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Review

Pharmacological chaperone approaches for rescuing GPCR mutants: Current state, challenges, and screening strategies

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ABSTRACT

A substantial number of G-protein coupled receptors (GPCRs) genetic disorders are due to mutations that cause misfolding or dysfunction of the receptor product. Pharmacological chaperoning approaches can rescue such mutant receptors by stabilizing protein conformations that behave similar to the wild type protein. For example, this can be achieved by improving folding efficiency and/or interaction with chaperone proteins. Although efficacy of pharmacological chaperones has been demonstrated *in vitro* for a variety of GPCRs, translation to clinical use has been limited. In this paper we discuss the history of pharmacological chaperones of GPCR's and other membrane proteins, the challenges in translation to the clinic, and the use of different assays for pharmacological chaperone discovery.

Contents

Abbreviations: AVP, Arginine Vasopressin; B1R, Bradykinin B1 receptor; CFTR, Cystic fibrosis transmembrane regulator; cNDI, congenital nephrogenic diabetes insipidus; D4R, Dopamine D4 receptor; DERET, diffusion-enhanced resonance energy transfer; ELISA, enzyme- linked immunosorbent assays; FAP, fluorogen-activated protein; HTS, high throughput screening; FSHR, Follicle stimulating hormone receptor; κ/δ OR, κ/δ opioid receptors; HTRF, Homogenous Time Resolved Fluorescence; LHR, Lutenizing hormone receptor; MCR4, Melanocortin 4 receptor; NAchR, Nicotinic acetylcholine receptor; PC, pharmacological chaperone; P-pg, P-glycoprotein; RhR, Rhodopsin; SERT, Serotonin transporter; V2R, Vasopressin 2 receptor.

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1. Introduction

Folding of newly translated polypeptides is a complex and inherently inefficient process, the failing of which results in protein aggregation or degradation instead of formation of functional proteins. Membrane proteins, including G protein-coupled receptors (GPCRs), appear to be particularly prone to misfolding, as a number of studies have demonstrated protein folding efficiencies of 50% or lower [1–4]. The energetic balance between folding and misfolding can be delicate and easily perturbed due to the relatively small change in free energy between folded and unfolded states [5]. In fact, single nucleotide mutations leading to single amino acid changes can alter energy of folding enough to reduce folding efficiency by several-fold [6]. It is therefore not surprising that a large number of diseases can be traced back to single point mutations. Recently, a study of several thousand missense mutations associated with a spectrum of Mendelian genetic diseases estimated that 28% could be due to poor protein folding and stability [7].

Pathologies due to misfolding can occur in one of two ways: i) reduction of functional protein levels or ii) formation of toxic aggregates. To prevent the maturation of improperly folded receptors and potential aggregation, cells have stringent quality control mechanisms that block protein maturation unless it satisfies certain conditions. Membrane protein quality control is accomplished by a series of cytosolic, endoplasmic reticulum (ER), and Golgi-resident chaperone proteins. Chaperone proteins recognize folding states based on exposed hydrophobic regions, which are uncharacteristic of properly folded membrane proteins, exposure of retention or export motifs, and finally the addition of post-translational modifications such as glycosylation [8]. Proteins that do not properly fold are exported from the ER and degraded by the ER-associated degradation (ERAD) system [9]. Although ER-quality control is essential for maintaining proteostasis, the stringency of ER quality control can lead to ER-retention of misfolded mutants that may otherwise be partially or fully functional. This provides an opportunity for pharmacological intervention by targeting the folding and maturation process. A pharmacological chaperone (pharmacochaperone or pharmacoperone, PC) is a small molecule that selectively binds to a target protein and increases maturation efficiency by stabilizing a favorable conformation that can pass the cell's quality control system [10–12]. PCs have been demonstrated to rescue mutations in membrane proteins, and have been particularly successful for rescuing GPCRs. GPCRs are flexible proteins that explore a wide range of conformational spaces with multiple energetic minima [13]. Such flexibility makes receptors susceptible to conformational defects due to mutations, but also allows for ligand interactions that stabilize distinct conformations for the same receptor [14]. This is reflected in the wide variety of possible ligand-GPCR interactions, including biased agonism and allosteric modulation. The wealth of GPCR ligands identified through drug development efforts and screening programs provide a large pool of candidate compounds that could act as PCs. Drug screening has also resulted in the development of assays to measure GPCR activity, allowing straightforward screening for ligands that increase mutant receptor function. Another consideration is that GPCR mutations can have a dominant negative phenotype due to receptor dimerization. Improperly folded mutant GPCRs can dimerize with their wild-type (WT) counterparts and result in ER-retention of the dimer/oligomer, as has been shown for the vasopressin 2 receptor (V2R) and the follicle stimulating hormone receptor (FSHR) [15,16]. In the cases of these dominant negative mutants, rescuing a fraction of misfolded mutant proteins can significantly increase the total amount of functional receptors at the cell surface, as the PC will augment the surface expression of both the mutant and retained wild type protein.

There have been numerous demonstrations of PC approaches *in vitro* with potential for therapeutic applications, including rescue of V2R for nephrogenic diabetes insipidus, and GnRHR for hypogonadism [1,17]. However, clinical success of these approaches has thus far been limited (Table 1). In this review we discuss some of the PCs currently identified for GPCRs and other membrane proteins, the hurdles of translating *in vitro* results to clinical application, and screening approaches for discovering novel PCs.

2. Demonstrations of pharmacological chaperoning

2.1. Vasopressin 2 receptor

Arginine vasopressin (AVP), also known as antidiuretic hormone, promotes water reabsorption in the collecting duct of the kidney via binding to the Gs-coupled V2R. Over 200 mutations causing X-linked congenital nephrogenic diabetes insipidus cNDI have been identified in the V2R, the majority of which are missense mutations that likely impair protein folding [37]. Misfolded V2Rs are retained in the ER or the ER/Golgi intermediate compartment (ERGIC), and are not expressed on the cell surface, resulting in insufficient functional V2Rs. In 2000, Morello and colleagues were the first to demonstrate rescue of an intracellularly retained mutant GPCR by showing rescue of the Δ62-64 V2R mutant using the cell-permeant antagonist SR121463A [17]. SR121463A was shown to act intracellularly by the observation that cell-impermeant peptide V2R ligands could not block its effect on rescuing cell surface expression of mutant V2R. Importantly the SR compound also rescued 7 other cNDI-causing V2R mutants, and therefore its action was not unique to rescuing Δ62-64. The authors finally showed that another cell-permeant nonpeptidic V2R antagonist, VPA985, could also rescue the Δ62-64 V2R. Similarly, Bernier et al. reported that the surface expression of another V2R mutant, R137H, could be rescued with a different SR antagonist, SR49059 [38]. Treatment of cells transiently expressing R137H with SR49059 increased AVP-stimulated cAMP accumulation by 1.9-fold for the mutant receptor. The effect could be blocked using monensin, an agent known to block protein transport from the Golgi to the plasma membrane. Using metabolic labeling, the authors showed that very little immature R137H is processed to maturity under basal conditions, but that treatment with SR49059 resulted in 40% of the precursor converting to the mature form. The study concluded that SR49059 acted intracellularly as a PC to rescue the surface expression and signaling efficacy of mutant R137H V2R. Two years later, the same team tested the chaperoning activity of SR49059 in cNDI patients having one of the following V2R mutations, W164S, Δ185-193 or R137H [39]. Two-day administration of SR49059 at increasing doses significantly decreased urine volume and increased urine osmolality, consistent with increased functional V2R in the three tested patients [39]. Interestingly the *in vivo* effects of the SR49059 in the patients continued to persist up to twelve hours after the washout period. Despite these promising clinical results with SR49059, further trials with this compound were abandoned because of adverse effects that involved potential interference with the cytochrome P450 metabolic pathway [39].