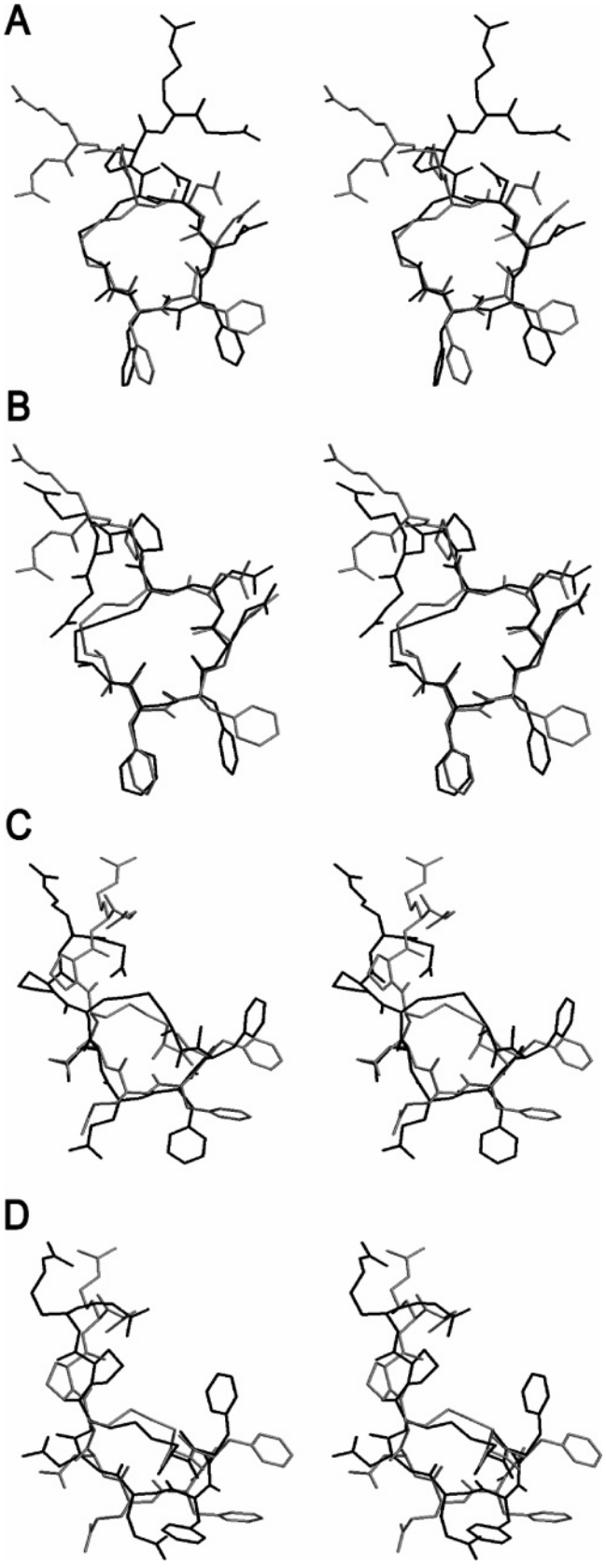

Figure 3. Stereodiagrams of superimposition of the analogue conformations inside the respective receptors. NMR-based structures before docking are gray, and receptor-bound conformations are black. Panel A, DL in OTR; panel B, DL in V1aR; panel C, DD in OTR; panel D, DD in V1aR.

D-MePhe^2 and D-MePhe^3 aromatic side chains in the unbounded analogue (NMR-determined structure) is not retained in the

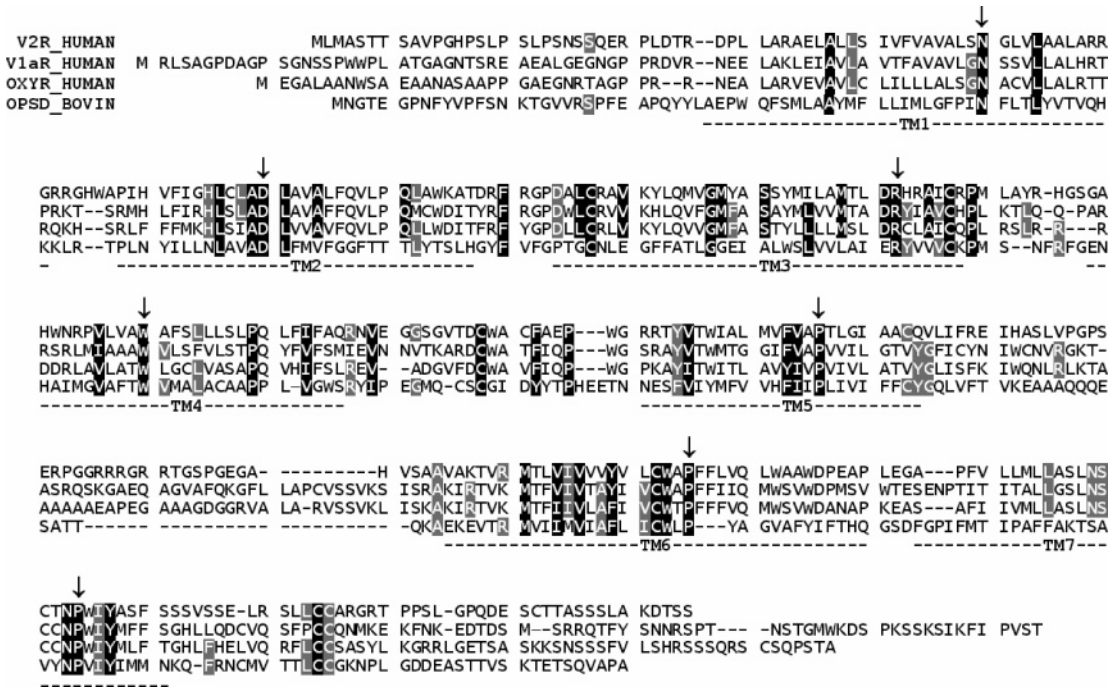

Figure 4. Primary sequence alignment of the human neurohypophyseal hormone receptors (OTR, V1aR, and V2R) and bovine rhodopsin, obtained using Multalin.[44] The putative transmembrane helices 1−7 are underlined. The TM 'N'50 residues are marked with an arrow.[25]

receptor-bound conformations. Thus, in both OTR-bound and V1aR-bound conformations of DD the reciprocal location of aromatic side chains at positions 2 and 3 excludes their $\pi-\pi$ interaction (see Figure 3, panel C and D). Therefore, it seems that the location of the aromatic rings at positions 2 and 3 in receptor-bound conformations results from forming some crucial aromatic receptor−ligand interaction (see below), which are more energetically favorable than an intramolecular $\pi-\pi$ interaction would be.

The Interactions Responsible for DL and DD Binding in OTR and V1aR. The residues proposed as interacting with the two biologically active DL and DD analogues are given in Table 3. As one can see, the OTR residues interacting with either DL or DD analogues are nearly the same (see Table 3), in agreement with the similar ligand locations inside the receptor binding cavity, as well as with nearly identical antagonistic activity (see Table 1). Regarding V1aR, although both ligands are situated differently, similar residues are still involved in interactions; however, some strong interactions that might be responsible for receptor−ligand selectivity do not appear (see below and Table 3).

All investigated complexes are stabilized by networks of hydrogen bonds (see Figure 2), which appear to be important anchors keeping the ligand inside the binding site and stabilizing the receptor−ligand complexes. Very important interactions in all four analyzed complexes involve the highly conserved (for sequence alignment of neurohypophyseal hormone receptors see Figure 4) 7TM Gln residues ($Q^{2.57}$, $Q^{2.61}$, $Q^{3.32}$, and $Q^{6.55}$). These residues make important parts of the receptor binding pockets and have been previously identified as interacting with the neurohypophyseal hormones and their analogues.[26,27,40,42,43,49] They are involved in stabilization of the receptor−ligand complexes, but they do not pertain to selectivity toward the respective receptors.

A crucial interaction for selectivity might be the salt bridge between the guanidinium group of Arg^8 and the carboxyl of the nonconserved $D^{2.65}$ (100, 112). This interaction occurring in the V1aR−DL complex is also present in both complexes with OTR but not in the nonactive V1aR−DD complex. Remarkably, in V2R (which binds neither of the investigated analogues) the equivalent position is occupied with the positively charged $K^{2.65}$ 100 (see Figure 4), incapable of forming a salt bridge with Arg^8. It is known that Arg^8 is crucial for selectivity in the vasopressin receptors.[3,28] Thus, identification of interactions of its guanidinium group via a salt bridge with the $D^{2.65}$ carboxyl might be critical for ligand binding. In addition, this acidic residue is located at the extracellular ends of the TM2; hence, it may be also involved in the ligand recognition. Another important interaction involving the Arg^8 guanidinium group is the hydrogen bond formed with the carboxyl of $E^{1.35}$ 54, in the V1aR−DL complex and in both complexes with OTR (with the equivalent residue $E^{1.35}$ 42). This interaction is not present in the V1aR−DD complex. Moreover, in the V1aR−DD complex does not appear any interaction with the conserved $N^{7.49}$ 344 interacting with the ligand in three remaining complexes. The nonconserved $M^{2.62}$ 109, $T^{7.38}$ 333, and EL2 R201 interact with the ligand exclusively in the V1aR−DL complex. Consequently, the lack of some polar interactionsin the V1aR−DD complex could partially explain the differences between the DL and DD activities with respect to V1aR (see Table 1).

In all complexes, besides the polar contacts, strong aromatic−aromatic receptor−ligand interactions can be observed as listed in Table 3. Thus, the interactions of the aromatic residues at positions 2 and 3 with $F^{6.44}$ 284, $Y^{6.44}$ 300, and $W^{6.48}$ (288, 304), $F^{6.51}$ (291, 307), and $F^{6.52}$ (292, 308) in OTR and V1aR, respectively, can be observed (see Table 3 and Figure 2). The most important seem to be the interactions with $W^{6.48}$ (288, 304) present in all complexes and with $F^{6.44}$ 284 ($Y^{6.44}$ 300), found in three complexes, again with the exception of the V1aR−DD complex. The strong aromatic−aromatic interactions with $W^{6.48}$ (288, 304), $F^{6.44}$ 284 ($Y^{6.44}$ 300), and other aromatic residues result in strong binding of TM6 and might prevent from receptor activation. The hypothesis that the TM6 cluster of aromatic residues is involved in stabilization of an inactive state of the receptor has been formulated for thyrotropin-releasing hormone

(TRH) receptor[29] and also confirmed in our former investigations.[30,40−43] Moreover, the interaction of the aromatic TM6 residues with ligands appears to be a feature typical of members of the GPCRs family A.[15,31−34]

Conclusion

In this study four cyclic AVP analogues substituted at positions 2 and 3 with combinations of enantiomers of *N*-methylphenylalanine were docked into OTR, V1aR, and V2R. During the docking, the N-methylated residues at positions 2 and 3 act as a structural restraint, determining the conformation of analogues, their location inside the receptor cavity, and mutual arrangement of the aromatic side chains. For the completely inactive LL and LD analogues, in general, it seems to be energetically unfavorable to adopt a conformation that allows efficient docking. For the biologically active DL and DD analogues, the receptor-bound conformations have been determined, showing that the conformation of the tocin ring does not change considerably, contrary to the highly flexible three-residue C-terminal tails. The location of the aromatic side chains at positions 2 and 3 is determined by their strong $\pi-\pi$ interaction with the receptor residues. These aromatic interactions with the TM6 cluster of aromatic residues are probably responsible for antagonistic activity of investigated analogues. The conserved polar residues constitute the handles keeping the biologically active DL and DD analogues inside the binding cavity, whereas the Arg^{8}-$D^{2.65}$ salt bridge might be responsible for their selective binding in OTR and V1aR versus V2R. In V2R, which does not bind any of the investigated analogues, this interaction would be impossible due to presence of positively charged $K^{2.65}100$ at the equivalent position.

The biological activity of investigated analogues is determined by the receptor-bound conformation and location, making the interactions with several crucial receptor residues (im)possible. The detailed knowledge about the receptor−ligand interactions at the molecular level will allow optimization of the pharmacological profile of investigated compounds by guiding the synthesis of the next generation of AVP analogues. Therefore, the amino acids at positions 2, 3, and 8 have been determined in this study to be especially crucial for binding due to their interaction with key receptor residues. Specifically, the simultaneous interaction of these residues with TM6 cluster and $D^{2.65}$, respectively, appears to be responsible for activity of investigated analogues, as proposed. The differences in chirality of N-methylated residues at positions 2 and 3 result in different analogue selectivity. However, the amino acid residue at position 8 is occupied by arginyl in all investigated analogues. The substitution of this arginyl may provide new drugs with improved antagonistic activity and selectivity toward oxytocin receptors.

In summary, this molecular modeling study is in good agreement with experimental data.[22,24,39] Moreover, our results complete these data, providing the detailed information about the receptor-bound conformations of analogues and their interaction with vasopressin and oxytocin receptors at the molecular level. Molecular modeling of receptor−ligand interactions in the conjuction with pharmacological and NMR data appear to be valuable tool to determine the relationships between the structure and activity of newly synthesized compounds and thus may be a successful approach to designing a new generation of potent drugs with improved selectivity.

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