HERG Is Protected from Pharmacological Block by α-1,2-Glucosyltransferase Function*†‡¶

Received for publication, June 22, 2006, and in revised form, December 7, 2006 Published, JBC Papers in Press, December 21, 2006, DOI 10.1074/jbc.M605976200

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The HERG (human ether-a-go-go-related gene) protein, which underlies the cardiac repolarizing current $I_{Kr}$, is the unintended target for many pharmaceutical agents. Inadvertent block of $I_{Kr}$, known as the acquired long QT syndrome (aLQTS), is a leading cause for drug withdrawal by the United States Food and Drug Administration. Hence, an improved understanding of the regulatory factors that protect most individuals from aLQTS is essential for advancing clinical therapeutics in broad areas, from cancer chemotherapy to antipsychotics and antidepressants. Here, we show that the K$^+$ channel regulatory protein KCR1, which markedly reduces $I_{Kr}$ drug sensitivity, protects HERG through glucosyltransferase function. KCR1 and the yeast α-1,2-glucosyltransferase ALG10 exhibit sequence homology, and like KCR1, ALG10 diminished HERG block by dofetilide. Inhibition of cellular glycosylation pathways with tunicamycin abrogated the effects of KCR1, as did expression in Lec1 cells (deficient in glycosylation). Moreover, KCR1 complemented the growth defect of an alg10-deficient yeast strain and enhanced glycosylation of an Alg10 substrate in yeast. HERG itself is not the target for KCR1-mediated glycosylation because the dofetilide response of glycosylation-deficient HERG(N598Q) was still modulated by KCR1. Nonetheless, our data indicate that the α-1,2-glucosyltransferase function is a key component of the molecular pathway whereby KCR1 diminishes $I_{Kr}$, drug response. Incorporation of in vitro data into a computational model indicated that KCR1 expression is protective against arrhythmias. These findings reveal a potential new avenue for targeted prevention of aLQTS.

$I_{Kr}$, an important component of the cardiac delayed rectifier K$^+$ current, is required for repolarization of the human cardiac action potential. Mutations in HERG (human ether-a-go-go-related gene), which encodes the α-subunit of the channel complex, are responsible for the chromosome 7-linked form (LQT2) of the congenital long QT syndrome (LQTS),2 an arrhythmia syndrome characterized by action potential prolongation and delayed cardiac repolarization. LQTS causes the atypical polymorphic ventricular tachycardia torsade de pointes, leading to sudden cardiac death (1–3). A far more commonly acquired form of LQTS (aLQTS) is precipitated by exposure to a wide range of therapeutic compounds that block HERG. aLQTS represents a significant problem for public health and the pharmaceutical industry3 and is typically observed in predisposed patients receiving treatment for non-cardiac illnesses. Indeed, about half of all drug withdrawals since 1998 were in response to potential pro-arrhythmic effects linked to aLQTS (5–7).

Numerous drug families bind the HERG channel and suppress $I_{Kr}$, in vitro, yet, surprisingly, few patients experience aLQTS. Clinically silent ion channel gene mutations have been characterized in families with cases of aLQTS, indicating that even the drug-induced form of the disorder may carry a genetic component (8–15). However, mutations in the gene that encodes HERG are associated with only a small percentage of the aLQTS cases, suggesting that genetic variations in an array of other molecules modulate HERG pharmacology, either enhancing or reducing HERG block and aLQTS risk.

The K$^+$ channel regulator KCR1 (38) protects heterologously expressed HERG current ($I_{HERG}$) from block by the antiarrhythmic drugs dofetilide, quinidine, and sotalol over clinically relevant concentration ranges (16). Moreover, a polymorphism associated with a reduced risk for aLQTS was shown to enhance KCR1-induced protection of $I_{HERG}$ from drug block in vitro (17). Here, we show that KCR1 protects $I_{HERG}$ through an unanticipated biochemical function, α-glucosyltransferase activity. The closest known homolog of KCR1, the endoplasmic reticulum (ER)-resident yeast α-glucosyltransferase Alg10 (Die2) (18), protects HERG from drug block in a manner identical to that of KCR1, and conversely, KCR1 functionally substitutes for Alg10 in yeast. Additionally, KCR1 modulation of HERG pharmacology is dependent upon a fully functioning cellular glycosylation machinery. These findings reveal an unprecedented mechanism for HERG pharmacological regulation and suggest an important new avenue for developing targeted interventions for preventing aLQTS.

*This work was supported by NHLBI Grants HL69914 (to S. K.) and P01 PPG HL46681 (to J. R. B.) from the National Institutes of Health and by a grant-in-aid from the American Heart Association (to S. K.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†The on-line version of this article (available at http://www.jbc.org) contains supplemental Fig. 1.

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1 The abbreviations used are: LQTS, long QT syndrome; aLQTS, acquired long QT syndrome; ER, endoplasmic reticulum; GFP, green fluorescent protein; CHO, Chinese hamster ovary; TM, tunicamycin; PBS, phosphate-buffered saline; CPY, carboxypeptidase Y; WT, wild-type.

2 Vincent, G. M. (2005) Romano-Ward Syndrome, www.genetests.org.


Plasmid Constructs—The HERG cDNA was subcloned into the mammalian expression vector pSI (Promega Corp., Madison, WI) as described previously (19). The HERG(N598Q) mutation was introduced into the same backbone using the Stratagene QuikChange site-directed mutagenesis kit. The human KCR1 cDNA was subcloned into the pcDNA vector for bicistronic expression with enhanced green fluorescent protein (GFP) (16). ALG10 (kindly provided by Dr. Markus Aebi, ETH Zurich, Zurich, Switzerland) was inserted into the same vector for mammalian cell expression. For expression in yeast, KCR1 and ALG10 were subcloned into the pGPD246 shuttle vector (a kind gift from Dr. Tony Weil, Vanderbilt University), which allows for selection on uracil-deficient medium (20). The 3’-end of the KCR1 cDNA was fused in-frame with cyan fluorescent protein by insertion into the Accl and SmaI sites of the pECFP-1 vector (Clontech). For in-frame fusion with the DsRed protein, KCR1 was subcloned into the Xhol/SacII sites of the pDsRed2-C1 vector (Clontech). pEYFP-ER was purchased from Clontech. The KCNE2 cDNA was amplified by PCR from human genomic DNA using the upstream primer 5’-TTAAATTGCTGGCCATGTCTATTTATCCAAATTTCACACAG-3’ and the downstream primer 5’-AACTGCA- GTCAAGGGGACATTGAACC-5’. For subcloning, HindIII and PstI sites (boldface) were built into the primer sequence. The start and stop codons are shown in italics. The T8A mutation was included in the upstream primer by substituting the underlined A for G in a separate upstream primer. cDNAs were subcloned into the pcGI vector for bicistronic expression with enhanced GFP.

Electrophysiology—Chinese hamster ovary (CHO) K1 cells (American Type Culture Collection, Manassas, VA) or Lecl cells (ATCC CRL-1735) were cultured in 6-cm Petri dishes and transiently transfected with HERG (1 μg) plus the pcGI vector (1 μg), with HERG (1 μg) plus KCR1 (1 μg), or with HERG (1 μg) plus ALG10 (1 μg) using FuGene 6 transfection reagent (Roche). The same conditions were used for experiments performed with the HERG(N598Q) mutant. The cells were cultured as described (16). Cells expressing green fluorescence were chosen for current recordings. Membrane currents were recorded essentially as described (16). Data acquisition was carried out using an Axopatch 200B amplifier and pCLAMP8.0 software (Axon Instruments, Foster City, CA).

A bath solution containing dofetilide was prepared from a 100 μM dofetilide stock in water on the day of experimentation. Tunicamycin (TM) was purchased from Sigma. After HERG current was recorded under control conditions, dofetilide (at the concentrations listed in each figure legend) was applied to the bath solution, followed by repetitive depolarizing pulses to +20 mV (2 s) from a holding potential of −80 mV (6 s) and by a hyperpolarizing pulse of −50 mV (2 s). The decline of relative tail currents during repetitive pulses in the presence of dofetilide was fitted to a single exponential function: 

\[ y = y_0 + Ae^{-t/\tau} \]

where \( y_0 \) is the offset, \( A \) is the current amplitude under the control condition, \( t \) is the time, and \( \tau \) is the time constant. All data are expressed as the means ± S.E., and statistical comparisons were made using one-way analysis of variance (Origin, MicroCal, LLC, Northampton, MA), with \( p < 0.05 \) indicating significance.

Yeast Transformation—The culture and transformation of yeast were accomplished essentially as described previously (21). KCR1 or ALG10 cDNA contained in the pGPD426 vector or pGPD426 alone was transformed into the Alg10-deficient wbp1Δ/Δ alb1Δ/Δ yeast strain YG649. Transformed cells (2 × 10^6) were counted in a hemocytometer and inoculated into uracil-deficient synthetic dextrose medium plus 1 M sorbitol. In parallel, the same numbers of SS328 and untransformed YG649 control cells were grown in uracil-deficient synthetic dextrose medium plus uracil and 1 M sorbitol. 54.5 h post-transformation, yeast protein extracts were prepared as described below.

Western Blotting—Whole cell protein extracts were prepared from transfected CHO or Lecl cells 2 days post-transfection as described previously (16). The membranes were probed with anti-HERG antibody (1:400 dilution; Alomone Labs) in phosphate-buffered saline (PBS) and 5% nonfat dry milk, and proteins were visualized with horseradish peroxidase-linked anti-rabbit F(ab’)2 antibody (1:10,000 dilution; Amersham Biosciences) and detected by ECL (Amersham Biosciences). Protein extracts from yeast cells were prepared by pelleting yeast cultures. The samples were heated to 70 °C in lysis buffer (1% SDS, 50 mM Tris-HCl [pH 7.5], and 2 mM phenylmethylsulfonyl fluoride) for 10 min in the presence of glass beads, and vortexed. The pellet was extracted again by boiling, and supernatants were saved. 20 μg of the sample was loaded onto 10% SDS-polyacrylamide gel; Western blot analysis was performed using anti-carboxypeptidase Y (CPY) monoclonal antibody (1:5000 dilution; Molecular Probes) in 1× PBS/Tween plus 5% nonfat dry milk and 2% bovine serum albumin; and proteins were detected with horseradish peroxidase-linked anti-mouse secondary antibody (1:10,000 dilution; Sigma) using ECL.

Confocal Imaging—A triple-FLAG epitope was fused in-frame with amino acid 2 of the N terminus of rat KCR1 contained on the p3XFLAG-CMV vector (Sigma). Mouse atrial tumor myocyte HL-1 cells (22) were transfected with FLAG-KCR1 and split onto fibronectin-coated chamber slides 24 h post-transfection. 48 h post-transfection, cells were fixed in 4% paraformaldehyde in PBS and permeabilized for 20 min with 0.2% Triton X-100 in blocking solution (0.16 g of bovine serum albumin and 0.4 ml of goat serum in 10 ml of PBS). Cells were washed with PBS and incubated with fluorescein isothiocyanate-linked anti-FLAG monoclonal antibody M2 (Sigma) in PBS and 2% bovine serum albumin for 1 h. This was followed by three PBS washes and a 1-h incubation with rabbit anti-calnexin polyclonal antibody (1:200 dilution; Stressgen Bioreagents, Victoria, British Columbia, Canada) and by three PBS washes and a 45-min incubation with Cy5-conjugated AffiniPure donkey anti-rabbit F(ab’)_2 antibody (1:150 dilution; Jackson ImmunoResearch Laboratories, Inc. West Grove, PA) to reveal calnexin localization. The slides were washed with PBS and mounted in AquaMount (Lerner Laboratories). Images were acquired on a Zeiss Axioskop LSM 510 inverted confocal microscope with a Plan Apochromat ×63/1.4 oil differential interference contrast objective (ScanZOOM 1.0). The absorption/emission wavelengths used were 650/670 nm for Cy5 detection, 492/520 nm for fluorescein iso-
**RESULTS**

**KCR1 Shares Sequence and Functional Characteristics with the Yeast Alg10 Protein**—KCR1 (GenBankTM accession number AY845858) shows 28% amino acid identity (43% similarity) to ALG10 (DIE2; accession number D38049), which encodes the *Saccharomyces cerevisiae* α-1,2-glucosyltransferase (Fig. 1). This enzyme is responsible for the addition of the terminal α-1,2-glucose residue to lipid-linked oligosaccharides in the yeast ER, a step that is thought to be necessary for the efficient transfer of oligosaccharides to nascent proteins (Fig. 2). Confocal imaging of FLAG-tagged KCR1 transfected into the mouse atrial tumor-derived cell line HL-1 (22) and the endogenous ER marker calnexin showed that the distribution pattern of KCR1 was consistent with ER localization (Fig. 3). Similar findings were obtained when DsRed-linked KCR1 and the ER marker enhanced yellow fluorescent protein were transfected into HL-1 cells (supplemental Fig. 1).

To test whether KCR1 and Alg10 also share functional characteristics in mammalian cells, we examined whether Alg10, like KCR1 (16), modulates drug block of $I_{\text{HERG}}$. To this end, we transfected HERG alone or cotransfected HERG with KCR1 or ALG10 into CHO cells and assessed drug block of $I_{\text{HERG}}$ using the pulse protocol shown in Fig. 4A. As in our previous experiments, we added the antiarrhythmic dofetilide to the bath solution, applied repetitive pulses, and recorded $I_{\text{HERG}}$ tail currents (Fig. 4B). The pulse-dependent reduction of tail current amplitude was fitted to a single exponential function as described under “Experimental Procedures.” Analogous to KCR1, coexpressing ALG10 and HERG significantly slowed drug block of $I_{\text{HERG}}$ (Fig. 4C), suggesting that KCR1 modulates drug block of $I_{\text{HERG}}$ via glycosylation.

We incorporated the data from Fig. 4C into the Luo-Rudy dynamic model of the mammalian action potential (23). The results show that HERG block sufficient to evoke action potential prolongation and development of early afterdepolarizations (the cellular precursor to torsade de pointes) was reversed by KCR1 effects on $I_{\text{HERG}}$ equivalent to those measured *in vitro* (Fig. 4D).

**KCR1-dependent HERG “Protection” Requires Intact Glycosylation Pathways**—To pursue this idea, we manipulated the cellular glycosylation pathway at multiple points (Fig. 2). TM is an antibiotic that blocks the initial step of glycoprotein synthesis, thus inhibiting the synthesis of all N-linked glycoproteins. HERG-transfected or HERG/KCR1-cotransfected cells were

![FIGURE 1. Amino acid sequence alignment of the yeast α-glucosyltransferase Alg10 and human KCR1. Identities are indicated in yellow, and similarities are indicated in green.](image)

![FIGURE 2. Schematic overview of the processes investigated in this study.](image)
preincubated with or without TM (1 μg/ml) for 24 h prior to current recording, and the drug response of \( I_{\text{HERG}} \) to dofetilide was assessed using the pulse protocol shown in Fig. 4A. Preincubating HERG-transfected cells with TM did not influence the drug response of \( I_{\text{HERG}} \) (Fig. 5A). The lack of effect of TM on HERG drug block allowed us to examine whether KCR1 is still able to influence \( I_{\text{HERG}} \) drug block when \( N \)-linked cellular glycosylation is inhibited. Preincubating HERG/KCR1-cotransfected cells with TM prevented KCR1 from diminishing the drug sensitivity of \( I_{\text{HERG}} \) effectively abrogating the effects of KCR1 (Fig. 5B). Western blot analysis of total cell extracts derived from transfections done with or without 1 μg/ml TM at various time points confirmed the effectiveness of the TM treatment in preventing the glycosylation of HERG, which was used as an indicator of glycosylation status (Fig. 5C).

Lec1 cells are derived from CHO cells with defective \( N \)-acetylgalactosamine transferase activity in the Golgi apparatus, resulting in a defect in \( N \)-linked glycosylation and producing only truncated cell-surface glycoproteins (Fig. 2) (24–26). If \( N \)-linked glycosylation is a requirement for the KCR1-mediated modulation of drug block of \( I_{\text{HERG}} \) the KCR1 effect should not be observed in Lec1 cells. HERG alone or with KCR1 was transfected into Lec1 cells, and drug block of \( I_{\text{HERG}} \) was assessed using the pulse protocol shown in Fig. 4A. Coexpressing KCR1 and HERG in Lec1 cells did not influence drug block of \( I_{\text{HERG}} \) (Fig. 6A). We used the glycosylation status of HERG to verify that Lec1 cells do not support glycosylation. As Western blot analysis showed (Fig. 6B), both the core and fully glycosylated forms of HERG are produced in CHO cells, whereas the fully glycosylated form is not produced in Lec1 cells. These findings further suggest that KCR1 modulates drug block of \( I_{\text{HERG}} \) through glycosylation.

**Human KCR1 Rescues the Growth Defect Caused by alg10 Deficiency in Yeast**—Although we have shown that Alg10 functionally mimics KCR1 to modulate \( I_{\text{HERG}} \) drug block in CHO cells, it is still possible that the two proteins yield effects on HERG pharmacology through distinct mechanisms. Hence, we sought to determine whether KCR1 can supply Alg10 function in yeast. The alg10 mutant yeast strain YG651 does not have a detectable growth defect, whereas the alg10/wbp1-2 double mutant strain YG649 is growth-defective at 30 °C. The growth defect of YG649 can be complemented by the ALG10 gene (18). We subcloned the yeast ALG10 and human KCR1 cDNAs into a yeast expression vector with a URA selectable marker and transformed them into the YG649 yeast strain, selecting for growth on uracil-deficient medium. Fig. 7A shows that YG649 transformed with either KCR1 or ALG10 exhibited robust growth on uracil-deficient synthetic dextrose medium after 2 days at 30 °C, whereas untransformed cells did not grow, and cells transformed with vector alone grew poorly. This suggests that, like Alg10, KCR1 can rescue the defect of the YG649 strain.

In yeast, Alg10 functions as an \( \alpha \)-glucosyltransferase, the activity
of which can be assessed biochemically using the endogenous substrate CPY (18). CPY is a yeast vacuolar protease with four N-linked oligosaccharides (27) and therefore migrates at five different apparent molecular masses on a Western blot. We prepared yeast protein extracts from the wild-type (WT) strain SS328; YG649 mutants; and YG649 transformed with human KCR1, yeast ALG10, or the empty vector alone and performed Western blot analysis using anti-CPY antibody. Fig. 7B shows that, as expected, SS328 produced the mature form of CPY, whereas YG649 contained primarily the incompletely glycosylated forms of CPY. The addition of TM to the growth medium did not alter the extent of CPY glycosylation in YG649-transformed cells, whereas KCR1 expression in YG649 abolished the effects of TM on CPY glycosylation. The results of these experiments indicate that KCR1 does not modulate the drug response of HERG in CHO cells probed with anti-HERG antibody and assessed unglycosylated and fully glycosylated forms. After incubation with TM (1 μg/ml) for the indicated time points (24, 30, and 48 h), HERG was no longer glycosylated.

A

B

C

Transcytosis

205 kDa

130 kDa

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glycosylated

unglycosylated

FIGURE 5. Inhibiting the glycosylation pathway with TM does not alter basal drug response, but abrogates the effects of KCR1 on HERG. A, HERG-transformed CHO cells were preincubated with or without TM (1 μg/ml) for 24 h prior to current recording. Drug block (60 nM dofetilide) of HERG was assessed using the pulse protocol shown in Fig. 4A. Preincubating HERG-transformed cells with TM did not alter the $I_{\text{HERG}}$ drug response. The time constants ($\tau$) of the decline of peak tail currents were 485 ± 57 s ($n$ = 7) for HERG without TM (□) and 520 ± 78 s ($n$ = 6, p = not significant) for HERG with TM (△). B, HERG/KCR1 cotransfected CHO cells were preincubated with or without TM (1 μg/ml) for 24 h prior to current recording. Preincubating HERG/KCR1 cotransfected cells with TM abolished the effects of KCR1 on $I_{\text{HERG}}$. The time constants ($\tau$) of the decline of peak tail currents were 489 ± 60 s ($n$ = 6) for HERG without TM (□), 790 ± 85 s ($n$ = 6, $p < 0.05$ versus HERG without TM) for HERG plus KCR1 without TM (△), and 486 ± 47 s ($n$ = 7, $p < 0.05$ versus HERG plus KCR1 without TM) for HERG plus KCR1 with TM (○). C, using the glycosylation status of HERG as a surrogate marker for the effectiveness of the TM treatment, we performed Western blot analysis of HERG-transformed CHO cells probed with anti-HERG antibody and assessed unglycosylated and fully glycosylated forms. After incubation with TM (1 μg/ml) for the indicated time points (24, 30, and 48 h), HERG was no longer glycosylated.

A

B

FIGURE 6. KCR1 does not modulate $I_{\text{HERG}}$ pharmacology in a glycosylation-deficient CHO cell line (Lec1). A, HERG cDNA and the GFP vector (HERG alone) or HERG and KCR1 cDNAs were transfected into Lec1 cells, and drug block (60 nM dofetilide) of HERG was assessed using the pulse protocol shown in Fig. 4A. Coexpressing KCR1 and HERG in Lec1 cells no longer modulated the drug response of $I_{\text{HERG}}$. The time constants ($\tau$) of the decline of peak tail currents were 442 ± 33 s ($n$ = 7) for HERG alone (□) and 540 ± 66 s ($n$ = 8, p = not significant) for HERG plus KCR1 (○). B, Western blot analysis of HERG showed both core (C) and fully (F) glycosylated forms in CHO cells. In contrast, a fully glycosylated form did not appear in Lec1 cells.

A

B

FIGURE 7. KCR1 rescues the alg10 defect in yeast. A, yeast strain YG649 (wbp1-2/alg10) was transformed with the empty vector (quadrant 1), vector plus KCR1 (quadrant 2), or vector plus ALG10 (quadrant 3) or left untransformed (quadrant 4) and plated on uracil-deficient synthetic dextrose selective growth medium. B, whole cell protein extracts were prepared from the transformed and control yeast strains and processed by Western blot analysis (10% SDS-PAGE) using an anti-CPY antibody. First lane, YG649 transformed with KCR1; second lane, transformed with ALG10; third lane, transformed with empty vector; fourth lane, untransformed; fifth lane, SS328 (WT). Molecular mass markers (kilodaltons) are indicated on the left. The positions of the −4, −3, −2, −1, and mature (mCPY) glycosylated forms of CPY are indicated on the right.

A

B

FIGURE 8. Inhibiting the glycosylation pathway with TM does not alter basal drug response, but abrogates the effects of KCR1 on HERG. A, HERG-transformed CHO cells were preincubated with or without TM (1 μg/ml) for 24 h prior to current recording. Drug block (60 nM dofetilide) of HERG was assessed using the pulse protocol shown in Fig. 4A. Preincubating HERG-transformed cells with TM did not alter the $I_{\text{HERG}}$ drug response. The time constants ($\tau$) of the decline of peak tail currents were 485 ± 57 s ($n$ = 7) for HERG without TM (□) and 520 ± 78 s ($n$ = 6, p = not significant) for HERG with TM (△). B, HERG/KCR1 cotransfected CHO cells were preincubated with or without TM (1 μg/ml) for 24 h prior to current recording. Preincubating HERG/KCR1 cotransfected cells with TM abolished the effects of KCR1 on $I_{\text{HERG}}$. The time constants ($\tau$) of the decline of peak tail currents were 489 ± 60 s ($n$ = 6) for HERG without TM (□), 790 ± 85 s ($n$ = 6, $p < 0.05$ versus HERG without TM) for HERG plus KCR1 without TM (△), and 486 ± 47 s ($n$ = 7, $p < 0.05$ versus HERG plus KCR1 without TM) for HERG plus KCR1 with TM (○). C, using the glycosylation status of HERG as a surrogate marker for the effectiveness of the TM treatment, we performed Western blot analysis of HERG-transformed CHO cells probed with anti-HERG antibody and assessed unglycosylated and fully glycosylated forms. After incubation with TM (1 μg/ml) for the indicated time points (24, 30, and 48 h), HERG was no longer glycosylated.
HERG itself is not a likely target for KCR1-mediated glycosylation. A, WT HERG cDNA and the GFP vector, HERG(N598Q) cDNA and the GFP vector (N598Q), or HERG(N598Q) and KCR1 cDNAs was transfected into CHO cells, and drug block (60 nm dofetilide) of \( I_{\text{HERG}} \) was assessed using the pulse protocol shown in Fig. 4A. The drug response of HERG(N598Q) was diminished in the presence of KCR1. The time constants \( \tau \) of the decline of peak tail currents were 525 ± 56 s (\( n = 5 \)) for WT HERG alone (■), 436 ± 51 s (\( n = 7 \), \( p = 0.05 \) versus WT HERG alone and HERG(N598Q) alone) for HERG(N598Q) alone (○), 1177 ± 126 s (\( n = 6 \), \( p < 0.05 \) versus WT HERG alone and HERG(N598Q) alone) for HERG(N598Q) alone (○), and 1177 ± 126 s (\( n = 6 \), \( p < 0.05 \) versus WT HERG alone and HERG(N598Q) alone) for HERG(N598Q) plus KCR1 (△). B, Western blot analysis showed that WT HERG supported both core (C) and fully (F) glycosylated forms in CHO cells. In contrast, HERG(N598Q) supported only the unglycosylated form.

HERG alone, HERG(N598Q) alone, or HERG(N598Q) plus KCR1 were transfected into CHO cells, and drug block of \( I_{\text{HERG}} \) was assessed using the protocol shown in Fig. 4A. As shown in Fig. 8A, drug block of HERG(N598Q) was still modulated by KCR1. To confirm the glycosylation status of HERG(N598Q), we performed Western blot analysis, which showed a lack of the fully glycosylated form (Fig. 8B). This indicates that, although N-linked glycosylation is essential for KCR1-mediated protection of \( I_{\text{HERG}} \), glycosylation of HERG alone does not render this protection.

KCNE2 (MiRP1) co-assembles with HERG (31, 32), although the functional implications have been debated (33), and it has been difficult to demonstrate expression of KCNE2 in human heart (34). The KCNE2 polymorphism T8A, which abrogates complex glycosylation of KCNE2, mildly increases \( I_{\text{Kr}} \) sensitivity to the antibiotic sulfamethoxazole (35). To address whether KCNE2 can modulate intracellular block of HERG by dofetilide in response to glycosylation, we first tested the HERG drug response in the presence of WT KCNE2 or KCNE2(T8A), but observed no significant differences (Fig. 9A). Moreover, KCR1 still attenuated drug block of \( I_{\text{HERG}} \) regardless of whether WT KCNE2 or KCNE2(T8A) was coexpressed (Fig. 9B). Hence, N-linked glycosylation of neither HERG nor KCNE2 underlies the glycosylation-dependent protection of HERG from dofetilide block.

**DISCUSSION**

The most frequent cause of aLQTS is the unintended block of \( I_{\text{Kr}} \) by commonly used pharmaceutical agents (7, 36), leading to impaired repolarization of cardiac myocytes, action potential prolongation, and potentially fatal ventricular arrhythmias. It is now recognized that aLQTS has a genetic component, as patients with increased vulnerability for the syndrome and carrying mutations in LQTS-linked genes have been identified (11). However, not all family members who carry specific gene mutations develop aLQTS, suggesting “incomplete penetrance” and implying that hidden factors influence the pathology in individual patients (37). Moreover, the vast majority of patients do not experience aLQTS despite well documented in vitro block of HERG at clinically relevant concentrations of numerous therapeutic agents. Hence, it is likely that constitutive regulatory mechanisms usually protect patients from aLQTS, and disruption of such pathways could constitute risk.

We have shown that KCR1, a protein of previously unknown function, diminishes the effects of the HERG blockers dofetilide, sotalol, and quinidine on \( I_{\text{Kr}} \). In a follow-up study, the human “gain-of-function” KCR1 polymorphism I447V was associated with reduced risk of aLQTS (17). Here, we have identified a surprising mechanism whereby KCR1 influences the pharmacology of \( I_{\text{HERG}} \). The closest homolog of KCR1 in the public data bases is the yeast Alg10/Die2 protein (Fig. 1), the gene of which encodes an ER-resident α-1,2-glucosyltransferase (18, 38). In fact, based on sequence homology to the yeast enzyme, KCR1 is identified as an α-1,2-glucosyltransferase (accession number CAC41349) in the NCBI Protein Database, although, to the best of our knowledge, biochemical evidence in support of this classification has not been reported. This enzyme is responsible for the addition of the terminal α-1,2-
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glucose residue to the nascent lipid-linked oligosaccharide (Fig. 2). Lack of the terminal α-1,2-glucose residue impedes substrate recognition of the next enzyme in the pathway, oligosaccharyltransferase, thereby resulting in reduced transfer of the pre-assembled oligosaccharide trees to nascent polypeptides.

In this study, we approached the hypothesis that glycosylation impacts KCR1-mediated drug regulation of \( I_{\text{HERG}} \) from multiple directions. (i) We showed that the yeast α-1,2-glucosyltransferase Alg10 modulated drug block of \( I_{\text{HERG}} \) in the same manner as KCR1. (ii) Inhibition of the glycosylation pathway with TM abrogated the effects of KCR1 on drug block of \( I_{\text{HERG}} \), indicating that \( N \)-glycosylation is required for KCR1 to exert an effect on HERG. The drug sensitivity of HERG alone was not altered in the presence of TM, and \( KCR1 \) must be coexpressed with \( HERG \) for the diminished drug sensitivity to manifest. (iii) Similar to results in the presence of TM, \( KCR1 \) also did not influence the pharmacological properties of \( I_{\text{HERG}} \) in Lec1 cells, a CHO cell line with a defect in the Golgi-resident \( N \)-acetylglucosamine transferase activity. This corroborates our conclusion that intact \( N \)-glycosylation is required for KCR1 to influence \( I_{\text{HERG}} \) pharmacology.

Having demonstrated that the yeast \( ALG10 \) gene product is functionally homologous to KCR1 with respect to channel-drug interactions, we assessed the biochemical activity of KCR1 in \( alg10 \)-deficient yeast. Our results showed that KCR1 was able to rescue the growth characteristics of an \( alg10 \)-deficient yeast strain. Moreover, we demonstrated enzyme activity by providing evidence that KCR1 biochemically modified a test substrate of Alg10 in yeast (Fig. 7), leading us to conclude that KCR1 is a functional homolog of Alg10.

When we exogenously expressed epitope-tagged KCR1 in cardiac HL-1 cells (22), we noted expression in the ER (Fig. 3 and supplemental Fig. 1), consistent with its proposed function as an \( \alpha \)-glucosyltransferase. However, KCR1 was not distributed evenly throughout the entire ER. This pattern may reflect compartmentalization within the ER, but is most likely a function of relatively weaker KCR1 expression levels compared with the ER marker.

The glycosylation status of HERG and its processing and maturation within the ER and Golgi are under intense scrutiny (39–46) largely because of numerous congenital LQT2 mutations judged to be “trafficking-defective” (47, 48). Such mutants never reach the plasma membrane because of abortive intracellular processing and cause a loss of \( \bar{I}_{\text{kr}} \) function in afflicted LQT2 patients. Nevertheless, we have excluded the possibility that glycosylation of HERG alone or in complex with KCNE2 explains the \( \alpha \)-1,2-glucosyltransferase effect of reducing \( I_{\text{HERG}} \) drug block (Figs. 8 and 9).

In summary, our experiments strongly support the notion that KCR1 modulates drug block of \( I_{\text{HERG}} \) through enhancing cellular glycosylation by acting as an \( \alpha \)-1,2-glucosyltransferase. Incorporation of our data derived in vitro into a computational model of the ventricular action potential potential corroborated our conclusion that KCR1 can play a protective role in aLQTS (Fig. 4D).

We consider it unlikely that KCR1 simply causes a protective “umbrella” of increasingly glycosylated surface membrane proteins, which then keep drugs from accessing the interior of the cell, because a prior experiment indicated that KCR1 maintains its protective effect even if the drug (dofetilide) is applied through an intracellular pipette solution (16). Rather, our data suggest that the overall glycosylation efficiency of the cell impacts \( I_{\text{HERG}} \) drug sensitivity. This implies that the status of the cellular glycosylation pathway may constitute a risk factor for aLQTS, motivating future studies of this biochemical pathway as a means of targeted prevention of drug-induced arrhythmias.

Acknowledgments—We thank Dr. Christina Petersen for helpful discussions throughout this work, Dr. Tony Weil for advice about the yeast experiments and the gift of vector pGD426, and Dr. Markus Aebl for the generous gift of mutant and WT yeast strains. HL-1 cells were a kind gift from Dr. William Claycomb (Louisiana State University).

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