Pharmacological chaperone action on G-protein-coupled receptors

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An increasing number of genetic diseases are found to result from mutations that lead to retention of the affected proteins in the endoplasmic reticulum, where they are recognized as misfolded by the quality control system. Several of these conformational diseases involve mutations in G-protein-coupled receptors. Recent studies demonstrated that pharmacologically selective compounds, termed pharmacological chaperones, can stabilize the misfolded receptors, facilitating their export from the endoplasmic reticulum to the plasma membrane, where they can be active. Such functional rescue suggests that pharmacological chaperones could represent novel therapeutic agents for the treatment of conformational diseases. Although only a few examples are currently available, the observation that pharmacological chaperones can also favour the folding of wild-type G-protein-coupled receptors indicates that these compounds could have wide applications.

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Current Opinion in Pharmacology 2004, 4:528–533

This review comes from a themed issue on New technologies
Edited by Alan Cuthbert
Available online 23rd August 2004

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DOI 10.1016/j.coph.2004.08.001

Abbreviations
AVP arginine-vasopressin
CFTR cystic fibrosis transmembrane conductance regulator
ER endoplasmic reticulum
GnRHR gonadotropin-releasing hormone receptor
GPCR G-protein-coupled receptor
NDI nephrogenic diabetes insipidus
V2R V2 vasopressin receptor

Introduction
Conformational diseases often result from mutations in proteins that are recognized as misfolded by quality control systems [1,2]. Such recognition can lead to two different phenotypes: some misfolded proteins can be efficiently ubiquitinated and degraded by the proteosome, leading to a loss of function [3], whereas others accumulate in cells, forming aggregates that can have toxic consequences and are often referred to as gain of functions [4]. Studies carried out in the past decade have linked these two types of quality control outcomes to the aetiology of a growing list of congenital and acquired conformational diseases. In parallel, efforts to overcome these defects have led to the development of various interventions that successfully rescue proteins from both aggregation and degradation pathways. In particular, treatments with chemical compounds known as either chemical or pharmacological chaperones have been found to stabilize some conformational mutants, promoting their proper transport to their site of action where, in many cases, they can be functional [5–7]. Identifying compounds that can bind to the mutant proteins has been easier for proteins such as channels and receptors for which selective ligands have already been characterized. Because of their involvement in many pathophysiological conditions and the rich pharmacological diversity generated through various drug screening campaigns, G-protein-coupled receptors (GPCRs) have attracted considerable attention for the identification of pharmacological chaperones. At least ten congenital diseases have been linked to mutations in GPCRs that lead to their retention in the endoplasmic reticulum (ER) (Table 1), and pharmacological chaperones have been identified for three of these (Table 2). Here, we review the studies that led to the discovery of these potential therapeutic agents, with a special emphasis on their proposed mechanisms of action.

The discovery of pharmacological chaperones acting on GPCRs
The first demonstration that pharmacologically selective agents could rescue cell surface expression and function of GPCR mutants, which were otherwise retained in the ER, came from work carried out on V2 vasopressin receptor (V2R) mutants responsible for nephrogenic diabetes insipidus (NDI). NDI is a rare X-linked disease characterized by a loss of antidiuretic response to the hormone arginine-vasopressin (AVP) that results in the inability of the affected patients to concentrate their urine, leading to large urinary output [8]. In infants, the water losses can lead to severe episodes of dehydration, resulting in growth and mental retardation and even death in the most extreme cases. To date, more than 175 different mutations distributed throughout the primary
sequence of the receptor have been identified in NDI patients. Although some mutations were linked to losses of either hormone binding or G protein coupling, the majority of the mutant receptors (38 out of the 53 tested in heterologous expression systems; see Figure 1) appear to be recognized as misfolded proteins that are retained in the ER by the quality control system before being degraded [9–13]. Because many of these mutations are relatively modest and would not be predicted to grossly affect the functional sites of the protein, our group posed the hypothesis that promoting the escape of mutant proteins from the ER could be sufficient to restore AVP responsiveness. On the basis of previous findings that non-specific binding of chemical compounds such as DMSO, TMAO and glycerol (known as chemical chaperones) could stabilize the folding of other mutant proteins [14,15], we tested the idea that binding to the receptor is sufficient for the chaperoning action of the ligands. By contrast, antagonists of the β2-adrenergic or δ-opioid receptors were without effect on the cell surface expression of the V2R mutants [18], confirming that receptor binding selectivity is required.

The restored cell surface expression promoted by V2R antagonist treatment led to functional recovery of AVP responsiveness in all cases (although to different extents)

<table>
<thead>
<tr>
<th>GPCR</th>
<th>Disease-related</th>
<th>Ligand used</th>
<th>Type</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Adrenocorticotropic hormone receptor</td>
<td>Familial adrenocorticotropic hormone resistance</td>
<td>SR121463</td>
<td>Antagonist</td>
<td>[16]</td>
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<tr>
<td>Calcium sensing receptor</td>
<td>Familial hypocalciuric hypercalcemia</td>
<td>VPA-985</td>
<td>Antagonist</td>
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<tr>
<td>Endothelin-B</td>
<td>Neonatal hyperparathyroidism</td>
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<td>GnRHR</td>
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<td>Indoles</td>
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<td>Luteinizing hormone receptor</td>
<td>Male pseudohermaphroditism</td>
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<td>Melanocortin 4 receptor</td>
<td>Obesity</td>
<td>Erythromycin-derived macrolides</td>
<td>Antagonist</td>
<td>[25*]</td>
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<td>Rhodopsin</td>
<td>Retinitis pigmentosa</td>
<td>Buprenorphine</td>
<td>Agonist</td>
<td>[18**]</td>
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<td>V2R</td>
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<td>TAN-67</td>
<td>Agonist</td>
<td>[18**]</td>
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<td>Tonazocine</td>
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<td>Naltirene</td>
<td>Antagonist</td>
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<td>Natirindole</td>
<td>Antagonist</td>
<td>[18**]</td>
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<td>Naloxone</td>
<td>Antagonist</td>
<td>[18**]</td>
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<td>11-cis-(7)-ring-retinal</td>
<td>Agonist</td>
<td>[30**]</td>
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<td></td>
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<td>9- or 11-cis-retinal</td>
<td>Agonist</td>
<td>[29]</td>
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following removal of the antagonist [16], confirming the hypothesis that the quality control system detected and retained mutant forms of the V2R that would otherwise be active. Therefore, NDI patients harbouring these mutations are victims of the extreme sensitivity of the quality control system that can detect subtle mutations that would not, on their own, lead to complete loss of receptor function. The contribution of specific components of the quality control apparatus in the retention of partially misfolded mutant receptors was further confirmed by the observation that mutant V2Rs remained associated for longer times with the molecular chaperone calnexin than did wild-type receptors [19].

These observations led us to propose that vasopressin antagonists acted by binding to the mutant receptors in the ER, stabilizing them in intermediate states of their folding path that more closely resembled the native state of the wild-type receptor, thus allowing the escape from the stringent quality control. Such a mechanism of action that would involve an intracellular site of action was supported by the observation that a peptidic V2R ligand which could not penetrate the cell was unable to mimic or block the rescuing effect of the non-peptidic ligands [16]. Biochemical analysis of the receptor species detected was also consistent with an ER site of action. Indeed, whereas ER-retained mutant receptors appeared largely as immature core-glycosylated receptor precursors, they were processed to fully mature receptors harbouring complex carbohydrate arborisation following treatment with the cell permeable V2R antagonists [16]. The antagonist treatment also increased the turnover rate of the precursor
form of the receptor without affecting the half-life of the mature receptor, which is indicative of an action on the biosynthetic processes. Taken together with the pharmacological specificity of action described above, these observations contributed to the emergence of the concept that ‘pharmacological chaperones’ assist the folding and ER export of mutant GPCRs.

Generalization of the pharmacological chaperone action on GPCRs

Pharmacological chaperones were soon identified for another GPCR, the gonadotropin-releasing hormone receptor (GnRHR). Indeed, ER-retained mutant forms of GnRHR, which are responsible for hypogonadotropic hypogonadism, were rescued by treatment with selective non-peptidic GnRHR antagonists [20]. As with the V₃R, treatment with an antagonist (an indole known as IN3) was first shown to restore both cell surface expression and signaling activity of 11 of the 13 disease-linked mutant GnRHRs tested [21**,22]. In addition, a cell impermeable GnRHR peptidic antagonist could not mimic or block the beneficial effects of IN3, consistent with an intracellular site of action for pharmacological chaperones [21**]. Interestingly, the kinetics of agonist-promoted internalization observed following stimulation of the rescued receptor was identical to that of the wild-type GnRHR [21**], confirming that the mutant receptor has normal pharmacological and biochemical properties once it has been released form the ER. This contrasts with the report that ΔF508 cystic fibrosis transmembrane conductance regulator (CFTR; the most frequent mutant form of CFTR found in cystic fibrosis patients) has a significantly reduced half-life at the plasma membrane following its rescue with chemical chaperones when compared with that of wild-type CFTR [23,24]. Whether the difference is a reflection of the different nature of chemical versus pharmacological chaperones, or is protein-dependent, remains to be determined. Conn and collaborators then extended their study to show that ten additional non-peptidic GnRHR antagonists, belonging to three chemically distinct classes of compounds (indoles, quinolones and erythromycin-derived macrolides), all displayed pharmacological chaperone action on mutant GnRHRs [25*]. Once again, the same set of mutations was rescued with all pharmacological chaperones tested. In addition, the efficacy of the rescue was proportional to the binding affinity of the different ligands [25*], further stressing the link between the binding energy and the pharmacological chaperone action of the ligands.

In the two cases discussed so far, pharmacological chaperones rescued the function of ER-retained and readily degraded GPCR mutants. In the case of another GPCR, rhodopsin, pharmacological treatment was found to prevent the cytotoxic aggregation that results from the most common mutation (P23H-rhodopsin) associated with autosomal dominant retinitis pigmentosa [26,27]. For this mutant form of rhodopsin, which causes loss of peripheral and night vision as a result of retinal degeneration [28], treatment with retinoids, such as 9- or 11-cis-retinal [29] or a 7-membered ring variant of the natural ligand 11-cis-retinal (11-cis-7-ring-retinal) [30**], restored the synthesis, cell surface expression and function of the mutant photo-receptor. Although the precise mechanism of action of the retinal variants has not been fully characterized, a pharmacological chaperone mode of action is likely.

Pharmacological chaperones can act on wild-type receptors

Although pharmacological chaperones have been described mainly in the context of mutant proteins, their action has also been reported for a few wild-type GPCRs. For example, the wild-type δ-opioid receptor is inefficiently processed, with less than 40% of the synthesized receptors reaching the mature form and being targeted to the plasma membrane under basal conditions [31]. Treatment with non-peptidic selective opioid ligands (Table 2) significantly increased the maturation efficacy, leading to the complete maturation and cell surface targeting of almost 100% of the produced receptors [18**]. This effect could not be attributed to the stabilization of the receptor at the cell surface, as peptidic ligands, which cannot penetrate the plasma membrane, were without effect on receptor processing. In this study, both δ-opioid agonists and antagonists were found to act as pharmacological chaperones, indicating that the binding properties of the ligands rather than their signaling efficacy underlie their action. Pharmacological chaperone effects were also reported for the wild-type GnRHR. Indeed, in addition to rescuing the natural mutant form of GnRHR, the antagonist IN3 was also found to enhance cell surface expression of the wild-type receptor [21**].

The fact that the maturation efficacy of wild-type GPCRs can be manipulated by pharmacological treatments opens the door to the use of pharmacological chaperones as regulators of tissue responsiveness in normal individuals. In the case of the δ-opioid receptor, this could have important implications in the design of anti-nociceptive drugs. However, whether inefficient maturation and its regulation by pharmacological chaperones will be limited to a few receptors, or represents a general trait of GPCRs, remains to be investigated.

Mechanistic features of pharmacological chaperones

The emerging hypothesis for the action of pharmacological chaperones suggests that selective lipophilic ligands can penetrate the plasma and ER membranes to bind to the partially folded receptor early during biosynthesis. In this context, ligand binding might alter the thermodynamic equilibrium in favour of the correctly folded protein, increasing the likelihood of the protein escaping the
stringent ER quality control, and ultimately leading to an increase in the steady-state level of functional receptors at the cell surface. This hypothesis is consistent with the observation that there is a clear correlation between the magnitude of ligand-mediated rescue and the binding affinity of ligands that mediate the effects. Such ‘built-in’ specificity of action for the targeted receptor presents the advantage of not having a general inhibitory effect on the quality control system, with the undesirable effects that this could cause. Pharmacological chaperone action should be distinguished from the cell surface stabilizing effects reported for some GPCR antagonists, which are believed to act by binding to receptors at the plasma membrane and preventing their spontaneous endocytosis and/or downregulation. Indeed, pharmacological chaperones need to act intracellularly during the biosynthesis of the receptors and, in contrast to cell surface stabilization effects, cannot be mimicked by cell impermeable peptide ligands. The difference between the two mechanisms of action is further emphasized by the fact that both agonists and antagonists can act as pharmacological chaperones, whereas only antagonists can prevent the spontaneous endocytosis and downregulation processes.

When considering their action as clinically relevant pharmacological chaperones, both agonists and antagonists could have drawbacks. For agonists, their ability to promote receptor folding and cell surface targeting can be countered by their propensity to promote rapid receptor endocytosis and downregulation, two phenomena that could greatly reduce their efficacy. For antagonists, high affinity binding to the receptor that persists for long periods of time once the receptor has reached the cell surface could prevent the binding of the natural hormone, thus inhibiting receptor action and defeating the purpose of rescuing the cell surface expression. The most appropriate drug candidates for the clinical use of pharmacological chaperones would therefore be either antagonists with moderate affinities or partial agonists that have little propensity to cause endocytosis or downregulation.

Conclusions
Pharmacological chaperones represent a promising avenue for the treatment of conformational diseases, such as familial hypocalciuric hypercalcaemia, hirschspring disease, hypogonadotropic hypogonadism, hypothyroidism, neonatal hyperparathyroidism, NDI, obesity and retinitis pigmentosa (see Table 1), that result from GPCR misfolding. Although proof-of-principle for their action has been obtained for only three of these GPCR-related diseases, the apparent generality of the concept, even for wild-type receptors, will certainly lead to other examples in the near future. Whether such an approach will have general clinical implications for conformational diseases remains to be investigated, and the answer will need to wait for the results of clinical trials that are currently being conducted with pharmacological chaperones.

Acknowledgements
We are grateful to Dr Monique Lagacé for critical reading of the manuscript.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest
18. Petaja-Repo UE, Hogue M, Bhalla S, Laperriere A, Morello JP, **Bouvier M: Ligands act as pharmacological chaperones and increase the efficiency of delta opioid receptor maturation. EMBO J 2002, 21:1628-1637. This is the first demonstration that pharmacological chaperones can increase the maturation efficacy of wild-type GPCRs using the δ-opioid receptor as a model. It also shows that both antagonists and agonists can act as pharmacological chaperones.


This study generalized the concept of pharmacological chaperones. It showed that treatment with selective GnRHR antagonists rescued surface expression and function of GnRHR mutants linked to the development of hypogonadotropic hypogonadism. It also demonstrated that the rescued receptors had normal pharmacological and biochemical properties.


This study demonstrated that GnRHR antagonists belonging to different chemical classes can all act as pharmacological chaperones for the GnRHR. This confirmed that the binding affinity of the ligands to the receptor, rather than other specific chemical properties, determines the pharmacological chaperone action.


This study extended the concept of pharmacological chaperone to a non-hormonal GPCR, the visual pigment rhodopsin. It also showed that pharmacological chaperones can rescue the expression of a rhodopsin mutant that would otherwise form cytotoxic aggregates leading to retinitis pigmentosa.