Functional Rescue of Kallmann Syndrome-associated Prokineticin Receptor 2 (PKR2) Mutants Deficient in Trafficking*

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Dan-Na Chen‡, Yan-Tao Ma§, Huadie Liu‡, Qun-Yong Zhou‡, and Jia-Da Li¶

From the ‡State Key Laboratory of Medical Genetics, Central South University, Changsha, Hunan 410078, China, the §Department of Basic Medical Sciences, Changsha Medical University, Changsha, Hunan 410219, China, the ¶Department of Pharmacology, Hubei University of Science and Technology, 88 Xiangming Road, Xianning, Hubei 437100, China, and the §§Department of Pharmacology, University of California, Irvine, California 92697

Background: Some Kallmann syndrome-associated PKR2 receptors are retained intracellularly.

Results: A PKR2 antagonist A457 rescued P290S PKR2, and glycerol rescued P290S and W178S PKR2.

Conclusion: Certain disease-associated PKR2 can be functionally rescued by chemical or pharmacological chaperones.

Significance: Our results provide a potential therapeutic strategy for patients bearing such mutations.

Mutations in the G protein-coupled prokineticin receptor 2 (PKR2) are known to cause Kallmann syndrome and idiopathic hypogonadotropic hypogonadism manifesting with delayed puberty and infertility. Some of the mutant receptors are not routed to the cell surface; instead, they are trapped in the cellular secretory pathway. The cell-permeant agonists/antagonists have been used to rescue some membrane receptors that are not targeted onto the cell membrane. Here, we chose three disease-associated mutations (W178S, G234D, and P290S), which all resulted in retention of PKR2 intracellularly. We show that a small molecule PKR2 antagonist (A457) dramatically increased cell surface expression and rescued the function of P290S PKR2, but had no effect on W178S and G234D PKR2. Furthermore, we also tested chemical chaperone glycerol on the cell surface expression and function of PKR2 mutants. Treatment with 10% glycerol significantly increased the cell surface expression and signaling of P290S and W178S PKR2. These data demonstrate that some Kallmann syndrome-associated, intracellularly retained mutant PKR2 receptors can be functionally rescued, suggesting a potential treatment strategy for patients bearing such mutations.

Kallmann syndrome (KS) is a disease that combines hypogonadotropic hypogonadism and anosmia/hyposmia (1, 2). The hypogonadism results from disruption in the embryonic migration of neuroendocrine gonadotropin-releasing hormone-synthesizing cells from the nasal cavity to their final destiny in the hypothalamus of the brain (3, 4), whereas anosmia or hyposmia is related to the absence or hypoplasia of the olfactory bulbs and tracts (5). KS is a highly heterogeneous genetic disease: more than 10 genes have been identified in KS patients, but mutations in these genes only account for ~40% of KS patients (5–7).

PKR2 and PK2, which encode the G protein-coupled prokineticin receptor 2 and its ligand prokineticin 2, respectively, are two KS-associated genes (8–10). PKR2 and PK2 were identified by a candidate gene strategy based on the phenotypes of the mice deficient in the corresponding genes. Indeed, mice deficient in either PKR2 or PK2 phenocopy many of the KS-like features, i.e. atrophy of both olfactory bulb and the reproductive system, due to markedly reduced number of gonadotropin-releasing hormone-synthesizing cells in the hypothalamus (11–13). The prevalence of PKR2 mutations is approximately 5% in KS patients from the general population; however, PKR2 mutations are found in 23.3% of the Maghrebian patients, presented with monogenic recessive or digenic/oligogenic transmission (14). Interestingly, one of PKR2 mutations, P290S, is identified in >5% of the Maghrebian KS patients (14).

PKR2 is a G protein-coupled receptor that regulates diverse biological processes (15, 16). KS-associated PKR2 mutations may result in disruption of ligand binding, G protein coupling, or deficiency in the trafficking onto the cell membrane (17–20). Mutant receptors with misfolding can be retained in the endoplasmic reticulum (ER) of the cellular secretory pathway. Some cell-permeant small molecule antagonist/agonists, however, can interact with intracellularly retained receptors and therefore aid their folding and transport to the plasma membrane. Such compounds, namely pharmacological chaperones, therefore have important therapeutic potential (21–24). Moreover, a group of so-called “chemical chaperones” may facilitate protein refolding by stabilizing the conformation of a mutant protein or by manipulating the chaperones residing in the ER (25, 26).

In this study, we investigated the effect of a small molecule PKR2 antagonist and a chemical chaperone (glycerol) on the
surface expression and signaling of mutant PKR2 which are deficient in the membrane expression. Our data indicated that some of these mutants can be rescued, opening a new therapeutic avenue for KS patients.

EXPERIMENTAL PROCEDURES

**Plasmids**—The human PKR2 tagged with a FLAG epitope at the N terminus was constructed as described (20). Plasmids encoding PKR2 harboring various mutations were engineered using the QuikChange mutagenesis kit (Stratagene, La Jolla, CA) according to the manufacturer’s instructions. All the constructs were confirmed by Sanger sequencing.

**Immunofluorescence Staining**—Human embryonic kidney (HEK) 293 cells were plated on coverslips and transfected with wild-type (WT) or respective mutant PKR2-expressing plasmids. After various treatments, cells were fixed in 4% paraformaldehyde for 30 min at room temperature. For permeabilization, cells were treated with 1% Triton X-100 for 1 h at room temperature. Cells were then incubated with blocking solution (PBS, 3% bovine serum albumin, 5% goat serum) for 1 h. Incubation with mouse anti-FLAG M2 (1:200 dilution; Sigma) was performed at 4 °C overnight. After being washed with PBS, fluorescence-labeled secondary anti-mouse antibodies (1:400 dilution; Invitrogen) were incubated for 2 h at room temperature in a dark room. After incubation with 4,6-diamidino-2-phenylindole (DAPI; Invitrogen) for 5 min, cells were mounted in Fluoromount medium (Sigma), and fluorescence images were examined with a laser scanning confocal system installed on a Carl Zeiss microscope (Zeiss, Gottingen, Germany) with a 63× oil immersion objective.

**Quantification of the Fluorescence Density**—To quantify the cell surface expression level, nonpermeabilized cells were immunostained as described above. For each condition, 50 fields were randomly selected, and images were taken. The fluorescence density of each image was calculated using the Metaphor software package. The average fluorescence density from cells expressing WT PKR2 was set as 100. All experiments were performed at least twice with a different batch of cells.

**Calcium Mobilization Assay**—An aequorin-based luminescent assay was used to measure mobilization of intracellular Ca$^{2+}$. Briefly, Chinese hamster ovary cells stably expressing the photoprotein aequorin were transiently transfected with WT or respective mutant PKR2-expressing plasmids. Two days after transfection, the cells were charged in Opti-MEM (Invitrogen) containing 8 μM coelenterazine cp (Invitrogen) at 37 °C for 2 h. Cells were detached by brief trypsinization and maintained in assay buffer (Hanks’ balanced salt solution plus 10 mM HEPES, pH 7.5, and 0.1% bovine serum albumin) at approximately 5 × 10^5 cells/ml. Luminescence measurements were made using a Sirius Single Tube Luminometer (Berthold Detection Systems GmbH, Pforzheim, Germany). PK2 was serially diluted in assay buffer and mixed with cells. The luminescence was monitored for 35 s (peak 1), then 100 μl of 1% Triton X-100 was injected to lyse the cells, and the luminescence was monitored continuously for another 20 s (peak 2). The area under the curve of the peak 1 was divided by the total area under the curve of peaks 1 and 2, and the maximal responses from WT receptors were normalized to 100.

**Molecular Modeling**—Three-dimensional structures of PKR2 were modeled with the intensive mode of the Phyre2 server. The sequences of WT and respective mutant PKR2 were submitted to the Phyre2 server, and six templates (c2ksaa (human substance-P receptor), c2rh1A (human β2-adrenergic receptor), c3emLA (human adenosine A2A receptor), c3ulnA (human M2 muscarinic acetylcholine receptor), c4djhA (human κ-type opioid receptor), and c3pdsA (human β2-adrenergic receptor)) were selected to model the protein based on heuristics to maximize the confidence, percentage identity, and alignment coverage.

**Statistical Analyses**—A repeated measures ANOVA followed by unpaired t test was used to analyze the data for differences. All statistical analyses were performed using Prism 4.4 (GraphPad Software, San Diego, CA).

RESULTS

**PKR2 Mutants (W178S, G234D, and P290S) Are Retained Intracellularly**—We chose three KS-associated PKR2 mutants that are reported to be deficient in the cell surface expression, including W178S, G234D, and P290S (10, 18). These mutations are located at the fourth, fifth, and sixth transmembrane domains, respectively (Fig. 1A). The subcellular localization of N-terminally FLAG-tagged WT or mutant PKR2 was determined by immunofluorescence using an anti-FLAG antibody. As shown in Fig. 1B, the WT receptor was localized predominantly on the cell surface. In contrast, all of the mutant receptors were undetectable on the cell surface, consistent with previous report using radioligand binding and cell surface enzyme-linked immunosorbent assay (18). Instead, they were trapped intracellularly as detected with immunofluorescence in the permeabilized cells (Fig. 1B).

Structural modeling of WT and three mutant PKR2 was made to understand how these mutations affect the likely three-dimensional structure of PKR2. As shown in Fig. 2A, the W178S mutation did not lead to significant structure change. However, the G234D mutation resulted in disruption in transmembrane domain III (amino acids 120–160, Fig. 2, A and B), whereas the P290S mutation led to disruption in transmembrane domain IV (amino acids 165–190) and subtle distortion in transmembrane domain VI (amino acids 270–300, Fig. 2, A, C, and D).

**Rescue of Cell Surface Expression of P290S PKR2 with a Small Molecule Antagonist**—Some small molecule agonists/antagonists, so called pharmacological chaperones, can interact with intracellularly retained receptors and aid their folding and/or transport onto the plasma membrane (21–24, 26). We recently synthesized a group of compounds that can specifically inhibit ligand-induced PKR2 signaling (27–29). As shown in Fig. 3A, a small molecule, A457, suppressed the PKR2 signaling with an IC$_{50}$ of 102 nM (PK2: 40 nM, EC$_{50}$ 7.8 nM).

By using immunofluorescence, we showed that treatment with A457 (1 μM) for 16 h dramatically increased the cell surface expression of P290S PKR2 (5.87 ± 0.66 versus 101.88 ± 7.61, p < 0.001, n = 50) whereas it did not affect that of W178S (4.36 ± 0.42 versus 6.08 ± 0.48, p > 0.5, n = 50) and G234D PKR2 (1.30 ± 0.18 versus 5.09 ± 0.51, p > 0.05, n = 50) (Fig. 3, B and C). The effect of A457 on the cell surface expression of...
P290S PKR2 was dose- and time-dependent (Fig. 3, D and E). A subtle increase was observed after incubation with A457 (1 μM) for 1–2 h (0 h: 8.02 ± 0.64; 1 h: 18.19 ± 2.13, p > 0.05; 2 h: 18.45 ± 1.48, p > 0.05, n = 50), and longer incubation (>4 h) steadily increased the cell surface expression of P290S PKR2 (4 h: 27.52 ± 4.67, p < 0.05; 8 h: 51.45 ± 4.26, p < 0.0001; 16 h: 123.58 ± 9.53, p < 0.0001; n = 50; Fig. 3D). Starting from 10 nM, A457 dose-dependently increased the cell surface expression of P290S PKR2 (0 nM: 8.94 ± 0.71; 1 nM: 21.28 ± 3.02, p > 0.05; 10 nM: 60.43 ± 4.32, p < 0.001; 100 nM: 83.34 ± 3.88, p < 0.001; 1 μM: 100.10 ± 5.65, p < 0.001; n = 50; Fig. 3E).

**A457 Rescues the Signaling of P290S PKR2**—As A457 potently inhibited PKR2 signaling (Fig. 3A), we washed A457-treated cells with PBS three times and measured the PKR2 signaling 8 h later, which efficiently eliminated the inhibitory effect of A457 on PKR2 signaling (data not shown). PK2 dose-dependently increased the intracellular calcium concentration in cells expressing WT, but not three mutant PKR2 (Fig. 4, A–C). However, incubation with A457 (1 μM) for 8 h significantly promoted the signaling of P290S PKR2 (Fig. 4A). No signaling was observed in cells transfected with W178S or G234D PKR2 after A457 treatment (Fig. 4, B and C).

The rescue of signaling of P290S PKR2 by antagonist was also dose- and time-dependent. Starting from 100 nM, a significant increase in the signaling was observed after A457 treatment (0 nM: 7.61 ± 2.48; 1 nM: 8.44 ± 3.33, p > 0.05; 10 nM: 23.55 ± 8.34, p > 0.05; 100 nM: 46.84 ± 9.28, p < 0.05; 1 μM: 63.60 ± 5.60, p < 0.01; n = 3; Fig. 4D), consistent with the cell surface expression (Fig. 3E). Moreover, the rescue of signaling could be observed as early as 2 h after incubation with 1 μM A457 (0 h: 7.16 ± 2.01; 1 h: 29.12 ± 1.75, p > 0.05; 2 h: 38.66 ± 0.04, p < 0.01; 4 h: 41.08 ± 0.67, p < 0.01; 8 h: 49.48 ± 1.37; 16 h: 90.69 ± 15.98, p < 0.001; n = 3; Fig. 4E). It is interesting that incubation with 1 μM A457 for 2 h only subtly increased the cell surface expression (Fig. 3D). We thus speculated that some of the mutant receptors may traffic onto the cell surface during the washout period (8 h) after A457 treatment, especially considering that 10 nM A457 was able to promote the cell surface of P290S PKR2 (Fig. 3E). We therefore compared the cell surface expression of P290S PKR2 with or without 8 h of washout after treatment with 1 μM A457 for 1–4 h. As shown in Fig. 4F, 8 h of washout indeed significantly increased the cell surface expression (0 h: 6.49 ± 0.52 versus 10.41 ± 0.65, p > 0.05; 1 h: 14.72 ± 1.72 versus 28.22 ± 2.66, p < 0.01; 2 h: 14.93 ± 1.20 versus 46.28 ± 2.88, p < 0.01; 4 h: 22.27 ± 3.78 versus 65.84 ± 3.68, p < 0.01; n = 50).

New Protein Synthesis Is Not Required for the Rescue of Mutant PKR2 by A457—The folding and synthesis of a protein may occur simultaneously. To see whether the pharmacologi-
cal chaperone A457 has to be present at the time of protein synthesis to function, we incubated cells with 100 nM cycloheximide (CHX) to inhibit protein synthesis. As shown in Fig. 5, CHX treatment (16 h) only slightly affected the signaling of WT PKR2. PK2 was not able to induce calcium immobilization in cells transfected with P290S PKR2 regardless of the presence or absence of CHX. Preincubation with A457 led to significant signaling of P290S PKR2 even in the presence of CHX. Our data thus indicated that new protein synthesis is not required for A457 to rescue P290S PKR2. Instead, A457 may stabilize the conformation of the previously misfolded/retained PKR2 receptors and aid their route onto the plasma membrane.

**Glycerol Functionally Rescues W178S and P290S PKR2**—The differential effect of A457 on the cell surface expression and signaling of PKR2 mutants may be attributable to either the drug or the mutations, or both. The so-called chemical chaperones, such as glycerol and dimethyl sulfoxide, may broadly promote the protein folding by decreasing the solvent-accessible surface area of the protein (30, 31). To dissect the mechanism underlying the differential effect of A457 on mutant PKR2, we further investigated the effect of glycerol on the cell surface and signaling of above-mentioned PKR2 mutants.

As shown in Fig. 6, A and B, 10% glycerol increased the cell surface expression of P290S (5.87 ± 0.66 versus 38.25 ± 3.34, p < 0.001, n = 50) and W178S (4.36 ± 0.42 versus 41.04 ± 2.82, p < 0.001, n = 50) PKR2, but not G234D (1.30 ± 0.18 versus 3.23 ± 0.86, p > 0.05, n = 50) PKR2. We therefore used P290S PKR2 as a model to study the incubation time on the cell surface expression. As shown in Fig. 6C, no significant increase of cell surface expression was observed until incubation with glycerol for 4 h (0 h: 10.77 ± 1.23; 1 h: 13.33 ± 1.67, p > 0.05; 2 h: 10.34 ± 1.03, p > 0.05; 4 h: 24.74 ± 3.07, p < 0.01; 8 h: 36.40 ± 4.25, p < 0.001; 16 h: 58.06 ± 5.10, p < 0.001; n = 50). Both W178S and P290S PKR2 rescued by glycerol were functional as measured with an aequorin-based calcium mobilization assay (Fig. 7).

The effect of glycerol is concentration-dependent. As shown in Fig. 8, A, 1% glycerol showed minimal effect on the cell surface expression of P290S PKR2 (control: 6.12 ± 0.96; 1%: 14.87 ± 0.93, p > 0.05, n = 50); however, treatment with 4 and 10% glycerol for 8 h significantly increased cell surface expression of P290S PKR2 (4%: 29.70 ± 3.18, p < 0.001; 10%: 46.84 ± 3.89, p < 0.001; n = 50). Consistent with the cell surface expression, 4 and 10% glycerol significantly increased the PK2-induced calcium mobilization in cells expressing P290S PKR2 (control: 11.91 ± 1.62; 1%: 19.76 ± 1.59, p > 0.05; 4%: 39.25 ± 5.67, p < 0.001; 10%: 54.41 ± 2.49, p < 0.001; n = 3; Fig. 8B).

**DISCUSSION**

The ER and Golgi apparatus are the cellular organelles where synthesis and processing of secretory and membrane protein occur. The ER provides an environment for newly synthesized polypeptide to fold properly. The correctly folded proteins are rescued by chemical chaperones such as glycerol and dimethyl sulfoxide.
then allowed to enter the processing pathway at the Golgi apparatus, leading to their final destinations within the cell. However, if a polypeptide does not fold correctly, the incorrect protein conformation must be recognized by the quality control system in the ER (32–34). The proteins that do not fulfill the criteria of the ER quality control system are retained in the ER and/or submitted to degradation (35). These general principles are applicable for the trafficking of G protein-coupled receptors, including some unique chaperones (36, 37). A few examples of diseases caused by conformational defects of proteins include cystic fibrosis (38), hypogonadotropic hypogonadisms (39), nephrogenic diabetes insipidus (40), retinitis pigmentosa (41), hypercholesterolemia (42), cataracts and neurodegenerative diseases (43).

Several strategies, including genetic, chemical, and pharmacological approaches, have been used to correct folding and rescue the function of trafficking-defective proteins, especially membrane receptors. Among these, pharmacological chaperones, i.e. small compounds that bind selectively to misfolded proteins and correct their folding and routing onto the plasma membrane, seems to be the most promising (44, 45). In the case of G protein-coupled receptors, most pharmacological chaperones are antagonists or agonists to the receptors. In a pioneer work, Morello et al. showed that treatment with a selective, cell-permeant vasopressin V2 receptor antagonist SR121463A rescue the cell surface targeting and function of several mutant V2 receptors associated with nephrogenic diabetes insipidus (21). Recently, Newton et al. showed that an allosterically binding small molecule agonist functionally rescue the mutant luteinizing hormone receptors that cause Leydig cell hypoplasia (24). The in vivo application of such strategy was demonstrated by Conn and colleagues (46). They showed that pharmacological chaperones could restore the testis function in hypogonadotropic hypogonadal mice harboring a misfolded gonadotropin-releasing hormone receptor mutant (46).

More than 20 different PKR2 mutations have been identified to be associated with KS idiopathic hypogonadotropic hypogonadism, and six (R80C, L173R, W178S, G234D, V274D, and

FIGURE 3. A small molecule PKR2 antagonist rescued the cell surface expression of P290S PKR2. A, A457 dose-dependently inhibited PK2-induced PKR2 signaling as assayed with an aequorin-base calcium mobilization assay. The structure of A457 is shown on the top. B, preincubation with A457 (1 μM) for 16 h significantly increased the cell surface expression of P290S, but not W178S or G234D PKR2. ***, p < 0.001, two-way ANOVA analysis followed by post hoc Bonferroni’s t analysis. Error bars, S.E. C, cells expressing FLAG-tagged WT or mutant (W178S, G234D, and P290S) PKR2 were treated with A457 (1 μM) for 16 h, and immunofluorescence staining was performed on nonpermeabilized cells. The receptors are shown in green, and nuclei are stained with DAPI (blue). Scale bar, 10 μm. D, the relative cell surface expression of P290S PKR2 was quantified after being treated with A457 (1 μM) for various periods of time (0–16 h). *, p < 0.05; ***, p < 0.001, one-way ANOVA analysis followed by post hoc Dunnett’s t analysis. E, the relative cell surface expression of P290S PKR2 was quantified after 16-h treatment with A457 at various concentrations (0–1 μM). ***, p < 0.001, one-way ANOVA analysis followed by post hoc Dunnett’s t analysis.
were reported to affect the trafficking (10, 18). We chose three mutations that are located in the transmembrane domain and deficient in trafficking to study the rescuing effect of a small molecule PKR2 antagonist, A457. Our results indicated that A457 can rescue the cell surface expression and function of P290S PKR2, but not W178S and G234D PKR2. It should be noted that G234D cannot be rescued by A457 or glycerol, implying that G234D mutation may result in a distorted conformation unfavorable for either strategy. We speculate that the introduction of a negatively charged aspartic acid may cause conformational changes and/or damage intramolecular interaction in PKR2 receptor. Indeed, the structural modeling indicated that G234D mutation can cause the disruption of transmembrane domain III (amino acids 120–160, Fig. 2, A and B).

Another strategy to prevent misfolding or correct a mutant protein conformation is to influence the protein folding environment inside the cell. Studies suggest that chemical chaperones, such as glycerol and dimethyl sulfoxide, are effective in inhibiting the formation of misfolded structure by decreasing the solvent-accessible surface area of the protein. In this study, we showed that 4–10% glycerol increased the cell surface expression of W178S were rescued by glycerol, but not A457. We speculate that the incapability of A457 to rescue W178S PKR2 may be attributable to its binding pocket on the receptor, i.e. A457 may only affect the conformation around its binding site. We thus propose that A457 binds to a site close to the fourth transmembrane domain, which was predicted to be disrupted in P290S mutant. Actually, A457 was also able to subtly rescue V274D PKR2 (data not shown), which may also distort the fourth transmembrane domain (20). Nevertheless, the precise mechanism will not be clear until a co-crystal structure is solved.

FIGURE 4. A457 rescued the signaling of P290S PKR2. A–C, PK2-induced signaling of P290S (A), W178S (B), and G234D (C) PKR2 before and after treatment with 1 μM A457 for 8 h measured with an aequorin-based calcium mobilization assay. Cells expressing WT PKR2 were used as a control. D, PK2-induced P290S PKR2 signaling before and after 8-h treatment with A457 of various concentrations. *, p < 0.05; **, p < 0.01, one-way ANOVA analysis followed by post hoc Dunnett’s t analysis. In A–E, cells were treated with A457 or vehicle for indicated period, and then washed three times with PBS. The PK2-induced calcium mobilization was measured at 8 h after washout. Experiments were performed at least three times with a different batch of cells with similar results.

FIGURE 5. New protein synthesis was not required for A457 to rescue P290S PKR2. Cells transfected with WT or P290S PKR2 were either left untreated or treated with A457 (1 μM), CHX (100 nM), or A457 (1 μM) plus CHX (100 nM) for 8 h. Cells were then washed three times with PBS and cultured in the medium for 8 h before PK2-induced calcium mobilization was measured. In the CHX-treated group, 100 nM CHX was included the culture medium until calcium mobilization assay. Experiments were performed at least three times with a different batch of cells with similar results.
Rescue of Trafficking-defective PKR2

**FIGURE 6.** 10% glycerol increased the cell surface expression of W178S and P290S PKR2. A, cells expressing FLAG-tagged WT or mutant (W178S, G234D, and P290S) PKR2 were treated with 10% glycerol for 8 h, and immunofluorescence staining was performed on nonpermeabilized cells. The receptors are shown in green, and nuclei are stained with DAPI (blue). Scale bar, 10 μm. B, 10% glycerol significantly increased the cell surface expression of P290S and W178S PKR2. ***, \( p < 0.001 \); two-way ANOVA analysis followed by post hoc Bonferroni’s analysis. C, the relative cell surface expression of P290S PKR2 was quantified after treated with 10% glycerol for various periods of time (0–16 h). **, \( p < 0.01 \); ***, \( p < 0.001 \), one-way ANOVA analysis followed by post hoc Dunnett’s t analysis.

**FIGURE 7.** 10% glycerol rescued the function of W178S and P290S PKR2. Cells expressing W178S (A), G234D (B), and P290S (C) PKR2 were treated with 10% glycerol for 8 h and washed three times with PBS, and PK2-induced calcium mobilization was measured 8 h later. Cells expressing WT PKR2 were used as a control. Experiments were performed at least three times with a different batch of cells with similar results.

**FIGURE 8.** Glycerol rescued the cell surface expression and signal transduction of P290S PKR2 in a concentration-dependent manner. A, cells expressing P290S PKR2 were treated with 0, 1, 4, and 10% glycerol for 8 h, and the relative cell surface expression of P290S PKR2 was quantified. B, after 8-h treatment with glycerol of various concentrations, cells expressing P290S PKR2 were washed three times with PBS, and PK2-induced calcium mobilization was measured 8 h later. Experiments were performed at least three times with a different batch of cells with similar results. ***, \( p < 0.001 \), one-way ANOVA analysis followed by post hoc Dunnett’s t analysis.
cued W178S showed signaling activity almost identical to that of the WT PKR2 receptor, implying that W178S mutation only affects the trafficking. However, although the signaling of P290S PKR2 can be rescued by A457 and glycerol, the efficiency (EC$_{50}$) and maximal response were lower than WT PKR2, indicating that the P290S mutation somehow interferes with the ligand binding and/or G protein coupling, in addition to trafficking. The structural modeling indicated that W178S mutation only disrupted two transmembrane helices (amino acids 165–190 and 270–300, respectively).

In summary, we demonstrated that a small molecule PKR2 antagonist and glycerol can rescue the cell surface expression and signaling of certain Kallmann syndrome-associated PKR2 mutants with trafficking deficiency. Our study thus provides a proof of concept for using pharmacological chaperone to treat patients harboring trafficking-defective PKR2 mutations.

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