A Molecular Model of Agonist and Nonpeptide Antagonist Binding to the Human V₁ Vascular Vasopressin Receptor¹

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Accepted for publication March 14, 2000

This paper is available online at http://www.jpet.org

ABSTRACT

The affinity of the nonpeptide antagonist OPC-21268 is greater for the rat V₁ arginine vasopressin (AVP) receptor (V₁R) than for the human V₁R. Site-specific mutagenesis was carried out to identify the residues that determine interspecies selectivity for nonpeptide antagonist binding. The introduction of rat amino acids in position 224, 310, 324, or 337 of the human V₁R sequence dramatically altered OPC-21268 affinity for the receptor, whereas binding of AVP, the peptide V₁R antagonist d(CH₂)₅Tyr(Me)AVP, and the nonpeptide V₁R antagonist SR49059 was not altered by these mutations. Computer modeling explained the mutagenesis results. Docking of OPC-21268 onto a homology-built model of the V₁R receptor yielded a model for the bound ligand in which the hydrophobic part is deeply embedded in the transmembrane region, whereas the polar part is located on the surface of the extracellular side. The increased affinity of the G337A mutant is due to two additional van der Waals contacts of the alanine methyl group with carbon atoms on the antagonist. The I310V mutant reduces the hydrophobicity in the vicinity of the polar oxygen atom of the antagonist. The I224V mutant relieves overcrowding in a hydrophobic binding pocket involving the aromatic residues Trp¹⁷⁶, Phe¹⁷⁹, Phe³⁵⁷, and Trp³⁰⁴. Finally, the E324D mutant enables the formation of a hydrogen bond of the carboxylate side chain with the amide side chain of Gin³¹¹, which in turn forms a hydrogen bond with the N57 nitrogen atom of OPC-21268. Thus, a few residues, distinct from those involved in agonist binding, control interspecies selectivity toward OPC-21268 nonpeptide antagonist binding.

The neurohypophysial hormone arginine vasopressin (AVP) is a cyclic nonapeptide (Fig. 1) whose actions are mediated by specific receptors. The first nonpeptide AVP antagonist found by random screening and optimization of chemical entities (Yamamura et al., 1991), OPC-21268 (Fig. 1), has an affinity for the human V₁R. Site-specific mutagenesis was carried out to identify the residues that determine interspecies selectivity for nonpeptide antagonist binding. The introduction of rat amino acids in position 224, 310, 324, or 337 of the human V₁R sequence dramatically altered OPC-21268 affinity for the receptor, whereas binding of AVP, the peptide V₁R antagonist d(CH₂)₅Tyr(Me)AVP, and the nonpeptide V₁R antagonist SR49059 was not altered by these mutations. Computer modeling explained the mutagenesis results. Docking of OPC-21268 onto a homology-built model of the V₁R receptor yielded a model for the bound ligand in which the hydrophobic part is deeply embedded in the transmembrane region, whereas the polar part is located on the surface of the extracellular side. The increased affinity of the G337A mutant is due to two additional van der Waals contacts of the alanine methyl group with carbon atoms on the antagonist. The I310V mutant reduces the hydrophobicity in the vicinity of the polar oxygen atom of the antagonist. The I224V mutant relieves overcrowding in a hydrophobic binding pocket involving the aromatic residues Trp¹⁷⁶, Phe¹⁷⁹, Phe³⁵⁷, and Trp³⁰⁴. Finally, the E324D mutant enables the formation of a hydrogen bond of the carboxylate side chain with the amide side chain of Gin³¹¹, which in turn forms a hydrogen bond with the N57 nitrogen atom of OPC-21268. Thus, a few residues, distinct from those involved in agonist binding, control interspecies selectivity toward OPC-21268 nonpeptide antagonist binding.

The combination of receptor three-dimensional modeling and site-directed mutagenesis experiments has suggested that the AVP agonist binding domain is made of a 15- to 20-Å-deep central cavity defined by the transmembrane helices and surrounded by the extracellular loops of the receptor (Moïlliac et al., 1995; Hibert et al., 1999). As shown for other families of GPCRs, residues that are critical for peptide agonist binding are not involved in antagonist binding to the AVP-OT receptors. Furthermore, the determinants of nonpeptide AVP receptor antagonist binding were unknown before this work.

The first nonpeptide AVP V₁R antagonist found by random screening and optimization of chemical entities (Yamamura et al., 1991), OPC-21268 (Fig. 1), has an excellent affinity for

ABBREVIATIONS: AVP, arginine vasopressin; OT, oxytocin; V₁, V₂, V₃, vascular vasopressin receptor; V₁R, V₂R, V₃R, renal vasopressin receptor; V₅R, V₆ pituitary vasopressin receptor; GPCR, G protein-coupled receptor; CHO, Chinese hamster ovary; TMS, transmembrane segment.
the rat V1R (25 nM) but a poor affinity for the human V1R (8800 nM) (Thibonnier et al., 1998a). The human and rat V1Rs share a high degree of structural homology with 96% sequence identity. The differing residues are presumably involved in species-related variations in antagonist binding. Comparison of the human and rat V1R sequences revealed that only 20 amino acid differences are present in the extracellular loops and the upper portions of the transmembrane segments (TMs; see Fig. 3). We reasoned that these interspecies differences in amino acid sequence modulate the receptor affinity for nonpeptide compounds. In this work, we produced a series of reverse mutations in which corresponding rat amino acids were introduced by site-directed mutagenesis into the human V1R sequence. The influence of these interspecies amino acid differences on nonpeptide antagonist binding was subsequently tested. A single amino acid substitution in the seventh TMS produced a 27-fold increase in the affinity toward OPC-21268. To gain information about the location of the OPC-21268 binding site, a model of this compound was docked onto a homology-built three-dimensional model of the human V1R. The hydrophobic moieties of this nonpeptide antagonist were found to be located deep within the transmembrane region, whereas the polar part is on the extracellular surface. This model of the ligand-receptor complex is consistent with the mutagenesis results and provides an explanation for the increased affinity of the mutants tested in this study.

**Experimental Procedures**

**Materials.** Standard reagents, unless stated otherwise, were purchased from Sigma Chemical Co. (St. Louis, MO). CHO-K1 cells were obtained from American Type Culture Collection (Rockville, MD). Cell culture media and geneticin were purchased from Life Technologies (Grand Island, NY). Fetal bovine serum was obtained from Entrogen (San Diego, CA). AVP and [3H]AVP were purchased from Amersham (Arlington Heights, IL). The nonpeptide V1R antagonists OPC-21268 and SR-49059 were purchased from Bachem California (Torrance, CA). The nonpeptide V1R antagonist OPC-21268 (batch no. 93F92 M) was provided by Dr. deil-Le Gal (Sanofi Recherche, Toulouse, France). The nonpeptide V1R antagonists OPC-21268 and SR-49059.

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The three extracellular and three intracellular loops of the V₁R were subsequently constructed with program Look v3.5 (Molecular Applications Group, Palo Alto, CA), using the spatial constraints for the ends of each loop provided by the coordinates of the helical bundle. Look v3.5 is a protein-modeling program that segmentally builds a protein by aligning short stretches of its sequence with homologous peptides of known structure and performs a full energy refinement of the model (Levitt, 1992). Because the N- and C-terminal domains of the V₁R are not involved in the binding of agonists or antagonists, they were not included in this model (Mouillac et al., 1995; Howl and Wheatley, 1996; Mendre et al., 1997; Thibonnier et al., 2000).

A disulfide bridge exists between cysteines 124 and 203 located on the second and third extracellular loops, respectively. Disruption of this disulfide bridge is known to cause a significant drop in binding affinity of ligands (Mouillac et al., 1995; Postina et al., 1996). Thus, it was necessary to ascertain that these cysteine residues were close enough in the model and that the sulfhydryl groups had the proper orientation to be able to form the disulfide bridge. This was achieved by performing an energy refinement in program X-PLOR (Brünger, 1993) with the constraint of forming this particular disulfide bridge. The sulfur-sulfur distance refined to a value of 2.03 Å, consistent with the formation of a disulfide bridge. The remainder of the structure was not significantly altered by this refinement procedure.

**Molecular Modeling of AVP and Antagonist Ligands.** Using program Look v3.5, a model of 8-AVP was built based on the structure of OT obtained from the crystallographically determined structure of the neurophysin-OT complex (Fig. 1; Rose et al., 1996). This model of AVP assumed a type I β-turn structure, containing a hydrogen bond between the carbonyl oxygen of Tyr² and the amide proton of Asn⁵ (Fig. 2A).

A model of the nonpeptide V₁R antagonist OPC-21268 was constructed with the program Alchemy 2000 (Fig. 2B; Tripos Inc., St. Louis, MO). This compound was first drawn in two dimensions and then extended into a three-dimensional model by a two-dimensional-to-three-dimensional builder incorporated in Alchemy 2000. Three possible rotations, each differing by 120°, of the bond between the lactam nitrogen and the piperidyl ring were considered. Each rotamer was subjected to an energy minimization, and the most stable of the three rotamers was chosen. A conformational search was performed on the resulting structure, systematically stepping through all possible rotations of the bonds of the piperidyl ring and of the bond connecting this ring to the lactam nitrogen. A rotational increment of 3° was set for the bonds in the ring, whereas an increment of 30° was used for the bond connecting the ring to the lactam nitrogen. Conformations with the lowest energy and devoid of any short contacts were saved. Three of the most stable conformations were subjected to yet another energy minimization, as were the corresponding conformations with a 180° rotation about the amide bond of the piperidyl nitrogen. Finally, the most stable conformation of these six was subjected to an optimization using the program Gaussian 98 (Gaussian, Inc., Pittsburgh, PA). A similar strategy was used to build a model of the nonpeptide V₁R antagonist SR-49059 (Fig. 2C).

**Docking of AVP and Antagonist Ligands onto V₁R.** Docking of the ligands was done with the program LiGIN (Sobolev et al., 1996), based on a built-in complementarity function. This function is
a sum of the surface areas of atomic contacts. These contacts are weighted according to the types of atoms in contact, and another term is included to prevent short contacts. After maximizing the complementarity function, LIGIN optimizes the lengths of possible hydrogen bonds. To take into account possible movements of the receptor on ligand binding, steric overlap between the ligand and a specified number of residues in the receptor can be allowed without energy penalty.

The model of AVP was docked onto V₃R by initially placing it in the upper portion of the transmembrane region (the expected binding pocket) and allowing LIGIN to search for the binding site within a 20-Å box around the original ligand position. A similar procedure was used for the docking of the antagonist OPC-21268. In the docking of both AVP and the antagonist, some steric overlap (one to three residues) was allowed between the ligand and receptor. Energy minimization with program X-PLOR relieved these short contacts.

**Molecular Modeling of Site-Directed Mutagenesis.** After docking the model of OPC-21268 onto wild-type V₃R, the receptor-ligand complex was subjected to an energy refinement using program X-PLOR. Interactions between this antagonist and four residues on the receptor (Ile²²⁴, Ile³¹⁰, Glu³²⁴, and Gly³³⁷) were analyzed. Based on the mutagenesis results, mutations of these four residues to Val²²⁴, Val³¹⁰, Asp³²⁴, and Ala³³⁷ were modeled in the program O (Jones et al., 1991). Interactions between the antagonist and the mutated residues, as well as any other residues in close contact to the ligand, were analyzed.

**Data Analysis.** Nucleotide and amino acid sequences were analyzed with the computer package MacVector (Oxford Molecular, Oxford, UK) on a Macintosh G3 computer. Binding parameters (Kᵢ and Bₘₐₓ) of AVP receptors were calculated by a nonlinear least-squares analysis program using the software package Kaleidagraph (Synergy Software, Reading PA; Thibonnier and Roberts, 1985). Data were expressed as mean ± S.E. Statistical analysis was performed with Kruskal-Wallis and Mann-Whitney nonparametric tests (StatView statistical package; Abacus Concepts, Berkeley, CA). P < .05 was considered statistically significant.

**Results**

**Radioligand Binding Characteristics of Wild-Type and Mutated Human V₃Rs.** The amino acid differences between human and rat V₃Rs are presented in Fig. 3. Mutations that altered the charge or shape were produced by introducing corresponding rat amino acid residues into the wild-type human V₃R sequence. Because mutations located into the first two extracellular loops do not affect the affinity of antagonists (Mouillac et al., 1995), we centered our attention on interspecies amino acid differences present in the other components of the ligand binding pocket. An extensive ligand binding characterization of these mutated human AVP receptors was carried out in stably transfected Chinese hamster ovary (CHO) cells. As shown previously, CHO cells do not express endogenous AVP-OT receptors (Thibonnier et al., 1994), and each clone tested in our experiments expressed a single AVP receptor clone.

The wild-type human V₃R expressed in CHO cells displayed a high affinity not only for the endogenous hormone AVP, but also for the reference V₃R peptide antagonist d(CH₂)₅Tyr(Me)AVP (“Maurice Manning’s V₃R antagonist”) and the V₃R nonpeptide antagonist SR-49059 (Table 1). As we observed previously, the affinity of the wild-type human V₃R for the OPC-21268 compound was quite weak.

None of the nine single mutations, two double mutations, and one triple mutation engineered in this study interfered with the normal folding of the V₃R within the plasma membrane, and the levels of expression of all of the mutated clones were similar to that of the wild-type human V₃R (Bₘₐₓ = 11,000–25,000 fmol/mg of protein).

For all of these mutated human V₃Rs, affinity for the natural hormone AVP, for the V₃R peptide antagonist d(CH₂)₅Tyr(Me)AVP, and for the V₃R nonpeptide antagonist SR-49059 remained in a close range (Kᵢ = 0.26–1.75 nM;
encountered for the rat V1R: 20 and 29 nM, respectively. The
compound (303-/440-fold improvement), similar to the values
each value is the mean of four to eight independent experiments.

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### Table 1

<table>
<thead>
<tr>
<th>Mutation</th>
<th>AVP</th>
<th>d(CH2)5Tyr(Me)AVP</th>
<th>SR49059</th>
<th>OPC21268</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>1.73 ± 0.12</td>
<td>1.59 ± 0.05</td>
<td>1.06 ± 0.08</td>
<td>8800 ± 1200</td>
</tr>
<tr>
<td>G134A</td>
<td>0.29 ± 0.04*</td>
<td>0.66 ± 0.02</td>
<td>0.46 ± 0.01</td>
<td>5046 ± 470</td>
</tr>
<tr>
<td>G222S</td>
<td>0.48 ± 0.02</td>
<td>0.59 ± 0.10</td>
<td>0.79 ± 0.04</td>
<td>4764 ± 398</td>
</tr>
<tr>
<td>I224V</td>
<td>0.63 ± 0.03</td>
<td>1.21 ± 0.07</td>
<td>0.79 ± 0.02</td>
<td>1242 ± 51*</td>
</tr>
<tr>
<td>I310V</td>
<td>0.52 ± 0.03</td>
<td>0.52 ± 0.03</td>
<td>0.34 ± 0.01*</td>
<td>3865 ± 304*</td>
</tr>
<tr>
<td>P318G</td>
<td>0.56 ± 0.01</td>
<td>0.54 ± 0.01</td>
<td>0.46 ± 0.02</td>
<td>5239 ± 305</td>
</tr>
<tr>
<td>M319N</td>
<td>0.87 ± 0.04</td>
<td>0.88 ± 0.04</td>
<td>0.68 ± 0.02</td>
<td>4780 ± 451</td>
</tr>
<tr>
<td>SS320F</td>
<td>0.73 ± 0.05</td>
<td>0.36 ± 0.05</td>
<td>0.59 ± 0.13</td>
<td>4755 ± 366</td>
</tr>
<tr>
<td>E324D</td>
<td>0.49 ± 0.05</td>
<td>0.48 ± 0.02</td>
<td>0.63 ± 0.06</td>
<td>2655 ± 204*</td>
</tr>
<tr>
<td>G337A</td>
<td>0.95 ± 0.05</td>
<td>1.36 ± 0.13</td>
<td>0.57 ± 0.07</td>
<td>328 ± 19*</td>
</tr>
<tr>
<td>G337A + I310V</td>
<td>0.95 ± 0.05</td>
<td>1.75 ± 0.11</td>
<td>1.01 ± 0.11</td>
<td>185 ± 25*</td>
</tr>
<tr>
<td>G337A + E324D</td>
<td>1.45 ± 0.03</td>
<td>1.27 ± 0.02</td>
<td>0.52 ± 0.05</td>
<td>20 ± 2*</td>
</tr>
<tr>
<td>G337A + I224V</td>
<td>0.62 ± 0.04</td>
<td>1.25 ± 0.03</td>
<td>0.49 ± 0.02</td>
<td>29 ± 2*</td>
</tr>
<tr>
<td>G337A + E324D +</td>
<td>0.98 ± 0.12</td>
<td>0.26 ± 0.01*</td>
<td>0.27 ± 0.03*</td>
<td>28 ± 3*</td>
</tr>
<tr>
<td>I224V</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P < .05 compared with the affinity of the wild-type receptor for the same ligand.

Table 1). These fluctuations are presumably not relevant from a pharmacological viewpoint. At variance, significant improvement in the human V1R affinity for OPC-21268 was obtained by introducing single mutations in positions 224 in the fifth TMS (7-fold improvement), 324 in the third extracellular loop 3-fold improvement, and 337 in the seventh TMS (27-fold improvement). Simultaneous mutations of E324D and G337A or I224V and G337A produced dramatic improvement of the human V1R affinity for the OPC-21268 compound (303-440-fold improvement), similar to the values encountered for the rat V1R: 20 and 29 nM, respectively. The combination of the three mutations G337A + E324D + I224V did not further improve the affinity for OPC-21268.

**Docking of Hormone AVP onto Human V1R.** AVP has a polar as well as a nonpolar surface. The exocyclic tripeptide Pro2-Arg8-Gly9 and one side of the hormone ring (Gln4, Asn25) are mainly hydrophilic, whereas the other part of the ring (Cys1, Cys6, Tyr2, and Phe3) is essentially hydrophobic. This dual surface property is reflected in the nature of the binding pocket that is formed by residues from TMSs 1, 3, 4, 5, 6, and 7, as well as the first extracellular loop (Fig. 4). The bottom of the cleft is mainly hydrophobic, closed by the aromatic and hydrophilic residues Met1, Phe135, Phe136, Phe179, Phe307, and Ile330. The entrance to the binding pocket and one side of it contain predominantly hydrophilic residues. The Arg guanido group at the entrance to the cleft forms a salt bridge with Asp112 of the receptor. A salt bridge between Arg6 of AVP and Asp112 of the receptor is shown by a broken line. Two hydrogen bonds, one between Asn1 of AVP and Lys125 and the other between Gln4 of AVP and Gln185 of the receptor, are shown by broken lines. Distances (in angstroms) are indicated near the broken lines. The secondary structure assignment of the interacting receptor residues is shown by small tube representations of helical segments H1, H4, H6, and H7, as well as extracellular loop 1 (el1, Met136, Phe138, Ser142, and Ser151) also interact with AVP. They are not shown here for reasons of clarity. The former two residues are within van der Waals contacts of Phe3, whereas the latter two form hydrogen bonds with the amide side chain nitrogen of Gln4 and the hydroxyl group of Tyr2, respectively.

**Docking of Nonpeptide Antagonist OPC-21268 onto Human V1R.** The location of the bound antagonist OPC-21268 is distinct from the AVP-binding pocket with only partial overlap near the extracellular surface (Fig. 5). The hydrophobic part is embedded in the transmembrane region far deeper than AVP, whereas the polar part is located on the surface of the extracellular side. The binding pocket is formed by residues from TMSs 4, 5, 6, and 7, as well as the third extracellular loop (Fig. 6). The 27-fold increase in the affinity of the G337A mutant is explained by the formation of two van der Waals contacts of the methyl carbon with carbon atoms C22 and C28 of the bicyclic ring structure of OPC-21268 at the bottom of the cleft (Fig. 7). The E324D mutant...
has an indirect effect. It enables the formation of a hydrogen bond of the carboxylate side chain with the amide side chain atom of Gln<sup>311</sup>. This causes a polarization of this amide nitrogen atom and enables it in turn to form another hydrogen bond to the N57 nitrogen atom of OPC-21268 (Fig. 7).

The I310V mutant reduces the hydrophobicity in the vicinity of the polar oxygen atom of the antagonist. The I224V mutant relieves overcrowding in a hydrophobic binding site involving the aromatic residues Trp<sup>175</sup>, Phe<sup>179</sup>, Phe<sup>307</sup>, and Trp<sup>304</sup>. The smaller valine side chain allows for better positioning of the aromatic residues to interact with the bicyclic ring structure of OPC-21268 (Fig. 8). Finally, the I310V mutant reduces the hydrophobicity in the vicinity of the polar oxygen atom of the antagonist. Thus, the model explains all of the mutations that significantly increase the affinity toward OPC-21268.

**Discussion**

AVP receptors represent a logical target for drug development in several therapeutic fields. As a new class of therapeutic agents, orally active AVP analogs could be used in several pathophysiological conditions. V<sub>1</sub>R agonists increase the reabsorption of free water in central diabetes insipidus. V<sub>2</sub>R agonists could reduce the systemic vascular resistances noted in arterial hypertension, congestive heart failure, and peripheral arteriopathy. V<sub>2</sub>R antagonists could reverse the hyponatremia of Schwartz-Bartter syndromes, congestive heart failure, and liver cirrhosis. Mixed V<sub>1</sub>/V<sub>2</sub>R antagonists may prevent thromboembolic events in surgical patients. V<sub>2</sub>R agonists and antagonists could be valuable additions to the diagnosis, imaging, localization, and medical treatment of adrenocorticotropic hormone-secreting tumors. Finally, OT receptor antagonists could be used in the treatment of primary dysmenorrhea and premature labor (Thibonnier, 1998).

Three different strategies can be contemplated to develop ligands with high affinity and selectivity for a given AVP receptor subtype: 1) the systematic or rationale alterations of the ligand structure, implemented by Maurice Manning and collaborators who designed numerous peptide AVP and OT analogs (Manning et al., 1995); 2) the random screening for new chemical compounds, developed by pharmaceutical companies who isolated the first nonpeptide V<sub>1</sub>R and V<sub>2</sub>R antagonists (Yamamura et al., 1991, 1992; Serradeil-Le Gal et al., 1993, 1996); and 3) structure-based drug design, requiring the knowledge of the three-dimensional structure of both the ligand and receptor. The AVP-OT receptors crystallographic structure has yet to be established. However, modeling by analogy based on the structure of bacteriorhodopsin has been done for the seven TMSs of many GPCRs and has yielded useful information (Ji et al., 1998).

These three strategies are complementary. For instance,
conformational energy calculations carried out on three non-peptide AVP-OT antagonists (OPC-21268, OPC-31260, and penicilide) found that the affinity of these compounds and their selectivity for AVP and OT receptors are probably connected with mimicking the aromatic rings of the Tyr² and the Ile³ OT residues or with mimicking the aromatic rings of the Tyr² and Phe³ AVP residues (Oldziej et al., 1995). Similarly, this study illustrates that strategies 2 and 3 are indeed complementary.

By random screening and subsequent optimization of chemical entities, nonpeptide compounds were recently shown to antagonize AVP receptors (Yamamura et al., 1991, 1992; Serradeil-Le Gal et al., 1993, 1996). They specifically antagonize the V₁R or the V₂R and have different chemical structures. The first AVP receptor antagonist OPC-21268 was found to be a potent entity in rat models but was subsequently found to display a poor affinity for human AVP receptors (Thibonnier et al., 1998b). To expand our understanding of the molecular characteristics of the ligand-binding pocket of AVP receptors, we used the amino acid differences among mammalian species to search for the rat versus human molecular determinants of nonpeptide V₁R binding.

Our data confirm that the molecular determinants of agonist and antagonist binding as well as peptide versus nonpeptide compounds are distinct (Mouillac et al., 1995). Amino acid residues that are important for peptide agonist binding are not critical determinants in binding of the cyclic peptide d(CH₂)₇Tyr(Me)AVP, of the linear peptide antagonist phenylacetyl¹-D-Tyr(Me)²-Phe³-Gln⁴-Asn⁵-Arg⁶-Pro⁷-Arg⁸-NH₂, and of the nonpeptide V₁R antagonist SR49059 (Mouillac et al., 1995). Similarly, the molecular determinants of peptide antagonist binding to the OT receptor are different from those involved in peptide agonist binding; they are TMSs 1, 2, and especially 7. The introduction of just seven amino acids of the upper part of the seventh TMS of the OT receptor into the V₂R sequence is sufficient to introduce high-affinity binding for an OT peptide antagonist into the V₂R.

All point mutations affecting peptide agonist binding to AVP receptors were found to have no or little effect on peptide antagonist binding, thus suggesting that peptide agonist and antagonist binding requirements are physically distinct (Phalipou et al., 1997). So far, there is little information about the molecular determinants of nonpeptide antagonists binding to AVP-OT receptors besides the fact that they are different from those involved in peptide agonist and antagonist binding. Cotte et al. (1998) found that residues 202 in the second extracellular loop and 304 in the seventh TMS of the V₂R which modulated species selectivity of cyclic peptide antagonists containing a D-isoleucyl at position 2, did not contribute to binding of nonpeptide antagonists OPC-31260 and SR-121463A.

The combination of site-directed mutagenesis and three-dimensional modeling in our study identified key residues involved in binding of the nonpeptide antagonist OPC-21268 to the V₁R. Our data clearly identified a single residue in the seventh TMS, explaining the different affinities of the human
The docking model developed for this study confirmed the importance of this single residue: Ala\(^{337}\). Furthermore, the model predicts that a serine residue at this position should cause an even tighter binding due to the formation of a hydrogen bond between the serine O\(\gamma\) atom with the quinoline oxygen atom of the antagonist. The glutamic acid-to-asparagine mutation at position 324 has an indirect effect. It enables the formation of a hydrogen bond between an oxygen atom of the carboxylate and the amide side chain nitrogen atom of Gln\(^{311}\). This has a polarizing effect on this nitrogen atom that in turn stabilizes another hydrogen bond of this atom with atom N\(\gamma\) of OPC-21268.

Combination of the three mutations in positions 224, 324, and 337 did not further improve the affinity of the antagonist for OPC-21268 compared with the two double mutations, suggesting that the field of GPCRs has a lack of experimentally determined structures. Therefore, molecular modeling is a very useful tool to derive structural information for the V\(_1\)R. It provides a framework to design and test new drugs, as well as site-specific mutations, in a rational way. However, one must keep in mind the limitations of molecular modeling. The approach is based on the assumption that the seven TM helices of this approach are the loops connecting the helical regions as well as the N- and C-terminal nonhelical segments. The former were built by sequence similarity to known protein segments from a database within the program LOOK, whereas the N- and C-terminal stretches were left.

Fig. 7. The stabilizing effect of the G337A and E324D mutations on antagonist binding. Portions of helices 6 and 7 as well as of the extracellular loop 3 (el3) are shown in tube representation; the antagonist as well as selected residues of the receptor are shown in ball-and-stick representation. Distances are indicated in angstroms. The 27-fold increase in affinity of this glycine-to-alanine mutant can be explained by the formation of two new van der Waals contacts of the methyl group with the antagonist. The glutamic acid-to-asparagine mutation at position 324 has an indirect effect. It enables the formation of a hydrogen bond between an oxygen atom of the carboxylate and the amide side chain nitrogen atom of Gln\(^{311}\). This has a polarizing effect on this nitrogen atom that in turn stabilizes another hydrogen bond of this atom with atom N\(\gamma\) of OPC-21268.

Fig. 8. The stabilizing effect of the G337A, I224V, and I310V mutations on antagonist binding. Portions of helices 4, 5, 6, and 7 are shown in tube representation; the antagonist as well as selected residues of the receptor are shown in ball-and-stick representation. Distances are indicated in angstroms. The isoleucine-to-valine substitution at position 310 puts a less hydrophobic residue in the vicinity of the polar O\(\gamma\) oxygen atom of the antagonist compound. This should have a slightly stabilizing effect, and it could explain the 2.3-fold increase in affinity. Residue 224 is located deep in the transmembrane region in a crowded environment, which constitutes a hydrophobic binding pocket for the antagonist. The pocket consists of residues Trp\(^{175}\), Phe\(^{179}\), Phe\(^{307}\), and Trp\(^{304}\) in addition to residue 224. Substitution of the smaller valine for the larger isoleucine relieves overcrowding and allows for better positioning of the aromatic residues to interact with the bicyclic ring structure of OPC-21268. This could explain the 7.1-fold increase in affinity for this mutant.

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out altogether from the model because they are not involved in ligand binding. The validity of the model is supported by the experimentally determined affinities for the drugs. The model explains very well all of our findings. It does not prove that the model is correct, but the model is certainly consistent with the data, and it provides a tool for designing new drugs and mutants.

In conclusion, this study provides for the first time the structural basis of species-selective binding of a nonpeptide antagonist to the V1R. These findings should generate new ideas for drug development of nonpeptide AVP receptor antagonists and for optimizing drug-receptor interactions.

Acknowledgment

The program LIGP was kindly provided by Vladimir Sobolev from the Weizmann Institute of Science in Rehovot, Israel.

References


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