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Functional rescue of vasopressin V2 receptor mutants in MDCK cells by pharmacochaperones: relevance to therapy of nephrogenic diabetes insipidus

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Robben JH, Sze M, Knoers NV, Deen PM. Functional rescue of vasopressin V2 receptor mutants in MDCK cells by pharmacochaperones: relevance to therapy of nephrogenic diabetes insipidus. Am J Physiol Renal Physiol 292: F253–F260, 2007. First published August 22, 2006; doi:10.1152/ajprenal.00247.2006.—Intracellular retention of a functional vasopressin V2 receptor (V2R) is a major cause of congenital nephrogenic diabetes insipidus (NDI) and rescue of V2R mutants by nonpeptide antagonists may restore their basolateral membrane (BM) localization and function. However, the criteria for efficient functional rescue of G protein-coupled receptor (GPCR) mutants at clinically feasible antagonist concentrations are unknown. We found that the four nonpeptide antagonists SR49059, OPC31260, OPC41061, and SR121463B induced maturation and rescued the BM expression of eight of nine different V2R mutants, stably expressed in physiologically relevant polarized cells. The extent of maturation and rescued BM expression correlated with the antagonists’ concentration and affinity for the V2R. Displacement of the antagonists by AVP and subsequent cAMP generation inversely correlated with the antagonists’ affinities for the V2R but is partially influenced by antagonist-specific aspects. Despite limited increases in maturation and cell-surface expression of V2R mutants, the low-affinity SR49059 optimally induced functional rescue at high concentrations, due to its easy displacement by vasopressin. At clinically feasible antagonist concentrations, however, only the high-affinity antagonists OPC31260 and OPC41061 induced functional rescue, as at these concentrations the extent of BM expression became limited. In conclusion, functional rescue of mutant V2Rs at clinically feasible concentrations is most effective with high-affinity antagonists. As OPC31260 and OPC41061 are clinically safe, they are promising candidates to relieve NDI. Moreover, as numerous other diseases are caused by endoplasmic reticulum-retained GPCRs for which cell-permeable antagonists become available, our finding that high-affinity antagonists are superior is anticipated to be important for pharmacotherapy development of these diseases.

Madin-Darby canine kidney cells; misfolding; antagonist; water transport

The synthesis, maturation, and routing of plasma membrane proteins are extremely complex processes that require specific interactions between many different intracellular components. It is not surprising, therefore, that flaws in these processes are responsible for many diseases, which are often caused by mutations in genes encoding membrane proteins. In the last two decades, numerous mutations have been identified in the coding sequences of such genes, of which ~50% are missense mutations involving only one or a few nucleotides. For example, in cystic fibrosis (CF), a severe disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, >300 unique missense mutations have been described (http://www.genet.sickkids.on.ca/cftr/). Cell expression studies revealed that most of these mutations lead to fully synthesized proteins that fail to pass the quality control mechanism of the endoplasmic reticulum (ER) as the protein is misfolded (10). Based on this cellular fate, these gene defects are so-called class II mutations, giving rise to “conformational diseases” (8). Usually, ER retention of such proteins is followed by their degradation by proteasomes (22).

As numerous studies revealed that ER-retained mutant proteins are often functional, research of the last decade has focused on the identification of compounds that can rescue the cell surface expression of such proteins. In this respect, the vasopressin type 2 receptor (V2R) is the prototypical protein, as it was the first receptor for which the exciting discovery was made that cell-permeable antagonists (CPAn, known as “pharmacological chaperones,” “pharmacochaperones,” or “pharma-coperones”) can promote cell surface trafficking of its ER-retained mutants (18).

V2R mutations cause the X-linked form of nephrogenic diabetes insipidus (NDI), a disorder in which patients are unable to concentrate their urine in response to the antidiuretic hormone arginine-vasopressin (AVP) (13). Morello and co-workers (18) showed that pretreatment with the high-affinity cell-permeable V2R antagonist SR121463A rescued the cell surface expression of 8 of 15 ER-retained V2R mutants (rescued cell surface expression), which could subsequently be activated by AVP (i.e., functional rescue). Since then, the concept by which CPAns rescue V2R mutants has been the subject of several studies (4, 31, 34). As indicated above, a crucial aspect necessary for functional rescue, besides rescued cell surface expression of the mutant, is displacement of the V2R-bound antagonist by AVP to generate a cAMP response. Likely based on this requirement, a V1 receptor CPAn, SR49059, was recently tested for its ability to increase the urine concentrating abilities in NDI patients (5). For three patients encoding the partially ER-retained V2R-R137H mutant, a significant urine volume reduction was obtained, thereby providing the proof of principle of the disease-curing effect of pharmacological chaperones in vivo. In patients encoding the fully ER-retained mutants V2R-W164S and V2R-D62–64, however, SR49059 was less effective.

To be of clinical value, functional rescue of V2R mutants should occur at low concentrations of antagonists and AVP and should last as long as possible. At present, however, it is unclear which features of CPAn are important to give the best...
functional rescue of V2R mutants under such conditions. Moreover, as the V2R is expressed in the basolateral membrane (BM) of renal principal cells, and proteins can traffic or function differently in nonpolarized vs. polarized cells (17), such studies are best performed in polarized renal epithelial cells.

Recently, we generated Madin-Darby canine kidney (MDCK) cells stably expressing V2R tagged with a green fluorescent protein (GFP) (23). In these cells, V2R-GFP was localized and regulated as can be anticipated to occur for V2R in vivo. Moreover, we found that several V2R mutants in NDI stably expressed in MDCK cells are ER retained (24). To determine which CPAn is likely the optimal pharmacological chaperone to relieve NDI in patients, we thoroughly tested a V1 receptor antagonist, a medium-affinity V2R antagonist, and two high-affinity V2R antagonists for their ability to rescue the cell surface expression and activity of several V2R mutants.

MATERIALS AND METHODS

Pharmacological chaperones. The V2R antagonist SR121463B (28) and the V1R antagonist SR49059 (29) were kindly supplied by C. Serradeil-Le Gal (Sanofi Synthélabo, Toulouse, France). The V2R antagonists OPC31260 and OPC41061 (14, 35) were kindly provided by Koji Komuro (Otsuka Pharmaceutical, Tokushima, Japan). All compounds were dissolved in dimethylsulfoxide as 0.01 M stock solutions and diluted in culture medium as indicated.

Expression constructs, cell culture, and transfection. Expression constructs encoding the wild-type V2R or the NDI-causing mutants -L44P, -I130F, -S167T, or -S167L fused at their COOH terminus to enhanced GFP were as described elsewhere (24). MDCK type II cells, which lack endogenous V2R expression, were kindly provided by Dr. Alexander Oksche (FMP, Berlin, Germany). MDCK type II cells were maintained in DMEM (Biowittaker, Verviers, Belgium) supplemented with 5% fetal bovine serum (PAA Laboratories, Karlsruhe, Germany), gentamicin, L-glutamine, sodium carbonate, and 1% nonessential amino acids. Calcium phosphate transfection and isolation of clones were done as described for MDCK type I cells (9).

Immunoblotting and immunocytochemistry. Polyacrylamide gel electrophoresis, Western blotting, and immunodetection were performed as described elsewhere (9, 23). For detection of V2R-GFP, 1:5,000 diluted rabbit anti-GFP serum was used (kindly provided by Student's t-test. Averaged data of more than three independent experiments are shown, and error bars represent 5D. Statistical analysis was performed using Microsoft Excel software.

RESULTS

Maturation of V2R mutants on antagonist treatment. During folding in the ER, V2R is expressed in its high-mannose glycosylated form. As it traverses the Golgi compartment on its way to the BM, it matures to complex- and O-glycosylated proteins. We have previously shown that missense V2R-GFP proteins in NDI that are trapped in the ER (class II) do not undergo maturation and are therefore visible as immature proteins of 60–63 kDa when expressed in MDCK I cells (24). Type I cells, however, endogenously express low levels of V2R and are thus not suitable for functional testing of mutant receptors. As MDCK II cells lack V2Rs (20), we stably expressed wild-type (wt) V2R, the functional mutants V2R-L44P, -I130F, and -S167T, and the nonfunctional V2R-S167L in these cells. As found for MDCK I cells, wt-V2R was mainly
expressed in the mature 75-kDa form, while the missense mutants were present as immature proteins of 60–63 kDa (Fig. 1, untreated samples). In addition, wt-V2R was mainly expressed in the BM, whereas the mutants were trapped in the ER (Fig. 2, untreated cells).

As maturation can serve as a read-out for translocation of mutant receptors to the BM, the cell-permeable antagonists were tested for their ability to induce receptor maturation. As shown in Fig. 1, overnight treatment with 1 μM V1R antagonist SR4 (9059) or the V2R antagonists OPC3 (1260), OPC4 (1061), and SR1 (21463) did not significantly (P > 0.05) increase the 75-kDa signal of wt-V2R. Treatment with OPC4 and SR1, however, caused a decrease or complete disappearance of the 60-kDa signals, respectively, suggesting that these compounds somewhat stabilize and increase receptor maturation of wt-V2R. Treatment of MDCK cells expressing V2R-L44P, -I130F, or -S167T (Fig. 1, middle 3 panels) with the four compounds resulted in increased maturation of all receptor mutants. However, the extent of maturation differed, as less matured V2R proteins were observed with SR4 compared with the other compounds. Moreover, especially for SR1, increased receptor maturation was accompanied by a decrease in the 60-kDa signal. Maturation of V2R-S167L, the nonfunctional mutant, was not increased by treatment with any of the compounds tested, although its expression was somewhat increased with SR1 (Fig. 1, bottom). In MDCK type I cells, similar effects on maturation for the four compounds were observed for V2R-L44P, -Δ62–64, -R113W, -I130F, -G201D, -T204N (not shown), and V2R-S167T and -V206D (26).

Rescue of V2R mutant plasma membrane expression on antagonist treatment. To determine whether increased receptor maturation coincided with increased BM localization, the cells were also subjected to CLSM analysis. As reported for MDCK type I cells (23), wt-V2R was predominantly present in the BM of untreated MDCK II cells. Its localization was not affected by treatment with any of the compounds (Fig. 2, top row). Without treatment, V2R-L44P, -I130F, -S167T, and -S167L were retained in the ER (Fig. 2 for V2R-L44P, -S167L), where they colocalized with the ER marker protein disulfide isomerase.

Treatment for 16 h with 1 μM SR4 did not visibly change the localization of V2R-L44P (Fig. 2, middle row). Treatment with OPC3, OPC4, or SR1, however, resulted in a clear translocation of V2R-L44P to the BM (Fig. 2, middle row), after which the localization was similar to that of wt-V2R. V2R-I130F and -S167T proteins responded similarly to the antagonist treatments as -L44P (not shown). V2R-S167L, however, did not translocate to the BM on antagonist treatment but remained trapped in the ER (Fig. 2, bottom row). The lack of a visible translocation of the V2R mutants by SR4, whereas maturation was clearly observed (Fig. 1), indicates that CLSM is less sensitive than immunoblotting.

Functional rescue of V2R mutants on antagonist treatment. Following rescue to the plasma membrane, the antagonists need to be displaced by an agonist to have functional rescue. To study the rate of displacement, we used radioactively labeled AVP, as this most closely resembles the natural ligand of the V2R. When untreated, the amount of [3H]AVP bound by mock-transfected cells was low compared with wt-V2R-expressing cells (Fig. 3A). Also, [3H]AVP binding to untreated MDCK II cells expressing V2R-L44P, -I130F, -S167T, or -S167L was not significantly (P > 0.05) different from binding to mock-transfected cells. To further exclude the presence of endogenous V2R in these cells, or the presence of low levels of V2R mutants in the plasma membrane, we determined whether DDAVP induces cAMP generation in these cell lines. However, treatment of mock-transfected MDCK II cells with 100 nM DDAVP did not result in a cAMP response, whereas cells stably expressing wt-V2R showed an ~10-fold increase in intracellular cAMP levels compared with untreated cells. Also, the cell lines expressing the mutants V2R-L44P, -I130F, -S167T, and -S167L did not respond to DDAVP treatment. In addition, basal cAMP levels were not significantly different between all cell lines and clones tested. Together, these data...
reveal that without rescued cell surface expression of V2R mutants, these cells lack the ability to bind AVP or generate cAMP in response to DDAVP.

This was different with rescued cell surface expression. Pretreatment of MDCK-V2R cells with 1 μM SR4 did not interfere with binding of AVP at all, as a similar amount of AVP was bound as found for nonpretreated control MDCK-V2R cells (Fig. 4A). In contrast, both OPC3 and OPC4 treatment reduced the amount of available binding sites for the wild-type receptor to ~30% of the nonpretreated control MDCK-V2R cells, indicating that both compounds are displaced by AVP to some extent. Finally, pretreatment with SR1 decreased the amount of available wt-V2R binding sites by 95% compared with control cells, indicating that this compound is hardly displaced with 100 nM [3H]AVP.

Subsequently, AVP binding was tested on the V2R mutants treated with the antagonists. Although we observed no BM localization, but some maturation, for V2R-L44P, -I130F, or -S167T on treatment with SR4, this compound increased the number of AVP binding sites 4.2-fold (Fig. 4B). OPC3 and

Fig. 3. Effect of DDAVP on MDCK cells expressing wt-V2R or mutants in NDI. A: MDCK II-wt-V2R, V2R-L44P, -I130F, -S167T, or -S167L (indicated) were seeded on filters, grown to confluence, washed with ice-cold PBS containing 1 mM MgCl2 and 0.1 mM CaCl2 (PBS-CM), and subsequently incubated for 1 h with 100 nM [3H]AVP for 1 h at 4°C to allow radioligand binding to cell-surface receptors. Subsequently, the filters with the cells were washed 3 times with ice-cold PBS-CM, excised, and counted in a scintillation counter. B: MDCK II-wt-V2R, V2R-L44P, -I130F, -S167T, or -S167L (indicated) were seeded on filters, grown to confluence, and subsequently treated with IBMX alone, or in combination with 100 nM DDAVP on the basolateral side. Subsequently, cells were lysed and cAMP accumulation was measured using a fluorescent cAMP assay kit. Triplicate samples were measured, and experiments were performed at least in triplicate.*Significantly different from untreated samples (P < 0.01).

Fig. 4. Functional rescue of V2R mutants. MDCK II-wt-V2R (A) or V2R-L44P, -I130F, -S167T, or -S167L (indicated; B) cells were seeded on filters, grown to confluence followed by 16 h treatment with 1 μM of the indicated pharmacological chaperones or left untreated. Next, cells were washed with PBS-CM followed by labeling with 100 nM [3H]AVP for 1 h at 4°C. Filters were washed 3 times with PBS-CM, excised, and counted in a scintillation counter. Values for untreated samples were set to 1. C: MDCK II-wt-V2R, MDCK-V2R-L44P, -I130F, -S167T, or -S167L cells (indicated) were seeded, grown, and treated as described above. Cells were subsequently washed in PBS-CM and challenged for 10 min with 100 nM DDAVP in the presence of IBMX. cAMP accumulation was measured using a fluorescent cAMP assay kit. All experiments were performed at least in triplicate.
OPC4 treatment, which clearly increased V2R mutant BM localization and maturation, increased the number of binding sites for these three mutants two- to threefold. In contrast, despite the clear BM localization and maturation of V2R-L44P, -I130F, or -S167T on treatment with SR1, incubation with this drug did not lead to a significant increase in binding sites for these mutants ($P > 0.05$, $n = 3$; Fig. 4B). No significantly increased numbers of AVP binding sites were measured for the nonfunctional mutant V2R-S167L with any of the treatments (Fig. 4B).

To test whether AVP binding also leads to intracellular signaling, cAMP measurements were performed following the same treatments as for the binding experiments. The relative cAMP levels generated (Fig. 4C) were in line with the obtained levels of AVP binding (Fig. 4B).

Functional rescue at reduced antagonist and AVP concentrations. In line with the choice for the use of a V1R antagonist in patients (5), our data above suggest that SR4 is most effective to functionally rescue mutant V2R in patients. However, the concentrations of the antagonists (1 μM) and AVP (100 nM) used will be difficult to obtain in patients. Therefore, we tested functional rescue of the V2R mutants at decreased antagonist concentrations and measured cAMP levels after stimulation with 0.1–10 nM concentrations of AVP. As shown in Fig. 5A, pretreatment of V2R-L44P-expressing cells with 1 or 0.1 nM SR4 did not yield a further cAMP response on stimulation with any of the AVP concentrations used. Pretreatment with 1 or 0.1 nM OPC3 or OPC4, however, led to a twofold increase in cAMP levels when cells were stimulated with 1 or 10 nM AVP, respectively. Similar results were obtained for V2R-I130F and -S167T (not shown). Pretreatment with SR1 did not result in significantly increased cAMP levels ($P > 0.05$) when tested in the conditions above (not shown).

To determine the level of rescued cell surface expression at lower CPA concentrations, cells expressing V2R-L44P, -I130F, -S167T were treated for 16 h with 100–3 nM concentrations of OPC3, OPC4, or SR1 and immunoblotted (Fig. 5B). At 100 and 30 nM concentrations, the extent of maturation (75–80-kDa signals) of V2R-L44P was highest for SR1 and OPC4, whereas OPC3 showed only a limited amount (100 nM) or no (30 nM) mature V2R. A further decrease in the concentration of the CPAns to 10 nM did not reveal any further maturation but showed an increased V2R-L44P expression for SR1-treated cells only. This SR1-specific effect on V2R-L44P expression was also found with 30 and 100 nM concentrations (Fig. 5B, 2 top panels). Treatment with 3 nM (Fig. 5B, bottom) of any of the CPAns showed no further effect on V2R-L44P maturation or expression level. Similar data were found for the mutants V2R-I130F and -S167T (not shown).

Time-resolved functional rescue of V2R mutants. On administration to patients, the blood concentrations of the antagonists will not be stable in time. Therefore, it is important to know how long it takes for the antagonists to confer a functionally rescued V2R phenotype, and whether this is different between antagonists. To study this, cells expressing V2R-L44P, -I130F, and -S167T were treated at different time points with 0.1 nM of the pharmacological chaperones, followed by [3H]AVP labeling to semiquantify the available AVP binding sites at the cell surface. Treatment of V2R-L44P, -I130F, or -S167T cells with OPC3 and OPC4 increased the available binding sites up to threefold, which became apparent after 8 h of treatment and did not further increase between 8 and 16 h of treatment (Fig. 6). Consistent with the absence of any rescue at the low concentrations used, SR4 treatment did not significantly ($P > 0.05$) affect [3H]AVP binding at any of the time points. These data indicated that in cell culture, between 4 and 8 h of treatment with a 0.1 nM antagonist is needed for a maximal functional rescue of the V2R mutants.

DISCUSSION

Pharmacological chaperones rescue a broad spectrum of V2R mutants. By definition, class II mutant proteins are ER retained due to misfolding. Binding of an antagonist to a mutant receptor can reverse the distorting effect of the mutation and thus aid in protein folding (3, 32). Indeed, our study reveals that all nine V2R mutants in NDI tested, except for V2R-S167L, are stabilized by the antagonists used, resulting in different levels of receptor maturation. In line with the finding of Tan et al. (31) that achieving the proper complex glycosylation state is necessary for V2R to reach the BM, maturation of the V2R mutants on immunoblotting coincides with BM ex-
expressing the GFP-tagged V2R mutants have no significant radioligand binding or cAMP signaling after agonist stimulation (Fig. 3). Following rescue to the cell surface, however, most of the V2R mutants are able to bind AVP and consequently elicit a cAMP response. Our data show that SR1 and OPC4 induce maturation of V2R-L44P, -I130F, and -S167T the best, followed by OPC3 and, much less, SR4 (Figs. 1 and 5B). Similarly, at 1 μM concentrations, SR1, OPC4, and OPC3 induce a robust cell surface rescue, whereas SR4 induced no detectable translocation (Fig. 2). A similarly reduced rescue for V2R-S167T and V2R-Δ62–64 by SR4 compared with SR1 was found by others (34). With the exclusion of severely distorted receptors (V2R-S167L), our data indicate that the level of maturation and translocation of V2R mutants in general is directly related to the antagonists’ affinities for V2R (Table 1), as similar relative effects were observed for eight of nine V2R mutants tested.

**Functional rescue is a balance between membrane expression and displacement by DDAVP.** Once at the plasma membrane, functional rescue can only occur if the pharmacological chaperone is displaced by an agonist, thereby allowing receptor activation and induction of the signaling cascade (12). Our data reveal that for functional rescue of V2R mutants, the pharmacological chaperone should fit two contradictory criteria: it should have a sufficient high affinity to facilitate the mutant receptor’s stabilization and translocation to the plasma membrane, but its binding should not be so strong as to interfere with its displacement by AVP. Moreover, our data confirm that the extent of functional rescue critically depends on the concentration used (12, 18).

At high concentrations (1 μM), SR4 showed a weak cell surface rescuing effect for the V2R mutants, whereas OPC3, OPC4, and SR1 rescued large amounts of receptor to the BM (Figs. 1 and 2). Nevertheless, subsequent AVP binding and cAMP generation are considerably lower for OPC3-, OPC4-, and SR1- compared with SR1- compared with SR4-treated cells and correlates largely with their affinities for the V2R (Fig. 4, B and C; Table 1). These data indicated that the extent of displacement by DDAVP is of major importance at these concentrations. A better functional rescue is obtained with a few rescued receptors which are fully available for AVP binding (SR4) than when many V2R mutants are rescued, which are liminally available for AVP binding (OPC3, OPC4, SR1). At low concentrations, however, pretreatment with (sub)nanomolar concentrations of OPC3, OPC4, or SR4, followed by stimulation with 1–10 nM AVP, only resulted in increased cAMP levels better functional rescue is obtained with a few rescued receptors which are fully available for AVP binding (SR4) than when many V2R mutants are rescued, which are liminally available for AVP binding (OPC3, OPC4, SR1). At low concentrations, however, pretreatment with (sub)nanomolar concentrations of OPC3, OPC4, or SR4, followed by stimulation with 1–10 nM AVP, only resulted in increased cAMP levels.

**Table 1. Characteristics of pharmacological chaperones**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Abbreviation</th>
<th>Kᵅ, nM</th>
<th>Class</th>
</tr>
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<tbody>
<tr>
<td>SR4059</td>
<td>SR4</td>
<td>275±50</td>
<td>V1R antagonist</td>
</tr>
<tr>
<td>OPC31260</td>
<td>OPC3</td>
<td>9.42±0.90</td>
<td>V2R antagonist</td>
</tr>
<tr>
<td>OPC41061</td>
<td>OPC4</td>
<td>0.43±0.06</td>
<td>V2R antagonist</td>
</tr>
<tr>
<td>SR121463</td>
<td>SR1</td>
<td>0.54±0.08</td>
<td>V2R antagonist</td>
</tr>
</tbody>
</table>

Values are means ± SE. V1R and V2R, V1 and V2 receptor; respectively. The values of the inhibitory constant (Kᵅ) for OPC3(1260), OPC4(1061), SR4(0959), and SR1(21463) on the human V2R are as described elsewhere (28–30, 36).
fore, at low antagonist concentrations, the extent of rescued cell surface expression becomes critical.

The absence of functional rescue on SR1 treatment seems to contradict data published by Morello et al. (18), who found that cAMP levels increased up to 15-fold in V2R mutants pre-treated with SR1. This difference is most likely due to the higher agonist/antagonist ratio and concentrations use by Morello et al., which were 10- and 100-fold higher compared with our “high concentration amounts,” respectively. This, however, provides additional support that the observed effect on cAMP generation depends on the concentrations used and ratios of antagonist to agonist.

Surprisingly, OPC4 and SR1 have similar affinities for the V2R (Table 1), but OPC4 was easier displaced by AVP than SR1 (Fig. 4A) and consequently yielded better functional rescue at any concentration used (Fig. 4, B and C). This difference was not caused by a reduced V2R mutant cell surface expression with SR1, as at low concentrations this was similar to, or better than, that of OPC4 or OPC3. Possibly, the different effects observed for SR1 and OPC4 might be due to differences in their V2R binding sites, as recently established (15). This is underscored by our finding that SR1, but not OPC4, stabilizes the ER-retained form of V2R-S167L (Fig. 1). These data reveal that compound-intrinsic factors other than their affinities influence their extent of displacement by AVP and ability to confer functional rescue.

Optimal pharmacological chaperone to treat congenital NDI. Treatment with SR4 showed a significant increase in urine concentration in three NDI patients encoding V2R-R137H, thereby providing proof of the principle that pharmacological chaperones can relieve NDI (5). In two other patients encoding V2R-W164S and Δ62–64 (185–193del), however, the response to SR4 treatment was weaker. Interestingly, V2R-R137H is only a partial class II mutant, as a considerable portion of this mutant is fully matured but is constitutively internalized from the plasma membrane (class V) (1), whereas V2R-W164S and Δ62–64 are fully retained in the ER (5). The reduced ER retention suggests a low level of misfolding of V2R-R137H, and the difference in the extent of ER-retention between V2R-R137H and other V2R mutants may underlie the observed effects of SR4 in NDI patients (4). Likely due to its low maximal blood plasma concentration of 30 nM (D. Bichet, personal communication), SR4 does not effectively rescue full class II mutants at low concentrations (Fig. 5A). As OPC3 and OPC4 allow functional rescue of fully ER-retained V2R mutants at nanomolar concentrations and NDI patients harboring full class II mutations are much more common (25), these compounds are anticipated to relieve NDI better than SR4 and in more NDI patients. Moreover, and in line with the adopted strategy by Bernier et al. (5), continuously elevated levels of the antagonists are needed, as it takes >4 h before a functional rescue is obtained (Fig. 6). Since nonpeptide antagonists remain active in vivo up to 8 h (11, 27), this would require the administration of at least three doses per day. The analyses in patients will be the subject of future studies.

In conclusion, we have demonstrated that cell-permeable V2R antagonists can rescue the cell surface expression of a broad spectrum of ER-retained V2R mutants and that functional rescue is a balance between a cell-permeable antagonist’s ability to rescue the cell surface expression of the V2R mutant and its ability to be displaced by AVP. Moreover, we show that at low concentrations the functional rescue occurs most efficiently by antagonists with a relatively high affinity for the receptor (Fig. 7). Our findings that a large number of V2R mutants are rescued by pharmacological chaperones and that functional rescue of mutant V2Rs at low antagonist concentrations is most effective with relatively high-affinity antagonists are anticipated to become important for other diseases, such as hypogonadotrophic hypogonadism (12), early-onset obesity (16), or hypothyroidism (7), in which mutations in G protein-coupled receptors are causal and for which cell-permeable antagonists are, or may become, available.

Concerning NDI patients with V2R mutations, of the four compounds tested, OPC3(1260) and OPC4(1061) combine cell surface rescue and displacement by AVP best when tested with low antagonist and near-physiological AVP concentrations. While other high-affinity V2R antagonists might be as suitable, OPC31260 is currently being tested as a treatment for polycystic kidney disease (2), while OPC41601 is under trial to treat hyponatremia and congestive heart failure in humans (19). Since negative side or toxicity effects have not been reported in these studies, OPC31260 and OPC41061 represent safe and promising candidates to treat NDI in patients with type II mutations in the V2R. (30, 36)

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References