Received: 7 January 2025 | Revised: 18 February 2025 | Accepted: 21 February 2025

DOI: 10.1111/andr.70018

ANDROLOGY

WILEY

REVIEW ARTICLE

Folding, misfolding, and regulation of intracellular traffic of G protein-coupled receptors involved in the hypothalamic–pituitary–gonadal axis

Alfredo Ulloa-Aguirre[1,2] | Ross C. Anderson[3,4] | Teresa Zariñán[1] | Rubén Gutiérrez-Sagal[1] | Eduardo Jardón-Valadez[5] | Claire L. Newton[3,6]

[1]Red de Apoyo a la Investigación, Universidad Nacional Autónoma de México (UNAM), Mexico City, Mexico

[2]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico

[3]Centre for Neuroendocrinology, Department of Immunology, Faculty of Health Sciences, University of Pretoria, Gezina, Pretoria, South Africa

[4]Department of Physiology, Faculty of Health Sciences, University of Pretoria, Gezina, Pretoria, South Africa

[5]Departamento de Recursos de la Tierra, Unidad Lerma, Universidad Auto´noma Metropolitana, Lerma de Villada, Estado de México, Mexico City, Mexico

[6]Deanery of Biomedical Sciences, University of Edinburgh, Edinburgh, UK

Correspondence
Alfredo Ulloa-Aguirre, Red de Apoyo a la Investigación, Universidad Nacional Autónoma de México (UNAM), Mexico City, Mexico.
Email: aulloaa@unam.mx

Claire L. Newton, Centre for Neuroendocrinology, Department of Immunology, Faculty of Health Sciences, University of Pretoria, Gezina, Pretoria, South Africa.
Email: claire.newton@up.ac.za

Funding information
South African National Research Foundation, Grant/Award Number: (94008); Universidad Nacional Autónoma de México, Grant/Award Number: (PAPIIT-DGAPA-IN208323)

Abstract

Background: G protein-coupled receptors are a large and functionally diverse family of membrane receptors involved in a number of biological processes. Like other proteins, G protein-coupled receptors need to be properly folded in order to traffic to the plasma membrane and interact with agonist.

Objective: Herein, we briefly review the process of folding and intracellular traffic of G protein-coupled receptors, with a focus on the gonadotropin-releasing hormone receptor and the gonadotropin receptors, whose variants can lead to misfolding, loss of plasma membrane trafficking and eventually to different forms of hypogonadism.

Results and Discussion: Pathogenic variants of G protein-coupled receptors may provoke loss-of-function of the receptor protein, thereby leading to disease. The presence of a stringent cellular quality control system promotes proper protein folding compatible with endoplasmic reticulum export and concomitantly prevents unfolded proteins accumulating within the cell. Molecular chaperones and companion factors are key elements of the quality control system that maintain the integrity of the proteostasis network by regulating, at different levels folding and assembly of nascent proteins and by promoting degradation of defective conformers, preventing aggregation and toxic accumulation. Due to the importance of the concept of molecular chaperoning in protein folding, pharmacoperone drugs emulating the role of endogenous chaperones as stabilizers of protein conformation currently represent a novel therapeutic opportunity for rescuing misfolded receptors and treating different diseases due to protein misfolding.

Conclusions: In vitro and in vivo studies in experimental animals and in humans have provided proof-of-principle of the beneficial effects of pharmacoperone drugs in modifying the course of human disease due to misfolding of G protein-coupled receptors.

KEYWORDS
G protein-coupled receptors, GPCR, hypogonadism, molecular chaperones, pharmacoperones, quality control system

1 | INTRODUCTION

Nascent proteins destined for membrane insertion or secretion are synthesized in the lumen of the endoplasmic reticulum (ER) where they undergo co- and post-translational modifications before trafficking to other cell compartments to continue their processing and eventually reach their final destination.[1,2] Folding, assembly, and other post-translational modifications (e.g., glycosylation) as well as dimerization and oligomerization can also occur in the ER.[3,4] If folding fails, then the misfolded protein is targeted for degradation in proteasomes.[5–7] Although some misfolded proteins may preserve function, they are usually retained and degraded, potentially leading to protein dyshomeostasis and cellular stress. Variants in genes are a frequent cause of misfolding of the encoded protein, that is, a defect in protein folding due to persistent number of non-native interactions that have an impact on the general architecture of the protein and/or its properties, hampering its ability to reach a minimal-energy configuration compatible with ER export.[8,9] Misfolding frequently results in loss-of-function of the conformationally defective protein,[9,10] which sometimes may be transcribed and translated at normal levels, but is unable to traffic to its final functional destination within the cell or to engage the secretory/PM insertion pathway.[9]

G protein-coupled receptors (GPCRs) are a large and functionally diverse family of membrane receptors involved in a number of biological processes. They are the largest class of membrane receptors in humans (more than 800 GPCRs, encompassing ~4% of the human genome). Their ligands are structurally diverse and highly variable in complexity, and include photons, odorants, lipids, enzymes, ions, peptides, and proteins.[11] These receptors primarily bind one or more intracellular heterotrimeric G proteins, which are activated upon agonist binding to the receptor,[12] and that act as the canonical mediators of effector activation and intracellular signaling. Five main families (glutamate, rhodopsin, adhesion, frizzled/taste2, and secretin) form the GPCR superfamily, with the rhodopsin family having the largest number of members.[13] These membrane receptors represent important targets for drug discovery, accounting for >30% of approved drugs in the pharmaceutical market.[14–18]

A number of human diseases (~40%) are caused by misfolding of GPCRs (and other proteins) and defective upward intracellular trafficking (for review see Refs. 10 and 19–22). These include neurodegenerative diseases (e.g., Parkinson's and Alzheimer's disease),[23,24] visual and developmental disorders (e.g., retinitis pigmentosa due to variants in rhodopsin[25]), malignant neoplasias,[26] and a number of endocrine diseases (e.g., X-linked nephrogenic diabetes insipidus due to variants in the vasopressin 2 receptor; V_2R[27,28]), familial hypocalciuric hypercalcemia (due to variants in the calcium-sensing receptor; CaSR[29]), congenital hypothyroidism (due to variants in the thyroid-stimulating hormone receptor; TSHR[30]), hypo- and hyper-gonadotropic hypogonadism (due to variants in the gonadotropin-releasing hormone receptor; GnRHR and gonadotropin receptors,[31] respectively), and obesity (due to variants in the melanocortin-3 and -4 receptors; MC3R and MC4R, respectively[32,33]), among others. In each of these cases, misfolding leads to ER retention, and increased aggregation/degradation, causing defective targeting of the variant receptor protein to the PM (reviewed in Ref. 34).

This review focuses on reproductive disorders caused by GPCR misfolding, mainly congenital hypogonadotropic hypogonadism (HypoH) due to variants in the human (h) GnRHR and hypergonadotropic hypogonadism (HyperH) provoked by variants in the gonadotropin receptors, and how emergent pharmacologic strategies have been experimentally applied to correct folding and routing of some misfolded receptors. Let us first briefly review some fundamental aspects of protein folding and how the quality control system (QCS) of the cell works to regulate folding of newly synthesized proteins destined for the exocytotic secretory pathway (which is also utilized by proteins destined for plasma membrane [PM] insertion, such as in the case of GPCRs).

2 | REGULATION OF PROTEIN FOLDING BY THE QCS OF THE CELL

Synthesis of proteins is a quite complex process regulated at several levels, and their folding, in particular, is a challenging task due to the high amount and physical proximity of these macromolecules that are continuously synthesized by the ER and that need to reach a distinct, low-energy three-dimensional conformation prior to their translocation to other domains within the cell. The folded state of a protein that occurs at co- and post-translational levels is usually the predominant state, given that its free energy is lower than that exhibited by the unfolded or intermediate states and that the ribosomes possess efficient mechanisms for entropic destabilization of the unfolded state (Figure 1). In addition, the cell also utilizes a QCS that, through a complex network of factors and mechanisms, continuously monitors the structural and folding states of newly synthesized proteins at different levels including the ER, the Golgi complex, and the PM. This is achieved by, employing a variety of strategies, of which the most important is the action of molecular chaperones (reviewed in Refs. 10 and 35).

Folding of a polypeptide chain is based on random iterations of inherent fluctuations of stable interactions between amino acid residues, which will progressively lead to the lowest energy conformational ensemble. In fact, protein folding follows a funnel-shaped landscape model (Figure 1) in which kinetically trapped conformations and intermediate structures traverse free-energy barriers with the aid, mainly, of molecular chaperones, in order to reach the most favorable,

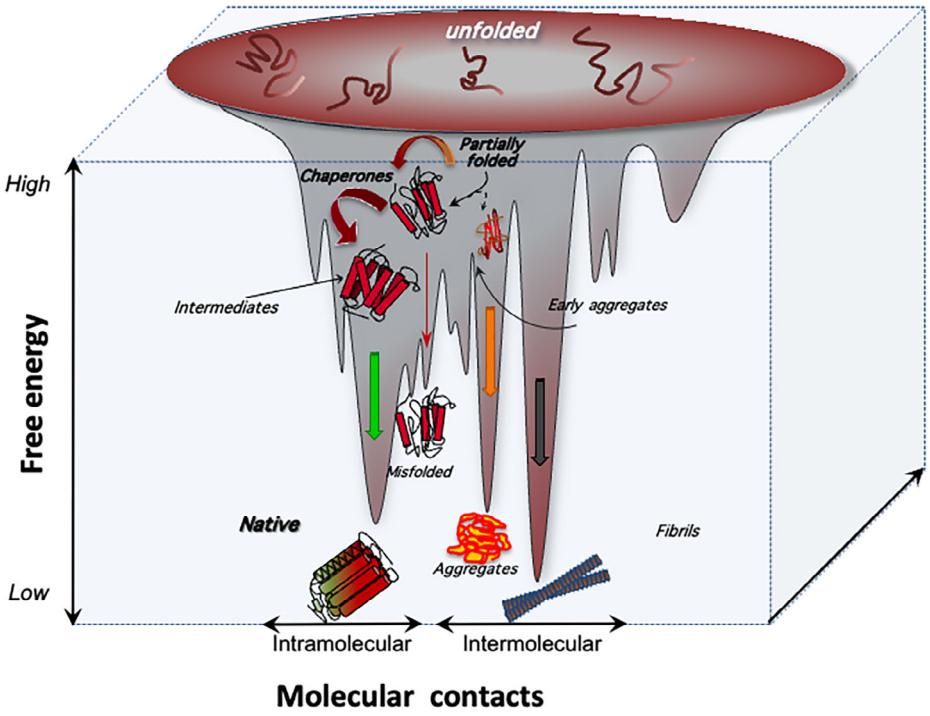

FIGURE 1 Schematic representation of the funnel-shaped free-energy surface (*y*-axis) of newly synthesized proteins; new proteins fold and approach the native state. Intermediate structures and kinetically trapped conformations traverse free-energy barriers helped by molecular chaperones to reach a favorable, low-energy downhill funnel. Potentially toxic aggregates or ordered amyloid fibrils may be formed as a result of overlapping between free-energy surface of folding and intermolecular aggregation (*x*-axis), when intermediates are folding simultaneously in the same compartment.

low-energy downhill funnel. Consequently, the number of possible conformations progressively decreases leading to a low number of conformations needed to be sampled. The final, three-dimensional, and complex protein structure is stabilized by a number of interactions that include non-covalent and covalent interactions. Although this mechanism is quite fast (microseconds for some proteins), errors in folding are frequent, given the complexity of the system; in fact, at least ~30% of the protein structures synthesized may contain errors that may potentially lead to misfolding and subsequent recognition by an efficient cellular QCS.[36,37]

Folding of proteins is assisted by molecular chaperones (Figure 2). These are key components of the ER QCS that contribute to protein homeostasis, preventing the accumulation and aggregation of misfolded proteins, from causing ER stress. Molecular chaperones are ER-resident proteins that bind to and stabilize unstable conformers to promote correct folding and assembly of the substrate polypeptide. Surveillance of the QCS for folding correctness and assembly of newly synthesized proteins through molecular chaperones relies more on general, non-native structural determinants of the folding protein. These include unpaired cysteine residues (necessary to reach a three-dimensional configuration of the protein), exposure of hydrophobic sequences and shapes, unprocessed glycans, and specific sequence motifs, allowing the recognition of proteins as misfolded or incompletely folded structures.[10] A few molecular chaperones also have the capability to disassemble protein aggregates, thereby preventing unproductive and potentially toxic interactions that may occur during the stages of folding.[38] The unfolded protein response (UPR) is a protective response to overcome ER stress and intracellular accumulation of reactive oxygen species and thereby cell toxicity and death.[39] The UPR modifies the biosynthetic capacity of the secretory pathway by upregulating ER chaperones and foldase expression and reducing the biosynthetic load.[40]

Incompletely folded or misfolded proteins are retained in the ER to attempt refolding by the chaperones or, if folding fails, to submit the defective structures to the ubiquitin–proteasome system degradation pathway.[41,42] For example, the calnexin–calreticulin cycle involved in glycoprotein folding relies on the action of glycosidases (I and II), which results in mono-glycosylated oligosaccharide structures that then associate with calnexin/calreticulin for folding, with the aid of Erp57, Erp29, and CypB (ER function-specific chaperones that bind to the calnexin/calreticulin chaperone system; for review, see Ref. 43). Since the action of these chaperones depends on substrate N-glycans added to the nascent protein, variants within glycosylation sites will result in protein misfolding and failure of this important quality control checkpoint in the ER. Prior to translocation to the Golgi complex, glycosidase II must remove the remaining glucose residue from the oligosaccharide chain, so that the glycoprotein can reach its native conformation and be directed to ER exit sites for translocation to the Golgi. Proteins that are incompletely folded are re-glycosylated to promote rebinding to calnexin/calreticulin and to retain the protein in the ER to attempt refolding. If multiple refolding cycles fail, then the misfolded protein is submitted to the ER-associated protein degrada-

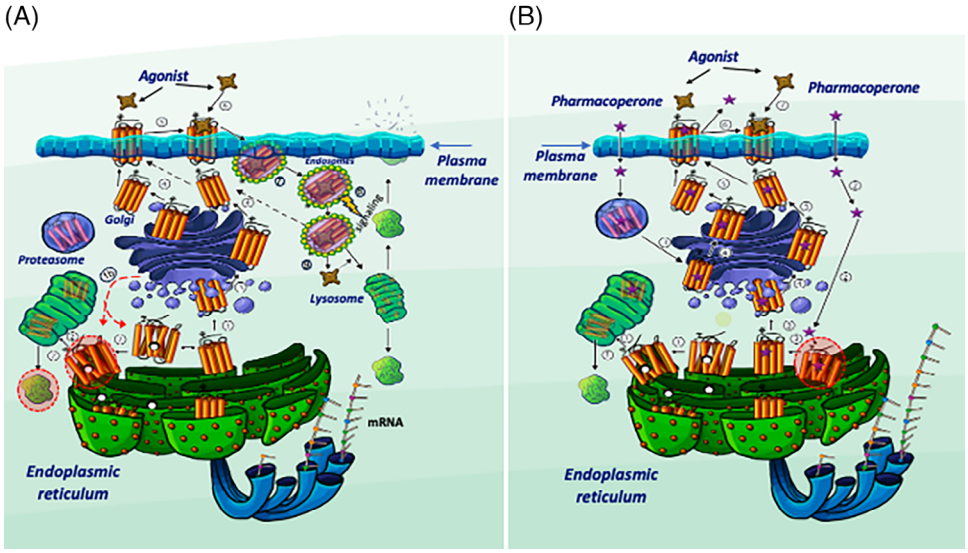

FIGURE 2 Intracellular trafficking of GPCRs belonging to the rhodopsin-like family of receptors. (A) Newly synthesized proteins fold in the endoplasmic reticulum (ER) (step 1), where misfolded proteins interact with molecular chaperones and co-chaperones (oval black and white structures, respectively), which attempt to correct folding and stabilize the protein in a conformation compatible with endoplasmic reticulum export. When correction of misfolding fails, the abnormal protein is dislocated into the cytoplasm for proteasomal degradation (step 2). Folded GPCRs are translocated to the Golgi apparatus to complete their maturation process including, for example, glycosylation (step 3), or they are translocated back to the ER to attempt re-folding of misfolded proteins that escaped the ER quality control system (step 1b). Mature receptors are then exported to the plasma membrane (step 4) where they interact with their specific ligands (steps 5 and 6). Phosphorylation and recruitment of β-arrestins follow activation of the receptor by the ligand (step 6), which promote endocytosis (step 7), internalization of the receptor–ligand complex, and further signaling (step 8). The receptor–ligand complex embedded in clathrin-coated vesicles may be either targeted to lysosomes for degradation (step 9) or dissociate with subsequent sorting of the receptor to the recycling pathway (step 4). (B) Rescue of misfolded receptors by pharmacoperones. Misfolded receptors that were not stabilized by molecular chaperones are submitted to degradation (step 1). The pharmacoperone crosses the plasma membrane, penetrates into the cell (step 2), and specifically binds the misfolded receptor (step 3). Pharmacoperone-stabilized receptors are then exported to the Golgi to complete processing (step 4) and finally to the plasma membrane (step 5), where the pharmacoperone (in the case of antagonists or agonists of the receptor) must dissociate from the rescued receptor to allow agonist binding and the subsequent activation of the receptor (steps 6 and 7). Interaction of pharmacoperones with misfolded receptors also may occur in other compartments before degradation (step 3, left). From Ref. 22, reproduced with permission from the authors).

tion (ERAD) pathway or to the ER lysosome-associated degradation system. Correctly folded proteins are transported in coat complex protein (COP)II-coated vesicles to the cis-Golgi, where misfolded proteins that escaped ER QCS retention are detected and can be retrotranslocated to the ER in COPI-coated vesicles or submitted to the lysosomal Golgi-associated degradation pathway. In the Golgi apparatus, glycoproteins complete processing by passing from a high mannose state, to a hybrid state, and finally to a complex state of glycosylation (at the cis-medial, and trans Golgi, respectively). After reaching the trans-Golgi network, proteins such as GPCRs, are targeted to the PM via the exocytotic–insertion pathway, where they are available to interact with agonist.[44]

Other examples of ER-resident molecular chaperones include the highly conserved HSP70 proteins, which are the best studied ATP-dependent chaperones.[45–47] BiP/Grp78 (chaperone Ig binding protein), which belongs to the HSP70 family, is one of the most important folding factors in lumen of the ER[48]; this chaperone interacts with a number of GPCRs, including the TSHR, the luteinizing hormone (LH)/choriogonadotropin receptor (LHCGR), and the angiotensin II AT1 receptor.[49–52] The effects of this essential molecular chaperone are assisted by members of the DnaJ family of cofactors to identify exposed hydrophobic regions, thereby keeping proteins in a conformation compatible for folding and oligomerization.[53]

A third checkpoint of the QCS surveillance for folding correctness occurs en route along the secretory and exosomal pathways (that is, the peripheral QCS; pQCS). This checkpoint occurs at the cell surface PM during or immediately after the insertion process.[54] As in the case of the ER QCS, chaperones, co-chaperones, and poly-ubiquitination-promoting enzymes are members of the pQCS, and thus both QCSs overlap in mechanisms to recognize misfolded conformers. This third checkpoint has the task of recognizing misfolded and dysfunctional proteins that escaped the ER and Golgi QCS. In addition, the pQCS also promotes degradation of misfolded, but sometimes still functional, proteins already expressed at the cell surface PM or that were rescued by pharmacological means through pharmacological chaperones (PCs/pharmacoperones; small-molecule, peptidomimetic agents that can aid in the folding of misfolded receptor proteins and facilitate their traffic to the PM). Still, functional misfolded proteins represent a therapeutic target for pharmacological interventions that prevent their degradation and allow restoration of function.

In addition to the mechanisms of QCS described above, some structural factors inherent to the protein itself also are important for the

successful trafficking. These include sequence motifs that determine N-linked glycosylation (the Asn-X-Ser/Thr sequence) and ER export of correctly folded proteins[55–57] (some of which may associate with components of the COPII transport machinery [and also small GTPases] to achieve exit from the ER[58]), highly conserved motifs among the rhodopsin-like subfamily of GPCRs (e.g., E/DRY motif and the N/DPxxY motif),[59–62] whose alteration may lead to altered intracellular trafficking, and retention sequences that restrict trafficking of the protein from the ER for further processing.[63,64,65]

In summary, upward trafficking of GPCRs and other proteins from the ER to other cell compartments is regulated by several factors, including mechanisms of quality control that check for proper folding of the protein molecule, presence of sequence motifs that promote or prevent trafficking of incompletely folded or misfolded conformers to the Golgi complex, and post-translational modifications such as glycosylation, palmitoylation, and oligomerization. Once trafficked to their final destination, GPCRs can be exposed to agonists that promote receptor activation and intracellular signaling.

Due to the importance of the concept of molecular chaperoning in protein folding and trafficking, PC drugs emulating the role of endogenous chaperones as stabilizers of protein conformation but with inherent target specificity, currently represent a unique and novel therapeutic opportunity for rescuing retained misfolded GPCRs and treating different diseases attributed to protein misfolding. This approach has been used in vitro and in vivo to successfully restore trafficking (and in many cases function) of misfolded GPCRs, including misfolded versions of the V_2R,[59,66–69] rhodopsin,[70] the CaSR,[29,71] the MC3R and MC4R,[34,72] the gonadotropin receptors,[73–75] and the GnRHR,[76–80] to name a few. The effects of some PCs associated with rescue of misfolded GnRH and gonadotropin receptors attributed to reproductive failure (hypogonadism) are discussed below.

3 | REPRODUCTIVE DISORDERS CAUSED BY GPCR MISFOLDING

Variants with inactivating mutations (inactivating variants) leading to misfolding and misrouting of GPCRs involved in reproductive function, include the neurokinin 3 receptor (NK3R, also known as TACR3), the prokineticin receptor 2 (PROKR2), and the GnRHR, which lead to HypoH,[81,82] as well as variants in the LHCGR and the follicle-stimulating hormone (FSH) receptor (FSHR), which cause HyperH.[83–87] Let us now briefly review how variants in the human GnRHR and the gonadotropín receptors may provoke misfolding of the receptor proteins and failure to traffic to the PM and describe novel pharmacologic strategies to rescue expression and function of these receptors.

3.1 | Misfolding and rescue of variant hGnRHRs

The GnRHR is the cognate receptor for the hypothalamic decapeptide GnRH. Gonadotropin-releasing hormone is secreted in a pulsatile manner through the hypothalamic–pituitary portal system and binds the GnRHR located in the PM of pituitary gonadotropes to stimulate synthesis and release of the gonadotropins, LH and FSH. Unlike other members of the rhodopsin-like family of GPCRs, the hGnRHR exhibits a number of unique features, including the absence of the carboxyl-terminal tail, which is present in GnRHRs from non-mammalian vertebral species such as avian and fish GnRHRs, and that is unique among the thousands of members of mammalian GPCRs.[82,88,89] Another feature of the hGnRHR is the presence of a lysine residue at position 191 (K191) in the extracellular loop (EL) 2, which is replaced by glutamic acid or glycine in non-primate mammals. Interestingly, in rodent (rat and mice) GnRHRs, K191 is absent, yielding a structure that is one residue smaller (327 amino acid residues) than the hGnRHR.[90] The absence of lysine in this position confers the rodent GnRHRs with an increased PM expression, whereas its presence in the hGnRHR limits the amount of receptor protein that reaches the PM after synthesis.[90,91] In fact, deletion of this lysine residue in primate GnRHR increases expression at the cell surface PM of a number of misfolded variants (see below) as well as the wild-type (WT) receptor, suggesting that a portion of WT GnRHR is normally inefficiently folded.[91] The mechanism(s) underlying the effect of K191 on PM expression of the primate receptor includes interfering with the formation of a disulfide bridge between residues 14 and 200, which is necessary to stabilize the hGnRHR in a conformation compatible with ER export. In fact, this particular disulfide bridge is not essential for rat GnRHR expression, as its disruption does not affect PM expression of the receptor.[92]

Resistance to GnRH by loss-of-function variants in the hGnRHR is the most common known genetic cause of normosmic congenital HypoH and leads to distinct forms of autosomal recessive HypoH.[82,93,94] Nevertheless, *GNRHR* variants may also occur in individuals with sporadic HypoH, but with a lower frequency.[93] Interestingly, patients harboring some hGnRHR variants also present alterations in other genes associated with HypoH, including the PROKR2 or the fibroblast growth factor receptor 1.[95–98] Nearly 55 loss-of-function variants (including deletions of large sequences and synonymous mutations) distributed along the entire coding sequence (except the intracellular loop [IL]-1 and the EL-3] of the hGnRHR have been described as a cause of partial or complete forms of congenital HypoH with variable phenotypes from complete to partial, depending on the particular variant. in vitro expression of variant hGnRH receptors in heterologous cell systems has shown that some of these variants may have an impact on ligand binding and/or intracellular signaling; these findings initially suggested that such variants altered motifs important for GnRH binding, receptor activation and/or interaction with effectors. However, studies on several variants showed that the loss-of-function was rather due to misfolding and mislocalization of the receptor protein that led to impaired PM expression[76] (termed Class II GPCR variants according to the classification system of Tao et al.[99]).

For a number of variant hGnRHRs, the function may be partially or completely restored in vitro and in vivo by genetic manipulation (e.g., deletion of K191 or addition of a carboxyl-terminal tail from fish GnRHR) and/or pharmacologic means, namely employing PCs,[100–102] which may be effective whenever the variant does not replace residues essential for receptor function.[77] Several small molecule PCs able

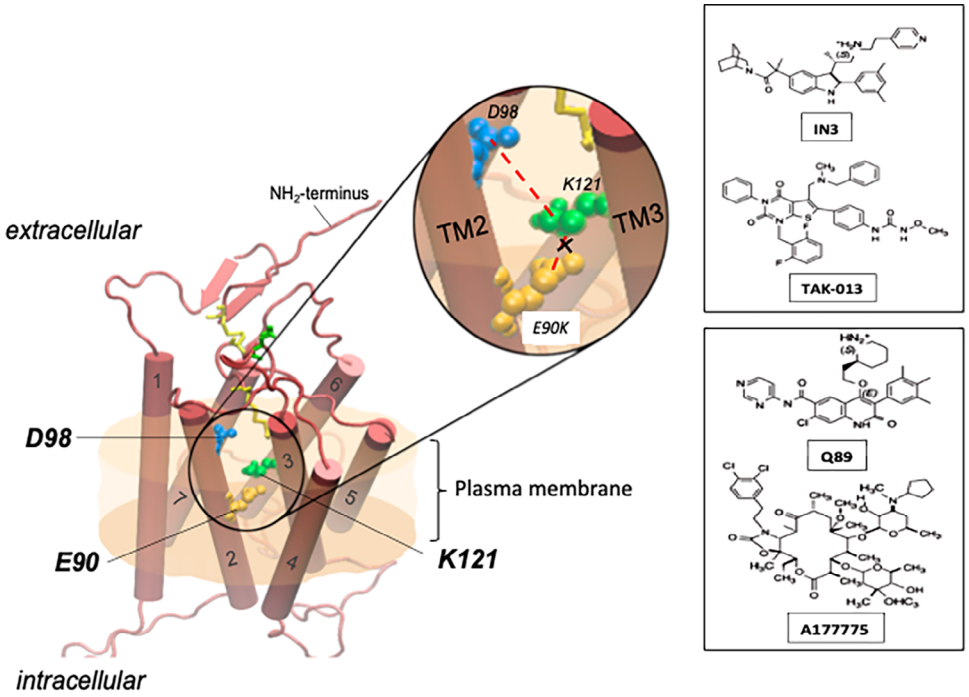

FIGURE 3 Mechanism of action of GnRHR pharmacoperones (right). Predicted molecule of the hGnRHR showing the seven transmembrane (TM) helices (displayed as rods) connected by the extracellular and intracellular loops. The C14–C200 and C114–196 disulfide bridges are shown as yellow sticks; K191 is represented by green sticks. E90 (at the TM helix 2; orange spheres) forms a salt bridge with K121 (at the TM helix 3; green spheres) (discontinuous red line), which in the case of the inactivating E90K mutation is eliminated (×) (magnified imagen). Pharmacoperones act to stabilize the misfolded mutant by bridging residues D98 (at the extracellular face of TM2; blue spheres) and K121 (discontinuous red line).

to correct trafficking of misfolded hGnRHRs leading to HypoH have been described. The peptidomimetics assessed as potential PCs came from four different chemical classes (indoles and quinolones [IN3 and Q89, respectively; Merck and Company], the erythromycin macrolide A177775 [Abbott Laboratories, Abbott Park, IL, USA], and TAK-013, which is a thienopyrimidine developed by Takeda Medical Industries, Ltd, Osaka, Japan)[80] (Figure 3). Originally designed as GnRH peptidomimetic *antagonists* identified through screening already existing chemical archives, these peptidomimetic molecules were selected as potential PCs considering both their permeability to the PM and specific binding to the hGnRHR with a known affinity, rather than for their original effect as antagonists.

The misfolded E90K variant, which has served as a paradigm to study the features of misfolded GnRHRs, severely affects PM expression as a consequence of misfolding and disruption of trafficking.[91] Apparently, the glutamic acid → lysine substitution at this position in transmembrane domain (TM) 2 prevents the formation of an E90-K121 salt bridge, leading to destabilization of the interaction between the TMs 2 and 3, which is essential to pass the ER QCS of the cell.[103] Consequently, the E90K variant GnRHR is retained intracellularly. However, either deletion of K191 or exposure to PCs, efficiently restored membrane expression and agonist-induced, receptor-mediated intracellular signaling of this variant in vitro and in vivo.[76,78,91,104] Exposure of cells expressing this variant to small molecule, peptidomimetic antagonists of GnRH creates a surrogate salt bridge between D98 (at the extracellular face of the TM1) and K121 (at the TM3), allowing stabilization of the receptor molecule and correcting its trafficking to the PM (Figure 3).[103] Further studies on knock-in male homozygous hypogonadal mice expressing the E90K GnRHR variant showed that homozygous E90K mice exhibited small testes and few or no Leydig cells or elongated spermatids.[78,79] However, when exposed to intermittent intravenous administration of the indole IN3 for 30 days, animals bearing the GnRHR E90K variant showed increased testis size and seminiferous tubules diameter, restored spermatogenesis, presence of nests of interstitial cells, and increased expression of the 17-β HSD mRNA and serum testosterone levels, whereas those treated with placebo did not show any effect.[78,79]

Gaining or losing a disulfide bridge also may result in misfolded GnRHRs, which is the case of the C200Y GnRHR variant, which prevents formation of the C14–C200 bridge. This particular bridge is required in the hGnRHR to pass the ER QCS. In the case of the Y108C variant, formation of an aberrant disulfide bridge between C108 and C200 occurs, leading to gross receptor distortion, thereby severely impairing its PM expression.[105] The function of the Y108C variant can be partially rescued by deleting K191 or by PC exposure, and complete rescue is attained only when both deletion of K191 and PC treatment are simultaneously applied.[105,106] Interestingly, not all variants are responsive to these approaches. In the particular case of the S168R and S217R variants, both abnormal GnRHRs are almost completely refractory to genetic (e.g., deletion of K191) or PC approaches[76] due to the thermodynamically unfavored

substitutions that lead to twisting of the corresponding α-helices moving the EL-2 away from the NH_2-terminal domain, interfering with the formation of the C14–C200 bridge essential for correct folding of the GnRHR protein and thereby for export to the ER to the PM.[107]

The observation that the PM expression of misfolded, but potentially functional variants of the hGnRHR and other GPCRs[21,22,108] may be rescued by pharmacological strategies and that the WT hGnRHR as well as other receptors with decreased expression in normal conditions may also respond to PCs, offers a unique therapeutic opportunity for manipulating the ER QCS of the cell to correct misfolding caused by sequence variants and to improve PM expression of WT receptors that may, in turn, allow increased interaction with agonist. Development of high throughput screening assay techniques to identify allosteric molecules that bind and rescue misfolded hGnRHRs will undoubtedly facilitate translation of pharmacological strategies based on PCs to the clinical arena.[80,102,109,110]

3.2 | Misfolding and pharmacological rescue of gonadotropin receptors

The gonadotropin receptors (LHCGR and FSHR) are members of the glycoprotein hormone receptor subfamily of GPCRs. The glycoprotein hormone receptors are characterized by large (>300 amino acid) N-terminal ectodomains (ECDs) comprising a series of leucine-rich repeats (LRRs), that provide an interface for docking of their large dimeric glycoprotein hormone ligands.[111] A "hinge" region links the ECDs to the 7-TM domain, and appears to play an important role in receptor activation following hormone binding.[112,113]

The cognate gonadotropin ligand for the FSHR is pituitary FSH, while the LHCGR interacts with both pituitary LH and placental human chorionic gonadotropin (hCG). Gonadotropin receptor signaling is essential to gonadal function, with the co-ordinated activities of LHCGR and FSHR and their cognate ligands stimulating sex steroid production in addition to trophic effects important for gonadal development and gamete production and maturation, and, in the case of hCG, maintenance of early pregnancy.[114] Inactivating genetic variants of the gonadotropin receptors can result in primary hypogonadism (HyperH) and infertility, usually characterized by low circulating levels of sex steroid hormones, with elevated gonadotropin secretion.[115] While puberty and the development of secondary sexual characteristics can be induced by administration of sex steroids, gametogenesis requires the co-ordinated non-steroidogenic activities of gonadotropins in addition to sex steroids, and thus these patients often (with the exception of males bearing inactivating FSHR variants) remain infertile with limited treatment options. The identification and development of gonadotropin receptor PCs able to restore function to these receptors may therefore offer a novel approach to inducing fertility in these patients, with the added benefit of restoring spatial and temporal receptor activation profiles in response to secretion of endogenous gonadotropin hormones.

Many non-peptide, low molecular weight (LMW) gonadotropin receptor analogues spanning a wide range of chemical scaffolds have been developed, primarily in response to the enormous increase in use of assisted reproductive technologies.[116] Although none have yet been approved for clinical application, there are an abundance of molecules that could potentially be repurposed/repositioned for application as gonadotropin receptor-targeted PCs.

3.2.1 | LHCGR dysfunction and rescue

Inactivating variants of the LHCGR result in reduced or absent fertility across a phenotypic spectrum in both males and females, although the effects on pubertal induction appear sexually dimorphic, with females harboring inactivating LHCGR variants usually undergoing normal pubertal development, followed by oligomenorrhea and infertility, in contrast to males who can often have altered primary sexual development and fail to undergo puberty.[117]

At least 40 naturally occurring inactivating variants of the LHCGR gene coding region have been associated with primary hypogonadism (HyperH).[84] These variants are located throughout all structural domains of the LHCGR protein, with intracellular retention being the most common mechanism contributing to their loss of function (Class II variants).[81,83,84,118–121] For example, one study that examined 20 missense, small deletion and frame-shift LHCGR variants indicated that 70% displayed some reduction in cell surface expression relative to total cellular expression.[83] Of these, 14 had severe loss of cell surface expression (<25% of WT levels)[83] and co-localized with ER markers.[74] Not surprisingly, the degree of intracellular retention often correlates with severity of the patient phenotype with those having moderately decreased cell surface expression (such as the homozygous variant I625K) often being associated with "milder" phenotypes (micropenis, hypospadias), and those with severely reduced cell surface expression (such as the homozygous variant A593P) associated with more complex disorders of sexual development.[51,75,122]

Many non-peptide compounds comprising a range of different chemical scaffolds have been identified that target the gonadotropin receptors, including pyrazoles and thienopyrimidines (reviewed in Refs. 123 and 124), primarily for use in assisted reproductive therapies to replace hCG/LH injections. While none have entered the clinic, some have reached advanced stages of development with the best characterized series of LHCGR-targeting small-molecules being the thienopyrimidines. One of these, LHR–Chap (also known as Org42599/Org43553; Figure 4A) is an allosteric agonist that has been extensively examined in vitro, where it displays high affinity and nanomolar potency at the LHCGR and, in a small cohort clinical study, was able to induce ovulation in GnRH suppressed, FSH-primed healthy females when administered orally.[125,126]

LHR–Chap was shown to act as an LHCGR PC able to restore cell surface expression to two intracellularly retained LHCGR variants (A593P and S616Y) in a time- and dose-dependent manner.[75] A subsequent study extended these observations to demonstrate rescue of cell surface expression (and function) of a number of intracellularly

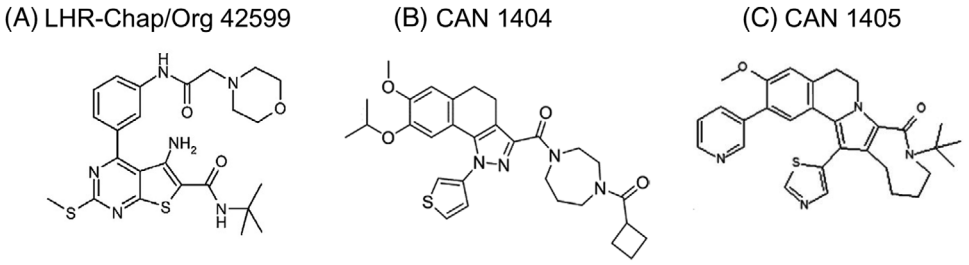

FIGURE 4 Small molecule structures that act as pharmacological chaperones at the LHCGR (A) or FSHR (B and C).

retained LHRCR variants.[74] Not all retained variants responded to treatment, and those variants that were rescuable had increased cell surface expression that spanned a continuum from marginal (13–17% of WT) to substantial increases (53–68% of WT)[74,75] and correlated with an increase in "mature" fully glycosylated LHCGR (a phenomenon also observed for PC-rescued FSHR variants[87]). Pertinently, a significant proportion (60%) of the rescued receptors were functional following rescue with LHR–Chap and stimulation with hCG. Although it should be noted that the hormone responses did not fully correlate with degree of rescue, presumably due to additional defects in ligand binding or signal transduction, or alternatively, that PC-bound receptor adopts a conformation that elicits sub-maximal hormone responses.[74]

Variant location-specific differences in PC responsiveness were observed. No ECD-located variants were rescuable, yet >60% of variants located in the TM helices showed increased cell surface expression. Together, these observations suggest that localized interactions between the PC and the receptor are important determinants of whether a variant can be rescued. The binding pocket for LHR–Chap is buried deep within the transmembrane bundle where interactions with residues from TM3, TM5, TM6, TM7, EL2, EL3, and the hinge region form a relatively stable binding interaction.[127] LHCGR TM variants T461I (at TM7), L502P (TM4), A593P (TM6), Del L608-V609 (TM7), and S616Y (TM7) have been shown to be severely retained and, following treatment with LHR–Chap, exhibited significantly increased cell surface trafficking, as determined by increased hCG-binding.[74] Molecular modeling studies indicate that LHR–Chap-induced stabilization could occur either through direct interactions of the chaperone with variant side chains, or via indirect stabilization of the TM helices harboring the variants.[74] Interestingly, indirect stabilization of TMs that harbor variants appear to result in more effective rescue when compared with the residues predicted to interact directly with LHR–Chap. This possibly indicates that disruption of the allosteric pocket is affecting LHR–Chap rescue potency. This would suggest that some TM variants located close to, or forming part of the allosteric pocket may be unrescuable, as they could possibly result in attenuation/ablation of LHR–Chap binding. Furthermore, variants located in the ECD or other locations distant from the allosteric pocket could simply be located too far from the LHR–Chap binding site for effective conformational stabilization (e.g., LHCGR variants C543R, T392I, and I374T).[74] Thus, screening libraries of small molecules for rescue of misfolded LHCGR variants may identify more efficacious LHR chaperones that engage with the receptor through different interaction sites.

3.2.2 | FSHR dysfunction and rescue

Approximately 30 naturally occurring inactivating coding-region variants of the FSHR have been described.[84] The vast majority of these have been identified in hypogonadal infertile female patients with primary ovarian insufficiency and HyperH, with a smaller number described in males. This discrepancy is possibly the result of the milder phenotypes described in males, where inactivating FSHR variants frequently result in reduced sperm quantity and quality, but retention of fertility.[128,129]

In vitro studies have, again, demonstrated that the majority of these inactivating variants result in trafficking defects (Class II variants).[81,85–87] For example, Hanyroup et al.[86] examined 17 missense FSHR variants, and 15 were determined to have trafficking defects (approximately 90%), with 11 having severe reductions in PM expression (≤18% of WT receptor cell surface expression). The intracellular retention of several of these variants has been corroborated in additional studies.[87] Another variant, I160T,[130] has also been demonstrated to have reduced cell surface expression.

Ovarian stimulation for promotion of follicular development during assisted reproduction protocols requires multiple FSH injections prior to oocyte retrieval. Blocking FSHR stimulation with antagonists also has potential novel non-steroidal contraceptive application.[131] Therefore, much effort has been dedicated to developing LMW orally bioavailable analogues targeting the FSHR, and multiple chemical classes have been shown to have activity, including thiazolidinones, diketopiperazines, hexahydroquinolines and isoquinolinones (reviewed in Ref. 132).

The LHCGR thienopyrimidine allosteric agonist, LHR–Chap, and the related Org 41841, both have agonist activity at the FSHR, albeit at a lower potency than at the LHCGR.[126,133] Our unpublished data have indicated that LHR–Chap can rescue intracellularly retained FSHR variants, with the potency of rescue being proportional to the potency of activation at both of the gonadotropin receptors. Org 41841 has also been proposed to act as an FSHR PC.[73] When a panel of intracellularly retained FSHR variants were tested, one (A189V) displayed partial rescue. However, this is perhaps surprising as A189 is located in the FSHR ECD, and the closely related thienopyrimidine, LHR–Chap was unable to rescue ECD-located variants in either LHCGR[74] or FSHR (unpublished data). Indeed, this study represents the first (and only) example of an intracellularly retained gonadotropin receptor ECD variant that is rescuable using a PC approach. This observation possibly relates to

interactions between the two receptor domains (i.e., ECD–TM), and further examination of the Org 41841–FSHR interaction may be able to shed additional light.

The low potency of rescue of FSHR variants and high potency activation of the LHCGR by LHR–Chap and Org 41841 possibly preclude their application in a therapeutic context and utilization of FSHR-specific LMW agonists and antagonists would thus be preferable. Indeed, two FSHR-selective allosteric agonists, a dihydrobenzoindazole, CAN 1404 (Figure 4B),[134] and a benzoindazolmethanone, CAN 1405 (Figure 4C), have been shown to be highly effective at rescuing FSHR TM variants.[86,87] For CAN 1404, six out of seven TM variants with severely reduced cell surface expression showed significant increases in receptor PM expression with a concurrent increase in FSH-induced responsiveness for four of these (A419T [at TM2], P519T [ECL2], A575V [TM6], and F591S [TM6]).[86] However, again, the FSH response of the rescued variants did not always correlate with the degree of rescue, implying additional functional deficits in receptor activation/signaling, or ligand-binding, or alternatively, that CAN 1404 stabilizes a differential conformation through which submaximal FSH responses are elicited. In a panel of seven FSHR variants, all four of the TM-located variants and none of the three ECD-located variants showed an increase in the ratio of mature (PM-expressed) to immature (intracellular) receptor forms following incubation with CAN 1405.[87] Again, the increase in PM expression was concomitant with an increase in hormone-induced signaling, demonstrating variant receptor functionality.

4 | GENERAL OBSERVATIONS REGARDING PC EFFECTS ON THE hGnRH AND GONADOTROPIN RECEPTORS

As described above for many other GPCRs (e.g., NK3R, V_2R, CaSR, and MC4R, among others[27–29,33,135–137]), the hGnRHR and gonadotropin receptors have been demonstrated to be amenable to PC rescue, and in the case of the hGnRHR and FSHR, by multiple LMW compounds across different chemical scaffolds. Dose–response analyses have indicated that, in general, the potency of rescue of cell surface expression of gonadotropin receptor variants is lower than the potency of receptor activation.[75,86] This is in agreement with previous observations that PC activity usually requires a dose of at least 100-fold higher than the EC_{50} observed in signaling assays,[138] which is not surprising considering that upward trafficking through the secretory pathway to the PM (which depends on the presence of a stable conformation compatible with ER export) and receptor activation (which is associated with rearrangement of TMs in response to agonist) are independent events.

Although PCs have now been identified for many GPCRs, the mechanism(s) of variant intracellular retention and PC rescue remain to be fully elucidated. Studies with the LHCGR (our unpublished data) and hGnRHR[139] have demonstrated that PC rescue occurs at a post-translational level, as pre-treatment with cycloheximide to inhibit de novo protein synthesis does not affect the rescue. Taken together with the observation that retained gonadotropin receptor variants co-localize with ER markers,[74] it is tempting to speculate that this is the cellular compartment where PCs exert their effects. However, it has been noted that misfolded, retained GPCR variants can be shuttled from the ER to the Golgi apparatus and back, possibly to prevent ER stress due to accumulation of misfolded proteins[140,141] or to attempt refolding when misfolded proteins escaped the ER QCS when ERAD is saturated.[142] Therefore, whether PCs rescue misfolded, retained GPCR variants from ER or non-ER compartments of the secretory pathway remains to be conclusively determined.

It is interesting to note that, while the WT hGnRHR demonstrates a relatively large increase in cell surface expression in response to PC treatment,[143] the effect of PCs on the WT gonadotropin receptors appears to be minor in comparison. Some studies have demonstrated no effect on WT LHCGR[74,75] or FSHR[86] surface expression, with other studies indicating marginal increases in WT FSHR cell surface expression.[73,87] This possibly reflects either a higher inherent stability of the WT gonadotropin receptors compared with the hGnRHR (for which a limited proportion of WT receptor reaches the cell surface[144]), that the allosteric PCs employed do not disrupt the trimeric configuration of the gonadotropin receptor, thereby preventing detection of an increase in binding sites,[145] or alternatively that PC-rescued WT gonadotropin receptors internalize faster, masking the actual rescuing effect of the PC. In fact, following PM insertion of rescued variants, removal of the PC ultimately results in loss of receptor at the cell surface[75,87] presumably through turnover, and a failure to replenish from the ER, although the kinetics of this effect are yet to be fully elucidated. Interestingly, LHR–Chap appears to prevent loss of LHCGR from the cell surface at high concentrations of hCG, which cause desensitization of LHCGR in non-PC-treated cells (unpublished data). Whether this is LHR–Chap preventing LHCGR desensitization, driving rapid LHCGR recycling, or simply reflects increased ER trafficking of the LHCGR to replenish desensitized receptor remains to be determined.

It also remains to be established why some variants are not amenable to rescue by PCs despite being located in, or near, the allosteric or orthosteric binding pocket of the PC. It is tempting to speculate that some amino acid substitutions, or small deletions/insertions result in such major reorientations of the allosteric or orthosteric pocket that the PC cannot bind, or cannot stabilize receptor folding to a degree that permits evasion of the ER QCS.[60,76] If this were true, it might be expected that severity of retention might correlate with propensity for rescue, but some gonadotropin and hGnRHR variants contradict this theory, as some severely retained variants demonstrate significant rescue whereas others not.[74,76,86] In a similar vein, it is not clear why some LMW compounds cannot rescue retained variants, despite being predicted to bind in the same allosteric pocket as effective PCs with a similar binding affinity (Ref. 146 and our unpublished observations on FSHR variants), which may depend on the varying ability of the LMW compound to stabilize the receptor variant in a conformation compatible with ER export. Future molecular docking studies may prove useful in better understanding the interactions between PCs and variant receptors.

5 | FUNCTION OF CELL SURFACE EXPRESSION RESCUED VARIANTS

It may be expected that if domains important for agonist binding, receptor activation and coupling to effectors are compromised by a change in the structure of a variant, functional rescue would not be possible despite good PM expression. However, this is not necessarily the case. For example, loss-of-function LHCGR variants C131R and I152T, both of which are located within the LRR region of the ECD and affect hormone binding to the receptor, do not respond to hormone, yet exhibit a robust response to LHR–Chap.[74] This is presumably due to LHR–Chaps' mechanism of activation of the receptor not involving the defective ECD region. Similarly, the variant E354K, which is located in the intramolecular agonist motif of the hinge region, has poor responsiveness to hormone, but in the presence of LHR–Chap, there is a significant increase in signal.[74] From a mechanistic standpoint, in this case, LHR–Chap appears to promote stabilization of the active conformation, in the absence of LH/hCG-induced unveiling of the tethered agonist. Thus, the repurposing of an allosteric agonist as a PC has utility beyond simply restoring PM trafficking.

6 | CONCLUSIONS

It is clear that misfolding and misrouting of pathogenic variant G protein-coupled receptors is more common than previously considered, and that pharmacological chaperones represent a promising therapeutic strategy to restore function of such misfolded receptors, as experimentally demonstrated for the hGnRHR and gonadotropin receptors. Identification of new pharmacological chaperone compounds that exhibit optimized properties (e.g., increased receptor selectivity and/or biased effects), by high-throughput screening assays and pharmacologic studies, is still necessary in order to translate this therapeutic strategy to the clinical setting. Furthermore, coupling such assays with in silico computer-aided structural approaches and artificial intelligence tools may provide useful information regarding the mechanism of action of pharmacological chaperones and how they interact with defective proteins to rescue expression and function.

Pharmacoperones that correct trafficking-defective G protein-coupled receptors via allosteric interactions are particularly attractive as they do not interfere with the orthosteric, active site of the receptor, permitting restoration of the spatial and temporal stimulation profile of the native hormone and to provide better therapeutic treatment at the lowest effective dose of the compound to prevent potential side effects.[102] It should, however, be noted that, as some of these compounds might stabilize the conformation of the receptor protein in an inactive state (antagonists), they would need to be removed to allow the receptor to become activated by hormone (although, in the case of allosteric biased antagonists, it has been shown that binding to the receptor may still allow for certain pathways of hormone activation[147]). In contrast, development of agonist pharmacological chaperones that concurrently rescue and activate the receptor may be complicated if the pharmacological chaperone causes rapid receptor internalization and turnover (as seen with many G protein-coupled receptor agonists). Thus, use of compounds that limit receptor internalization would be desirable.

In conclusion, in vivo studies on experimental animals[78] and in humans[59] have provided proof-of-principle of the beneficial effects of pharmacological chaperone drugs in modifying the course of human disease and set the scene for the potential future clinical use of pharmacological chaperones targeting G protein-coupled receptors, including the hGnRHR and gonadotropin receptors.

AUTHOR CONTRIBUTIONS

Conceived and designed the review; wrote the paper: A. U.-A. *Contribute to designing the review; contribute to writing the paper*: R. C. A. *Contributed to writing the paper; reviewed the first draft and final version*: T. Z. *Reviewed the first draft and final version; contribute to designing Figure 1*: R. G.-S. *Reviewed the final version of the paper; designed Figure 3*: E. J.-V. *Conceived and designed the review; wrote the paper*: C. L. N.

ACKNOWLEDGMENTS

The studies performed in the laboratory of Alfredo Ulloa-Aguirre are supported by grants from the Coordinación de la Investigación Científica and PAPIIT-DGAPA grant IN208323, UNAM, Mexico City, Mexico. Claire Newton's research in this area has been supported by the South African National Research Foundation (94008).

ORCID

Alfredo Ulloa-Aguirre https://orcid.org/0000-0002-9532-3886
Claire L. Newton https://orcid.org/0000-0001-6050-556X

REFERENCES

1. Castro-Fernandez C, Maya-Nunez G, Conn PM. Beyond the signal sequence: protein routing in health and disease. *Endocr Rev*. 2005;26(4):479-503. doi:10.1210/er.2004-0010
2. Hartl FU, Hayer-Hartl M. Converging concepts of protein folding in vitro and in vivo. *Nat Struct Mol Biol*. 2009;16(6):574-581. doi:10.1038/nsmb.1591
3. Helenius A, Aebi M. Roles of N-linked glycans in the endoplasmic reticulum. *Annu Rev Biochem*. 2004;73:1019-49.
4. Tao YX, Conn PM. Pharmacoperones as novel therapeutics for diverse protein conformational diseases. *Physiol Rev*. 2018;98(2):697-725. doi:10.1152/physrev.00029.2016
5. Hiller MM, Finger A, Schweiger M, Wolf DH. ER degradation of a misfolded luminal protein by the cytosolic ubiquitin-proteasome pathway. *Science*. 1996;273(5282):1725-1728.
6. Ravid T, Hochstrasser M. Diversity of degradation signals in the ubiquitin-proteasome system. *Nat Rev Mol Cell Biol*. 2008;9(9):679-690. doi:10.1038/nrm2468
7. Schubert U, Anton LC, Gibbs J, Norbury CC, Yewdell JW, Bennink JR. Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. *Nature*. 2000;404(6779):770-774.
8. Dobson CM. Protein folding and misfolding. *Nature*. 2003;426(6968):884-890.
9. Dobson CM. Principles of protein folding, misfolding and aggregation. *Semin Cell Dev Biol*. 2004;15(1):3-16.
10. Ulloa-Aguirre A, Janovick JA, Brothers SP, Conn PM. Pharmacologic rescue of conformationally-defective proteins: implications for the treatment of human disease. *Traffic*. 2004;5(11):821-837.

11. Ulloa-Aguirre A, Conn PM. G Protein-coupled receptors and the G protein family. In: Conn PM, ed. Handbook of Physiology. Section 7: The Endocrine System. *Vol 1: Cellular Endocrinology*. New York: Oxford University Press; 1998. pp. 87-124.
12. Oldham WM, Hamm HE. Heterotrimeric G protein activation by G-protein-coupled receptors. *Nat Rev Mol Cell Biol*. 2008;9(1):60-71. doi:10.1038/nrm2299
13. Fredriksson R, Lagerstrom MC, Lundin LG, Schioth HB. The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. *Mol Pharmacol*. 2003;63(6):1256-1272.
14. Downey ML, Peralta-Yahya P. Technologies for the discovery of G protein-coupled receptor-targeting biologics. *Curr Opin Biotechnol*. 2024;87:103138. doi:10.1016/j.copbio.2024.103138
15. Insel PA, Sriram K, Wiley SZ, et al. GPCRomics: GPCR expression in cancer cells and tumors identifies new, potential biomarkers and therapeutic targets. *Front Pharmacol*. 2018;9:431. doi:10.3389/fphar.2018.00431
16. Retamal JS, Ramirez-Garcia PD, Shenoy PA, Poole DP, Veldhuis NA. Internalized GPCRs as potential therapeutic targets for the management of pain. *Front Mol Neurosci*. 2019;12:273. doi:10.3389/fnmol.2019.00273
17. Sriram K, Insel PA. G protein-coupled receptors as targets for approved drugs: how many targets and how many drugs? *Mol Pharmacol*. 2018;93(4):251-258. doi:10.1124/mol.117.111062
18. Yang D, Zhou Q, Labroska V, et al. G protein-coupled receptors: structure- and function-based drug discovery. *Signal Transduct Target Ther*. 2021;6(1):7. doi:10.1038/s41392-020-00435-w
19. Ulloa-Aguirre A, Janovick JA., Zariñán T, Hanyaloglu AC. Intracellular trafficking of G protein-coupled receptors to the cell surface plasma membrane in health and disease. In: Ulloa-Aguirre A, Tao, Y-X., ed. *Cellular Endocrinology in Health and Disease*. Elsevier Inc.; 2021. pp. 375-412.
20. Ulloa-Aguirre A, Hanyaloglu AC, Zarinan T, Janovick JA. Protein homeostasis and regulation of intreacellular trafficking of G protein-coupled receptors. In: Pey AL (Ed) *Protein Homeostasis Diseases*, Elsevier Inc 2020: pp. 247–277.
21. Ulloa-Aguirre A, Janovick JA. Modulation of proteostasis and protein trafficking: a therapeutic avenue for misfolded G protein-coupled receptors causing disease in humans. *Emerg Top Life Sci*. 2019;3:39-52.
22. Ulloa-Aguirre A, Zarinan T, Gutierrez-Sagal R, Tao YX. Targeting trafficking as a therapeutic avenue for misfolded GPCRs leading to endocrine diseases. *Front Endocrinol (Lausanne)*. 2022;13:934685. doi:10.3389/fendo.2022.934685
23. Forloni G, Terreni L, Bertani I, et al. Protein misfolding in Alzheimer's and Parkinson's disease: genetics and molecular mechanisms. *Neurobiol Aging*. 2002;23(5):957-976.
24. Ramanan VK, Saykin AJ. Pathways to neurodegeneration: mechanistic insights from GWAS in Alzheimer's disease, Parkinson's disease, and related disorders. *Am J Neurodegener Dis*. 2013;2(3):145-175.
25. Hollingsworth TJ, Gross AK. Defective trafficking of rhodopsin and its role in retinal degenerations. *Int Rev Cell Mol Biol*. 2012;293:1-44. doi:10.1016/B978-0-12-394304-0.00006-3
26. Bruning A, Juckstock J. Misfolded proteins: from little villains to little helpers in the fight against cancer. *Front Oncol*. 2015;5:47. doi:10.3389/fonc.2015.00047
27. Bichet DG. [Nephrogenic diabetes insipidus]. *Nephrol Ther*. 2006;2(6):387-404.
28. Conn PM, Ulloa-Aguirre A, Ito J, Janovick JA. G protein-coupled receptor trafficking in health and disease: lessons learned to prepare for therapeutic mutant rescue in vivo. *Pharmacol Rev*. 2007;59(3):225-250. doi:10.1124/pr.59.3.2
29. Huang Y, Breitwieser GE. Rescue of calcium-sensing receptor mutants by allosteric modulators reveals a conformational checkpoint in receptor biogenesis. *J Biol Chem*. 2007;282(13):9517-9525.
30. Calebiro D, de Filippis T, Lucchi S, et al. Intracellular entrapment of wild-type TSH receptor by oligomerization with mutants linked to dominant TSH resistance. *Hum Mol Genet*. 2005;14(20):2991-3002. doi:10.1093/hmg/ddi329
31. Ulloa-Aguirre A, Janovick JA, Leanos-Miranda A, Conn PM. Misrouted cell surface GnRH receptors as a disease aetiology for congenital isolated hypogonadotrophic hypogonadism. *Hum Reprod Update*. 2004;10(2):177-192.
32. Tao YX. The melanocortin-4 receptor: physiology, pharmacology, and pathophysiology. *Endocr Rev*. 2010;31(4):506-543. doi:10.1210/er.2009-0037
33. Tao YX, Huang H. Ipsen 5i is a novel potent pharmacoperone for intracellularly retained melanocortin-4 receptor mutants. *Front Endocrinol (Lausanne)*. 2014;5:131. doi:10.3389/fendo.2014.00131
34. Tao YX, Conn PM. Chaperoning G protein-coupled receptors: from cell biology to therapeutics. *Endocr Rev*. 2014;35(4):602-47. doi:10.1210/er.2013-1121
35. Laurin S-A, Ahrari S, Bouvier M. Pharmacological chaperones: therapeutic potential for diseases resulting from GPCR misfolding. In: Kostic M, Jones LH, eds. *Protein Homeostasis in Drug Discovery: A Chemical Biology Perspective*. 1st ed. 2023. 65-134.
36. Garofalo R, Wohlgemuth I, Pearson M, Lenz C, Urlaub H, Rodnina MV. Broad range of missense error frequencies in cellular proteins. *Nucleic Acids Res*. 2019;47(6):2932-2945. doi:10.1093/nar/gky1319
37. Drummond DA, Wilke CO. The evolutionary consequences of erroneous protein synthesis. *Nat Rev Genet*. 2009;10(10):715-724. doi:10.1038/nrg2662
38. Duennwald ML, Echeverria A, Shorter J. Small heat shock proteins potentiate amyloid dissolution by protein disaggregases from yeast and humans. *PLoS Biol*. 2012;10(6):e1001346. doi:10.1371/journal.pbio.1001346
39. Chen X, Shi C, He M, Xiong S, Xia X. Endoplasmic reticulum stress: molecular mechanism and therapeutic targets. *Signal Transduct Target Ther*. 2023;8(1):352. doi:10.1038/s41392-023-01570-w
40. Schroder M, Kaufman RJ. ER stress and the unfolded protein response. *Mutat Res*. 2005;569(1-2):29-63. doi:10.1016/j.mrfmmm.2004.06.056
41. Hartl FU, Bracher A, Hayer-Hartl M. Molecular chaperones in protein folding and proteostasis. *Nature*. 2011;475(7356):324-332. doi:10.1038/nature10317
42. Hartl FU, Hayer-Hartl M. Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science*. 2002;295(5561):1852-1858.
43. Kozlov G, Gehring K. Calnexin cycle - structural features of the ER chaperone system. *FEBS J*. 2020;287(20):4322-4340. doi:10.1111/febs.15330
44. Lippincott-Schwartz J, Roberts TH, Hirschberg K. Secretory protein trafficking and organelle dynamics in living cells. *Annu Rev Cell Dev Biol*. 2000;16:557-89. doi:10.1146/annurev.cellbio.16.1.557
45. Genest O, Wickner S, Doyle SM. Hsp90 and Hsp70 chaperones: Collaborators in protein remodeling. *J Biol Chem*. 2019;294(6):2109-2120. doi:10.1074/jbc.REV118.002806
46. Mayer MP, Bukau B. Hsp70 chaperones: cellular functions and molecular mechanism. *Cell Mol Life Sci*. 2005;62(6):670-84. doi:10.1007/s00018-004-4464-6
47. Mayer MP, Gierasch LM. Recent advances in the structural and mechanistic aspects of Hsp70 molecular chaperones. *J Biol Chem*. 2019;294(6):2085-2097. doi:10.1074/jbc.REV118.002810
48. Pobre KFR, Poet GJ, Hendershot LM. The endoplasmic reticulum (ER) chaperone BiP is a master regulator of ER functions: Getting by with a little help from ERdj friends. *J Biol Chem*. 2019;294(6):2098-2108. doi:10.1074/jbc.REV118.002804

49. Anukanth A, Khorana HG. Structure and function in rhodopsin. Requirements of a specific structure for the intradiscal domain. *J Biol Chem*. 1994;269(31):19738-19744.
50. Lanctot PM, Leclerc PC, Escher E, Guillemette G, Leduc R. Role of N-glycan-dependent quality control in the cell-surface expression of the AT1 receptor. *Biochem Biophys Res Commun*. 2006;340(2):395-402. doi:10.1016/j.bbrc.2005.12.016
51. Mizrachi D, Segaloff DL. Intracellularly located misfolded glycoprotein hormone receptors associate with different chaperone proteins than their cognate wild-type receptors. *Mol Endocrinol*. 2004;18(7):1768-1777. doi:10.1210/me.2003-0406me.2003-0406
52. Siffroi-Fernandez S, Giraud A, Lanet J, Franc JL. Association of the thyrotropin receptor with calnexin, calreticulin and BiP. Efects on the maturation of the receptor. *Eur J Biochem*. 2002;269(20):4930-4937. doi:10.1046/j.1432-1033.2002.03192.x
53. Qiu XB, Shao YM, Miao S, Wang L. The diversity of the DnaJ/Hsp40 family, the crucial partners for Hsp70 chaperones. *Cell Mol Life Sci*. 2006;63(22):2560-2570. doi:10.1007/s00018-006-6192-6
54. Okiyoneda T, Apaja PM, Lukacs GL. Protein quality control at the plasma membrane. *Curr Opin Cell Biol*. 2011;23(4):483-491. doi:10.1016/j.ceb.2011.04.012
55. Duvernay MT, Dong C, Zhang X, Zhou F, Nichols CD, Wu G. Anterograde trafficking of G protein-coupled receptors: function of the C-terminal F(X)6LL motif in export from the endoplasmic reticulum. *Mol Pharmacol*. 2009;75(4):751-761. doi:10.1124/mol.108.051623
56. Duvernay MT, Filipeanu CM, Wu G. The regulatory mechanisms of export trafficking of G protein-coupled receptors. *Cell Signal*. 2005;17(12):1457-6145.
57. Duvernay MT, Zhou F, Wu G. A conserved motif for the transport of G protein-coupled receptors from the endoplasmic reticulum to the cell surface. *J Biol Chem*. 2004;279(29):30741-30750.
58. Wang G, Wu G. Small GTPase regulation of GPCR anterograde trafficking. *Trends Pharmacol Sci*. 2012;33(1):28-34. doi:10.1016/j.tips.2011.09.002
59. Bernier V, Morello JP, Zarruk A, et al. Pharmacologic chaperones as a potential treatment for X-linked nephrogenic diabetes insipidus. *J Am Soc Nephrol*. 2006;17(1):232-243. doi:10.1681/ASN.2005080854
60. Leanos-Miranda A, Janovick JA, Conn PM. Receptor-misrouting: an unexpectedly prevalent and rescuable etiology in gonadotropin-releasing hormone receptor-mediated hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*. 2002;87(10):4825-4828.
61. Topaloglu AK, Lu ZL, Farooqi IS, et al. Molecular genetic analysis of normosmic hypogonadotropic hypogonadism in a Turkish population: identification and detailed functional characterization of a novel mutation in the gonadotropin-releasing hormone receptor gene. *Neuroendocrinology*. 2006;84(5):301-308.
62. Topaloglu AK, Reimann F, Guclu M, et al. TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. *Nat Genet*. 2009;41(3):354-358. doi:10.1038/ng.306
63. Benke D, Zemoura K, Maier PJ. Modulation of cell surface GABA(B) receptors by desensitization, trafficking and regulated degradation. *World J Biol Chem*. 2012;3(4):61-72. doi:10.4331/wjbc.v3.i4.61
64. Angelotti T, Daunt D, Shcherbakova OG, Kobilka B, Hurt CM. Regulation of G-protein coupled receptor traffic by an evolutionary conserved hydrophobic signal. *Traffic*. 2010;11(4):560-578. doi:10.1111/j.1600-0854.2010.01033.x
65. Ma D, Zerangue N, Lin YF, et al. Role of ER export signals in controlling surface potassium channel numbers. *Science*. 2001;291(5502):316-319. doi:10.1126/science.291.5502.316
66. Albright JD, Reich MF, Delos Santos EG, et al. 5-Fluoro-2-methyl-N-[4-(5H-pyrrolo[2,1-c]-[1, 4]benzodiazepin-10(11H)-ylcarbonyl)-3-chlorophenyl]benzamide (VPA-985): an orally active arginine vasopressin antagonist with selectivity for V2 receptors. *J Med Chem*. 1998;41(14):2442-2444.
67. Hawtin SR. Pharmacological chaperone activity of SR49059 to functionally recover misfolded mutations of the vasopressin V1a receptor. *J Biol Chem*. 2006;281(21):14604-14614. doi:10.1074/jbc.M511610200
68. Robben JH, Knoers NV, Deen PM. Cell biological aspects of the vasopressin type-2 receptor and aquaporin 2 water channel in nephrogenic diabetes insipidus. *Am J Physiol Renal Physiol*. 2006;291(2):F257-F270. doi:10.1152/ajprenal.00491.2005
69. 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*. 2007;292(1):F253-F260. doi:10.1152/ajprenal.00247.2006
70. Li T, Sandberg MA, Pawlyk BS, Rosner B, Hayes KC, Dryja TP, Berson EL. Effect of vitamin A supplementation on rhodopsin mutants threonine-17 → methionine and proline-347 → serine in transgenic mice and in cell cultures. *Proc Natl Acad Sci USA*. 1998;95(20):11933-11938.
71. Riccardi D, Martin D. The role of the calcium-sensing receptor in the pathophysiology of secondary hyperparathyroidism. *NDT Plus*. 2008;1(Suppl 1):i7-i11. doi:10.1093/ndtplus/sfm038
72. Huang H, Wang W, Tao YX. Pharmacological chaperones for the misfolded melanocortin-4 receptor associated with human obesity. *Biochim Biophys Acta*. 2017;1863(10 Pt A):2496-2507. doi:10.1016/j.bbadis.2017.03.001
73. Janovick JA, Maya-Nunez G, Ulloa-Aguirre A, et al. Increased plasma membrane expression of human follicle-stimulating hormone receptor by a small molecule thienopyr(im)idine. *Mol Cell Endocrinol*. 2009;298(1-2):84-88. doi:10.1016/j.mce.2008.09.015
74. Newton CL, Anderson RC, Kreuchwig A, Krause G, Katz AA, Millar RP. Rescue of function of mutant luteinising hormone receptors with deficiencies in cell surface expression, hormone binding, and hormone signalling. *Neuroendocrinology*. 2021;111(5):451-464. doi:10.1159/000508000
75. Newton CL, Whay AM, McArdle CA, et al. Rescue of expression and signaling of human luteinizing hormone G protein-coupled receptor mutants with an allosterically binding small-molecule agonist. *Proc Natl Acad Sci USA*. 2011;108(17):7172-7176. doi:10.1073/pnas.1015723108
76. Conn PM, Ulloa-Aguirre A. Trafficking of G-protein-coupled receptors to the plasma membrane: insights for pharmacoperone drugs. *Trends Endocrinol Metab*. 2010;21(3):190-197. doi:10.1016/j.tem.2009.11.003
77. Conn PM, Ulloa-Aguirre A. Pharmacological chaperones for misfolded gonadotropin-releasing hormone receptors. *Adv Pharmacol*. 2011;62:109-141. doi:10.1016/B978-0-12-385952-5.00008-7
78. Conn PM, Smithson DC, Hodder PS, et al. Transitioning pharmacoperones to therapeutic use: in vivo proof-of-principle and design of high throughput screens. *Pharmacol Res*. 2014;83:38-51. doi:10.1016/j.phrs.2013.12.004
79. Janovick JA, Stewart MD, Jacob D, et al. Restoration of testis function in hypogonadotropic hypogonadal mice harboring a misfolded GnRHR mutant by pharmacoperone drug therapy. *Proc Natl Acad Sci USA*. 2013;110(52):21030-21035. doi:10.1073/pnas.1315194110
80. Ulloa-Aguirre A, Conn PM. Pharmacoperones as a New Therapeutic Approach: In Vitro Identification and In vivo Validation of Bioactive Molecules. *Curr Drug Targets*. 2016;17(13):1471-1481.
81. Ulloa-Aguirre A, Zarinan T, Dias JA, Conn PM. Mutations in G protein-coupled receptors that impact receptor trafficking and reproductive function. *Mol Cell Endocrinol*. 2014;382(1):411-423. doi:10.1016/j.mce.2013.06.024
82. Ulloa-Aguirre A, Janovick JA, Conn PM, Lira-Albarrán S. Mutations of gonadotropin-releasing hormone receptor gene. In: Huhtaniemi IML, ed. *Encyclopedia of Endocrine Diseases*. 3th. Edition. Elsevier Inc; 2024.

Chapter Mutations of Gonadotropin-releasing hormone receptor gene:704-712.
83. Newton CL, Anderson RC, Katz AA, Millar RP. Loss-of-function mutations in the human luteinizing hormone receptor predominantly cause intracellular retention. *Endocrinology*. 2016;157(11):4364-4377. doi:10.1210/en.2016-1104
84. Narayan P, Ulloa-Aguirre A, Dias JA. Gonadotropin hormones and their receptors. In: Strauss JF 3rd, Barbieri RL, Dokras A, Williams CJ, Williams Z, eds. *Yen & Jaffe's Reproductive Endocrinology*. 9th ed.: Elsevier; 2024. 23-55.
85. Ulloa-Aguirre A, Zarinan T, Gutierrez-Sagal R, Dias JA. Intracellular trafficking of gonadotropin receptors in health and disease. *Handb Exp Pharmacol*. 2018;245:1-39. doi:10.1007/164_2017_49
86. Hanyroup S, Anderson RC, Nataraja S, Yu HN, Millar RP, Newton CL. Rescue of cell surface expression and signalling of mutant follicle-stimulating hormone receptors. *Endocrinology*. 2021;162(12):bqab134 doi:10.1210/endocr/bqab134
87. Ulloa-Aguirre A, Zariñán T, Gutiérrez-Sagal R, Nataraja S, Yu HN, Jardón-Valadez E. OR03-01 functional rescue of trafficking-defective mutant follicle-stimulating hormone receptors (FSHR) leading to premature ovarian failure with the pharmacoperone CAN1405. *J Endocr Soc*. 2023;7(Supplement 1). doi:10.1210/jendso/bvad114.1557
88. Millar RP, Lu ZL, Pawson AJ, Flanagan CA, Morgan K, Maudsley SR. Gonadotropin-releasing hormone receptors. *Endocr Rev*. 2004;25(2):235-275.
89. Flanagan CA, Manilall A. Gonadotropin-releasing hormone (GnRH) receptor structure and GnRH binding. *Front Endocrinol (Lausanne)*. 2017;8:274. doi:10.3389/fendo.2017.00274
90. Arora KK, Chung HO, Catt KJ. Influence of a species-specific extracellular amino acid on expression and function of the human gonadotropin-releasing hormone receptor. *Mol Endocrinol*. 1999;13(6):890-896. doi:10.1210/mend.13.6.0291
91. Maya-Nunez G, Janovick JA, Ulloa-Aguirre A, Soderlund D, Conn PM, Mendez JP. Molecular basis of hypogonadotropic hypogonadism: restoration of mutant (E(90)K) GnRH receptor function by a deletion at a distant site. *J Clin Endocrinol Metab*. 2002;87(5):2144-2149.
92. Ulloa-Aguirre A, Janovick JA, Miranda AL, Conn PM. G-protein-coupled receptor trafficking: understanding the chemical basis of health and disease. *ACS Chem Biol*. 2006;1(10):631-638.
93. Beranova M, Oliveira LM, Bedecarrats GY, et al. Prevalence, phenotypic spectrum, and modes of inheritance of gonadotropin-releasing hormone receptor mutations in idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*. 2001;86(4):1580-1588.
94. Chevrier L, Guimiot F, de Roux N. GnRH receptor mutations in isolated gonadotropic deficiency. *Mol Cell Endocrinol*. 2011;346(1-2):21-28. doi:10.1016/j.mce.2011.04.018
95. Pitteloud N, Quinton R, Pearce S, et al. Digenic mutations account for variable phenotypes in idiopathic hypogonadotropic hypogonadism. *J Clin Invest*. 2007;117(2):457-463. doi:10.1172/JCI29884
96. Silveira LF, Trarbach EB, Latronico AC. Genetics basis for GnRH-dependent pubertal disorders in humans. *Mol Cell Endocrinol*. 2010;324(1-2):30-38. doi:10.1016/j.mce.2010.02.023
97. Sykiotis GP, Plummer L, Hughes VA, et al. Oligogenic basis of isolated gonadotropin-releasing hormone deficiency. *Proc Natl Acad Sci USA*. 2010;107(34):15140-15144. doi:10.1073/pnas.1009622107
98. Goncalves CI, Aragues JM, Bastos M, Barros L, Vicente N, Carvalho D, Lemos MC. GNRHR biallelic and digenic mutations in patients with normosmic congenital hypogonadotropic hypogonadism. *Endocr Connect*. 2017;6(6):360-366. doi:10.1530/EC-17-0104
99. Tao YX. Inactivating mutations of G protein-coupled receptors and diseases: structure-function insights and therapeutic implications. *Pharmacol Ther*. 2006;111(3):949-973. doi:10.1016/j.pharmthera.2006.02.008
100. Conn PM, Smith E, Hodder P, Janovick JA, Smithson DC. High-throughput screen for pharmacoperones of the vasopressin type 2 receptor. *J Biomol Screen*. 2013;18(8):930-937. doi:10.1177/1087057113483559
101. Conn PM, Ulloa-Aguirre A, Janovick JA. "Pharmacoperone": what's in a word? *Pharmacol Res*. 2013;83:1-2. doi:10.1016/j.phrs.2013.11.005
102. Smith E, Janovick JA, Bannister TD, et al. Rescue of mutant gonadotropin-releasing hormone receptor function independent of cognate receptor activity. *Sci Rep*. 2020;10(1):10579. doi:10.1038/s41598-020-67473-w
103. Janovick JA, Patney A, Mosely R, et al. Molecular mechanism of action of pharmacoperone rescue of misrouted GPCR mutants: the GnRH receptor. *Mol Endocrinol*. 2009;23(2):157-168.
104. Brothers SP, Cornea A, Janovick JA, Conn PM. Human loss-of-function gonadotropin-releasing hormone receptor mutants retain wild-type receptors in the endoplasmic reticulum: molecular basis of the dominant-negative effect. *Mol Endocrinol*. 2004;18(7):1787-1797.
105. Maya-Nunez G, Janovick JA, Aguilar-Rojas A, et al. Biochemical mechanism of pathogenesis of human gonadotropin-releasing hormone receptor mutants Thr104Ile and Tyr108Cys associated with familial hypogonadotropic hypogonadism. *Mol Cell Endocrinol*. 2011;337(1-2):16-23. doi:10.1016/j.mce.2011.01.016
106. Maya-Nunez G, Ulloa-Aguirre A, Janovick JA, Conn PM. Pharmacological chaperones correct misfolded GPCRs and rescue function: protein trafficking as a therapeutic target. *Subcell Biochem*. 2012;63:263-289. doi:10.1007/978-94-007-4765-4_14
107. Janovick JA, Conn PM. Use of pharmacoperones to reveal GPCR structural changes associated with constitutive activation and trafficking. *Methods Enzymol*. 2010;485:277-292. doi:10.1016/B978-0-12-381296-4.00016-6
108. Hou ZS, Ulloa-Aguirre A, Tao YX. Pharmacoperone drugs: targeting misfolded proteins causing lysosomal storage-, ion channels-, and G protein-coupled receptors-associated conformational disorders. *Expert Rev Clin Pharmacol*. 2018;11(6):611-624. doi:10.1080/17512433.2018.1480367
109. Janovick JA, Ulloa-Aguirre A. Cellular high-throughput screening. In: Pey AL (Ed) *Protein Homeostasis Deseases*, Elsevier Inc 2020:343-358.
110. Smithson DC, Janovick JA, Conn PM. Therapeutic rescue of misfolded/mistrafficked mutants: automation-friendly high-throughput assays for identification of pharmacoperone drugs of GPCRs. *Methods Enzymol*. 2013;521:3-16. doi:10.1016/B978-0-12-391862-8.00001-6
111. Jiang X, Liu H, Chen X, et al. Structure of follicle-stimulating hormone in complex with the entire ectodomain of its receptor. *Proc Natl Acad Sci USA*. 2012;109(31):12491-12496. doi:10.1073/pnas.1206643109
112. Bruser A, Schulz A, Rothemund S, Ricken A, Calebiro D, Kleinau G, Schoneberg T. The activation mechanism of glycoprotein hormone receptors with implications in the cause and therapy of endocrine diseases. *J Biol Chem*. 2016;291(2):508-520. doi:10.1074/jbc.M115.701102
113. Schulze A, Kleinau G, Neumann S, Scheerer P, Schöneberg T, Brüser A. The intramolecular agonist is obligate for activation of glycoprotein hormone receptors. *Faseb J*. 2020;34(8):11243-11256. doi:10.1096/fj.202000100R
114. Casarini L, Simoni M. Recent advances in understanding gonadotropin signaling. *Fac Rev*. 2021;10:41. doi:10.12703/r/10-41
115. Huhtaniemi IT, Themmen AP. Mutations in human gonadotropin and gonadotropin-receptor genes. *Endocrine*. 2005;26(3):207-217.
116. Adamson GD, Zegers-Hochschild F, Dyer S. Global fertility care with assisted reproductive technology. *Fertil Steril*. 2023;120(3 Pt 1):473-482. doi:10.1016/j.fertnstert.2023.01.013

117. Latronico AC, Arnhold IJ. Inactivating mutations of the human luteinizing hormone receptor in both sexes. *Semin Reprod Med*. 2012;30(5):382-386. doi:10.1055/s-0032-1324721
118. Kossack N, Troppmann B, Richter-Unruh A, Kleinau G, Gromoll J. Aberrant transcription of the LHCGR gene caused by a mutation in exon 6A leads to Leydig cell hypoplasia type II. *Mol Cell Endocrinol*. 2013;366(1):59-67. doi:10.1016/j.mce.2012.11.018
119. Lu X, Yan Z, Cai R, et al. Pregnancy and live birth in women with pathogenic LHCGR variants using their own oocytes. *J Clin Endocrinol Metab*. 2019;104(12):5877-5892. doi:10.1210/jc.2019-01276
120. Rivero-Muller A, Potorac I, Pintiaux A, et al. A novel inactivating mutation of the LH/chorionic gonadotrophin receptor with impaired membrane trafficking leading to Leydig cell hypoplasia type 1. *Eur J Endocrinol*. 2015;172(6):K27-K36. doi:10.1530/EJE-14-1095
121. Yuan P, He Z, Zheng L, et al. Genetic evidence of 'genuine' empty follicle syndrome: a novel effective mutation in the LHCGR gene and review of the literature. *Hum Reprod*. 2017;32(4):944-953. doi:10.1093/humrep/dex015
122. Toledo SP, Brunner HG, Kraaij R, et al. An inactivating mutation of the luteinizing hormone receptor causes amenorrhea in a 46,XX female. *J Clin Endocrinol Metabol*. 1996;81(11):3850-3854. doi:10.1210/jcem.81.11.8923827
123. Anderson RC, Newton CL, Anderson RA, Millar RP. Gonadotropins and their analogs: current and potential clinical applications. *Endocr Rev*. 2018;39(6):911-937. doi:10.1210/er.2018-00052
124. Nataraja SG, Yu HN, Palmer SS. Discovery and development of small molecule allosteric modulators of glycoprotein hormone receptors. *Front Endocrinol (Lausanne)*. 2015;6:142. doi:10.3389/fendo.2015.00142
125. Gerrits M, Mannaerts B, Kramer H, Addo S, Hanssen R. First evidence of ovulation induced by oral LH agonists in healthy female volunteers of reproductive age. *J Clin Endocrinol Metab*. 2013;98(4):1558-1566. doi:10.1210/jc.2012-3404
126. van Koppen C, Zaman G, Timmers C, et al. A signaling-selective, nanomolar potent allosteric low molecular weight agonist for the human luteinizing hormone receptor. *Naunyn-Schmiedeberg's Arch Pharmacol*. 2008;378(5):503-514. doi:10.1007/s00210-008-0318-3
127. Duan J, Xu P, Cheng X, et al. Structures of full-length glycoprotein hormone receptor signalling complexes. *Nature*. 2021;598(7882):688-692. doi:10.1038/s41586-021-03924-2
128. Rivero-Muller A, Huhtaniemi I. Genetic variants of gonadotrophins and their receptors: Impact on the diagnosis and management of the infertile patient. *Best Pract Res Clin Endocrinol Metab*. 2022;36(1):101596. doi:10.1016/j.beem.2021.101596
129. Tapanainen JS, Aittomaki K, Min J, Vaskivuo T, Huhtaniemi IT. Men homozygous for an inactivating mutation of the follicle-stimulating hormone (FSH) receptor gene present variable suppression of spermatogenesis and fertility. *Nat Genet*. 1997;15(2):205-206. doi:10.1038/ng0297-205
130. Beau I, Touraine P, Meduri G, et al. A novel phenotype related to partial loss of function mutations of the follicle stimulating hormone receptor. *J Clin Invest*. 1998;102(7):1352-1359.
131. Nataraja S, Sriraman V, Palmer S. Allosteric regulation of the follicle-stimulating hormone receptor. *Endocrinology*. 2018;159(7):2704-2716. doi:10.1210/en.2018-00317
132. Anderson RC, Newton CL, Millar RP. Small molecule follicle-stimulating hormone receptor agonists and antagonists. *Front Endocrinol (Lausanne)*. 2018;9:757. doi:10.3389/fendo.2018.00757
133. van Straten NC, Schoonus-Gerritsma GG, van Someren RG, et al. The first orally active low molecular weight agonists for the LH receptor: thienopyr(im)idines with therapeutic potential for ovulation induction. *Chembiochem*. 2002;3(10):1023-1026. doi:10.1002/1439-7633(20021004)3:10⟨1023::AID-CBIC1023>3.0.CO;2-9
134. Timmers CM, Loozen HJJ, inventors; Dihydrobenzoindazoles. Patent WO/2011/012674. 2011.
135. Fan J-Q, Valenzano, K., Lee G., Bouvier, M. Pharmacological chaperones for treating obesity. US Patent application number 20090312345 (2009). 2009.
136. Morello J, Salahpour A, Laperrière A, et al. Pharmacological chaperones rescue cell-surface expression and function of misfolded V2 vasopressin receptor mutants. *J Clin Investig*. 2000;105(7):887-895.
137. Anderson RC, Hanyroup S, Song YB, et al. Functional rescue of inactivating mutations of the human neurokinin 3 receptor using pharmacological chaperones. *Int J Mol Sci*. 2022;23(9). doi:10.3390/ijms23094587
138. Leidenheimer NJ, Ryder KG. Pharmacological chaperoning: a primer on mechanism and pharmacology. *Pharmacol Res*. 2014;83:10-19. doi:10.1016/j.phrs.2014.01.005
139. Janovick JA, Brothers SP, Cornea A, et al. Refolding of misfolded mutant GPCR: post-translational pharmacoperone action in vitro. *Mol Cell Endocrinol*. 2007;272(1-2):77-85. doi:10.1016/j.mce.2007.04.012
140. Song YB, Park SY, Park K, Hwang H, Carroll RS, Hsu VW, Kaiser UB. Trafficking-defective mutant PROKR2 cycles between endoplasmic reticulum and Golgi to attenuate endoplasmic reticulum stress. *Proc Natl Acad Sci USA*. 2022;119(8). doi:10.1073/pnas.2102248119
141. Kincaid MM, Cooper AA. ERADicate ER stress or die trying. *Antioxid Redox Signal*. 2007;9(12):2373-2387. doi:10.1089/ars.2007.1817
142. Kincaid MM, Cooper AA. Misfolded proteins traffic from the endoplasmic reticulum (ER) due to ER export signals. *Mol Biol Cell*. 2007;18(2):455-463. doi:10.1091/mbc.E06-08-0696
143. Janovick JA, Knollman PE, Brothers SP, et al. Regulation of G protein-coupled receptor trafficking by inefficient plasma membrane expression: molecular basis of an evolved strategy. *J Biol Chem*. 2006;281(13):8417-8425.
144. Finch AR, Sedgley KR, Armstrong SP, Caunt CJ, McArdle CA. Trafficking and signalling of gonadotrophin-releasing hormone receptors: an automated imaging approach. *Br J Pharmacol*. 2010;159(4):751-760. doi:10.1111/j.1476-5381.2009.00413.x
145. Jiang X, Fischer D, Chen X, et al. Evidence for follicle-stimulating hormone receptor as a functional trimer. *J Biol Chem*. 2014;289(20):14273-14282. doi:10.1074/jbc.M114.549592
146. Janovick JA, Goulet M, Bush E, Greer J, Wettlaufer DG, Conn PM. Structure-activity relations of successful pharmacologic chaperones for rescue of naturally occurring and manufactured mutants of the gonadotropin-releasing hormone receptor. *J Pharmacol Exp Ther*. 2003;305(2):608-614.
147. Dias JA, Campo B, Weaver BA, et al. Inhibition of follicle-stimulating hormone induced preovulatory follicles in rats treated with a nonsteroidal negative allosteric modulator of follicle-stimulating hormone receptor. *Biol Reprod*. 2014;9019:1-11. doi:10.1095/biolreprod.113.109397

How to cite this article: Ulloa-Aguirre A, Anderson RC, Zariñán T, Gutiérrez-Sagal R, Jardón-Valadez E, Newton CL. Folding, misfolding, and regulation of intracellular traffic of G protein-coupled receptors involved in the hypothalamic–pituitary–gonadal axis. *Andrology*. 2025;1-14. https://doi.org/10.1111/andr.70018