Annu. Rev. Pharmacol. Toxicol. 2001. 41:175–202


# THE BASIC AND CLINICAL PHARMACOLOGY OF NONPEPTIDE VASOPRESSIN RECEPTOR ANTAGONISTS

M Thibonnier, P Coles, A Thibonnier, and M Shoham
*Departments of Medicine and Biochemistry, Case Western Reserve University School of Medicine and University Hospitals of Cleveland, Cleveland, Ohio 44106–4951; e-mail: mxt10@po.cwru.edu*

**Key Words** vasopressin receptors, nonpeptide antagonists, SIADH, G protein–coupled receptors, aquaretics, hyponatremia, congestive heart failure, dysmenorrhea, liver cirrhosis

■ **Abstract** The neurohypophysial hormone arginine vasopressin (AVP) is a cyclic nonpeptide whose actions are mediated by the stimulation of specific G protein–coupled membrane receptors pharmacologically classified into $V_1$-vascular ($V_1R$), $V_2$-renal ($V_2R$) and $V_3$-pituitary ($V_3R$) AVP receptor subtypes. The random screening of chemical compounds and optimization of lead compounds recently resulted in the development of orally active nonpeptide AVP receptor antagonists. Potential therapeutic uses of AVP receptor antagonists include (*a*) the blockade of $V_1$-vascular AVP receptors in arterial hypertension, congestive heart failure, and peripheral vascular disease; (*b*) the blockade of $V_2$-renal AVP receptors in the syndrome of inappropriate vasopressin secretion, congestive heart failure, liver cirrhosis, nephrotic syndrome and any state of excessive retention of free water and subsequent dilutional hyponatremia; (*c*) the blockade of $V_3$-pituitary AVP receptors in adrenocorticotropin-secreting tumors. The pharmacological and clinical profile of orally active nonpeptide vasopressin receptor antagonists is reviewed here.

## INTRODUCTION[1]

The neurohypophysial antidiuretic hormone arginine vasopressin (AVP) is a peptide actively involved in the regulation of free water reabsorption, body fluid

[1]Since the original pharmacological studies and designation of AVP/OT receptor subtypes, their recent cloning and molecular characterization call for the revision of their nomenclature. For the sake of clarity and in reference to their main site of expression, in this review we call the $V_{1a}$ receptor the $V_1$-vascular receptor, the $V_2$ receptor the $V_2$-renal receptor, and the $V_{1b}$ or $V_3$ receptor the $V_3$-pituitary receptor.

osmolality, blood volume, blood pressure, cell contraction, cell proliferation, and adrenocorticotropin (ACTH) secretion via the stimulation of specific G protein–coupled receptors (GPCRs) currently classified into $V_1$-vascular ($V_1R$), $V_2$-renal ($V_2R$), and $V_3$-pituitary ($V_3R$) subtypes having distinct pharmacological profiles and intracellular second messengers (1, 2).

The nonosmolar release of AVP that activates the $V_2$-renal receptors is responsible for the hyponatremia noted in the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) and is instrumental in the development of dilutional hyponatremia observed in congestive heart failure, liver cirrhosis, and several renal pathologies (3). Hyponatremia is the most common electrolyte disorder noted in hospitalized patients, and mortality rate dramatically increases 60-fold in its presence (4). Moreover, currently available therapeutic modalities of dilutional hyponatremia are unsatisfactory and often associated with significant complications and toxicity.

AVP has been shown in vitro to be one of the most powerful vasoconstrictor substances in isolated vascular preparations through activation of the $V_1$-vascular receptors (5). AVP also stimulates blood platelet aggregation, coagulation factor release, and cellular proliferation. AVP vasoconstrictor and mitogenic actions may contribute to the pathogenesis of arterial hypertension, heart failure, and atherosclerosis (6, 7).

The secretion of ACTH by the anterior pituitary is predominantly regulated by corticotropin-releasing hormone but also by AVP through activation of the $V_3$-pituitary receptors. Indeed, AVP is an important physiological modulator of the hypothalamo-pituitary-adrenal axis (8). Moreover, the $V_3$-pituitary receptor is a corticotrophic phenotypic marker that is overexpressed in ACTH-hypersecreting tumors (9, 10).

The potential usefulness of AVP receptor antagonists in treating human diseases still remains an unanswered question because of the lack of orally active agents approved by the Food and Drug Administration. However, the review of the aforementioned actions of AVP calls for the development of orally active AVP receptor antagonists for use in the treatment of various human diseases.

Besides AVP, oxytocin (OT) is another neurohypophysial peptide involved in uterine contractions and milk ejection. Studies performed in knock-out mice confirmed that the OT receptor is a major player in parturition. As premature labor and delivery represent a significant health and financial burden, the development of OT receptor (OTR) antagonists may offer a specific and selective tocolytic treatment. This topic has been recently reviewed by Freidinger & Pettibone (11) and will not be covered in this review. By the same token, blockade of the $V_1$-vascular AVP receptors present in the nonpregnant uterus may alleviate the symptoms of primary dysmenorrhea, a major cause of lost wages in the female population.

## SIGNAL TRANSDUCTION PATHWAYS OF THE $V_1R$, $V_2R$, $V_3R$, AND OXYTOCIN RECEPTOR

### $V_1$-Vascular Receptor

The $V_1R$ expressed in the liver, vascular smooth muscle cells, and the testis is the product of the same gene, undergoing identical splicing (12). The $V_1R$ is also expressed in several tissues or organs, including blood platelets, adrenal cortex, kidney, reproductive organs, spleen, adipocytes, brain, and various immortalized cell lines (3T3, A10, WRK-1, and A7r5). AVP binding to the $V_1R$ leads to the activation of phospholipases C, D, and $A_2$; the production of inositol 1,4,5-triphosphate and diacylglycerol; the simultaneous activation of protein kinase C, p42/p44 MAP kinase, PI 3-kinase, and calcium/calmodulin-dependent kinase II; the mobilization of intracellular calcium; the influx of extracellular calcium via receptor-operated $Ca^{++}$ channels; and the activation of the $Na^{+}$-$H^{+}$ exchanger (13–17). No stimulation of cAMP accumulation is noted after stimulation of the $V_1R$. The secondary nuclear signal mechanisms triggered by activation of $V_1Rs$ include induction of immediate-early response genes expression and protein synthesis, leading to cellular hypertrophy, increased cell protein content, and cell proliferation (17, 18). Indeed, activation of $V_1Rs$ leads to a mitogenic response in vascular smooth muscle cells, 3T3 cells, renal mesangial cells, hepatocytes, and adrenal glomerulosa cells. These responses are specifically blocked by $V_1R$ antagonists of peptide and nonpeptide nature. The G proteins coupled to the $V_1R$ are mainly members of the $G_q$ family but also of the $G_i$ family, as some of the signals activated by $V_1Rs$ stimulation (e.g. phospholipase $A_2$ activation) are reduced by pertussis toxin pretreatment (12). Shortly after agonist binding, AVP receptor internalization occurs and may contribute to receptor desensitization (19, 20). Phosphorylation and dephosphorylation of AVP/OT receptors and their role in the desensitization/resensitization processes are currently being unraveled (21, 22). Examination of the structure of AVP receptors and OT receptors suggests that G protein–coupled receptor kinases (GRKs) and protein kinase C (PKC) are involved in their signal transduction. To explore the physical association of AVP receptors and OT receptors with GRKs and PKC, we stably expressed wild types and mutated forms of these receptor subtypes as green fluorescent fusion proteins and analyzed them by fluorescence, immunoprecipitation, and immunoblotting (23). Addition of a C-terminus green fluorescent protein tag did not interfere with ligand binding, internalization, and signal transduction. After agonist stimulation PKC dissociated from the $V_1R$ did not associate with the $V_2R$, but associated with the $V_3R$ and the OTR. After AVP stimulation, only GRK5 briefly associated with AVP receptors following a time course that varied with the receptor subtype. No GRK associated with the OT receptor. Exchanging the $V_1R$ and $V_2R$ C-termini altered the time course of PKC and GRK5 association. Deletion of the $V_1R$ C-terminus resulted in no PKC association, but did result in a ligand-independent, sustained association of GRK5

with the receptor. Deletion of the GRK motif prevented association and reduced receptor phosphorylation. Thus, agonist stimulation of AVP/OT receptors leads to receptor subtype–specific interactions with GRK and PKC through specific motifs present in the receptors' C-termini.

## $V_2$-Renal Receptor

The $V_2R$ is expressed on the basolateral membrane of the collecting duct in the medullary portion of the kidney (as well as in the MDCK and LLC-$PK_1$ cell lines), where it mediates the antidiuretic effect of AVP. AVP binding to the $V_2R$ leads to the sequential coupling of the cholera toxin–sensitive G protein $G_s$, activation of adenylyl cyclase, production of cAMP, and activation of protein kinase A, promoting the insertion of aquaporin 2 water channels (AQP-2) into the luminal surface of the renal collecting tubule cells and later the enhanced synthesis of AQP-2 mRNA and protein (24, 25). Agonist binding to the $V_2R$ triggers receptor phosphorylation by GRK5 and internalization (21, 23). An elegant work by Fahrenholz and colleagues (26) suggests that the enzymatic cleavage of the ligand-occupied $V_2R$ by a metalloprotease produces a major alteration of the binding site, which contributes to the termination of signal transmission. Further work by the same authors indicates that the proteolytic cleavage of the $V_2R$ requires a defined conformation, especially of the first two extracellular domains, that is induced by agonist binding (27). More than 150 mutations of the human $V_2R$ gene (AVPR2) in Xq28 were found to be the cause of X-linked congenital nephrogenic diabetes insipidus (28). The remaining 5–10% of hereditary nephrogenic diabetes insipidus cases appear to be due to mutations in the AQP-2 water channel (29).

## $V_3$-Pituitary Receptor

The $V_3R$ was described initially in corticotroph cells where it potentiates the release of ACTH. Moreover, recent RT-PCR experiments indicate its presence in other tissues, such as the brain, the kidney, the pancreas, and the adrenal medulla (10, 30–32). Prior to the cloning and functional expression of the human pituitary $V_3R$, studies of the binding characteristics and signal transduction pathways activated following binding of AVP to this receptor were hampered by its limited availability. Initial observations were made using either freshly isolated animal (rat, pig, sheep) cells (8, 33–36) or samples of human corticotroph adenomas (37). In these studies, occupancy of $V_3Rs$ by agonists triggered the sequential activation of phospholipase C and protein kinase C, the mobilization of intracellular free calcium, the phosphorylation of the myristoylated alanine-rich C kinase substrate, and secretion of ACTH (37–39). Conflicting data regarding coupling of the $V_3R$ to adenylyl cyclase have been reported (35, 36, 40). No information was available regarding the nature of the G-protein(s) and the kinases-phosphatases coupled to the $V_3R$, or the eventual mitogenic role of this receptor. Studies of ligand binding profile, coupling to phospholipase C and adenylyl cyclase, revealed a unique pharmacological profile for this pituitary receptor, distinct from those of the $V_1R$ and

the $V_2R$ subtypes. Thus, this AVP receptor subtype was designated as $V_3$ or $V_{1b}$ (34, 41). A recent pharmacological characterization of the porcine pituitary AVP receptor with cyclic and linear peptide AVP receptor antagonists confirmed that the pituitary and liver AVP binding sites were dissimilar, both cyclic and linear $V_1R$ antagonists having in general a much lower affinity for the pituitary receptor than for the liver receptor (42). We have recently completed a comprehensive characterization of the signal transduction pathways linked to the human $V_3R$ expressed in Chinese hamster ovary cells (43). Depending on the level of expression of the receptor, the $V_3R$ couples to members of the $G_{q/11}$ family, alone or in combination with $G_i$, and may also recruit $G_s$. Thus, the human $V_3R$ has a pharmacological profile clearly distinct from that of the human $V_1R$ and $V_2R$ and activates several signaling pathways via different G proteins, depending on the level of receptor expression. The increased synthesis of DNA and cAMP levels observed in cells expressing medium and high levels of $V_3Rs$, respectively, may represent important events in the tumorigenesis of corticotroph cells (43).

### OT Receptor

The OTR is expressed in the uterus, the mammary gland, the ovary, the brain, the kidney, and lactotroph cells. OT binding to its receptor leads to phospholipase C activation, calcium mobilization, and stimulation of phosphatidyl inositol turnover (44). A recent publication by Ohmichi et al indicates that stimulation of the OTR of human uterine myometrial cells induces MAP kinase phosphorylation through a pertussis toxin–sensitive G protein (45). In human myometrial cells, the OTR activates phospholipase $C_\beta$ by interacting with at least two types of G proteins, a member of the pertussis toxin–sensitive $G_i$ family and a member of the pertussis toxin–insensitive $G_{q/11}$ family (46).

## MOLECULAR BIOLOGY OF ARGININE VASOPRESSIN/OXYTOCIN RECEPTORS

Complementary DNAs coding for the human, rat, mouse, ovine, porcine, bovine, toad, and fish AVP/OT receptors have been cloned, sequenced, and expressed. These receptors share a common structure made of a single polypeptide chain containing seven hydrophobic membrane-spanning domains with a high degree of homology across species for a given subtype (Figure 1).

Alignment of the amino acid sequences of the AVP/OT receptors reveals that several amino acid sequences are remarkably conserved. The typical Asp-Arg motif present at the C-terminus of the third transmembrane domain of the superfamily of G protein–coupled receptors is also present in all members of the AVP/OT family of receptors. Mutation of $Asp^{136}$ in the human $V_2R$ led to an agonist-independent activation of cyclic AMP production, mimicking the situation found in constitutively activated adrenergic receptors (47).

All AVP/OT receptors share the sequence FQVLPQ present at the C-terminus of the second transmembrane domain. This sequence is thought to play a major role in ligand binding, signal transduction, and receptor proteolysis (26). The three-dimensional (3D) modeling of the cleavage and agonist binding site of the $V_2R$ (upper part of the transmembrane helix 2 containing the cleavage site and the first extracellular loop that is involved in agonist binding) suggests that $Pro^{95}$ causes a kink of helix 2 between $Phe^{91}$ and $Pro^{95}$, providing more space for the interior cleft. Agonist binding to $Asp^{103}$, $Gln^{96}$, and $Gln^{92}$ induces a conformational change allowing $Glu^{92}$ and $Val^{93}$ to become more accessible to the $V_2R$-degrading enzyme (27).

The sequence FXGPDXLCRXVK, present at the C-terminus of the first extracellular loop, and the sequence DCWXXFXXPWG, located in the second extracellular loop, are present in all AVP/OT receptor sequences. These two extracellular sequences are not found in other members of the superfamily of G protein–coupled receptors, and these conserved residues and motifs presumably represent the backbone of the functional structure of AVP receptors in terms of ligand-binding specificity and signal transduction.

All AVP/OT receptor sequences have in common the motif NPWIY, present in their seventh transmembrane domain. We studied the possible role of the conserved tyrosine residue within this motif in the ligand-binding characteristics, internalization pattern, phosphorylation, and signal transduction of the human $V_1$-vascular AVP receptor by replacing it with an alanine residue (Y348A). Wild-type and mutant receptors with and without a green fluorescent protein tag were stably expressed in Chinese hamster ovary cells. The Y348A mutation did not alter the membrane insertion of the receptor and its ligand-binding characteristics. The internalization kinetics of the agonist-occupied mutated receptor were unaltered. $Tyrosine^{348}$ was briefly phosphorylated after agonist stimulation, and its replacement by an alanine residue led to a dramatic reduction of the mitogenic cascade (inositol phosphate production, DNA synthesis, kinases phosphorylation, and cell proliferation) observed with the wild-type receptor. All AVP/OT receptor sequences also share a di-cysteine motif in the proximal portion of their C-terminus. Based on the work done by Birnbaumer and colleagues with the human $V_2R$, these two adjacent cysteines that are presumably palmitoylated are not required for ligand-binding affinity, AVP-stimulation of adenylyl cyclase, receptor internalization, and desensitization (48). However, mutation of these palmitoylated cysteine residues reduced receptor intracellular transport (49).

Conversely, other regions within the AVP/OT receptor sequences bear very little similarity to each other and are presumably responsible for subtype specificity. For instance, the third intracytoplasmic loop that plays a key role in G protein–coupling is drastically different between the $V_1R$ (coupled to $G_{q/11}$) and $V_2R$ (coupled to $G_s$) sequences. The systematic exchange of the $V_1R$ and $V_2R$ intracellular domains performed by Liu & Wess revealed that the $V_1R$ second intracellular loop was required for activation of the phosphatidylinositol pathway, whereas the $V_2R$ third intracellular loop was required for activation of the adenylyl cyclase pathway (50). In a subsequent article the same group reported that other AVP receptor domains besides the intracellular loops were also critical for optimum G protein–coupling

efficiency (51). Substitution of the 21 amino acids of the proximal portion of the $V_2R$ C-terminus in the $V_1R$ sequence resulted in a chimeric receptor that gained the ability to stimulate cAMP production to a significant extent (29% of maximum effect of the wild-type $V_2R$). Insertion into the $V_1R$ sequence of both the third intracellular loop and the proximal portion of the $V_2R$ C-terminus led to an agonist-induced production of cAMP similar to that of the wild-type $V_2R$. These observations suggest that different segments of the $V_2R$ cooperate to produce a maximal activation of adenylyl cyclase, presumably through $G_s$ coupling.

It also comes as no surprise that the amino-terminal and carboxy-terminal regions differ widely between the different AVP/OT receptor sequences. The C-terminal region of the $V_1R$ contains four protein kinase C (PKC) phosphorylation sites, whereas the $V_2R$ C-terminus has none. We studied the role of the $V_1R$ cytoplasmic tail in internalization and signal transduction by creating truncated, mutated, and chimeric forms of human $V_1Rs$ stably expressed in Chinese hamster ovary cells (52). Deletions, mutations, or chimeric alterations of the $V_1R$ C-terminus did not alter ligand-binding characteristics. Rate and extent of $V_1R$ internalization were much reduced in the absence of the proximal region of the $V_1R$ C-terminus. Deletion of the $V_1R$ C-terminus or its replacement by the $V_2R$ C-terminus prevented AVP stimulation of DNA synthesis, progression through the cell cycle, and cell proliferation. DNA synthesis, cell cycle progression, and cell proliferation were progressively restored in the truncated forms of the $V_1R$ C-terminus containing one, two, and three PKC sites. Coupling to phospholipase C was assessed by assay of inositol phosphate production. AVP stimulation of all truncated forms of $V_1Rs$ produced a normal maximal inositol phosphate production, but a reduced potency was noted for the truncations $V_1R359X$ ($EC_{50}$ = 5.5 nM) and $V_1R399X$ ($EC_{50}$ = 1.68 nM) versus the wild-type receptor ($EC_{50}$ = 0.31 nM). Thus, the $V_1R$ cytoplasmic C-terminus is not involved in ligand specificity but is instrumental in receptor internalization. The $V_1R$ C-terminus facilitates the interaction between intracellular loops of the receptor, G protein, and phospholipase C. It is absolutely required for transmission of the mitogenic action of AVP, probably via kinase phosphorylation sites. Similarly, a $V_2R$ fragment consisting of the N-terminus, the first transmembrane helix, and the first intracellular loop is sufficient to establish correct membrane targeting and transmembrane topology (49). Truncation of the last 14 and 27 amino acids of the human $V_2R$ ($V_2R345X$ and $V_2R358X$ mutants) did not alter membrane insertion and binding affinity of the mutant receptors (21). However, complete deletion of the C-terminus of the human $V_2R$ ($V_2R337X$ mutant) reduced the membrane integration of the $V_2R$, which was retained inside the endoplasmic reticulum (53). Moreover, a chimeric $V_2R$ with a $\beta_2$-adrenergic receptor C-terminus also did not bind AVP and was retained inside the cells. These data suggest that the C-termini of the $V_1R$ and the $V_2R$ play different roles in terms of receptor folding and membrane processing: The $V_1R$ receptor C-terminus is not required, whereas specific amino acids in the proximal portion of $V_2R$ receptor C-terminus are required for these functions. As a matter of fact, the $Glu^{335}/Leu^{339}Leu^{340}$ motif within the proximal portion of the $V_2R$ C-terminus is essential for receptor transport from the reticulum endoplasmic

apparatus to the Golgi apparatus (54). Residue $Leu^{339}$ may be required for folding back the intracellular C-terminus to residue $Leu^{62}$ of the first cytoplasmic loop.

The genomic characteristics, tissue expression, chromosomal localization, and regional mapping of the human AVP/OT receptor genes are now established. The genes for the human $V_1$-vascular, $V_2$-renal, $V_3$-pituitary, and OT receptors are single-copy genes derived from a common ancestor, but they are located on different chromosomes: respectively, chromosomes 12, X, 1, and 3. Similarly, the genes encoding the rat $V_1$-vascular, $V_2$-renal, $V_3$-pituitary, and OT receptors, as well as the gene coding the sheep $V_1$-vascular receptor were isolated. All these genes share the unique feature among G protein–coupled receptors of an intron located before the seventh transmembrane domain of the receptor sequences.

## DEVELOPMENT OF VASOPRESSIN RECEPTOR ANTAGONISTS

Three different strategies could be considered to design AVP receptor antagonists. They are:

1. The systematic or rational modifications of the ligand structure. This approach has been elegantly and efficiently implemented by Maurice Manning and collaborators who designed numerous peptide AVP and OT agonists and antagonists (55). However, the lack of oral bioavailability and the short half-life of these peptide compounds have limited their utilization in clinical medicine.
2. The random screening for new chemical compounds. This approach is time consuming and labor intensive, but has been successfully developed by pharmaceutical companies as described below (Figure 2; Figure 3).
3. The structure-based drug design. This requires the knowledge of the 3D structure of both the ligand and receptor. The AVP/OT receptors have been cloned, but their crystallographic structure remains to be established in order to move forward with this approach, which relies upon computer modeling.

These three strategies are complementary. Consequently iterative interactions between random screening and structure-based drug design ought to produce highly potent and selective compounds.

## NONPEPTIDE $V_1$-VASCULAR ARGININE VASOPRESSIN RECEPTOR ANTAGONISTS

Recently, nonpeptide AVP $V_1$R antagonists were discovered by random screening of chemical entities (56, 57). The availability of such orally active compounds

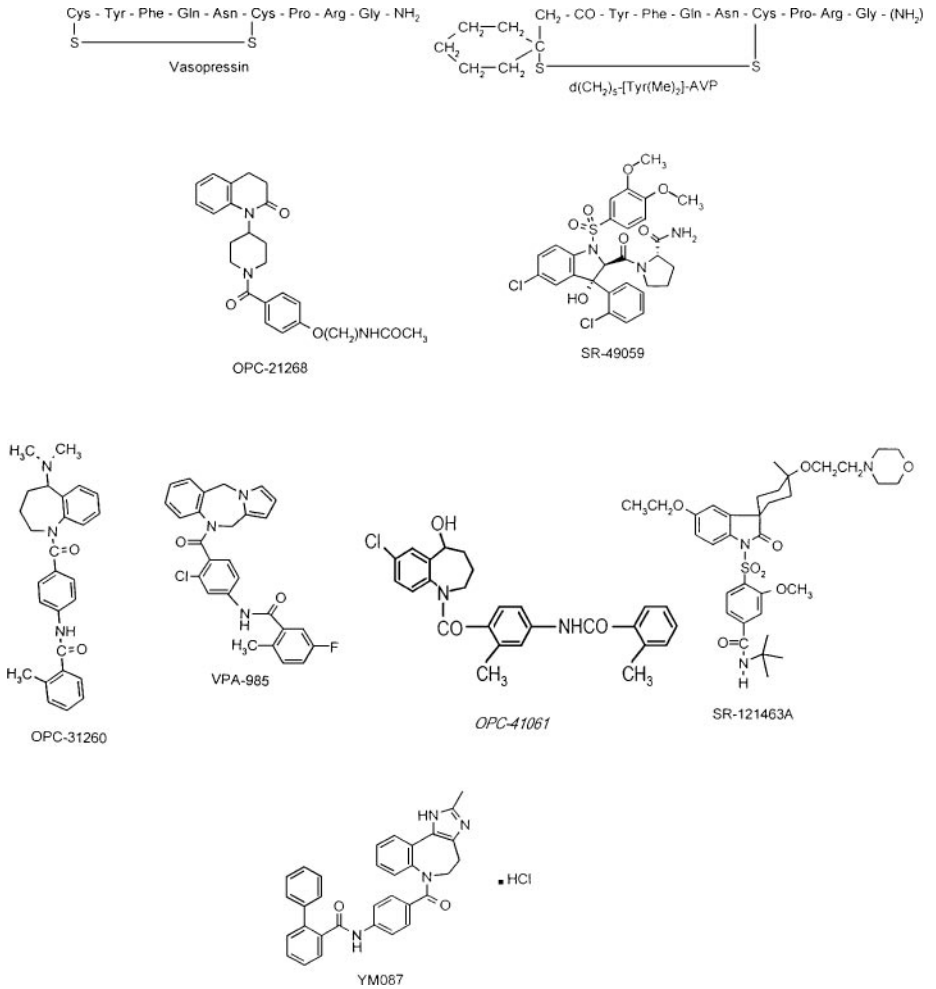



**Figure 2** Chemical structure of AVP and related antagonists. The structure of AVP; the reference peptide $V_1$-vascular receptor antagonist $d(CH_2)_5$Tyr(Me)AVP; the nonpeptide $V_1$-vascular receptor antagonists OPC-21268 and SR-49059; the $V_2$-renal receptor antagonists OPC-31260, VPA-985, OPC-41061, and SR-121463A; and the dual $V_1$-vascular/$V_2$-renal receptor antagonist YM087 are shown.

now allows assessment of the potential therapeutic applications of $V_1$-vascular receptors blockade in human diseases.

## OPC-21268

In 1991 Yamamura et al described the characteristics of a nonpeptide $V_1R$ antagonist, OPC-21268 or 1-(1-[4-(3-acetylaminopropoxy)benzoyl]-4-piperidyl)-3,4-dihydro-2(1H)-quinolinone (Figure 2) (56). This molecule was developed by optimization of a lead compound that was found by screening of several thousand compounds. OPC-21268 is orally active and is a selective $V_1R$ antagonist in the rat, in which it blocks exogenous AVP-induced vasoconstriction and calcium mobilization (56). The amount of OPC-21268 ($IC_{50}$) required for displacement of [$^3$H]AVP is 5 nM in rat smooth muscle cells and 400 nM in rat hepatocytes. Affinity of OPC-21268 for rat $V_2Rs$ is much weaker ($IC_{50} > 10^{-4}$ M). OPC-21268 does not alter the pressor response to angiotensin II or norepinephrine. In the rat the inhibitory effect of OPC-21268 on AVP-induced vasoconstriction is dose dependent and lasts more than 8 hours for an oral dose of 30 mg/kg. OPC-21268 has been shown to acutely and chronically reduce the blood pressure of the deoxycorticosterone acetate–salt rat model of hypertension (58) and to reduce the blood pressure of spontaneously hypertensive rat animals when given to young animals (57). In a model of congestive heart failure by rapid ventricular pacing in conscious dogs, OPC-21268 significantly increased cardiac output and reduced total peripheral resistances and mean arterial blood pressure (59).

Unfortunately, the affinity of OPC-21268 for the human $V_1R$ is rather weak (43, 60), again underlining the vexing problem of interspecies variability for a given AVP/OT compound. In healthy male human volunteers 10 to 600 mg oral doses of OPC-21268 did not alter plasma AVP levels, urine output, or hemodynamic parameters (61). OPC-21268 failed to prevent the AVP-induced contraction of human internal mammary arteries harvested from patients undergoing coronary artery bypass surgery (60).

## SR-49059

SR-49059 ((2S)1-[(2R3S)-5-chloro-3-(2-chloro-phenyl)-1-(3,4-dimethoxybenzene-sulfonyl)-3-hydroxy-2,3-dihydro-1H-indole-2-carbonyl]-pyrrolidine-2-carboxamide) (Figure 2) was also developed by chemical optimization of a lead compound found by random screening (62). It has a marked affinity, selectivity, and efficacy toward both animal and human $V_1$-vascular receptors, and is devoid of partial agonist activity (63–65). This compound inhibits AVP-induced vascular smooth muscle cell contraction and blood pressure elevation for at least 8 hours. In healthy human volunteers, SR-49059 inhibits ex vivo AVP-induced platelet aggregation and skin blanching (66). SR-49059 antagonism of AVP aggregating effect has been shown to be competitive (63). In healthy subjects SR-49059 inhibits

exogenous AVP-induced changes in skin blood flow and vasoconstriction of the radial artery dose dependently (67).

We studied the clinical and pharmacological profile of SR-49059 before and during the osmotic stimulation of AVP release in 24 male hypertensive patients (68). SR-49059 (300 mg) did not alter blood pressure or heart rate at baseline and did not prevent the blood pressure increment induced by the hypertonic saline infusion. However, in the presence of SR-49059, blood pressure peak at the end of the saline infusion was lower and blood pressure return to baseline after the saline infusion happened faster. Heart rate was faster between H + 3 and H + 12 after SR-49059 administration. The rise of plasma sodium, osmolality, and AVP triggered by the saline infusion was not significantly modified by SR-49059. AVP-induced in vitro aggregation of blood platelets was significantly reduced by SR-49059, with a peak effect two hours after drug administration that coincided with the drug peak plasma concentration. Plasma renin and aldosterone levels before and after the saline infusion were not modified by SR-49059. Urine volume, osmolality, and electrolytes were not altered by SR-49059 administration. SR-49059 effects were similar in African Americans and Caucasians, as well as in salt-sensitive versus salt-resistant patients. Thus, we concluded that in a situation of osmotic release of AVP and volume expansion in hypertensive patients, a single oral dose of the $V_1$-vascular receptor antagonist SR-49059 able to block AVP-induced platelet aggregation exerted a transient vasodilating effect that was not associated with a sustained blood pressure reduction. SR-49059 is a pure $V_1$-vascular receptor antagonist because the analysis of the renal parameters assessed in our study indicates that SR-49059 does not block the AVP $V_2$-renal receptors.

## Nonpeptide $V_1$-Vascular Arginine Vasopressin Receptor Antagonists as a Treatment of Dysmenorrhea

The endometrial blood flow in women with primary dysmenorrhea fluctuates in a pattern that coincides with pulsatile AVP secretion, whereas AVP circulating levels are often increased in this condition (69). Furthermore, receptor density and in vivo effects of AVP, but not those of OT, increase premenstrually (70). These observations and studies performed with peptide antagonists suggest that $V_1$R antagonists could be used in the treatment of primary dysmenorrhea. In fact, in healthy nonpregnant women, SR-49059 (300 mg) antagonized vasopressin-induced uterine contractions, thus suggesting that this type of antagonist could be explored in the treatment of dysmenorrhea (71). Furthermore, in women suffering from primary dysmenorrhea, SR-49059 produced a dose-related reduction of intensity of menstrual pain, as recorded by visual analog scale and Sultan pain score in a double-blind, placebo-controlled, cross-over trial (72). No significant effect on the bleeding pattern was observed. This pilot study showed for the first time a therapeutic effect of an orally active $V_1$R antagonist in the symptomatic treatment of dysmenorrhea, which needs to be confirmed by larger trials.

## NONPEPTIDE $V_2$-RENAL ARGININE VASOPRESSIN RECEPTOR ANTAGONISTS

Several $V_2$-renal nonpeptide AVP receptor antagonists are currently being developed by various pharmaceutical companies and have entered various stages of clinical trials development. The goal is to develop a new class of orally active medications that selectively block the $V_2$Rs, thus leading to the elimination of free water without modifying electrolyte urine excretion (at variance with classical natriuretic agents). The term aquaretic drugs has been coined to describe this class of new agents. They are described below in chronological order of development.

### OPC-31260

By modifying the structure of the first $V_1$-vascular nonpeptide antagonist, OPC-21268, Yamamura et al developed a potent $V_2$-renal nonpeptide antagonist identified as OPC-31260 (5-dimethylamino-1-[4-(2-methylbenzoylamino)benzoyl]-2,3,4,5-tetrahydro-1H-benzazepine hydrocloride) (73). OPC-31260 displaces [$^3$H] AVP more potently from its $V_2$-renal receptors ($IC_{50} = 14$ nM) than from its $V_1$-vascular receptors ($IC_{50} = 1.2\ \mu$M). In the rat OPC-31260 blocks the antidiuretic action of AVP in a dose-dependent fashion. OPC-31260 blocks AVP-induced production of cAMP and AQP-2. This compound has no partial agonist activity in the Brattleboro rat, but inhibits the antidiuretic effect of dDAVP. Furthermore, when measuring the cyclic AMP production of a constitutively active D136A $V_2$R mutant, OPC-31260 behaved like an inverse agonist or antagonist with negative intrinsic activity, whereas the two peptide antagonists d$(CH_2)_5$[D-Tyr(Et)$^2$,Val$^4$, Tyr-$NH_2{}^9$]AVP and d$(CH_2)_5$[D-Ile$^2$,Ile$^4$,Tyr-$NH_2{}^9$]AVP displayed partial agonist properties (47). In the conscious rat, 1 to 30 mg/kg oral doses of OPC-31260 produce a dose-dependent increase of urinary volume and decrease of urine osmolality without significantly altering urine electrolytes. In rats having free access to water, OPC-31260 did not alter plasma osmolality, hematocrit, or body weight, despite the production of abundant hypotonic urine. In experimental models of liver cirrhosis, congestive heart failure, SIADH, nephrotic syndrome, and acute hyponatremia, OPC-31260 was able to increase urine flow and free water clearance while restoring plasma sodium and osmolality (57, 74). In healthy humans OPC-31260 administration (0.017 to 1 mg/kg) results in hypotonic urine excretion with mild sodium excretion. The effect on sodium excretion is limited, especially when compared to the effect of natriuretic agents. OPC-31260 also has a weak $V_1$-vascular antagonist effect in humans. OPC-31260 was administered intravenously to 11 patients with SIADH (75). Single i.v. doses of 0.25 and 0.50 mg/kg increased urine volume and decreased urine osmolality. The aquaretic effect of OPC-31260 lasted 4 hours when given intravenously. The efficacy of a single 30-mg oral dose of OPC-31260 was tested in 8 patients with biopsy-proven liver

cirrhosis, edema, and ascites (76). Significant increase of urine volume and decrease of urine osmolality were noted. Urinary sodium excretion did not change in these cirrhotic patients, whereas it increased two- to threefold in normal subjects. Administration of OPC-31260 to rats and humans increases plasma AVP dose-dependently, therefore raising the issue of loss of aquaretic effect during chronic treatment.

## VPA-985

VPA-985 (5-Fluoro-2-methyl-N-[4-(5H-pyrrolo[2,1-c][1,4]benzodiazepin-10 (11H)-yl carbonyl)-3-chlorophenyl]benzamide) is another specific and selective nonpeptide $V_2$-renal receptor antagonist that has been shown to be a potent aquaretic compound in rats and dogs (77). VPA-985 competitively inhibits [$^3$H]AVP binding to $V_2$Rs of rat and dog renal medulla ($K_i = 0.48$ and 0.82 nM). It is devoid of partial agonist activity. Its affinity for rat and human $V_2$Rs is in the nanomolar range and it is 100-fold more selective for the $V_2$R than for the $V_1$R and the OTR. VPA-985 produces a dose-dependent increase in urine volume and free water clearance with simultaneous decrease in urine osmolality and increase in serum osmolality and sodium. Oral administration of 100 to 200 mg/kg/day for 3 consecutive days did not alter the arterial pressure of normotensive, spontaneously hypertensive, and deoxycorticosterone acetate–salt hypertensive rats (78). VPA-985 is currently being evaluated in clinical trials. Preliminary results in human heart failure demonstrated a dose-dependent increase of urine flow and decrease of urine osmolality in patients with New York Heart Association class II or III heart failure (79). VPA-985 was also effective in patients with liver cirrhosis.

## OPC-41061

(±)-7-chloro-5-hydroxy-1-[2-methyl-4-(2-methylbenzoylamino)benzoyl]-2,3,4, 5-tetrahydro-1H-1-benzazepine) is another specific and selective nonpeptide $V_2$-renal receptor antagonist that has been shown to be a potent aquaretic compound in rats and dogs (80). OPC-41061 inhibits [$^3$H]AVP binding to rat $V_2$Rs with a $K_i = 1.33 \pm 0.30$ nM, and its selectivity for the $V_2$R over the $V_1$R is about 250 fold. When compared to furosemide (10–100 mg/kg) in conscious male rats, OPC-41061 (1–10 mg/kg) markedly increased the electrolyte-free water clearance, whereas furosemide elevated only the electrolyte clearance (81). The high dose of OPC-41061 also increased urinary sodium excretion, albeit much less than furosemide, presumably by inhibition of sodium reabsorption at the AVP-sensitive segment in the thick ascending limb of the loop of Henle. OPC-41061 dose-dependently elevated serum sodium, furosemide tended to decrease it. Opposite to furosemide, OPC-41061 did not alter the renin-angiotensin-aldosterone system. The high doses of both drugs increased serum AVP concentrations to the same extent. OPC-41061 produced an additive diuretic effect in the presence of

furosemide. This compound is now tested in patients with hyponatremia of various etiologies, including congestive heart failure and liver cirrhosis.

## SR-121463A

SR-121463A (1-[4-(N-tert-butylcarbamoyl)-2-methoxybenzene sulfonyl]-5-ethoxy-3-spiro-[4-(2-morpho-linoethoxy)cyclohexane]indol-2-one fumarate) is a potent and highly selective $V_2R$ antagonist in several species without any agonistic property (82). Its selectivity for the $V_2R$ over the other AVP receptor subtypes is of several orders of magnitude. In normally hydrated rats, SR-121463A produces a powerful dose-dependent aquaresis without altering sodium and potassium excretion. No antidiuretic action is detected in Brattleboro rats. This compound has yet to be tested in human subjects.

## Selective $\kappa$-Opioid Receptor Agonists as an Alternative to Nonpeptide $V_2$-Renal Arginine Vasopressin Receptor Antagonists

Besides the specific nonpeptide $V_2R$ antagonists described above, another class of pharmacologic agents was tested to inhibit AVP actions. These compounds act by reducing the circulating levels of AVP, presumably by interacting with $\kappa$-opioid receptors at hypothalamic sites with a subsequent inhibition of AVP secretion. The renal and hormonal effects induced by a 10-day administration of OPC-31260 (5 mg/kg) or the $\kappa$-opioid receptor agonist niravoline (3 mg/kg) that reduces AVP secretion were compared in cirrhotic rats with ascites and water retention (83). Niravoline increased water excretion, peripheral resistances, serum osmolality, and sodium excretion. Niravoline reduced creatinine clearance, AVP and aldosterone excretion, and AVP mRNA expression. OPC-31260 also increased water and sodium excretion and reduced aldosterone excretion with no effect on serum osmolality, renal filtration, and systemic hemodynamics. In 12 healthy volunteers a single 30-min i.v. infusion of a 2-mg dose of niravoline produced a potent aquaretic effect accompanied by a stimulation of the sympathetic and renin-angiotensin-aldosterone systems and an increase in blood pressure (84). Because of their central mechanism of action, the clinical development of the $\kappa$-opioid receptor agonists has been limited.

## Nonpeptide $V_2$-Renal Arginine Vasopressin Receptor Antagonists as a Treatment of Cerebral Edema

László et al (85) studied the effect of the $V_2$-renal nonpeptide antagonist OPC-31260 on the cerebral edema induced by subarachnoid hemorrhage. Significant water retention, an increase in the brain content of water and sodium, and an elevation of plasma AVP levels are observed in this condition. OPC-31260 administered at doses of 10–30 mg/kg produced a dose-dependent diuresis and prevented water retention and alterations of brain water and sodium contents. OPC-31260

administration further enhanced plasma AVP levels. The blockade of the $V_2$-renal receptors can explain the action of OPC-31260, although a direct effect on brain capillary permeability cannot be ruled out.

### Nonpeptide $V_2$-Renal Arginine Vasopressin Receptor Antagonists as a Treatment of Congenital Nephrogenic Diabetes Insipidus

To date, 155 mutations within the $V_2R$ gene have been reported in 239 families afflicted with the X-linked form of nephrogenic diabetes insipidus. Some mutations produce severely truncated or altered receptor proteins that are not functional. For more subtle mutations (e.g. single amino acid alteration), the receptor protein is not properly folded and is retained intracellularly by the endoplasmic reticulum (28). In a recent seminal work, Morello et al demonstrated that selective, nonpeptidic $V_2R$ antagonists (SR121463A and VPA-985) that are cell-permeant dramatically increased cell surface expression and rescued the function of 8 out of 15 $V_2R$ mutants by promoting their proper folding and maturation (86). Cyclic AMP production by these "rescued" $V_2R$ mutants was as high as 30% of that observed for the wild-type $V_2R$. A peptidic cell-impermeant $V_2R$ antagonist could not mimic these effects and was unable to block the rescue mediated by the permeant antagonists, indicating that the nonpeptide antagonists act intracellularly, presumably by binding to and stabilizing partially folded mutants. This important observation suggests that small ligands can act as pharmacological chaperones that promote the proper folding and maturation of receptors, their insertion into the cell surface, and the restoration of their intracellular signal coupling. Interestingly, molecules that were developed to block wild-type receptors may turn out to represent a unique way to re-establish the functionality of mutated receptors.

## DUAL NONPEPTIDE $V_1$-VASCULAR AND $V_2$-RENAL ARGININE VASOPRESSIN RECEPTOR ANTAGONISTS

A combined $V_1$-vascular and $V_2$-renal receptor blockade could lead to synergetic effects on systemic hemodynamic and renal parameters. The respective $V_1R/V_2R$ antagonist ratio is of importance when one considers the "ideal" profile of an AVP antagonist to be developed as an antihypertensive agent. Based on the elegant studies performed by Sladek et al (87, 88) with peptide antagonists in the spontaneously hypertensive rat, a pure $V_1R$ antagonist is expected to not produce a sustained decrease in blood pressure in well-hydrated animals, whereas a dual $V_1R/V_2R$ antagonist will achieve a reduction in blood pressure via alterations of both peripheral resistances and circulating blood volume. However, the effective and safe ratio of $V_1R/V_2R$ antagonism remains to be established, as shown by Hofbauer et al's study of deoxycorticosterone acetate–salt hypertensive rats (89):

Administration of a dual $V_1R/V_2R$ peptide antagonist led to a greater blood pressure reduction than a pure $V_1R$ antagonist, but at the expense of water loss and hypernatremia.

### YM087

(4′-[(2-methyl-1,4,5,6-tetrahydroimidazo[4,5-d][1]benzazepin-6-yl)-carbonyl]-2-phenylbenzanilide monochloride) is a newly discovered nonpeptide AVP antagonist that has a high affinity for both rat $V_1R$ and $V_2R$, the $K_i$ values in radioligand competition binding assays being respectively 0.48 and 3.04 nM (90). Its affinity for the OTR is weaker ($K_i = 44.4$ nM) and it does not bind to the $V_3R$. YM087 blocks both AVP-induced calcium mobilization in cultured vascular smooth muscle cells and cAMP production in cultured renal epithelial cells. YM087 has no agonistic properties. Oral administration of YM087 (0.1–3 mg/kg) dose-dependently inhibited AVP binding to rat liver $V_1R$ and renal $V_2R$ over 24 hours (91). In vivo YM087 antagonized the pressor response to exogenous AVP in pithed rats and produced an aquaretic effect in dehydrated rats in a dose-dependent manner. In normally hydrated and normotensive rats oral YM087 (1–3 mg/kg/day) for 7 days produced a dose-dependent aquaresis with no effect on systolic blood pressure. In the conscious dog YM087 increases urine output dose dependently over a dose range of 0.03–0.3 mg/kg orally without altering sodium and potassium excretion. Preliminary results in humans suggest that YM087 is a well-tolerated $V_1R/V_2R$ antagonist, the $V_2R$ blockade being more pronounced than the $V_1R$ blockade (92). In a group of 6 healthy volunteers, a single oral dose of 60 mg YM087 and a single i.v. dose of 50 mg YM087 produced a significant sevenfold increase in urine flow rate and a fall in urine osmolality from 600 to less than 100 milliosmoles/l, with a peak effect 2 hours after drug administration (93). Simultaneously, plasma osmolality and plasma AVP levels increased significantly. The aquaretic effect of these single doses lasted at least 6 hours. When administered intravenously YM087 inhibited AVP-induced skin vasoconstriction via blockade of the $V_1Rs$. However, antagonism of the $V_1Rs$ was less marked than blockade of the $V_2Rs$. No significant change in blood pressure or heart rate was found in these single-dose studies.

## NONPEPTIDE $V_3$-PITUITARY ARGININE VASOPRESSIN RECEPTOR ANTAGONISTS

Currently, there is no available orally active compound that is selective for the $V_3R$, whether of agonist or antagonist nature. Considering the role of AVP in modulating ACTH secretion by corticotroph cells, one may envision the use of such compounds in dynamic hormonal testing, imaging, and medical treatment of ACTH-secreting tumors.

## THREE-DIMENSIONAL MOLECULAR DOCKING OF PEPTIDE ARGININE VASOPRESSIN ANALOGS TO ARGININE VASOPRESSIN RECEPTORS

Cloning of human and animal AVP/OT receptor subtypes and their stable expression in immortalized cell lines have allowed several investigators to begin defining the molecular determinants of AVP-receptor subtypes' peptide-ligand selectivity (94).

We built a 3-D model of the human $V_1R$ and successfully docked AVP within the structure of this receptor (95). The 3-D model of AVP was docked onto $V_1R$ by initially placing it in the upper portion of the transmembrane region (the expected binding pocket) and searching for the binding site with the program LIGIN within a $20 \times 20 \times 20$ Å box around the original ligand position. In the docking of AVP some steric overlap (1–3 residues) was allowed between the ligand and the receptor. Energy minimization with the program X-PLOR relieved these short contacts. AVP has a polar as well as a nonpolar surface. The exocyclic tripeptide $Pro^7$-$Arg^8$-$Gly^9$ and one side of the hormone ring ($Gln^4$, $Asn^5$) are mainly hydrophilic, whereas the other part of the ring ($Cys^1$, $Cys^6$, $Tyr^2$, and $Phe^3$) is essentially hydrophobic in nature. This dual surface property is reflected in the nature of the binding pocket that is formed by residues from transmembrane segments (TMS) 1, 3, 4, 5, 6, and 7, as well as by the first extracellular loop (Figure 4). The bottom of the cleft is mainly hydrophobic, closed by the aromatic and hydrophobic residues Met135, Phe136, Phe179, Phe307, and Ile330. The entrance to the binding pocket and one side of it contain predominantly hydrophilic residues. The $Arg^8$ guanido group at the entrance to the cleft forms a salt bridge, with Asp112 located on the first extracellular loop. Trp 111 forms van der Waals contacts with the hydrophobic part of $Arg^8$. The $\varepsilon$-amino group of Lys128 forms a hydrogen bond to the amide side chain nitrogen of $Asn^5$. Other hydrogen bonds are formed between the side chain moieties of Gln185 and Ser182 with $Gln^4$, and Ser213 O$\gamma$ with $Tyr^2$ OH. Another wall of the pocket is lined with the hydrophobic residues Ile55 and Ile330.

Some amino acid residues that are common to all AVP/OT receptor subtypes are important for peptide agonist binding. They are $D^{207}$, $Q^{214}$, $Q^{218}$, $K^{308}$, $Q^{311}$, $Q^{413}$, and $Q^{620}$. None of these residues is involved in peptide and nonpeptide antagonist binding (94). The presence of a disulfide bond between two conserved cysteine residues present in exoloops 1 and 2 is required to maintain the integrity of the receptor structure. Studies performed recently with the $V_1$-vascular, $V_2$-renal AVP and the OT receptors from several species revealed that a few key residues determine peptide ligand selectivity for a given receptor subtype. For instance, residue Tyr115, located in the first extra-cellular loop, is crucial for high affinity binding of peptide agonists and confers $V_1$-vascular receptor subtype specificity (96). The use of natural small synthetic peptides mimicking segments of the $V_1R$ revealed that the N-terminal part of the $V_1R$ is not involved in peptide agonist binding (97). At variance, natural peptides mimicking the external loops

of the $V_1R$, especially one peptide mimicking the 205-218 portion of the second extracellular loop, were able to inhibit specific AVP binding to the $V_1R$.

Site-directed mutagenesis experiments of the cloned bovine and porcine $V_2Rs$ revealed that $Asp^{103}$ in the first extracellular loop is responsible for high-affinity binding of the $V_2R$ peptide agonist dDAVP (98). Similarly, residues responsible for selective binding of peptide agonists and antagonists to the $V_2$-renal receptor were identified (99). Residues 202 (Arg versus Leu) in the second extracellular loop and 304 (Gly versus Arg) in the seventh transmembrane domain are responsible for species-selective cyclic peptide antagonists binding in an independent and additive manner. Residue 100 (Lys versus Asp) in the second transmembrane domain plays a similar role for peptide agonist discrimination.

For peptide agonist binding and selectivity of the OT receptor subtype, the first three extracellular domains are the most important (100). The N-terminal domain and the first extracellular loop of the OT receptor interact with the linear C-terminal tripeptidic part of the ligand OT, whereas the second extracellular loop of the OT receptor interacts with the cyclic part of OT. The molecular determinants of peptide antagonist binding to the OT receptor are different, i.e. the transmembrane helices 1, 2, and especially 7. Introduction of just seven amino acids of the upper part of the seventh TMS of the OT receptor into the $V_2R$ sequence is sufficient to introduce high-affinity binding for an OT peptide antagonist into the $V_2R$.

Thus, these studies suggest that the molecular determinants of peptide agonists and antagonists binding to AVP/OT receptors are distinct.

## THREE-DIMENSIONAL MOLECULAR DOCKING OF NONPETIDE ANTAGONISTS TO ARGININE VASOPRESSIN RECEPTORS

Examination of the 3-D structure of the nonpeptide AVP receptor antagonists (Figure 3) indicates that this is a rather heterogeneous class of compounds. As there was no knowledge of the molecular determinants of AVP receptors involved in nonpeptide antagonist binding, we studied this issue by site-directed mutagenesis and molecular modeling techniques. The first nonpeptide AVP $V_1R$ antagonist found by random screening and optimization of chemical entities, OPC-21268, has an excellent affinity for the rat $V_1R$ (25 nM), but has a poor affinity for the human $V_1R$ (8800 nM) (2). The human and rat $V_1Rs$ 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 $V_1R$ sequences revealed that only 20 amino acid differences are present in the extracellular loops and the upper portions of the transmembrane segments. We reasoned that these interspecies differences in amino acid sequence modulate the receptor affinity for nonpeptide compounds. Thus, we produced a series of reverse mutations in which corresponding rat amino acids were introduced by site-directed mutagenesis into the human $V_1R$ sequence (95). The influence of these

interspecies amino acid differences on nonpeptide antagonist binding was subsequently tested. The introduction of rat amino acids in positions 224, 310, 324, or 337 of the human $V_1R$ sequence dramatically altered OPC-21268 affinity for the receptor, whereas binding of AVP, the peptide $V_1R$ antagonist $d(CH_2)_5Tyr(Me)AVP$, and the nonpeptide $V_1R$ antagonist SR49059 was not altered by these mutations.

In order to gain information about the location of the OPC-21268 binding site, a model of this compound was docked onto a homology-built, 3D model of the human $V_1R$. Very little direct structural information is available for G protein–coupled receptors (GPCRs), and for many years molecular models of these receptors have been built based upon the crystal structure of bacteriorhodopsin. Although bacteriorhodopsin consists of the seven transmembrane helices by which GPCRs are characterized, it shares very little sequence homology with any of the GPCRs. Still, the use of bacteriorhodopsin to establish the orientation of the transmembrane domains of AVP receptors is the only way to build a model based on an experimentally determined high-resolution structure (101). Coordinates of bovine rhodopsin are also available, but only for the seven TMS without any loops. As the basis for our model building of the $V_1R$ receptor we used a model of the seven TMS of $V_1R$ generated by G Vriend with the program WHATIF (102) based upon the crystal structure of bacteriorhodopsin (G Protein–Coupled Receptor Data Base at http://swift.emblheidelberg.de/ 7tm/htmls/ consortium.html) (103).

The three extracellular and three intracellular loops of the $V_1R$ were subsequently constructed with the program Look v3.5 (Molecular Applications Group, Palo Alto, CA 94304), 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 also performs a full energy refinement of the model (104). As the N-terminal and C-terminal domains of the $V_1R$ are not involved in the binding of agonists or antagonists, they were not included in this model.

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 ligand. 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 in order to be able to form the disulfide bridge. This was achieved by performing an energy refinement in the program X-Plor 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 rest of the structure was not significantly altered by this refinement procedure.

Models of the nonpeptide AVP receptor antagonists were constructed with the program Alchemy 2000 (Tripos Inc., St. Louis, MO). The compounds were drawn in two dimensions and then extended into a 3D model by a 2D-to-3D builder incorporated in Alchemy 2000. Conformations with the lowest energy and devoid of any short contacts were saved. Finally, the most stable conformations

were subjected to an optimization using the program Gaussian 98 (Gaussian, Inc., Pittsburgh PA).

Docking of the nonpeptide ligand OPC-21268 to the receptors was done with the program LIGIN based on a built-in complementarity function (105). 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. In order to take into account possible movements of the receptor upon ligand binding, steric overlap between the ligand and a specified number of residues in the receptor can be allowed without energy penalty. The location of the bound antagonist OPC-21268 is distinct from the AVP-binding pocket with only partial overlap near the extracellular surface (Figure 4). The hydrophobic part is embedded in the transmembrane region far deeper than AVP is embedded, whereas the polar part is located on the surface of the extracellular side. The binding pocket is formed by residues from TMS 4, 5, 6, and 7, as well as the third extracellular loop. The 27-fold increase in the affinity of the Gly337Ala 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 (Figure 5). The Glu324Asp 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 Gln311. 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. The Ile310Val mutant reduces the hydrophobicity in the vicinity of the polar oxygen atom of the antagonist. The Ile224Val mutant relieves overcrowding in a hydrophobic binding site involving the aromatic residues Trp175, Phe179, Phe307, and Trp304. The smaller valine side chain allows for better positioning of the aromatic residues to interact with the bicyclic ring structure of OPC-21268. Finally, the Ile310Val mutant reduces the hydrophobicity in the vicinity of the polar oxygen atom of the antagonist. Thus, the model explains all the mutations that significantly increase the affinity towards OPC-21268.

The combination of site-directed mutagenesis and 3-D modeling in our study identified key residues involved in binding of the nonpeptide antagonist OPC-21268 to the $V_1$R. Our data clearly identified a single residue in the seventh TMS explaining the different affinities of the human and rat $V_1$R for OPC-21268. The docking model developed for this study confirmed the importance of this single residue, Ala337. Furthermore, the model predicts that a serine residue at this position should cause an even tighter binding owing to the formation of a hydrogen bond between the serine O$\gamma$ atom with the quinoline oxygen atom of OPC-21268, in addition to the van der Waals interaction of the serine $\beta$-carbon with carbon atoms 22 and 28 of this antagonist. This study also suggests modifications to the antagonist to increase the affinity for the receptor. For example, elimination of the quinoline oxygen atom should stabilize the interactions with the hydrophobic pocket deep inside the transmembrane region. However, this may cause adverse solubility

problems. A similar situation exists for residue 310 of the receptor and oxygen 47 of the antagonist. A hydrophobic residue in the vicinity of this polar atom is clearly unfavorable. A valine at this position, as found in the human sequence, is better than an isoleucine, the corresponding rat residue, but a threonine would be even better. Alternatively, replacement of oxygen 47 of the antagonist with a carbon atom should also increase the affinity. With respect to residue 224, a valine at this position seems to be optimal. This residue is located in a rather crowded hydrophobic environment into which a valine seems to fit better than the bulkier isoleucine.

Combination of the three mutations in positions 224, 324, and 337 did not improve further the affinity of the $V_1R$ for OPC-21268 when compared with the two double mutations, thus suggesting that alterations of the structure of the nonpeptide antagonist will be required to increase further the affinity of this compound.

The field of GPCRs suffers from a lack of experimentally determined structures. Therefore, molecular modeling is a very useful tool to derive structural information for the $V_1R$. It provides a framework to design and test new drugs as well as site-specific mutations in a rational way. However, one has to keep in mind the limitations of molecular modeling. The approach is based on the assumption that the seven transmembrane segments are similar in structure to bacteriorhodopsin. The Achilles' heel of this approach is 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 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 all of our findings very well. 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, our study provided for the first time the structural basis of species-selective binding of a nonpeptide antagonist to the $V_1R$. These findings should generate new ideas for drug development of nonpeptide AVP receptor antagonists and for optimizing drug-receptor interactions.

## CONCLUSIONS

In the near future, results of ongoing clinical studies testing the new orally active nonpeptide AVP receptor antagonists will tell us if these medications live up to their potential therapeutic indications. $V_1R$ antagonists may turn out to be an effective treatment of dysmenorrhea. $V_2R$ antagonists or aquaretics will undoubtedly facilitate the treatment of hyponatremia. However, because of their potency and the risk of rapid and excessive correction of serum sodium with potential neurologic complications, their use will require caution, especially in patients who do not have free access to water. Dual $V_1R/V_2R$ antagonists in a ratio that remains to be established may provide useful adjuvant treatment of arterial hypertension and

congestive heart failure. Finally, the design of specific $V_3R$ antagonists may offer diagnostic tools and medical treatment for ACTH-secreting tumors.

## ACKNOWLEDGMENTS

This article was supported by grants from the National Institutes of Health to Dr. Thibonnier and from the National Science Foundation to Dr. Shoham. Dr. Thibonnier has or has had research contracts with Otsuka, Parke-Davis, Sanofi, and Wyeth-Ayerst.



## LITERATURE CITED

1. Michell RH, Kirk JC, Billah MM. 1979. Hormonal stimulation of phosphatidylinositol breakdown with particular reference to the hepatic effects of vasopressin. *Biochem. Soc. Trans.* 7:861–65
2. Thibonnier M, Conarty DM, Preston JA, Wilkins PL, Berti-Mattera LN, et al. 1998. Molecular pharmacology of human vasopressin receptors. *Adv. Exp. Med. Biol.* 449:251–76
3. Andreoli TE. 1997. Edematous states: an overview. *Kidney Int.* 51:S2–10
4. Anderson RJ, Chung H, Kluge R, Schrier RW. 1985. Hyponatremia: a prospective analysis of its epidemiology and the pathogenetic role of vasopressin. *Ann. Intern. Med.* 102:164–68
5. Altura BM, Altura BT. 1977. Vascular smooth muscle and neurohypophyseal hormones. *Fed. Proc.* 36:1853–60
6. Johnston CI. 1985. Vasopressin in circulatory control and hypertension. *J. Hypertens.* 3:557–69
7. Goldsmith SR. 1987. Vasopressin as vasopressor. *Am. J. Med.* 82:1213–19
8. Aguilera G. 1994. Regulation of pituitary ACTH secretion during chronic stress. *Front. Neuroendocrinol.* 15:321–50
9. Dahia PLM, Ahmed-Shuaib A, Jacobs RA, Chew SL, Honegger J, et al. 1996. Vasopressin receptor expression and mutation analysis in corticotropin-secreting tumors. *J. Clin. Endocrinol. Metab.* 81:1768–71
10. De Keyser Y, Lenne F, Auzan C, Jégou S, René P, et al. 1996. The pituitary V3 vasopressin receptor and the corticotroph phenotype in ectopic ACTH syndrome. *J. Clin. Invest.* 97:1311–18
11. Freidinger RM, Pettibone DJ. 1997. Small molecule ligands for oxytocin and vasopressin receptors. *Med. Res. Rev.* 17:1–16
12. Thibonnier M, Jeunemaitre X, Graves MK, Wagner MS, Wilkins P, et al. 1995. Structure of the human V1a vasopressin receptor gene. See Ref. 106, pp. 553–71
13. Thibonnier M. 1992. Signal transduction of $V_1$-vascular vasopressin receptors. *Regul. Pept.* 38:1–11
14. Briley EM, Lolait SJ, Axelrod J, Felder CC. 1994. The cloned vasopressin V1a receptor stimulates phospholipase A2, phospholipase C, and phospholipase D through activation of receptor-operated calcium channels. *Neuropeptides* 27:63–74
15. Nishioka N, Hirai S, Mizuno K, Osada S, Suzuki A, et al. 1995. Wortmannin inhibits the activation of MAP kinase following vasopressin V1 receptor stimulation. *FEBS Lett.* 377:393–98
16. Granot Y, Erikson E, Fridman H, Van Putten V, Williams B, et al. 1993. Direct evidence for tyrosine and threonine phosphorylation and activation of mitogen-activated protein kinase by vasopressin in cultured rat vascular smooth muscle cells. *J. Biol. Chem.* 268:9564–69

17. Thibonnier M, Conarty DM, Plesnicher CL. 2000. Mediators of the mitogenic action of the human $V_1$-vascular vasopressin receptor. *Am. J. Physiol.* 279:H2529–39
18. Geisterfer AAT, Owens GK. 1989. Arginine-vasopressin-induced hypertrophy of cultured rat aortic smooth muscle cells. *Hypertension* 14:413–20
19. Lutz W, Sanders M, Salisbury J, Lolait S, O'Carroll AM, et al. 1993. Vasopressin receptor-mediated endocytosis in cells transfected with V1-type vasopressin receptors. *Kidney Int.* 43:845–52
20. Fishman JB, Dickey BF, Bucher NLR, Fine RE. 1985. Internalization, recycling, and redistribution of vasopressin receptors in rat hepatocytes. *J. Biol. Chem.* 260:12641–46
21. Innamorati G, Sadeghi H, Eberle AN, Birnbaumer M. 1997. Phosphorylation of the $V_2$ vasopressin receptor. *J. Biol. Chem.* 272:2486–92
22. Innamorati G, Sadeghi H, Birnbaumer M. 1998. Transient phosphorylation of the V1a vasopressin receptor. *J. Biol. Chem.* 273:7155–61
23. Berrada K, Plesnicher CL, Luo X, Thibonnier M. 2000. Dynamic interaction of human/oxytocin receptor subtypes with G protein-coupled receptor kinases and protein kinase C after agonist stimulation. *J. Biol. Chem.* 275:27229–37
24. Bichet DG, Lonergan M, Arthus MF, Fujiwara TM, Morgan K. 1995. Nephrogenic diabetes insipidus due to mutations in AVPR2 and AQP2. See Ref. 106, pp. 605–13
25. Hayashi M, Sasaki S, Tsuganezawa H, Monkawa T, Kitajima W, et al. 1994. Expression and distribution of aquaporin of collecting duct are regulated by vasopressin $V_2$ receptor in rat kidney. *J. Clin. Invest.* 94:1778–83
26. Kojro E, Fahrenholz F. 1995. Ligand-induced cleavage of the $V_2$ vasopressin receptor by a plasma membrane metalloproteinase. *J. Biol. Chem.* 270:6476–81
27. Kojro E, Postina R, Gilbert S, Bender F, Krause G, et al. 1999. Structural requirements for $V_2$ vasopressin receptor proteolytic cleavage. *Eur. J. Biochem.* 266:538–48
28. Bichet DG, Oksche A, Rosenthal W. 1997. Congenital nephrogenic diabetes insipidus. *J. Am. Soc. Nephrol.* 8:1951–58
29. Agre P, Bonhivers M, Borgnia MJ. 1998. The aquaporins, blueprints for cellular plumbing systems. *J. Biol. Chem.* 273:14659–62
30. Lolait SJ, O'Carroll AM, Mahan LC, Felder CC, Button DC, et al. 1995. Extrapituitary expression of the rat V1b vasopressin receptor gene. *Proc. Natl. Acad. Sci. USA* 92:6783–87
31. Grazzini E, Lodboerer AM, Perez-Martin A, Joubert D, Guillon G. 1996. Molecular and functional characterization of V1b vasopressin receptor in rat adrenal medulla. *Endocrinology* 137:3906–14
32. De Keyser Y, Auzan C, Lenne F, Beldjord C, Thibonnier M, et al. 1994. Molecular cloning, sequencing, and functional expression of a cDNA encoding the human $V_3$-pituitary vasopressin receptor. *FEBS Lett.* 356:215–20
33. Antoni F. 1984. Novel ligand specificity of pituitary vasopressin receptors in the rat. *Neuroendocrinology* 39:186–88
34. Baertschi AJ, Friedli M. 1985. A novel type of vasopressin receptor on anterior pituitary corticotrophs? *Endocrinology* 116:499–502
35. Giguere V, Labrie F. 1982. Vasopressin potentiates cyclic AMP accumulation and ACTH release induced by corticotropin-release factor in rat anterior pituitary cells in culture. *Endocrinology* 111:1752–54
36. Knepel W, Homolka L, Vlakovska M, Nutto D. 1984. In vitro adrenocortico tropin/$\beta$-endorphin-releasing activity of vasopressin analogs is related neither to pressor nor to antidiuretic activity. *Neuroendocrinology* 38:344–50

37. Levy A, Lightman SL, Hoyland J, Mason WT. 1990. Inositol phospholipid turnover and intracellular Ca++ responses to thyrotropin-releasing hormone, gonadotropin-releasing hormone and arginine vasopressin in pituitary corticotroph and somatotroph adenomas. *Clin. Endocrinol.* 33:73–79
38. Liu JP. 1994. Studies of the mechanisms of action of corticotropin-releasing factor (CRF) and vasopressin (AVP) in the ovine anterior pituitary: evidence that CRF and AVP stimulate protein phosphorylation and dephosphorylation. *Mol. Cell. Endocrinol.* 106:57–66
39. Liu JP, Engler D, Funder JW, Robinson PJ. 1994. Arginine vasopressin (AVP) causes the reversible phosphorylation of the myristoylated alanine-rich C kinase substrate (MARCKS) protein in the ovine anterior pituitary: evidence that MARCKS phosphorylation is associated with adrenocorticotropin (ACTH) secretion. *Mol. Cell. Endocrinol.* 105:217–26
40. Holmes MC, Antoni FA, Szentendrei T. 1984. Pituitary receptors for corticotropin-releasing factor: no effect of vasopressin on binding or activation of adenylate cyclase. *Neuroendocrinology* 39:162–69
41. Jard S, Gaillard RC, Guillon G, Marie J, Schoenenberg P, et al. 1986. Vasopressin antagonists allow demonstration of a novel type of vasopressin receptor in the rat adenohypophysis. *Mol. Pharmacol.* 30:171–77
42. Arsenijevic Y, Dubois-Dauphin M, Tribollet E, Manning M, Sawyer WH, et al. 1994. Vasopressin-binding sites in the pig pituitary gland: competition by novel vasopressin antagonists suggests the existence of an unusual receptor subtype in the anterior lobe. *J. Endocrinol.* 141:383–91
43. Thibonnier M, Preston JA, Dulin N, Wilkins PL, Wagner M, et al. 1997. The human V3-pituitary receptor: ligand-binding profile and density-dependent signaling pathways. *Endocrinology* 138:4109–22
44. Kimura T, Saji F. 1995. Molecular endocrinology of the oxytocin receptor. *Endocrine J.* 42:607–15
45. Ohmichi M, Koike K, Nohara A, Kanda Y, Sakamoto Y, et al. 1995. Oxytocin stimulates mitogen-activated protein kinase activity in cultured human puerperal uterine myometrial cells. *Endocrinology* 136:2082–87
46. Phaneuf S, Carrasco MP, Europe-Finner GN, Hamilton C, Lopez-Bernal A. 1996. Multiple G proteins and phospholipase C isoforms in human myometrial cells: implication for oxytocin action. *J. Clin. Endocrinol. Metab.* 81:2098–103
47. Morin D, Cotte N, Balestre MN, Mouillac B, Manning M, et al. 1998. The D136A mutation of the $V_2$ vasopressin receptor induces a constitutive activity which permits discrimination between antagonists with partial agonist and inverse agonist activities. *FEBS Lett.* 441:470–75
48. Sadeghi HM, Innamorati G, Dagarag M, Birnbaumer M. 1997. Palmitoylation of the $V_2$ vasopressin receptor. *Mol. Pharmacol.* 52:21–29
49. Schülein R, Rutz C, Rosenthal W. 1996. Membrane targeting and determination of transmembrane topology of the human vasopressin $V_2$ receptor. *J. Biol. Chem.* 271:28844–52
50. Liu J, Wess J. 1996. Different single receptor domains determine the distinct G protein coupling profiles of members of the vasopressin receptor family. *J. Biol. Chem.* 271:8772–78
51. Erlenbach I, Wess J. 1998. Molecular basis of $V_2$ vasopressin receptor/$G_s$ coupling selectivity. *J. Biol. Chem.* 273:26549–58
52. Thibonnier M, Preston J, Conarty D, Plesnicher C, Berti-Mattera L. 2000. Role of the C-terminus region of the human $V_1$-vascular vasopressin receptor in signal transduction. *Am. J. Physiol.* In press
53. Oksche A, Dehe M, Schülein R, Wiesner B, Rosenthal W. 1998. Folding and cell surface expression of the vasopressin V2

receptor: requirement of the intracellular C-terminus. *FEBS Lett.* 424:57–62
54. Krause G, Hermosilla R, Oksche A, Rutz C, Rosenthal W, et al. 2000. Molecular and conformational features of transport-relevant domain in the C-terminal tail of the vasopressin $V_2$ receptor. *Mol. Pharmacol.* 57:232–42
55. Manning M, Cheng LL, Stoev S, Sawyer WH, Tribollet E, et al. 1995. Novel potent and selective antagonists and radioiodinated ligands for oxytocin and vasopressin receptors. See Ref. 106, pp. 21–38
56. Yamamura Y, Ogawa H, Chihara T, Kondo K, Ongawa T, et al. 1991. OPC-21268, an orally effective nonpeptide vasopressin $V_1$ receptor antagonist. *Science* 252:572–74
57. Phillips PA, Burrell LM, Gow CB, Johnston CI, Grant S, et al. 1995. Vasopressin antagonism: physiological and pharmacological roles. See Ref. 106, pp. 643–58
58. Burrell LM, Phillips PA, Stephenson JM, Risvanis J, Rolls KA, et al. 1994. Blood pressure lowering effect of an orally active vasopressin $V_1$ receptor antagonist in mineralocorticoid hypertension in the rat. *Hypertension* 23:737–43
59. Naitoh M, Suzuki H, Murakami M, Matsumoto A, Arakawa K, et al. 1994. Effects of oral AVP receptor antagonists OPC-21268 and OPC-31260 on congestive heart failure in conscious dogs. *Am. J. Physiol. Heart Circ. Res.* 267:H2245–54
60. Burrell LM, Phillips PA, Rolls K, Buxton BF, Johnston CI, et al. 1994. Vascular responses to vasopressin antagonists in man and rat. *Clin. Sci.* 87:389–95
61. Ohnishi A, Ko Y, Fujihara H, Miyamoto G, Okada K, et al. 1993. Pharmacokinetics, safety, and pharmacologic effects of OPC-21268, a nonpeptide orally active vasopressin $V_1$ receptor antagonist in humans. *J. Clin. Pharmacol.* 33:230–38
62. Serradeil-Le Gal C, Bourrie B, Raufaste D, Carayon P, Garcia C, et al. 1994. Effect of a new potent, non-peptide $V_{1a}$ vasopressin antagonist, SR 49059, on the binding and the mitogenic activity of vasopressin on swiss 3T3 cells. *Biochem. Pharmacol.* 47:633–41
63. Serradeil-Le Gal C, Wagnon J, Guillon G, Garcia C, Lacour C, et al. 1993. Biochemical and pharmacological properties of SR49059, a new potent, non-peptide antagonist of rat and human vasopressin V1a receptors. *J. Clin. Invest.* 92:224–31
64. Serradeil-Le Gal C, Herbert JM, Delisee C, Schaeffer P, Raufaste D, et al. 1995. Effect of SR-49059, a vasopressin $V_{1a}$ antagonist, on human vascular smooth muscle cells. *Am. J. Physiol. Heart Circ. Physiol.* 37:H404–10
65. Serradeil-Le Gal C, Villanova G, Boutin M, Maffrand JP, Le Fur G. 1995. Effects of SR 49059, a non-peptide antagonist of vasopressin $V_{1a}$ receptors, on vasopressin-induced coronary vasoconstriction in conscious rabbits. *Fundam. Clin. Pharmacol.* 9:17–24
66. Brouard R, Chassard D, Hediard N. 1995. The advantages of surrogate markers during phase I for new exploratory compounds. Application for SR 49059, an orally active $V_{1a}$ vasopressin receptor antagonist. *Thérapie* 4:S34
67. Weber R, Pechère-Bertschi A, Hayoz D, Gerc V, Brouard R, et al. 1997. Effects of SR-49059, a new orally active and specific vasopressin V1 receptor antagonist, on vasopressin-induced vasoconstriction in humans. *Hypertension* 30:1121–27
68. Thibonnier M, Kilani A, Rahman M, Pilumeli DT, Warner K, et al. 1999. Effects of the nonpeptide V1 vasopressin receptor antagonist SR49059 in hypertensive patients. *Hypertension* 34:1293–300
69. Strömberg P, Akerlund M, Forsling ML, Granstrom E, Kindahl H. 1984. Vasopressin and prostaglandins in premenstrual pain and primary dysmenorrhea. *Acta Obstet. Gynecol. Scand.* 63:533–38
70. Bossmar T, Akerlund M, Szamatowicz J, Laudanski T, Fantoni G, et al. 1995. Receptor-mediated uterine effects of

vasopressin and oxytocin in nonpregnant women. *Br. J. Obstet. Gynaecol.* 102:907–12

71. Bossmar T, Brouard R, Döberl A, Akerlund M. 1997. Effects of SR 49059, an orally active $V_{1a}$ vasopressin receptor antagonist, on vasopressin-induced uterine contractions. *Br. J. Obstet. Gynaecol.* 104:471–477
72. Brouard R, Bossmar T, Fournie-Lloret D, Chassard D, Akerlund M. 2000. Effect of SR49059, an orally active $V_{1a}$ vasopressin receptor antagonist, in the prevention of dysmenorrhoea. *Br. J. Obstet. Gynaecol.* 107:614–19
73. Yamamura Y, Ogawa H, Yamashita H. 1992. Characterization of a novel aquaretic agent, OPC-31260, as an orally effective, nonpeptide vasopressin $V_2$-receptor antagonist. *Br. J. Pharmacol.* 105:787–92
74. Kitiyakara C, Wilcox CS. 1997. Vasopressin V2-receptor antagonists: panaceas for hyponatremia? *Curr. Opin. Nephrol. Hypertens.* 6:461–67
75. Saito T, Ishikawa S, Abe K, Kamoi K, Yamada K, et al. 1997. Acute aquaresis by the nonpeptide arginine vasopressin (AVP) antagonist OPC-31260 improves hyponatremia in patients with syndrome of inappropriate secretion of antidiuretic hormone (SIADH). *J. Clin. Endocrinol. Metab.* 82:1054–57
76. Inoue T, Ohnishi A, Matsuo A, Kawai B, Kunihiro N, et al. 1998. Therapeutic and diagnostic potential of a vasopressin-2 antagonist for impaired water handling in cirrhosis. *Clin. Pharmacol. Ther.* 63: 561–70
77. Chan PS, Coupet J, Park HC, Lai F, Hartupee D, et al. 1998. VPA-985, a nonpeptide orally active and selective vasopressin $V_2$ receptor antagonist. In *Vasopressin and Oxytocin. Molecular, Cellular, and Clinical Advances*, ed. HH Zingg, CW Bourque, D Bichet, pp. 439–43. Montréal: Plenum
78. Lai F, Sheperd C, Bailey T, Chan PS, Cervoni P. 1997. Effects of the vasopressin $V_2$ antagonist VPA-985 in rat caudal arteries and on arterial pressure in normotensive, SHR, and DOCA-salt hypertensive rats. Presented at *1997 World Congr. Neurohypophys. Hormones, Montréal*
79. Abraham WT, Oren RM, Crisman TS, Robertson AD, Shaker S, et al. 1997. Effects of an oral nonpepide, selective $V_2$ receptor vasopressin antagonist in patients with chronic heart failure. *J. Am. Coll. Cardiol.* 29:169A
80. Yamamura Y, Nakamura S, Ito S, Hirano T, Onogawa T, et al. 1998. OPC-41061, a highly potent human vasopressin $V_2$-receptor antagonist: pharmacological profile and aquaretic effect by single and multiple oral dosing in rats. *J. Pharmacol. Exp. Ther.* 287:860–67
81. Hirano T, Yamamura Y, Nakamura S, Onogawa T, Mori T. 2000. Effects of the $V_2$-receptor antagonist OPC-41061 and the loop diuretic furosemide alone and in combination in rats. *J. Pharmacol. Exp. Ther.* 292:288–94
82. Serradeil-Le Gal C, Lacour C, Valette G, Garcia G, Foulon L, et al. 1996. Characterization of SR-121463A, a highly potent and selective, orally active vasopressin V2 receptor antagonist. *J. Clin. Invest.* 98:2729–38
83. Bosch-Marcé M, Poo JL, Jiménez W, Bordas N, Leivas A, et al. 1999. Comparison of two aquaretic drugs (Niravoline and OPC-31260) in cirrhotic rats with ascites and water retention. *J. Pharmacol. Exp. Ther.* 289:194–201
84. Bellissant E, Denolle T, Sinnassamy P, Bichet DG, Giudicelli JF, et al. 1996. Systemic and regional hemodynamic and biological effects of a new kappa-opioid agonist, niravoline, in healthy volunters. *J. Pharmcol. Exp. Ther.* 278:232–42
85. László FA, Varga C, Nakamura S. 1999. Vasopressin receptor antagonist OPC-31260 prevents cerebral oedema after subarachnoid haemorrhage. *Eur. J. Pharmacol.* 364:115–22

86. Morello JP, Salahpour A, Laperrière A, Bernier V, Arthus MF, et al. 2000. Pharmacological chaperones rescue cell-surface expression and function of misfolded V2 vasopressin receptor mutants. *J. Clin. Invest.* 105:887–95
87. Sladek CD, Blair MI, Mangiapane M. 1987. Evidence against a pressor role for vasopressin in spontaneous hypertension. *Hypertension* 9:332–38
88. Sladek C, Blair ML, Sterling C, Mangiapane ML. 1988. Attenuation of spontaneous hypertension in rats by a vasopressin antagonist. *Hypertension* 12:506–12
89. Hofbauer KG, Mah SC, Baum HP, Hänni H, Wood JM, et al. 1984. Endocrine control of salt and water excretion: the role of vasopressin in DOCA-salt hypertension. *J. Cardiovasc. Pharmacol.* 6:S184–91
90. Tahara A, Tomura Y, Wada KI, Kusayama T, Tsukada J, et al. 1997. Pharmacological profile of YM087, a novel potent nonpeptide vasopressin V1a and V2 receptor antagonist, in vitro and in vivo. *J. Pharmacol. Exp. Ther.* 282:301–8
91. Risvanis J, Naitoh M, Johnston CI, Burrell LM. 1999. In vivo and in vitro characterisation of a nonpeptide vasopressin $V_{1a}$ and $V_2$ receptor antagonist (YM087) in the rat. *Eur. J. Pharmacol.* 381:23–30
92. Fricker AF, Burnier M, Brunner HR. 1997. Evaluation of the non-peptide, orally active, V1/V2 receptor antagonist YM087 in normal subjects. Presented at *1997 World Congr. Neurohypophysial Hormones, Montréal*
93. Burnier M, Fricker AF, Hayoz D, Nussberger J. 1999. Pharmacokinetic and pharmacodynamic effects of YM087, a combined $V_1/V_2$ vasopressin receptor antagonist in normal subjects. *Eur. J. Clin. Pharmacol.* 55:633–37
94. Mouillac B, Chini B, Balestre MN, Elands J, Trumpp-Kallmeyer S, et al. 1995. The binding site of neuropeptide vasopressin $V_{1a}$ receptor. *J. Biol. Chem.* 270: 25771–77
95. Thibonnier M, Coles P, Conarty DM, Plesnicher CL, Shoham M. 2000. A molecular model of agonist and nonpeptide antagonist binding to the human $V_1$ vascular vasopressin receptor. *J. Pharmacol. Exp. Ther.* 294:195–203
96. Chini B, Mouillac B, Ala Y, Balestre MN, Trumpp-Kallmeyer S, et al. 1995. Tyr115 is the key residue for determining agonist selectivity in the $V_{1a}$ vasopressin receptor. *EMBO J.* 14:2176–82
97. Mendre C, Dufour MN, Le Roux S, Seyer R, Guillou L, et al. 1997. Synthetic rat $V_{1a}$ vasopressin receptor fragments interfere with vasopressin binding via specific interaction with the receptor. *J. Biol. Chem.* 272:21027–36
98. Ufer E, Postina R, Gorbulev V, Fahrenholz F. 1995. An extracellular residue determines the agonist specificity of $V_2$ vasopressin receptors. *FEBS Lett.* 362: 19–23
99. Cotte N, Balestre MN, Phalipou S, Hibert M, Manning M, et al. 1998. Identification of residues responsible for the selective binding of peptide antagonists and agonists in the V2 vasopressin receptor. *J. Biol. Chem.* 273:29462–68
100. Postina R, Kojro E, Fahrenholz F. 1996. Separate agonist and peptide antagonist binding sites of the oxytocin receptor defined by their transfer into the $V_2$ vasopressin receptor. *J. Biol. Chem.* 271:31593–601
101. Henderson R, Baldwin JM, Ceska TA, Zemlin F, Beckmann E, et al. 1990. Model for the structure of bacteriorhodopsin based on high-resolution electron cryomicroscopy. *J. Mol. Biol.* 213:899–929
102. Vriend G. 1990. A molecular modeling and drug design program. *J. Mol. Graph. Model.* 8:52–56
103. Rodriguez R, Chinea G, Lopez N, Pons T, Vriend G. 1998. Homology modelling, model and software evaluation: three related sources. *Comput. Appl. Biosci.* 14:523–28

104. Levitt M. 1992. Accurate modeling of protein conformation by automatic segment matching. *J. Mol. Biol.* 226: 507–33
105. Sobolev V, Wade RC, Vriend G, Edelman M. 1996. Molecular docking using surface complementarity. *Proteins: Struct. Func. Genet.* 25:120–29
106. Saito T, Kurokawa T, Yoshida S, eds. 1995. *Neurohypophysis: Recent Progress of Vasopressin and Oxytocin Research.* Amsterdam: Elsevier

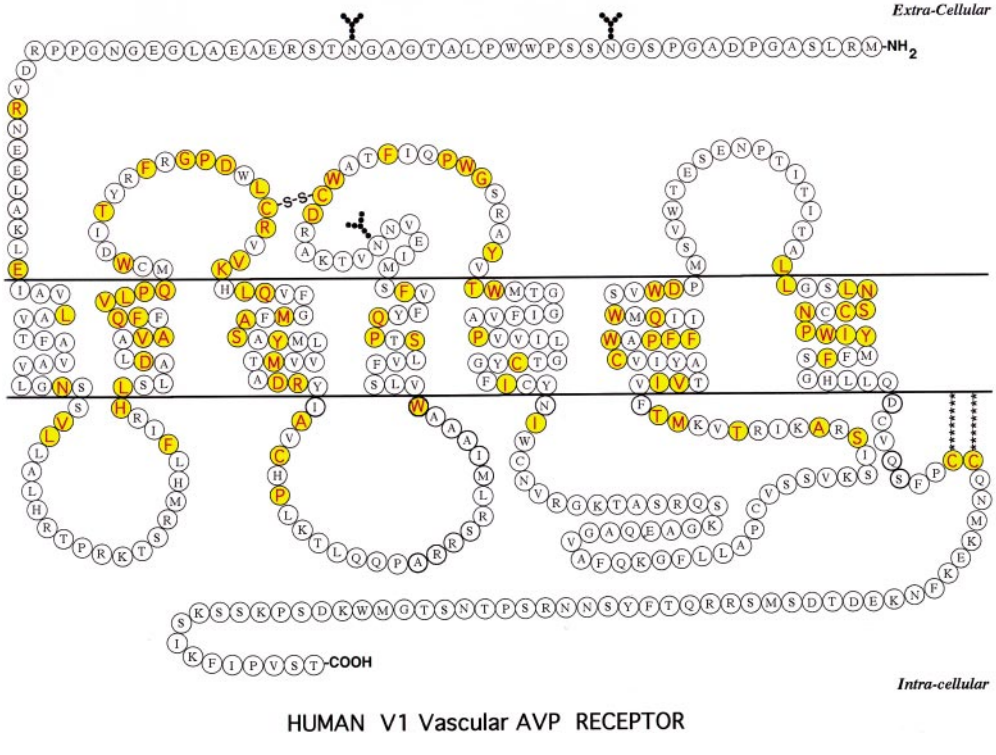
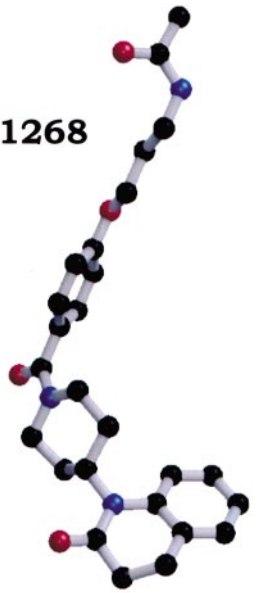



**Figure 1** Two-dimensional model of the human $V_1$-vascular AVP receptor. Conserved amino acids throughout the whole family of AVP/OT receptor subtypes are in red characters on yellow background.

(A)

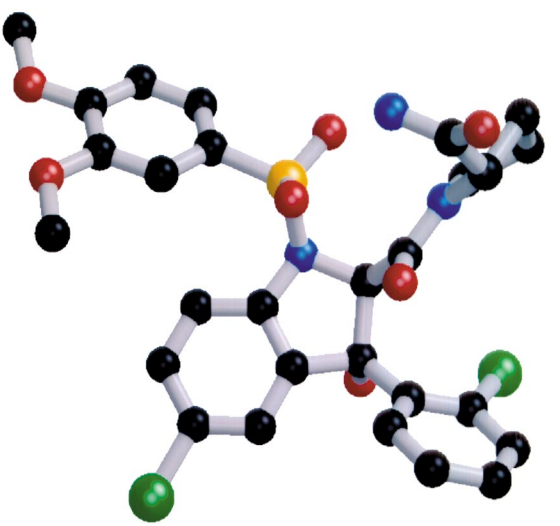
OPC-21268



1-{1-[4-(3-acetylaminopropoxy)benzoyl]-4-piperidyl}-3,4-dihydro-2(1H)-quinolinone

(B)

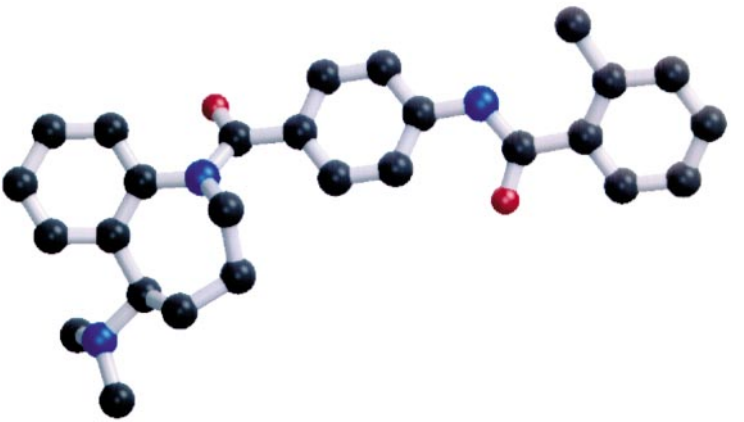
SR-49059



(2S)1-[(2R3S)-5-chloro-3-(2-chloro-phenyl)-1-(3,4-dimethoxybenzene-sulfonyl)-3-hydroxy-2,3-dihydro-1H-indole-2-carbonyl]-pyrrolidine-2-carboxamide

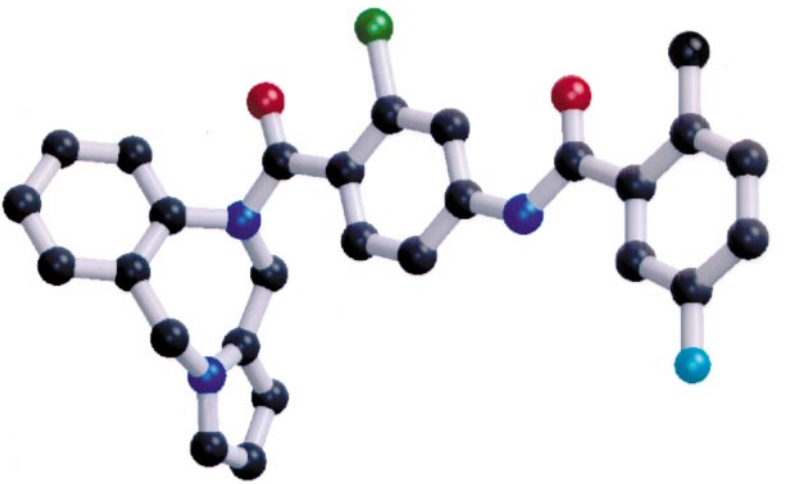
See legend with Figure 3G

(C) **OPC-31260**



**5-dimethylamino-1-[4-(2-methylbenzoylamino)benzoyl]-2,3,4,5-tetrahydro-1H-benzazepine hydrochloride**

(D) **VPA-985**

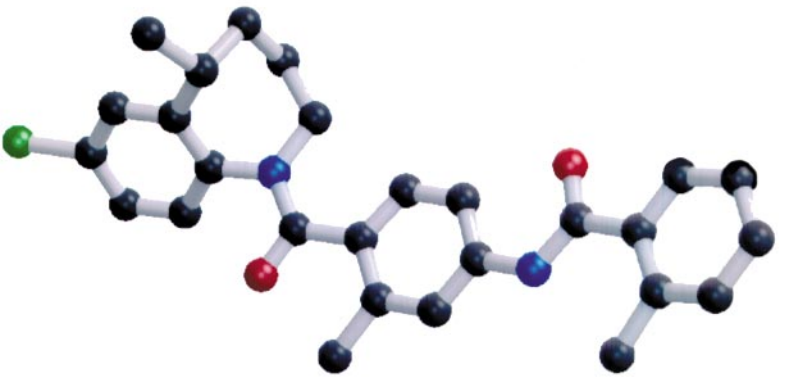


**5-Fluoro-2-methyl-N-[4-(5H-pyrrolo[2,1-c][1,4]benzodiazepin-10(11H)-yl)carbonyl)-3-chlorophenyl]benzamide**

See legend with Figure 3G

(E)

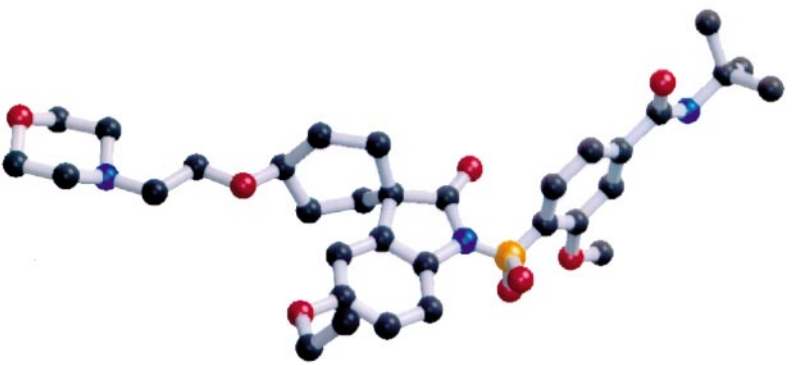
## OPC-41061



(+/-)-7-chloro-5-hydroxy-1-[2-methyl-4-(2-methylbenz oylamino)benzoyl[-2,3,4,5-tetrahydro-1H-1-benzazepine

(F)

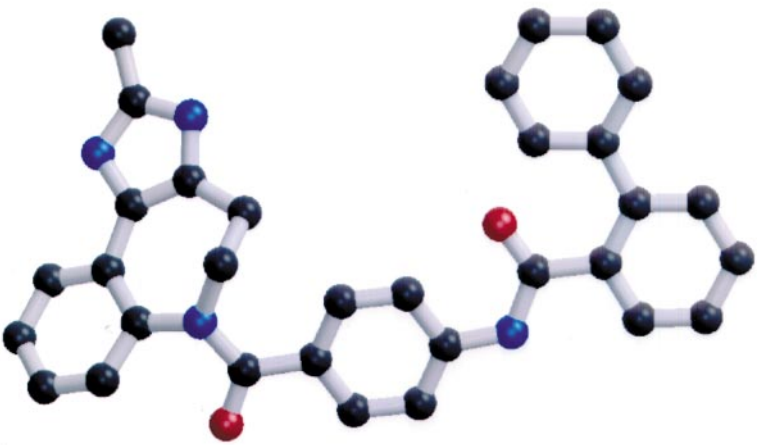
## SR-121463A



1-[4-(N-tert-butylcarbamoyl)-2-methoxybenzene sulfonyl]-5-ethoxy-3-spiro-[4-(2-morpho-linoethoxy) cyclohexane]indol-2-one fumarate

See legend with Figure 3G

(G) **YM087**



4′-[(2-methyl-1,4,5,6-tetrahydroimidazo[4,5-d][1]benzazepin-6-yl)-carbonyl]-2-phenylbenzanilide monochloride

**Figure 3** Three-dimensional structures of the nonpeptide $V_1$-vascular receptor antagonists (OPC-21268 and SR-49059), the $V_2$-renal receptor antagonists (OPC-31260, SR-121463A, VPA-985, OPC-41061), and the dual $V_1$-vascular/$V_2$-renal receptor antagonist YM087 in ball-and-stick representation. Carbon, nitrogen, oxygen, sulfur, chloride, and fluorine atoms are shown in black, blue, red, yellow, green, and cyanin respectively.

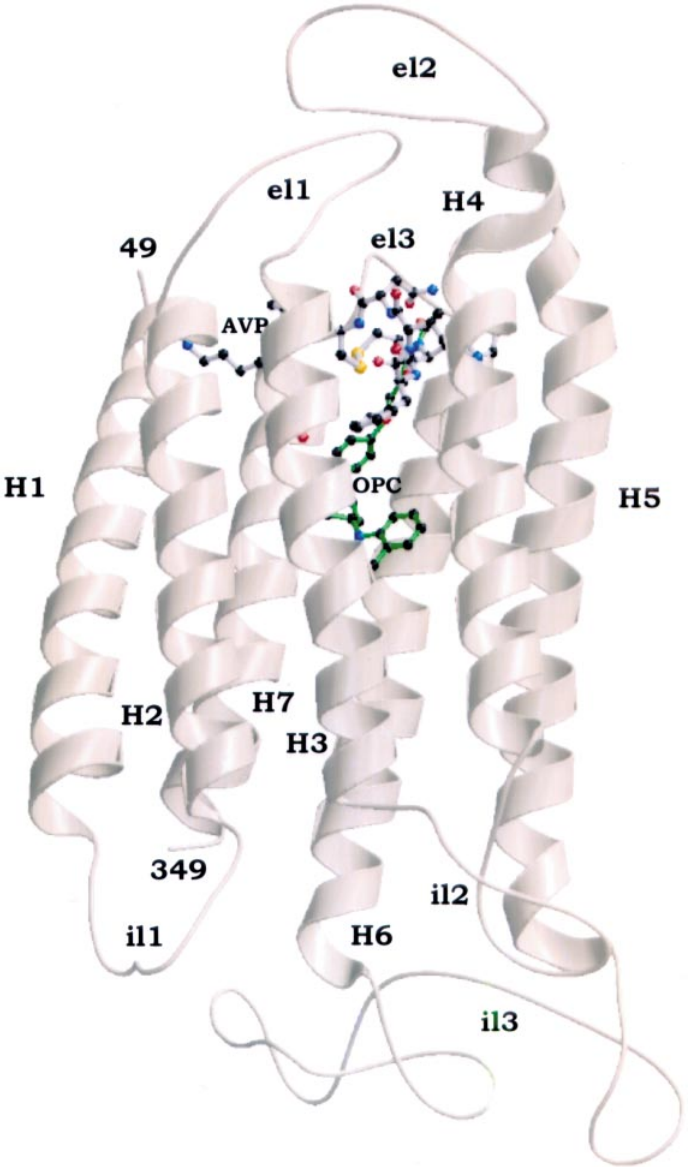



**Figure 4** Docking of AVP and the nonpeptide $V_1$-vascular receptor antagonist OPC-21268 to the human $V_1$-vascular AVP receptor. Superposition of the models of AVP and the nonpeptide antagonist OPC-21268 as bound to the human $V_1$-vascular AVP receptor. The loops are labeled il1, il2, and il3 for the intracellular loops and el1, el2 and el3 for the extracellular loops. The transmembrane segments are labeled H1 to H7. The different binding modes of agonist and antagonist are clearly shown.

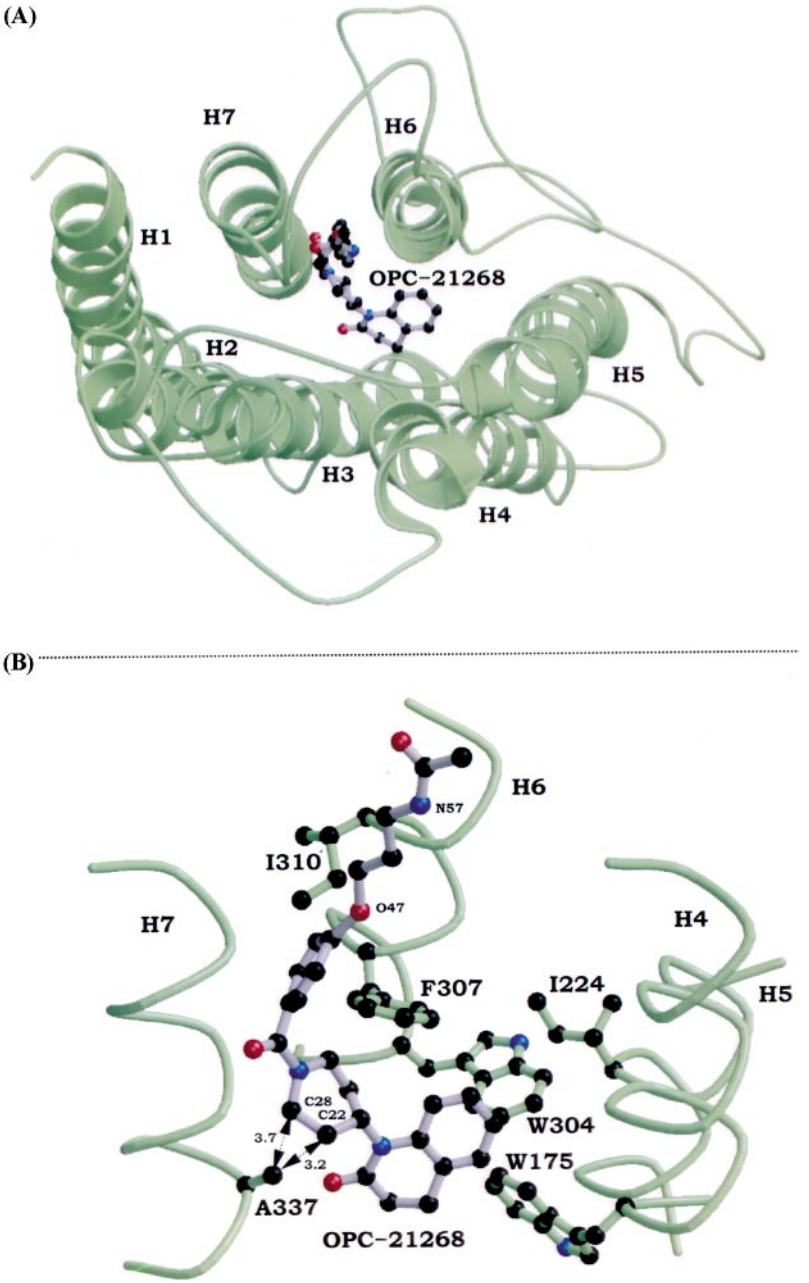



**Figure 5** Docking of the nonpeptide antagonist OPC-21268 onto the model of the human $V_1$-vascular AVP receptor. (a) top view, (b) stabilizing effect of the G337A, I224V, and I310V mutations on antagonist binding.