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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

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Abstract The substitution, in the human V_2 vasopressin receptor, of the aspartate at position 136 by alanine leads to agonist-independent activation of this mutant V_2 receptor. Pharmacological studies of the D136A V_2 receptor helped us in characterizing different V_2 receptor antagonists. SR-121463A and OPC-31260, two non-peptide antagonists, behaved as inverse agonists, while two cyclic peptides 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 known to be V_2 antagonists, demonstrated clear partial agonist properties. The finding of a constitutively activated human V_2 receptor represents a useful tool in characterizing V_2 receptor antagonist ligands.

Key words: Constitutively active mutant V_2 receptor; V_2 antagonist

1. Introduction

In recent years, it has been shown that many G protein-coupled receptors (GPCRs) may exist in an active form in the absence of agonist [1,2]. This constitutive (or agonist-independent) activity of GPCRs is now well documented and several human diseases such as familial precocious puberty in boys, hyperthyroidism and Janssen type chondrodysplasia are associated with mutations inducing constitutive activation of the corresponding receptor – e.g. LH, TSH and PTH/PTH-related peptide [3–6]. A constitutive activity of others GPCRs has also been reported for the α_{1B}-adrenergic, α_2-adrenergic, the β_2-adrenergic, the muscarinic cholinergic receptors, as well as the AT_{1A} angiotensin II, dopamine D5, 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}), 5-HT_{1B}, 5-HT_{1D}, and 5-HT_{2C} receptors in cell lines in which they are overexpressed or mutated [7–17]. In cells in which a constitutive receptor activity can be measured, antagonists that reduce the levels of activity and functional response are referred to as inverse agonists or antagonists with negative intrinsic activity, whereas antagonists that do not reduce activity are called neutral antagonists or antagonists with no intrinsic activity [18].

The vasopressin V_2 receptor belongs to the large family of the GPCRs and is responsible for the antidiuretic effect of the neurohypophyseal hormone arginine vasopressin (AVP). The binding of AVP to the V_2 receptor, located on the basolateral membrane of the principal cells of the renal collecting duct, represents the first step of the antidiuretic action of this hormone, activating adenylyl cyclase and then increasing the intracellular concentration of cyclic adenosine monophosphate (cAMP) [19]. This preliminary step initiates a cascade of intracellular events that lead to the exocytic insertion of specific water channels, aquaporin 2, into the luminal membrane of the renal principal cells [20].

Several different mutations in the V_2 receptor gene have been reported to be responsible for the loss of function or dysregulation of the V_2 receptor in patients with X-linked congenital nephrogenic diabetes insipidus, a disease characterized by resistance of the renal collecting duct to the antidiuretic action of AVP [21], but to date no mutation responsible for a gain of function – i.e. constitutive activity – of the V_2 receptor has been described, either in vivo or in vitro.

The main goal of our study was to find out whether such a constitutively active mutant V_2 receptor may exist in vitro and, if so, to use this mutant as a tool for a better characterization of different V_2 receptor antagonists. Antagonists of the V_2 receptor, also called aquaretic agents, are of significant clinical interest in several diseases characterized by an abnormal increase of circulating AVP plasma levels leading to the activation of the V_2 receptors, responsible for water retention and subsequent hypotonic hyponatremia (syndrome of inappropriate antidiuretic hormone secretion, liver cirrhosis, nephrotic syndrome, certain stages of congestive heart failure or hypertension) [22–25].

Intact and broken cell preparations of COS-7 cells expressing a mutated human V_2 receptor were used as our experimental model. We demonstrate that a specific mutation of aspartate 136 in the human V_2 receptor (Fig. 1), which belongs to the highly conserved DRH/Y sequence, causes its constitutive activity. Evidence is also presented that certain V_2 receptor antagonists have a partial agonist activity on the D136A mutant V_2 receptor whereas others behave as inverse agonists.

2. Materials and methods

2.1. Drugs

AVP was from Bachem. The V_2 receptor cyclic peptide antagonist ligands 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 were synthesized in the laboratory of Dr. M. Manning (MCO, Toledo, OH). SR-121463A and OPC-31260 was from Sanofi Recherche. [^{3}H]AVP (60–80 Ci/mmol) was from NEN Life Science Products.

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2.2. DNA construct

The cloning of the human V_2 receptor cDNA was performed as previously described [26]. The cDNA was then subcloned into the *Eco*RI/*Xba*I restriction sites of the eukaryotic expression vector pCMV [27]. The sequence of the human V_2 vasopressin receptor cDNA was verified by direct dideoxynucleotide sequencing of both strands (T7 sequencing kit, Pharmacia Biotech).

The D136A mutation was introduced in the human V_2 vasopressin receptor cDNA sequence directly on the eukaryotic expression vector pCMV with the QuickChange site-directed mutagenesis kit (Stratagene) and its identity confirmed by direct dideoxynucleotide sequencing (T7 sequencing kit, Pharmacia). The supercoiled DNA used for transfection was prepared with Qiagen columns (Qiagen, Chatsworth, CA).

2.3. Cell culture and transient expression

Expression vectors harboring the wild type or mutant receptors were transiently transfected into COS-7 cells (American Type Culture Collection) by electroporation. COS-7 cells were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL) supplemented with 10% fetal calf serum (Eurobio, France), 100 IU/ml penicillin and streptomycin (Gibco BRL), in 5% CO_2 in air at 37°C. Electroporation (280 V, 950 mF, Bio-Rad gene pulser electroporator) was performed in a total volume of 300 μl, with 20 μg of carrier DNA (vector without insert). Various amounts of plasmid DNA (vector with a subcloned insert) ranging from 10 to 800 ng for the wild type V_2 receptor and from 25 ng to 2000 ng for the D136A mutant V_2 receptor were added, as well as 10^7 cells in electroporation buffer (50 mM K_2HPO_4, 20 mM CH_3COOK, 20 mM KOH, pH 7.4). After electroporation, cells were split into 24-well clusters for determination of cAMP accumulation or for binding assays to intact cells.

2.4. Binding assays

Binding assays were performed either on intact cells or on cell membranes.

2.4.1. Binding assays to intact cells. After electroporation, cells were plated in 24-well clusters at a density of 2×10^5 cells/well. Binding assays were performed after 48 h. Cells were washed three times in phosphate-buffered saline (PBS), after which each well received 0.2 ml of ice-cold PBS with 1% BSA and phenylalanine 10^{-3} M and the appropriate dilution of [^{3}H]AVP. Plates were incubated for 4 h in the cold room before removal of the binding mixture by aspiration. After quickly rinsing four times with ice-cold PBS, 0.5 ml of 0.1 N NaOH was added to each well to extract radioactivity. The fluid from the well was then transferred to scintillation vials after neutralization with 0.5 ml HCl 0.1 N. Non-specific binding was determined under the same condition in the presence of 0.5 mM unlabeled AVP. The number of cells in each well was determined after trypsinization to normalize the results as binding capacity per million cells (fmol/million cells). All assays were performed in triplicate on at least three separate batches of electroporated cells.

2.4.2. Binding assays on cell membranes. Binding experiments were performed on COS-7 cell membrane preparations as previously described [28]. For saturation experiments, [^{3}H]AVP was used as the radioligand; membrane protein (20–30 μg) was incubated for 30 min at 30°C with 1–2 nM [^{3}H]AVP, a concentration equalling the K_d value determined in the saturation experiments, and a concentration range of unlabeled ligands (10^{-11} to 10^{-5}) for competition experiments. In each set of experiments, non-specific binding was determined in the presence of a 250–1000-fold excess of unlabeled ligand. Bound and free radioactivity were separated by filtration over Whatman GF/C filters presoaked with 10 mg/ml of BSA. All assays were performed in triplicate on at least three separate batches of electroporated cells.

The ligand binding data were analyzed by non-linear least-squares regression using the computer program LIGAND [29].

2.5. cAMP assays

Transfected cells were plated in supplemented DMEM using 24-well clusters and [^{3}H]adenine (Isotopchim) was added to the culture medium at a final concentration of 2 mCi/ml for the last day of culture (36 h after cell electroporation). Cells were washed twice in PBS, then PBS supplemented with glucose 5.5 mM and isobutylmethylxanthine 1 mM was added for 15 min. AVP (at concentrations ranging from 10^{-12} to 10^{-5} M) or the V_2 receptor antagonists, SR-121463A [30], OPC-31260 [31], d(CH_2)$_5$[D-Tyr(Et)^2,Val^4,Tyr-NH_2^{9}]AVP [32] and d(CH_2)$_5$[D-Ile^2,Ile^4,Tyr-NH_2^{9}]AVP [33] were added to the medium. After a further 10 min incubation period, the reaction was stopped by replacing the incubation medium with 1 ml of 5% trichloroacetic acid. ATP and cAMP were added in the acid extract at a concentration of 0.5 mM. Relative intracellular cAMP levels were determined by measuring the formation of [^{3}H]cAMP from the prelabeled adenine nucleotide pool. Labeled cAMP was separated by sequential chromatography on Dowex and alumina columns as previously described [34]. The radioactivity present in the cAMP fractions was expressed as a percentage of the sum of the radioactivity recovered in the cAMP fraction and the radioactivity which was not retained by the Dowex column. The latter mainly correspond to labeled ATP. All assays were performed in triplicate on at least three separate batches of electroporated cells. The data were analyzed by means of non-linear regression using a sigmoidal dose-response equation (KaleidaGraph software).

3. Results

We first mutated aspartate 136 of the DRH motif, which is highly conserved in GPCRs, to an alanine mutant. Then the wild type and the aspartate to alanine mutant (D136A) of the human V_2 receptor (Fig. 1), which mimics the situation found in constitutively activated α_{1B}-adrenergic receptor [8], were transfected into monkey kidney COS-7 cells for binding and cAMP assays. Receptor expression levels were studied by using increasing concentrations of cDNA encoding the D136A mutant V_2 receptor or the wild type V_2 receptor during sequential electroporation procedures. COS-7 cells were transfected with 10–800 ng cDNA/10^7 cells for the wild type and with 50–2000 ng cDNA/10^7 cells for the D136A mutant V_2 receptor and the receptor density was measured by binding assay of [^{3}H]AVP to intact cells (Fig. 2). For each value of cDNA used for both receptors, binding and cAMP assays were performed on transfected cells obtained during the same electroporation procedure.

Analysis of the data obtained for the wild type V_2 receptor demonstrated that the receptor density was positively correlated with the amount of cDNA used for transfection (Fig. 2). For the D136A V_2 receptor, the expression remained low and significantly higher levels of expression could not be achieved even using larger amounts of transfected cDNA (Fig. 2).

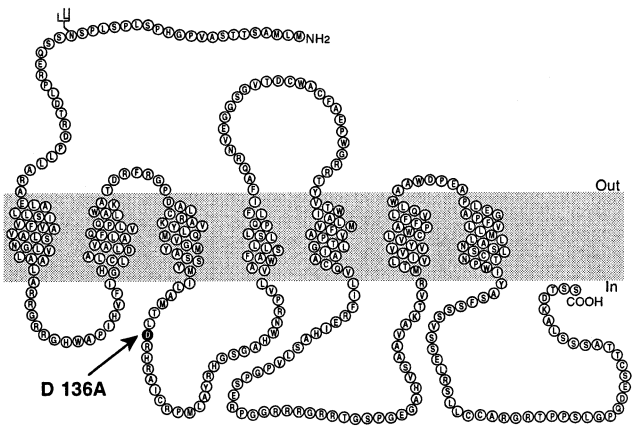

Fig. 1. Schematic representation of the human V_2 receptor showing the primary sequence and the possible transmembrane topology with the localization of the D136A mutation. The limits of the seven transmembrane domains correspond to the alignment of the seven putative transmembrane domains of vasopressin receptors published by Mouillac [21,28].

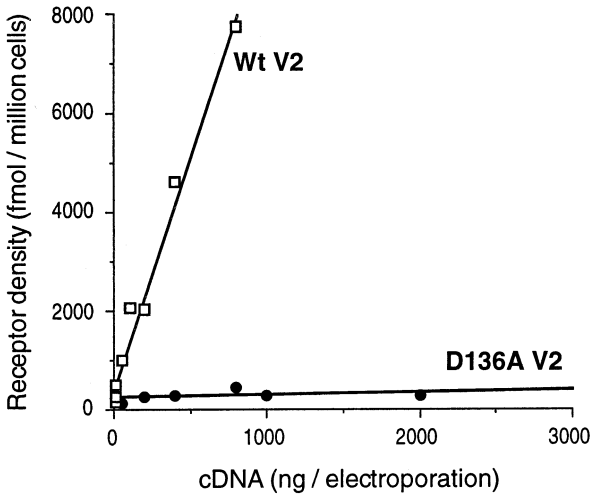

Fig. 2. Receptor density measured in COS-7 cells expressing the wild type (□) and D136A (●) V_2 receptors transfected with increasing amounts of cDNA which ranged from 10 to 800 ng/10^7 cells for the wild type and from 50 to 2000 ng/10^7 cells for the D136A V_2 receptors. The receptor density was measured during binding assays to intact cells, using [^{3}H]AVP. The results are from at least three independent experiments, each performed in triplicate.

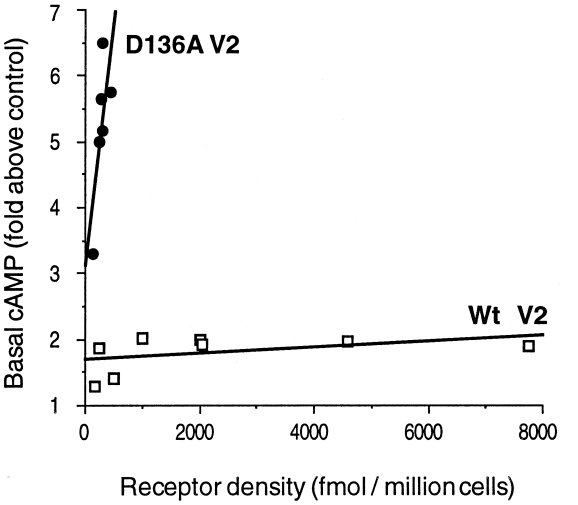

Fig. 3. The effect of expression level on basal level of cAMP production in COS-7 cells expressing the wild type (□) and D136A (●) V_2 receptors. COS-7 cells were transfected with increasing amounts of cDNA which ranged from 10 to 800 ng/10^7 cells for the wild type and from 50 to 2000 ng/10^7 cells for D136A. The results are from at least three independent experiments, each performed in triplicate.

Comparable levels of receptor expression were obtained with 15 ng cDNA/10^7 cells for the wild type and 2000 ng cDNA/10^7 cells for the D136A mutant receptor. In these conditions, the D136A and the wild type V_2 receptors bound AVP with an equivalent K_d (0.4 ± 0.27 nM and 0.57 ± 0.24 nM for the mutant and the wild type V_2 receptor, respectively) (Table 1). However, at this same level of expression, the basal cAMP production was found to be significantly increased (5–6-fold higher) for the D136A V_2 receptor when compared with the wild type V_2 receptor demonstrating that this mutant receptor is constitutively active (Fig. 3). For the wild type receptor, the basal cAMP production was not dependent on the receptor density and remained stable in spite of a significant increase in the receptor expression, up to 7500 fmol/10^6 cells. In contrast, within the range of cDNA used, the constitutive activity of the mutant receptor was always linearly dependent on receptor density and did not show any trend for saturation (Fig. 3).

Fig. 4 shows that the D136A V_2 receptor retained its ability to respond to the agonist and the EC_{50} value for adenylyl cyclase stimulation by AVP for the COS-7 cells expressing both receptors was not significantly modified (0.5 ± 0.15 nM and 0.7 ± 0.1 nM for the mutant and the wild type V_2 receptor, respectively). However, the cAMP production induced by 10^{-6} M AVP added on COS-7 cells expressing the same amount of receptors was found to be 3-fold higher for the D136A mutant than for the wild type V_2 receptor (Fig. 4).

To better characterize the properties of different V_2 receptor antagonists, we used the constitutively activated mutant V_2 receptor to study the effects of this class of compounds on the cAMP contents of intact COS-7 cells expressing the wild type or the D136A V_2 receptor. Two cyclic 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 and two non-peptide antagonists SR-121463A and OPC-31260 were used in these assays. The first two display a high [32] and a low [26] affinity for the human V_2 receptor (K_i: 1.12 ± 0.1 nM and 128 ± 6 nM, respectively), while the other two have a high and mild affinity for the human V_2 receptor (K_i: 1.20 ± 0.11 nM and 36.3 ± 0.4 nM, respectively) [30,31].

Table 1
Pharmacological properties of the wild type and D136A constitutively active human V_2 receptors: binding to AVP, cyclic peptide antagonists and non-peptide antagonists

		K_d (nM)	
		Wild type	D136A
A:	[^{3}H]AVP	0.57 ± 0.24	0.40 ± 0.27
		K_i (nM)	
		Wild type	D136A
B:	d(CH$_2$)$_5$[D-Ile^2,Ile^4,Tyr-NH$_2$^{9}]AVP	128 ± 6	157 ± 79
	d(CH$_2$)$_5$[D-Tyr(Et)^2,Val^4,Tyr-NH$_2$^{9}]AVP	1.12 ± 0.1	1.72 ± 0.47
	SR-121463A	1.2 ± 0.11	0.79 ± 0.18
	OPC-31260	36.3 ± 0.4	50.8 ± 10

A: The K_d values for [^{3}H]AVP were directly determined in saturation experiments. B: The K_i values for the unlabeled ligands were obtained in competition binding assays by displacement of [^{3}H]AVP (1–2 nM).
The binding assays were conducted on membrane preparations of COS-7 cells expressing the receptors. All values are the mean ± S.E.M. of three different experiments each performed in triplicate.

The affinities of these different antagonists, measured by competition experiments for both receptors, are summarized in Table 1. It demonstrates that the K_i values of these compounds for either the wild type or the D136A V_2 receptor are not statistically different. The effects of these four compounds on the cAMP production by COS-7 cells expressing the wild type and the D136A mutant V_2 receptors are given in Fig. 5A,B. It shows that the two cyclic 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 behaved as partial agonists for the D136A V_2 receptor with a maximal activity of nearly 30% of the response induced by 10 μM AVP. These partial agonist properties were also found for the wild type V_2 receptor but with a much lower potency, the maximum cAMP production being less than 5% of the response induced by 10 μM AVP.

Conversely, the non-peptide antagonist SR-121463A behaved as an inverse agonist. Indeed, increasing doses of this compound progressively suppressed the basal cAMP production of the constitutively active mutant receptor. OPC-31260, the non-peptide antagonist, also decreased the basal cAMP production of the mutant, but was found to be less potent and efficient than SR-121463. This may be related to the difference observed in the affinity of the two compounds for this mutant V_2 receptor.

Thus, the data shown in Fig. 5 suggest that antagonists with partial agonist activity and antagonists with inverse agonist activity were distinguished by analysis of their action on cAMP production by cells expressing the D136A V_2 receptor.

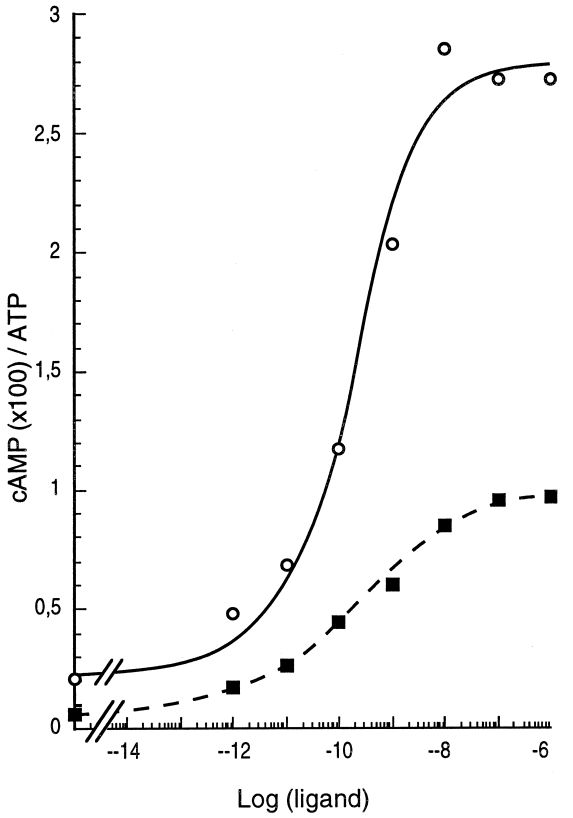

Fig. 4. Effects of increasing doses (10^{-12} to 10^{-6} M) of AVP on cAMP production in COS-7 cells expressing the wild type (■) and D136A (○) human V_2 receptors. COS-7 cells were transfected with 15 ng cDNA/10^7 cells for the wild type and 2000 ng/10^7 cells for the constitutively active D136A V_2 receptor. The receptor densities measured on intact cells were 251 ± 47 and 298 ± 7 fmol/10^6 cells for the wild type and D136A V_2 receptors respectively. The results are the means from at least three independent experiments, each performed in triplicate.

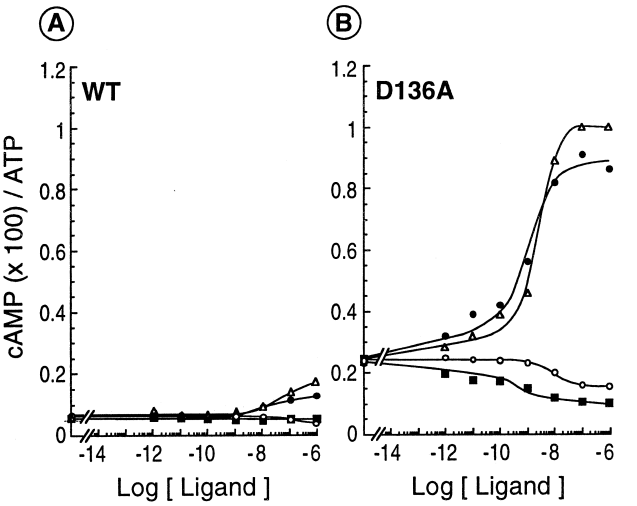

Fig. 5. cAMP production in COS-7 cells expressing the wild type (A) and the D136A (B) human V_2 receptor. Effects of increasing doses (10^{-12} to 10^{-6} M) on cAMP production of the non-peptide antagonist compounds SR-121463A (■) [30] and OPC-31260 (○) [31], and of the cyclic peptide antagonist ligands d(CH$_2$)$_5$[D-Tyr-(Et)^2,Val^4,Tyr-NH$_2$^{9}]AVP [32] (●) and d(CH$_2$)$_5$[D-Ile^2,Ile^4,Tyr-NH$_2$^{9}]AVP (△) [33]. The COS-7 cells were transfected with 15 ng cDNA/10^7 cells for the wild type and 2000 ng/10^7 cells for the constitutively active D136A V_2 receptor to obtain similar receptor densities. Each curve on graphs A and B is representative of at least three independent experiments, each performed in triplicate.

4. Discussion

The two major findings of this study were that replacement of aspartate 136 by alanine in the human V_2 receptor results in constitutive activation of the receptor with respect to cAMP production, and that two classes of V_2 receptor antagonists could be distinguished by their effects on cAMP content of intact COS-7 cells expressing a constitutively activated human mutant V_2 receptor. The functional and pharmacological characterization of the D136A V_2 receptor demonstrated that, for the first time, a mutant V_2 receptor should be included in the family of the constitutively active GPCRs mutant receptors. These findings are in good agreement with those shown previously for a similar mutation in the α_{1B}-adrenergic receptor [8] which is coupled to Gq-mediated stimulation of phospholipase C. In this receptor, D142 was mutated into different amino acids and the D142A α_{1B}-adrenergic receptor exhibited a significant constitutive activity.

We showed that the cAMP basal production was increased 5-fold when compared with the wild type V_2 receptor, in similar conditions of receptor expression. Analysis of the cAMP production after stimulation by 10^{-6} M AVP suggested that the D136A V_2 receptor behaves also as a superactive receptor, demonstrating a 3-fold increase in cAMP production, when compared with the wild type (Fig. 4). The constitutive activity of the D136A V_2 receptor was clearly receptor-mediated as indicated by its dependence on the expression levels of the receptor and its susceptibility to inhibition by the V_2 receptor antagonists SR-121463A and OPC-31260. In contrast to what was observed previously for different constitutively active mu-

tant adrenergic receptors [35–38], the affinity of the D136A V_2 receptor for AVP binding was not significantly improved when compared to the wild type V_2 receptor.

The D136A V_2 receptor, like other receptors mutated at DRH/Y [8], displays a low expression level when expressed in COS-7 cells. This lowered receptor density could be explained by the receptor down-regulation as a consequence of constitutive activation. It may also be related to a structural instability of the constitutively activated receptor, which appears more susceptible to denaturation [39]. By Western blot analysis, we showed that the migration patterns for the wild type and the D136A V_2 receptor were similar suggesting that the mature form of both receptors can be expressed at the cell surface (data not shown). Of course, one cannot exclude that a large portion of the mutated V2 receptor is retained in the endoplasmic reticulum or in the Golgi complex, or is not fully processed.

The mutated aspartate at position 136 of the human V_2 receptor belongs to the DRH/Y sequence highly conserved throughout the whole GPCR family. It has been proposed by Scheer et al., from data concerning the constitutively active D142A α_{1B}-adrenergic receptor, that the prototropic equilibrium between the deprotonated (anionic) and protonated (neutral) forms of this aspartate residue of the DRY motif seems to play a key role in the molecular events leading to its agonist-independent activation [8]. In our work, we showed that the replacement of the corresponding residue in the V_2 receptor, i.e. the aspartate in position 136, with the non-polar amino acid alanine also confers a constitutive activity to the V_2 receptor. Therefore, our data show that, if the molecular events accompanying the structural modification in constitutively active GPCRs remain largely unknown, some conclusions drawn from different GPCRs are, at least partly, reproducible from one type to another. From our findings, as we failed to find any significant changes in the binding affinity of the D136A V_2 receptor for the agonist, it is also suggested that for the V_2 receptor, the conformational link between receptor domains involved in ligand binding and the DRH sequence is different than for other GPCRs, i.e. an activating mutation at the DRH site does not have an effect on the ligand binding domain.

The observation that a number of GPCRs can be spontaneously active has led to a simple model in which receptors exist in an equilibrium between inactive, R, and active, R*, conformation states, knowing that in the absence of agonist and in basal conditions, the equilibrium between the two states markedly favors R over R*. The results of our experiments can also be interpreted with this model. The pharmacological study of the different compounds used demonstrated clearly that SR-121463A, which is known to be a human V_2 receptor antagonist, behaved as an inverse agonist. OPC-31260, another human V_2 receptor antagonist, also demonstrated inverse agonist properties. The discrepancies observed between these non-peptide antagonists and the two cyclic peptide antagonists studied demonstrated that the molecular interactions between these two classes of antagonists compounds and the V_2 vasopressin receptor are necessarily different. If the inverse agonists are thought to bind the inactive form of the receptor preferentially and then shift the equilibrium toward R suppressing the constitutive activity, the two peptide ligands, 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, which have a cyclic structure close to AVP and behave as partial agonists on the D136A mutant V_2 receptor, seem to bind preferentially the active form of the receptor, R*, as agonist ligands do.

It is worth noting at this point that the antagonist desGly-d(CH_2)$_5$[D-Tyr(Et)^2,Val^4]AVP [23], which acts as an aquaretic in the rat, was found to have agonist (antidiuretic) properties when tested in man [25]. The D136A V_2 receptor clearly shows in vitro this property that was otherwise difficult to observe and appears to be a useful tool to characterize the different human V_2 receptor antagonists which are thought to be promising therapeutic agents in several pathological conditions.

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References

[1] Costa, T., Ogino, Y., Munson, P.J., Onaran, H.O. and Rodbard, D. (1992) Mol. Pharmacol. 41, 549–560.
[2] Lefkowitz, R.J., Cotecchia, S., Samama, P. and Costa, T. (1993) Trends Pharmacol. Sci. 14, 303–307.
[3] Latronico, A.C., Anasti, J., Arnhold, I.J.P., Mendonca, B.B., Domenice, S., Alabno, M.C., Zachman, K., Wajchenberg, B.L. and Tsigos, C. (1995) J. Clin. Endocrinol. Metab. 80, 2490–2494.
[4] Parma, J., Duprez, L., Van Sande, J., Cochaux, P., Gervy, C., Mockel, J., Dumont, J. and Vassart, G. (1993) Nature 365, 649–651.
[5] Wonerow, P., Schöneberg, T., Schültz, G., Gudermann, T. and Paschke, R. (1998) J. Biol. Chem. 273, 7900–7905.
[6] Schipani, E., Jensen, G.S., Pincus, J., Nissenson, R.A., Gardella, T.J. and Jüppner, H. (1997) Mol. Endocrinol. 11, 851–858.
[7] Cotecchia, S., Exum, S., Caron, M.G. and Lefkowitz, R.J. (1990) Proc. Natl. Acad. Sci. USA 87, 2896–2900.
[8] Scheer, A., Fanelli, F., Costa, T., De Bennedetti, P.G. and Cotecchia, S. (1996) EMBO J. 15, 3566–3578.
[9] Hwa, J., Gaivin, R., Porter, J.E. and Perez, D.M. (1997) Biochemistry 36, 633–639.
[10] Samama, P., Pei, G., Costa, T., Cotecchia, S. and Lefkowitz, R.J. (1994) Mol. Pharmacol. 45, 390–394.
[11] Chidiac, P., Hebert, T.E., Valiquette, M., Dennis, M. and Bouvier, M. (1994) Mol. Pharmacol. 45, 490–499.
[12] Burstein, E.S., Spalding, T.A. and Brann, M.R. (1997) Mol. Pharmacol. 51, 312–319.
[13] Groblewski, T., Maigret, B., Larguier, R., Lombard, C., Bonnafous, J.C. and Marie, J. (1997) J. Biol. Chem. 272, 1822–1826.
[14] Kozell, L.B. and Neve, K.A. (1997) Mol. Pharmacol. 52, 1137–1149.
[15] Barr, A.J. and Manning, D.R. (1997) J. Biol. Chem. 272, 32979–32987.
[16] Thomas, D.R., Faruq, S.A., Balcarek, J.M. and Brown, A.M. (1995) J. Receptor Signal. Transduct. Res. 15, 199–211.
[17] Barker, E.L., Westphal, R.S., Schmidt, D. and Sanders-Bush, E. (1994) J. Biol. Chem. 269, 11687–11690.
[18] Costa, T. and Herz, A. (1989) Proc. Natl. Acad. Sci. USA 86, 7321–7325.
[19] Jard, S. (1983) in: Current Topics in Membranes and Transport (Kleinzeller, Ed.), Vol. 18, pp. 255–285, Academic Press, New York.
[20] Knepper, M.A. and Rector, F.C. (1996) in: The Kidney, (Brenner, B.M., Ed.), 5th edn., pp. 532–570, W.B. Saunders, Philadelphia, PA.
[21] Ala, Y., Morin, D., Mouillac, B., Sabatier, N., Vargas, R., Cotte,

N., Déchaux, M., Antignac, C., Arthus, M.F., Lonergan, M., Turner, M.S., Balestre, M.N., Alonso, G., Hibert, M., Barberis, C., Hendy, G.H., Bichet, D.G. and Jard, S. (1998) J. Am. Soc. Nephrol. 9, 1861–1972.
[22] Mah, S.C. and Hofbauer, K.G. (1987) Drugs Future 12, 1055–1070.
[23] Manning, M. and Sawyer, W.H. (1989) J. Lab. Clin. Med. 114, 617–632.
[24] Sorensen, J.B., Andersen, M.K. and Hansen, H.H. (1995) J. Intern. Med. 238, 97–100.
[25] Kinter, L.B., Caltabiano, S. and Huffman, W.F. (1993) Biochem. Pharmacol. 45, 1731–1737.
[26] Cotte, N., Balestre, M.N., Phalipou, S., Hibert, M., Manning, M., Barberis, C. and Mouillac, B. (1998) J. Biol. Chem 273, 29462–29468.
[27] Schall, T., Lewis, M., Koller, K.J., Lee, A., Rice, G., Wong, G.H.W., Gatanaga, T., Granger, G.A., Lentz, R., Raab, H., Kohr, W.J. and Goedel, D.V. (1990) Cell 61, 361–370.
[28] Mouillac, B., Chini, B., Balestre, M.N., Elands, J., Trumpp-Kallmeyer, S., Hoflack, J., Hibert, M., Jard, S. and Barberis, C. (1995) J. Biol. Chem. 270, 25771–25777.
[29] Munson, P.J. and Rodbard, D. (1980) Anal. Biochem. 107, 220–239.
[30] Serradeil-Le Gal, C., Lacour, C., Valette, G., Garcia, G., Foulon, L., Galindo, G., Bankir, L., Pouzet, B., Guillon, G., Barberis, C., Chicot, D., Jard, S., Vilain, P., Garcia, C., Marty, E., Raufaste, D., Brossard, G., Nisato, D., Maffrand, J.P. and Le Fur, G. (1996) J. Clin. Invest. 98, 2729–2738.
[31] Yamamura, Y., Ogawa, H., Yamashita, H., Chihara, T., Miyamoto, H., Nakamura, S., Onogawa, T., Yamashita, T., Hosokawa, T., Mori, T., Tominaga, M. and Yabuuchi, Y. (1992) Br. J. Pharmacol. 105, 787–791.
[32] Ala, Y., Morin, D., Mahé, E., Cotte, N., Mouillac, B., Jard, S., Barneris, C., Tribollet, E., Dreifuss, J.J., Sawyer, W.H., Wo, N.C., Kolodziejczyk, A.S., Cheng, L.L. and Manning, M. (1997) Eur. J. Pharmacol. 331, 285–293.
[33] Sawyer, W.H., Bankowski, K., Misicka, A., Nawrocka, E., Kruszynski, M., Stoev, S., Klis, W.A., Przybylski, J.P. and Manning, M. (1988) Peptides 9, 157–163.
[34] Salomon, Y., Londos, C. and Rodbell, M. (1974) Anal. Biochem. 58, 541–548.
[35] Kjelsberg, M.A., Cotecchia, S., Ostrowski, J., Caron, M.G. and Lefkowitz, R.J. (1992) J. Biol. Chem. 267, 1430–1433.
[36] Ren, Q., Kurose, H., Lefkowitz, R.J. and Cotecchia, S. (1993) J. Biol. Chem. 268, 16483–16487.
[37] Samama, P., Cotecchia, S., Costa, T. and Lefkowitz, R.J. (1993) J. Biol. Chem. 268, 4625–4636.
[38] Scheer, A., Fanelli, F., Costa, T., De Benedetti, P.G. and Cotecchia, S. (1997) Proc. Natl. Acad. Sci. USA 94, 808–813.
[39] Gether, U., Ballesteros, J.A., Seifert, R., Sanders-Bush, E., Weinstein, H. and Kobilka, B.K. (1997) J. Biol. Chem. 272, 2587–2590.