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QUARTERLY

Molecular modelling of the vasopressin V2 receptor/antagonist interactions*°

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We predict some essential interactions between the V2 vasopressin renal receptor (V2R) and its selective peptide antagonist desGly^9-[Mca^1,D-Ile^2,Ile^4]AVP, and compare these predictions with the earlier ones for the non-peptide OPC-36120 antagonist- and the [Arg^8]vasopressin (AVP) agonist-V2 receptor interactions. V2R controls antidiuresis in mammals and belongs to the superfamily of the heptahelical transmembrane (7TM) G protein-coupled receptors (GPCR)s. V2R was built, the ligands docked and the structures relaxed using advanced molecular modeling techniques. Both the agonist and the antagonists (no matter whether of peptide- or non-peptide type) appear to prefer a common V2R compartment for docking. The receptor amino-acid residues, potentially important in ligand binding, are mainly in the TM3-TM7 helices. A few of these residues are invariant for the whole GPCR superfamily while most of them are conserved in the subfamily of neurohypophyseal receptors, to which V2R belongs. Some of the equivalent residues in a related V1a receptor have been earlier reported as critical for the ligand affinity.

The nonapeptide hormone vasopressin (CYFQNCPRG-NH_2, AVP) regulates the renal water absorption *via* the interaction with the V2 receptor (V2R). V2R and the other three structurally related neurophyseal hormone receptors (NHRs), viz. the vascular V1a and the

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Abbreviations: OT, oxytocin; AVP, [arginine^8]vasopressin; Mca, β,β-cyclopentamethylene-β-mercaptopropionyl; OPC-31260, [5-dimethylamino-1-{4-(2-methylbenzoylamino)benzoyl}-2,3,4,5-tetrahydro-1*H*-benzazepine; GPCR, G protein-coupled receptor; V2R, vasopressin V2 receptor; V1a(b)R, vasopressin V1a(b) receptor; RD, rhodopsin; NHR, neurophyseal hormone receptor; 7TM, heptahelical transmembrane receptor or domain; EL, extracellular loop; IL, intracellular loop.

pituitary V1b AVP receptors (V1aR and V1bR, respectively), and the oxytocin receptor (OTR), form a subfamily within the large superfamily (of > 1000 members) of G protein-coupled receptors (GPCRs), the most abundant class of transmembrane mediators of information from external stimuli (neurotransmitters, hormones, odorants, light) to intracellular second messenger systems. Although no details on the GPCR architecture are known at the atomic resolution level, a recent significant progress, including both the low (6 Å) resolution structure of rhodopsin (RD) [1] and the multi-sequence analysis [2], prompted the development of perhaps the most rational under the circumstances strategy for the GPCR modeling [3, 4]. The strategy includes in a self-consistent way the 6 Å resolution structure of RD [1], the multiple sequence alignments [2] and the numerous experimental intramolecular geometrical constraints known for RD, and leads eventually to the 7TM sequences and arrangement characterized by the total root-mean-square (rms) deviation of 1.6 Å taking into account the C^{α} atoms [3]. The model, even if chiefly applying to RD, provides a good start for modeling other 7TM receptors, given the sequence homologies among various GPCR subfamilies.

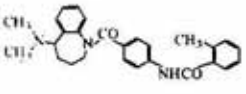
All four NHRs, while having their 7TM domains discernibly homologous with RD, share a high degree of sequence identity among themselves [5, 6], particularly within their transmembrane (TM) domains TM2, TM3, TM6 and TM7, the extracellular loop EL1 and the C-terminal part of EL2, where the identity reaches 87% [5, 6] and thus warrants an assumption of similar recognition and binding modes for the agonists in all NHRs. Major differences occur in the intracellular loops (IL)s and correlate with receptor's linking to a specific second messenger systems: V1aR, V1bR and OTR to the $G_{q/11}$ protein/phospholipase Cβ tract while V2R to the G_s protein/cAMP system [7].

In this work we intend, using molecular modelling, to analyse possible docking modes of one of the most selective V2R peptide antagonists, desGly^9-[Mca^1,D-Ile^2,Ile^4]AVP [8]; to compare it with the docking modes of the selective non-peptide V2R antagonist OPC-31260 [9] and the agonist AVP [10] and finally to locate the V2R residues potentially responsible for antagonist binding, for further verification of their biological role *via* mutagenesis-affinity studies in the future. The affinity data [11] for the ligands discussed in this work and the molecular topology of OPC–31260 (item 4) are given in Table 1.

METHODS

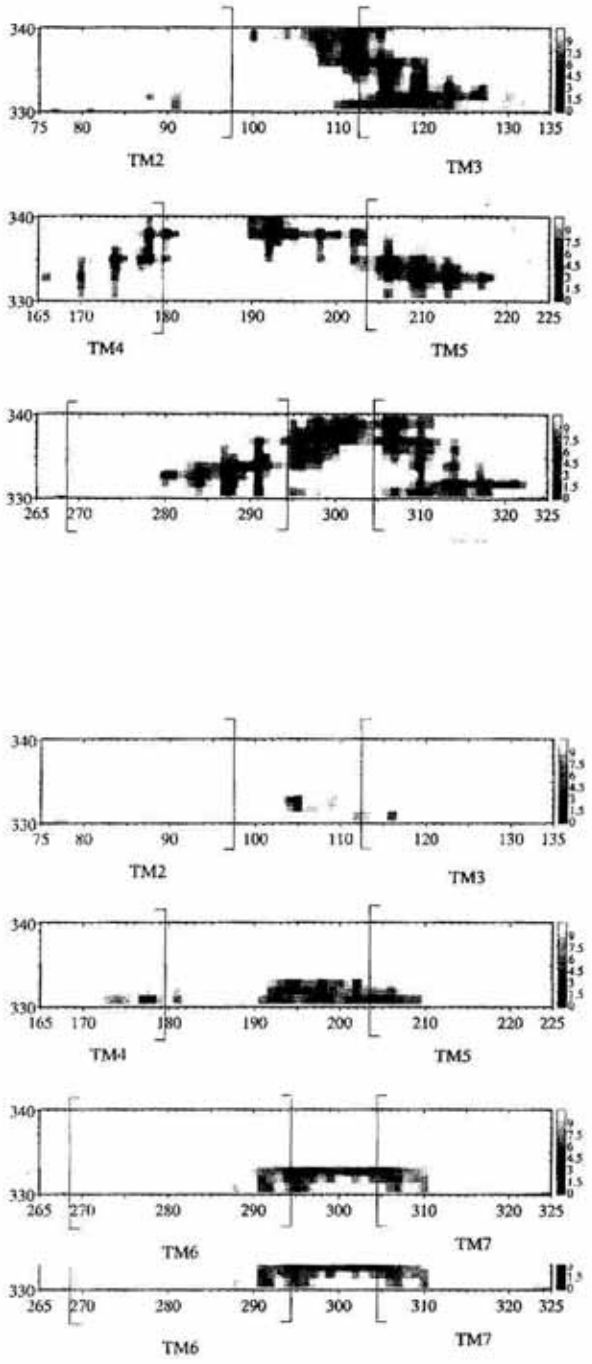
The 7TM domain of the human V2R was obtained from the Swiss-Model protein modeling server [4], given the helix alignments in agreement with those proposed by Baldwin [2,

Table 1. Affinity and selectivity [11] of the neurophyseal hormones and the antagonists discussed in this work toward the neurophyseal receptors. Affinities K_d (nM) for agonists are in bold, for antagonists in italic.

	Ligand [a]	V1a	V1b	V2	OT
1	AVP	**1.7**	**3.2**	**0.4**	**1.6**
2	OT	**56**	**251**	**89**	**1.9**
3	desGly^9-[Mca^1,D-Ile^2,Ile^4]AVP	*5.2(pA2)*	-	*2.8*	-
4		*1200*	-	*14*	-

[a] No highly selective V1b ligands or highly selective V1a/V2 agonists exist.

12]. The loops and the amino domain were built using the SYBYL suite of programs [13]. Initial ligand docking was attained in several ways, taking into account the complementarity of the electrostatic potentials in the V2R extracellular cleft (*vide* infra) and around the ligand. The systems were relaxed by the consecutive use of minimisation and constrained simulated annealing (CSA) protocols *in vacuo*, with all but the 7TM C^{α} atoms free to move. Optimal ligand docking modes were selected from a critical comparison of the ligand/receptor interaction energy terms (as those given in Fig. 1) against conclusions on biologically relevant V2R residues, arising from structure-activity data [8, 9]. All non-standard amino-acid residues (including OPC-31260, divided into 3 "residues", see Fig. 1B), were parametrized in accordance with the recommendations in the AMBER 4.1 manual [14]. In particular, the charges were optimised by fitting them to the *ab initio* molecular electrostatic potentials (6-31G* basis set, GAMESS molecular orbital program package [15] for several conformations of each new residue, followed by consecutive averaging the charges over all conformations, as recommended in the new RESP protocol [16]. Details of the modeling and computations are described



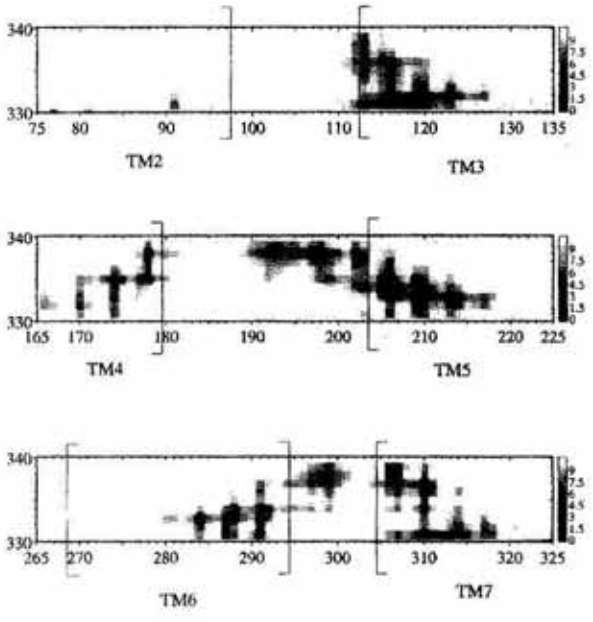


Figure 1. The maps of the receptor-ligand contacts, typical of the averaged structures.

elsewhere [10]. Computations were done using AMBER 4.1 suite of programs [14] on either an SGI Power Challenge 8xR10000 or an IBM SP2 15xPOWER2 supercomputers. The images for presentation were prepared using the MolMol program [17].

RESULTS

Any GPCR modelled to the RD template [3, 4] has a deep (about 21Å) cleft on the extracellular side, surrounded by TM3-TM7 with a narrower extension towards TM2. The cavity ends up with a floor made of mainly hydrophobic residues TM3:M123, TM4:L170, TM5:V213,F214 and TM6:W284,F287,F288 in V2R. The cleft is big enough to accomodate the pressin ring of AVP and its analogs, and even more so, to fit the OPC-31260 molecule.

Despite starting from several conformations and/or docking configurations for the ligand, most of the simulations ended with the docking modes converging to one typical of V2R/AVP [10]. Meanwhile, we have also found that the V2R/OPC-31260 docking mode implicates the same V2R cavity and a subset of the V2R amino-acid residues typically involved in the peptide ligand docking [10]. Since OPC-31260 is much thinner than the AVP pressin ring, CYFQNC, it cannot fill the entire V2R cleft and adheres anywhere to the TM3-TM7 cavity wall. Typical V2R/desGly^9-[Mca^1,D-Ile^2,Ile^4]AVP contacts are shown on the interaction map in Fig. 1C. For comparison, similar contacts for V2R/AVP and V2R/OPC-31260 are given (Fig. 1, Panels A and B, respectively). Notice that the maps clearly indicate that desGly^9-[Mca^1,D-Ile^2,Ile^4]AVP lacks the 9th amino-acid residue (Gly) and that OPC-31260 is partitioned into only 3 "amino-acid" residues. From Fig. 1 it is also clearly seen that desGly^9-[Mca^1,D-Ile^2,Ile^4]AVP and AVP mostly involve common V2R residues for similar pairwise interactions.

Thus, TM3:V115 is in a close contact with the AVP:C1-C5 disulfide and, similarly, with the desGly^9-[Mca^1,D-Ile^2,Ile^4]AVP:Mca1-C6 moiety; TM3:K116 is in a close contact with the peptide ligands' residues 1-6; likewise TM3:Q119 and M:124 contact ligands' residues 1,2 and 2,3, respectively. This list could be countinued up to TM7; a careful inspection of Figs. 1A and C should suffice instead. On the other hand, it is remarkable, as can be noticed from Fig. 1B, that the OPC-31260 antagonist, while being immersed in the V2R

Figure 1 (continued)

The contours represent the closest distances between pairs of residues with increasing shading for decreasing distance, in accordance with the scale on the right. Horizontal axis: successive residues of V2R, with the sequences interrupted where the receptor-ligand contacts exceed 9 Å. As the TM helices are marked, it is clear that EL1-EL3 correspond to the central sections of the strips from the top to the bottom, respectively. Vertical axis: ligand amino-acid sequence (numbered 331–340) with OPC-31260 being partitioned into three "amino-acid" residues at the two peptide bonds, $N(CH_3)^+_2$ marking the "C-terminus" i.e. the 333rd residue. Panel A: V2R/AVP. Panel B: V2R/OPC-31260. Panel C: V2R/desGly^9-[Mca^1,D-Ile^2,Ile^4]AVP. The V2R sequence, with the putative TM helices underlined, supplements the Figure.

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MLMASTTSAV PGHPSLPSLP SNSSQERPLD TRDPLLARAE LALLSIVFVA VALSNGLVLA      60
ALARRGRRGH WAPIHVFIGH LCLADLAVAL FQVLPQLAWK ATDRFRGPDA LCRAVKYLQM     120
VGMYASSYMI LAMTLDRHRA ICRPMLAYRH GSGAHWNRPV LVAWAFSLLL SLPQLFIFAQ     180
RNVEGGSGVT DCWACFAEPW GRRTYVTWIA LMVFVAPTLG IAACQVLIFR EIHASLVPGP     240
SERPGGRRRG RRTGSPGEGA HVSAAVAKTV RMTLVIVVVY VLCWAPFFLV QLWAAWDPEA     300
PLEGAPFVLL MLLASLNSCT NPWIYASFSS SVSSELRSLL CCARGRTPPS LGPQDESCTT     360
ASSSLAKDTS S
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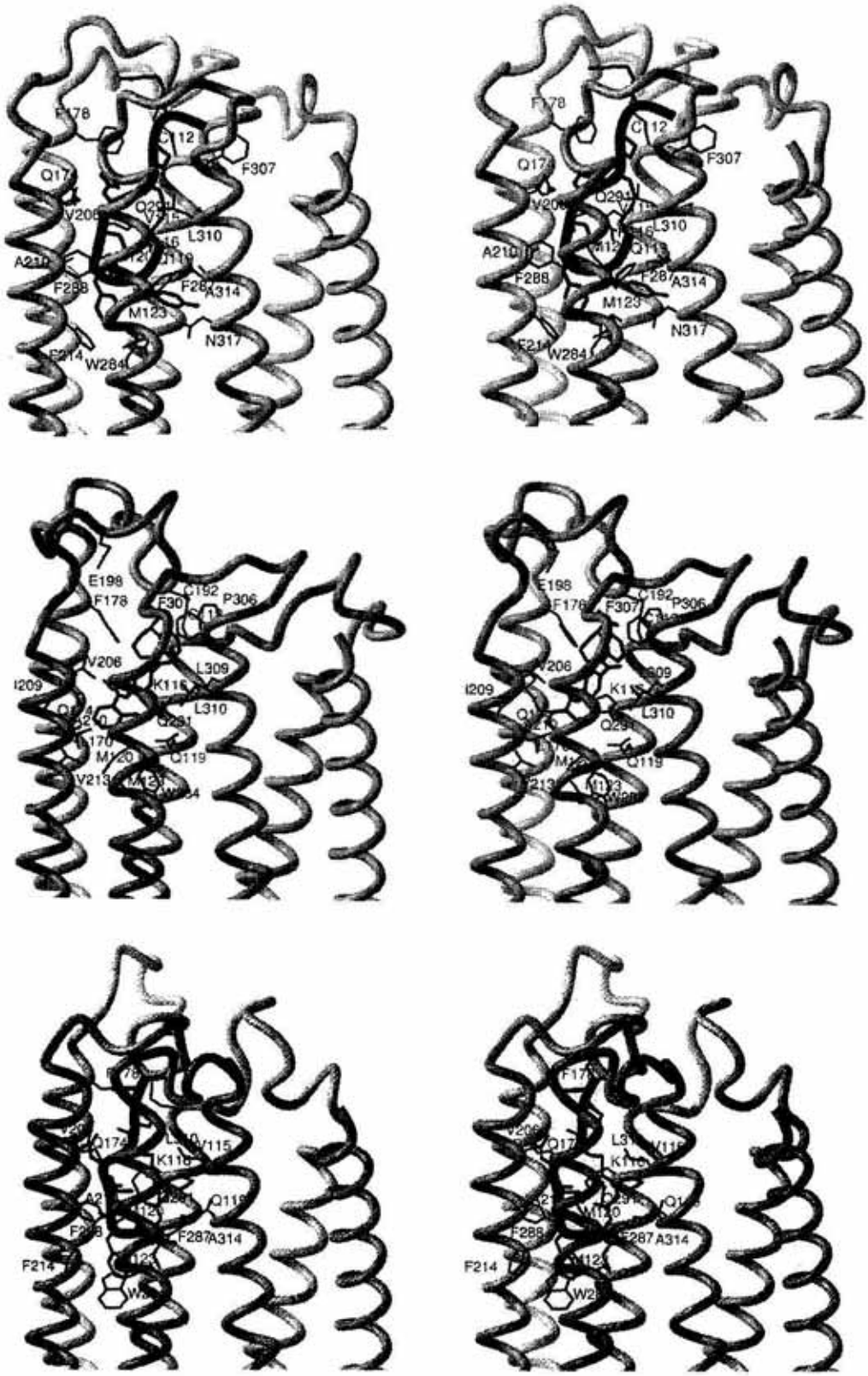

Figure 2. Stereodiagrams of the relaxed docking modes.

Only the receptor extracellular halves are shown. V2R is gray and the ligand black. The interacting receptor residues (see Fig. 1) are marked and their side chains exposed. Panel A: V2R/AVP. Panel B: V2R/OPC-31260. Panel C: V2R/desGly^{9}-[Mca^{1},D-Ile^{2},Ile^{4}]AVP.

cavity by approximately two helical turns shallower than the peptide ligands, still interacts with the subset of the same V2R residues (see Fig. 2).

In Fig. 2 are shown stereo images of the same V2/ligand complexes. All V2R interacting residues are marked so that the significant receptor-ligand interactions can be seen. Both the agonist and antagonist V2R/peptide complexes exploit their potentials for nonpolar interactions between the ligands' N-terminal 1–3 amino-acid triad and the hydrophobic floor at the bottom of the V2R extracellular cavity, and simultaneously develop numerous polar and nonpolar interactions with the walls of the cleft. Major interactions, common to both AVP and desGly^9-[Mca^1,D-Ile^2, Ile^4]AVP involve on the V2R part TM3:C112, V115-K116,Q119, M123, TM4:Q174, TM5: V206,A210,V213, TM6:W284,F287,F288, Q291 and TM7:F307,L310,A314, N317 (see Figs. 1 and 2.) The hydrophobic Mca^1 β,β-pentamethylene moiety fits snugly a hydrophobic pocket formed by TM3:V115 and TM7:L310 and A314.

DISCUSSION

Even though the ligand/ELs interactions are quite apparent in Fig. 1, we do not attribute to them much significance since the loop conformations resulting from CSA *in vacuo* appear to a great extent circumstantial [10]. We hope to cope with this feature in future simulations including the phospholipid membrane.

OPC-31260, despite being much smaller than a peptide ligand and thus fitting many places within the V2R cavity (including the extension toward TM2) prevailingly choses a compartment common with the peptide agonists. OPC-31260 typically orients itself so that its long axis is nearly vertical and its $HN(CH_3)^+_2$ involved in a (bifurcated) ion bridge with one (two) of the numerous negatively charged Asp and/or Glu residues in ELs (see Fig. 1B). In this regard, it is interesting to notice that EL2 contains three carboxylates in V2R and two in V1aR, which may bear on the increased V2R/V1aR selectivity of the OPC-31260 analogs having a cationic group in the equivalent place [18].

Regarding desGly^9-[Mca^1,D-Ile^2,Ile^4]AVP, it is seen that its Mca^1 β,β-pentamethylene moiety, a requisite for the antagonism towards both AVP and OT receptors [8], cannot confer a selectivity since the tight pocket it takes (TM3:V115 and TM7:L310 and A314, see above) is conservative among all NHRs. On the other hand, the two features potentially responsible for the V2R/V1aR selectivity could be: (i) an interaction between antagonist's I4 and TM4:A210 and (ii) a weakened (relative to the agonist) interaction between residue 2 (D-Ile in the antagonist) and TM7 (see Figs. 1 and 2).

The tendency for all three ligands to dock within the same compartment of the V2R extracellular cavity, suggests a simple competitive mechanism for the antagonism towards V2R by both desGly^9-[Mca^1,D-Ile^2,Ile^4]AVP and OPC-31260. The V2R amino acid residues involved in ligand binding are invariant or conservative (marked with a 'c' below) for the NHR subfamily: (TM3:V115-K116,Q119, M123, TM5:V206c, A210c,V213c, TM6:F288, Q291 and TM7:F307c,A314c) or even invariant over the whole GPCR superfamily (TM3: C112, TM4:Q174, TM6:W284,F287 and TM7: N317). The invariant (conservative) residues within the NHR subfamily may be pertinent to ligand binding while the invariant over the whole GPCR superfamily may have to do with the signal transduction, putatively universal for the whole GPCR superfamily. Our results on agonist docking agree with those obtained by Mouillac *et al.* [19] for a related AVP/V1aR system. Moreover, some of the equivalent V1aR residues have already been found critical for the ligand affinity.

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REFERENCES

1. Schertler, G.F.X. & Hargrave, P.A. (1995) Projection structure of frog rhodopsin in two crystal forms. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 11578–11582.
2. Baldwin, J.M. (1993) The probable arrangement of the helices in G protein-coupled receptors. *EMBO J.* **12**, 1693–1703.
3. Herzyk, P. & Hubbard, R.E. (1995) Automated method for modeling seven-helix transmembrane receptors from experimental data. *Biophys. J.* **69**, 2419–2442.
4. Peitsch, M.C., Herzyk, P., Wells, T.N.C. & Hubbard, R. (1995) Automated G protein-coupled receptor modeling by Swiss-Model: URL http://expasy.hcuge.ch/swissmod/SWISS-MODEL.html.
5. Sugimoto, T., Saito, M., Mochizuki, S., Watanabe, Y., Hashimoto, S. & Kawashima, H. (1994) Molecular cloning and functional expression of a cDNA encoding the human V1b vasopressin receptor. *J. Biol. Chem.* **269**, 27088–27092.
6. De Keyzer, Y., Auzan, C., Lenne, F., Beldjord, C., Thibonnier, M., Bertagna, X. & Clauser, E. (1994) Cloning and characterisation of the human V3 pituitary vasopressin receptor. *FEBS Lett.* **356**, 215–220.
7. Iismaa, T.P., Biden, T.J. & Shine, J. (1995) *G Protein-Coupled Receptors*, Chapter 1, Springer-Verlag, Heidelberg.
8. Manning, M. & Sawyer, W.H. (1993) Design, synthesis and some uses of receptor-specific agonists and antagonists of vasopressin and oxytocin. *J. Recept. Res.* **13**, 195–214.
9. Yamamura, Y., Ogawa, H., Yamashita, H., Chihara, T., Miyamoto, H., Nakamura, S., Onogawa, T., Yamashita, T., Hosokawa, T., Mori, T., Tominaga, M. & Yabuuchi, Y. (1992) Characterization of a novel aquaretic agent, OPC-31260, as an orally effective, nonpeptide vasopressin V2 receptor antagonist. *Br. J. Pharmacol.* **105**, 787–791.
10. Czaplewski, C., Kaźmierkiewicz, R. & Ciarkowski, J. (1998) Molecular modeling of the human vasopressin V2 receptor/agonist complex. *J. Comp.-Aided Molec. Design* **12**, in press.
11. Barberis, C. & Tribollet, E. (1996) Vasopressin and oxytocin receptors in the central nervous system. *Crit. Rev. Neurobiol.* **10**, 119–154.
12. Baldwin, J.M. (1994) Structure and function of receptors coupled to G proteins. *Curr. Opin. Cell. Biol.* **6**, 180–190.
13. SYBYL, v. 6.1 (1994) Tripos Inc., St. Louis, MO, U.S.A.
14. AMBER, v. 4.1 (1995) Pearlman, D.A., Case, D.A., Caldwell, J.W., Ross, W.S., Cheatham III, T.E., Ferguson, D.M., Seibel, G.L., Singh, U.C., Weiner, P.K. & Kollman, P.A., University of California, San Francisco, CA, U.S.A.
15 Schmidt, M.W., Baldridge, K.K., Boatz, J.A., Elbert, S.T., Gordon, M.S., Jensen, J.H., Koseki, S., Matsunaga, N., Nguyen, K.A., Su, S., Windus, T.L., Dupuis, M. & Montgomery, J.A. (1993) General atomic and molekular electronic structure system. *J. Comput. Chem.* **14**, 1347–1363.
16. Bayly, C.I., Cieplak, P., Cornell, W.D. & Kollman, P. (1993) A well-behaved electrostatic potential based method using charge restraints for deriving atomic charges: The RESP model. *J. Phys. Chem.* **97**, 10269–10280.
17. Koradi, R., Billeter, M. & Wüthrich, K. (1996) MOLMOL: A program for display and analysis of macromolecular structures. *J. Mol. Graphics* **14**, 51–55.

18. Ogawa, H., Yamashita, H., Kondo, K., Yamamura, Y., Miyamoto, H., Kan, K., Kitano, K., Tanaka, M., Nakaya, K., Nakamura, S., Mori, T., Tominaga, M. & Yabuuchi, Y. (1996) Orally active, nonpeptide vasopressin V_2 receptor antagonists: A novel series of 1-[4-(benzoylamino)benzoyl]-2,3,4,5-tetrahydro-1*H*-benzazepines and related compounds. *J. Med. Chem.* **39**, 3547–3555.

19. Mouillac, B., Chini, B., Balestre, M.-N., Elands, J., Trumpp-Kallmeyer, S., Hoflack, J., Hibert, M., Jard,S. & Barberis, C. (1995) The binding site of neuropeptide vasopressin V1a receptor. *J. Biol. Chem.* **270**, 25771–25777.