Thapsigargin Selectively Rescues the Trafficking Defective LQT2 Channels G601S and F805C*

Several mutations in the human ether-a-go-go-related K⁺ channel gene (HERG or KCNH2) cause long QT syndrome (LQT2) by reducing the intracellular transport (trafficking) of the channel protein to the cell surface. Drugs that bind to and block HERG channels (i.e. E4031) rescue the surface expression of some trafficking defective LQT2 mutations. Because these drugs potently block HERG current, their ability to correct congenital LQT is confounded by their risk of causing acquired LQT. We tested the hypothesis that pharmacological rescue can occur without HERG channel block. Thapsigargin (1 μM), a sarcoplasmic/endoplasmic reticulum Ca²⁺-ATPase inhibitor, rescued the surface expression of G601S, and it did so without blocking current. Thapsigargin-induced rescue and E4031-induced rescue caused complex glycosylation that was evident within 3 h of drug exposure. Disruption of the Golgi apparatus with brefeldin A prevented thapsigargin- and E4031-induced rescue of G601S. Confocal imaging showed that G601S protein is predominantly “trapped” intracellularly and that both thapsigargin and E4031 promote its relocation to the surface membrane. We also studied two other trafficking defective LQT2 mutations. Thapsigargin rescued the C terminus mutation F805C but not N470D, whereas E4031 rescued N470D but not F805C. Other sarcoplasmic/endoplasmic reticulum Ca²⁺-ATPase inhibitors did not rescue G601S or F805C. This study supports the hypothesis that the LQT2 trafficking defective phenotype can be reversed without blocking the channel; 2) demonstrates pharmacological rescue of a C terminus LQT2 mutation; and 3) shows that thapsigargin can correct trafficking defective phenotypes in more than one channel type and disease (i.e. LQT2 and cystic fibrosis).

MUTATIONS IN THE HUMAN ETHER-A-GO-GO-RELATED K⁺ CHANNEL GENE (HERG OR KCNH2) K⁺ CHANNEL CAUSE LONG QT SYNDROME (LQT2) BY REDUCING FUNCTIONAL HERG CURRENT (IHERG). SEVERAL LQT2 MUTATIONS DISRUPT ION CHANNEL TRANSPORT (TRAFFICKING) OUT OF THE ENDOPLECTIC RETICULUM (ER) (1). THE SURFACE MEMBRANE EXPRESSION IN SOME OF THESE TRAFFICKING DEFECTIVE LQT2 MUTATIONS CAN BE INCREASED (RESCUED) IN CELLS EXPRESSING MUTANT CHANNELS BY REDUCING CELL CULTURE TEMPERATURE (<30 °C) AND/OR INCUBATING IN DRUGS THAT CAUSE HIGH AFFINITY HERG CHANNEL BLOCK (2–6). THE RESCUE OF TRAFFICKING DEFECTIVE LQT2 CHANNELS CAN INCREASE FUNCTIONAL IHERG, AND THEREFORE HAS THERAPEUTIC POTENTIAL.

We tested the hypothesis that we could pharmacologically rescue trafficking defective LQT2 channels without using drugs that block IHERG. Our approach has similarity to cystic fibrosis, where the predominant mutation, ΔF508 (deletion of phenylalanine at position 508 in the CFTR transporter), prevents the trafficking of the CFTR channel protein to the surface membrane (for review, see Ref. 10). Several experimental drugs rescue surface membrane expression of ΔF508 CFTR without blocking the channel (for review, see Ref. 11). In recent animal and human trials drugs such as thapsigargin (12) and sodium 4-phenylbutyrate (4-PBA, Ref. 13) are hypothesized to rescue ΔF508 CFTR expression by altering its interaction(s) with molecular chaperone proteins (Refs. 12, 15, and 16, see also the editorial in Ref. 17). We speculated that if similar molecular chaperone proteins regulated LQT2 trafficking defective mutations, then these drugs might cause the pharmacological rescue in some LQT2 mutations without blocking IHERG. We first studied the mechanism of pharmacological rescue using the G601S LQT2 mutation, and then compared these findings with two additional LQT2 mutations, N470D and F805C.

EXPERIMENTAL PROCEDURES

Cell Lines and Drug Exposure—Human embryonic kidney 293 (HEK293) cell lines stably expressing WT HERG and the trafficking defective LQT2 mutations N470D (asparagine to aspartate at amino acid position 470), G601S (glycine to serine at position 601), Ref. 18, and F805C (phenylalanine to cysteine at position 805; Ref. 20) have been previously described (1, 2, 9). The cells were cultured in Dulbecco’s modified Eagle’s medium at 37 °C. Tunicamycin (Sigma), thapsigargin (Alomone, Jerusalem, Israel), 2,5-di-(di-tert)-butyl-1,4-hydroquinone (DBHQ) (Sigma), cyclopiazonic acid (Alomone), and 4-PBA (Sigma) were added to the Dulbecco’s modified Eagle’s medium after being dissolved in MeSO. The final MeSO concentration in Dulbecco’s modified Eagle’s medium was <0.1%. Incubating HEK293 cells expressing the N470D, G601S, or F805C mutations overnight...
Fig. 1. **Thapsigargin increases** \(I_{G601S}\) **and causes complex glycosylation.** A, whole cell current was recorded using the voltage-clamp protocol shown in the inset (see also “Experimental Procedures”). Representative current traces are shown for control (Con) and thapsigargin (Tha) exposure. Tull \(I_{G601S}\) measured at \(-120\) mV (dashed box) are shown on an expanded time base in the lower current traces. Incubation for \(8-10\) h in \(1 \mu\)M thapsigargin increased \(I_{G601S}\). B, the average peak tail \(I_{G601S}\) density for control cells (circles) and cells incubated in thapsigargin (squares) were plotted as a function of \(V_{m}\), and fit by Boltzmann functions (solid lines). C, Western blot analysis shows WT HERG protein (left panel), which undergoes core and complex glycosylation (Con, 135 and 155 kDa bands, respectively). Tunicamycin (Tuni, 5 \(\mu\)g/ml, 48 h exposure) prevents complex glycosylation. For G601S (right panel), under control (Con) conditions only core-glycosylated protein is detected, whereas complex glycosylation of G601S appears with 5 \(\mu\)M E4031 or 1 \(\mu\)M thapsigargin exposure. WT, wild type.

0.1% Me\_SO had no effect on \(I_{HERG}\) or complex glycosylation. E4031 (Eisai Ltd., Tokyo, Japan) was added to the Dulbecco’s modified Eagle’s medium after being dissolved in distilled H\_2O to make a 10 mM stock solution.

**Electrophysiology—**Functional analysis was done using the standard whole cell patch clamp technique as previously described (21). Ionic currents were measured using an external solution that contained (in mm) 137 NaCl, 4 KCl, 1.8 CaCl\_2, 1 MgCl\_2, 10 glucose, and 10 HEPES (pH 7.4 with NaOH), and an internal pipette solution that contained (in mm) 130 KCl, 1 MgCl\_2, 5 EGTA, 5 MgATP, 10 HEPES (pH 7.2 with KOH). An Axopatch-200 patch clamp amplifier (Axon Instruments, Union City, CA) was used to record membrane currents and measure capacitance. The uncompensated pipette resistance was 1–1.5 M\(\Omega\) and series resistance was compensated between 75 and 85%. After obtaining intracellular access, only cells with seal resistances >1 G\(\Omega\) were used. Computer software (pCLAMP 8.0; Axon Instruments) was used to generate voltage-clamp protocols, acquire current signals, and analyze data. Data were filtered at 10 kHz and were capacitance corrected. Origin (6.0, Microcal, Northampton, MA) was used for curve fitting and generating graphs. The holding potential in all experiments was \(-80\) mV, and the 0 current baseline is indicated as a dotted line in all figures. In Fig. 1, A and B, the peak tail current was measured at \(-120\) mV for 3 s following depolarizing steps \((V_{\text{Step}})\) to voltages from \(-70\) to 60 mV in 10-mV increments for 5 s. The voltage protocol was applied every 20 s. Data are given as current density (peak tail current normalized in each cell to its cell capacitance). Data were fit by the Boltzmann function (22, 23),

\[
I_{\text{Density}} = I_{\text{Density MAX}}/(1 + \exp(V_m - V_{\text{Step}})/k) \tag{Eq. 1}
\]

where \(I_{\text{Density}}\) is the current density, \(I_{\text{Density MAX}}\) is the maximal current density, \(V_m\) is the step potential where the current density is 50% of \(I_{\text{Density MAX}}\), and \(k\) is the mV-fold change in \(I_{\text{Density}}\). All voltage-clamp experiments were performed at 22–23 °C within 1–2 h of removing the cells from their culture conditions.

**Western Blot—**HEK293 cells from similarly confluent cultures were used to isolate cell proteins. The Western blot procedure was similar to that previously described (1, 2), except whole cell lysates were used. Whole cell lysates were obtained by treating cells with Nonidet P-40 buffer and determining protein concentration using a micro-BCA protein assay reagent kit (Pierce). Equal amounts of protein were subjected to SDS-polyacrylamide gel electrophoresis followed by electrophoretic transfer onto nitrocellulose membranes. The nitrocellulose membranes were incubated with HERG antibody directed against the C terminus (1). The antibody was detected with an ECL detection kit (Amersham Biosciences).

**Immunocytochemistry and Confocal Imaging—**HEK293 cells expressing the G601S mutation were plated in 35-mm Petri dishes containing collagen-coated coverslips. Cells were fixed with 4% buffered paraformaldehyde for 10 min, permeabilized with Triton X-100 (0.1%) for 10 min, and rinsed in 0.75% glycine buffer for 10 min to quench background fluorescence. The cells were incubated with 2\(\mu\)l of blocking solution (10% goat serum, 0.05% NaN\(_3\), in phosphate-buffered saline) for 1 h to block nonspecific binding sites, and the cells were subsequently incubated with the HERG antibody (1:3000) in blocking solution at room temperature. Excess antibody was washed off with three 10-min long rinses of blocking solution. The cells were then incubated with highly cross-absorbed Alexa Fluor 568 goat anti-rabbit IgG (H+L) antibody (1:500; Molecular Probes, Inc., Eugene, OR) in the blocking solution, and then were washed with four 10-min long treatments with blocking solution alone. After the final wash, the coverslips containing the stained cells were mounted on to a slide using 50% glycerol in phosphate-buffered saline. To determine nonspecific binding, control experiments with secondary antibody alone were also done. Imaging was performed with a Bio-Rad MRC 1024 laser scanning confocal microscope equipped with a mixed gas (Ar/Kr) laser operated by 24-bit Lasersharp software (Bio-Rad). Image acquisition utilized excitation at 568 nm with emission detected at 605 ± 18 nm. Cells were randomly selected and used for imaging and analysis. Z-scan sections were taken at 0.5 μm. The number of Z-scans required to image the full thickness of a cell ranged between 15 and 25 sections. Data are shown as single z-scan images and as superimposed (stacked) z-scan images.

**Statistics—**Data are presented as the mean ± S.E. Student’s t test is used for statistical analysis with \(p < 0.05\) considered significant.

**RESULTS**

**Incubating HEK293 Cells in Thapsigargin Increases \(I_{G601S}—**Thapsigargin alters cytoplasmic and ER \(Ca^{2+}\) by inhibiting the sarcoplasmic/ER \(Ca^{2+}\)-ATPase (SERCA, Ref. 24). Egan and colleagues (12) speculated that thapsigargin rescued \(F_{508}\)-CFTR by altering the activity of \(Ca^{2+}\)-dependent chaperone proteins in the ER. We tested if thapsigargin could also rescue the trafficking defective \(LQT2\) mutation G601S. We incubated HEK293 cells expressing G601S in 1 \(\mu\)M thapsigargin for 8–10 h and we compared the G601S current \((I_{G601S})\) to control (no drug treatment) current. Fig. 1A shows representative whole
Fig. 2. Incubation in thapsigargin for 2–3 h rescues \( I_{\text{G601S}} \) and complex glycosylation. \( A \), tail \( I_{\text{G601S}} \) was measured by using a holding potential of \(-80 \text{ mV}\), applying a depolarizing step to \(20 \text{ mV}\) for 3 s, followed by a hyperpolarizing step to \(-120 \text{ mV}\) for 3 s, and repeated every 20 s. Representative tail currents recorded during the hyperpolarizing step are shown for control (Con) cells, and \( I_{\text{G601S}} \) was increased in cells incubated in 1 \( \mu \text{M} \) thapsigargin for 2–3, 8–10, or 24 h. \( B \), thapsigargin increased the absolute mean peak tail \( I_{\text{G601S}} \) density in a time-dependent manner compared with control (Con) cells (*, \( p < 0.05 \)). \( C \), Western blot analysis showed that the mature protein band was detectable 3 h after incubating the cells in 5 \( \mu \text{M} \) E4031 or 1 \( \mu \text{M} \) thapsigargin (Thapsi) compared with control.

Cell currents with the voltage protocol shown in the inset. In control conditions there is only a low amplitude tail \( I_{\text{G601S}} \) (left panels). After 8–10 h of incubation in 1 \( \mu \text{M} \) thapsigargin, \( I_{\text{G601S}} \) during the depolarizing and hyperpolarizing (−120 mV) steps was increased (right panels). The tail \( I_{\text{G601S}} \) is shown in the lower panels on an expanded time base. Fig. 1B shows the absolute mean peak tail \( I_{\text{G601S}} \) densities recorded at −120 mV plotted as a function of \( V_{\text{Step}} \) (as in each group). The data were fit with a Boltzmann function (solid line) to calculate the maximal tail \( I_{\text{G601S}} \) density. Thapsigargin exposure significantly increased the maximal tail \( I_{\text{G601S}} \) density from −11.6 ± 1.5 pA/pF in control cells to −38.2 ± 5.2 pA/pF in thapsigargin-exposed cells (\( p < 0.05 \)).

HERG channels undergo core and complex glycosylation during normal biogenesis. The core-glycosylated (immature) form of the channel protein has a \( M_r \) of \(-135,000 \), and the complexly glycosylated (mature) form of the channel protein has a \( M_r \) of \(-155,000 \) (1, 25). Fig. 1C (left panel) shows a representative Western blot (\( n = 5 \)) of WT HERG channel protein from control cells (left lane) and cells incubated with 5 \( \mu \text{g/ml} \) tunicamycin for 48 h (right lane). Tunicamycin, which disrupts \( N \)-linked glycosylation, eliminated the 155-kDa protein band. Fig. 1C (right panel) shows a representative Western blot (\( n = 4 \)) of G601S channel protein from cells incubated in 5 \( \mu \text{M} \) E4031 for 10 h (left lane), control cells (middle lane), and cells incubated in 1 \( \mu \text{M} \) thapsigargin for 10 h (right lane). E4031 and thapsigargin caused the appearance of the 155-kDa protein band, although the effect of thapsigargin was less robust. The appearance of the 155-kDa band is commonly used as an indicator of channel rescue (3, 5, 6).

Fig. 2A shows the time course of \( I_{\text{G601S}} \) rescue with thapsigargin after 2–3 and 8–10 h of exposure. Cells expressing G601S were depolarized for 3 s to 20 mV to fully activate \( I_{\text{G601S}} \) and tail currents were recorded at −120 mV. Representative tail \( I_{\text{G601S}} \) from control cells and from cells exposed to 1 \( \mu \text{M} \) thapsigargin show a progressive, time-dependent increase in the tail \( I_{\text{G601S}} \) amplitude. Fig. 2B shows the absolute mean peak tail \( I_{\text{G601S}} \) density from control cells and cells incubated in thapsigargin. After incubating 2–3 h, thapsigargin increased \( I_{\text{G601S}} \) density (\( n = 5 \) cells in each group, \( p < 0.05 \)), which was further increased after 8–10 h. \( I_{\text{G601S}} \) did not increase further after 8–10 h of exposure. Fig. 2C shows that incubating in 1 \( \mu \text{M} \) thapsigargin or 5 \( \mu \text{M} \) E4031 for 3 h, compared with control conditions, was sufficient to cause the appearance of the 155-kDa protein band on Western blots.

**Fig. 3. Thapsigargin does not cause block of \( I_{\text{G601S}} \).** \( I_{\text{G601S}} \) was rescued by incubating cells overnight in 5 \( \mu \text{M} \) E4031, and E4031 was then removed. \( A \), the voltage-clamp protocol is shown in the inset. From the same cells, the records show control (Con) \( I_{\text{G601S}} \) and \( I_{\text{G601S}} \) after the application of 1 \( \mu \text{M} \) thapsigargin (+Thapsi, 4 min) or 5 \( \mu \text{M} \) E4031 (+E4031, 1 min) to the bath solution. Thapsigargin did not block \( I_{\text{G601S}} \), whereas E4031 blocked \( I_{\text{G601S}} \) leaving a small amplitude endogenous current native to HEK293 cells. \( B \), the averaged normalized peak tail \( I_{\text{G601S}} \) amplitude is plotted as a function of time before and after the application of 1 \( \mu \text{M} \) thapsigargin or 5 \( \mu \text{M} \) E4031 (drug applied at 0 time). Peak tail current in each cell was normalized to the control (pre-drug) value. \( I_{\text{G601S}} \) did not change after the application of thapsigargin (circles). In contrast, E4031 rapidly blocked \( I_{\text{G601S}} \) (triangles).
Thapsigargin Does Not Block $I_{G601S}$—We wanted to determine whether thapsigargin rescue of G601S protein involved drug block of the channel. We first pharmacologically rescued $I_{G601S}$ by incubating G601S expressing cells overnight with 5 $\mu$M E4031. Following this, cell culture media was replaced with E4031-free media for 1–2 h before cells were harvested for patch clamp recording (3). From the holding potential of $-80$ mV, $I_{G601S}$ was recorded by depolarizing cells to 0 mV for 3 s, then repolarizing to $-50$ mV for 5 s to record tail current. The voltage protocol was repeated every 20 s, and $I_{G601S}$ was recorded before and during the application of 1 $\mu$M thapsigargin or 5 $\mu$M E4031. Fig. 3A shows representative current traces of pharmacologically rescued $I_{G601S}$ before and during the application of 1 $\mu$M thapsigargin (upper panel) or 5 $\mu$M E4031 (lower panel). Thapsigargin, unlike E4031, did not cause block of $I_{G601S}$. Fig. 3B shows averaged normalized peak tail $I_{G601S}$ amplitude data recorded at $-50$ mV from G601S expressing cells, and the effect of thapsigargin or E4031. Thapsigargin did not affect the peak tail $I_{G601S}$ (circles). In contrast, 5 $\mu$M E4031 rapidly blocked $I_{G601S}$ (triangles). We conclude that thapsigargin-induced pharmacological rescue of G601S does not involve drug block of $I_{HERG}$.

**Pharmacological Rescue of G601S Protein Requires the Golgi Apparatus**—Thapsigargin and E4031 rescue $I_{G601S}$ and cause complex glycosylation of the protein. This suggests that pharmacological rescue by both drugs results in G601S trafficking through the Golgi apparatus where complex sugars are modified and added to the protein. Intracellular protein trafficking is a complex process that requires vesicular budding via the assembly of coat proteins. Coat protein I and the GTPase ADP ribosylating factor regulate the vesicular trafficking between the ER and Golgi apparatus. Brefeldin A (BFA) is a fungal metabolite that potently inhibits ADP ribosylating factor-mediated vesicular transport and disrupts the Golgi apparatus (26, 27). We tested the hypothesis that disruption of the Golgi apparatus with BFA would prevent pharmacological rescue of G601S. For this experiment, G601S expressing cells were incubated with 1 $\mu$M thapsigargin or 5 $\mu$M E4031 for 2–3 h to induce pharmacological rescue of the channel. As shown in Fig. 2, this incubation period is sufficient to rescue G601S channels. Thirty minutes after adding thapsigargin or E4031, 5 $\mu$g/ml BFA was added to some of the cell culture dishes. Fig. 4A shows representative tail $I_{G601S}$ recorded at $-120$ mV from cells cultured with and without BFA, and Fig. 4B shows the absolute mean peak tail $I_{G601S}$ density ($n = 4$ cell in each group). BFA reduced $I_{G601S}$ rescue in cells exposed to thapsigargin or E4031 ($p < 0.05$ in each group). For comparison, we incubated cells expressing WT HERG channels with 5 $\mu$g/ml BFA. BFA exposure for 3 h did not affect $I_{HERG}$; control peak tail $I_{HERG}$ was $157 \pm 20$ pA/pF ($n = 6$ cells) and after BFA exposure for 2–3 h peak tail $I_{HERG}$ was $168 \pm 21$ pA/pF ($n = 8$ cells). Because co-incubating BFA inhibited the increase of $I_{G601S}$ by either thapsigargin or E4031 exposure, the data suggest that pharmacological rescue is dependent on an intact Golgi apparatus.

**Pharmacological Rescue Redistributes Intracellular G601S Protein to the Cell Surface**—The results suggest that incubation in thapsigargin or E4031 mobilizes G601S protein that is present in an intracellular compartment(s). To test this, we used confocal microscopy to image G601S protein in control cells, and in cells incubated for 10 h with 1 $\mu$M thapsigargin or 5 $\mu$M E4031 to induce pharmacological rescue. Fig. 5 shows representative ($n =$...
Thapsigargin and E4031 selectively rescue different LQT2 mutations—Three methods to increase functional $I_{G601S}$ are reduced culture temperature (<30 °C, Ref. 2), and incubating cells in high affinity HERG blockers or thapsigargin. We compared findings obtained with the G601S mutation with two other trafficking defective LQT2 mutations. Fig. 6A shows representative tail currents recorded at $-120$ mV from cells stably expressing the G601S, F805C, or N470D mutations under control conditions; after incubating at 27 °C for 24 h; and after incubating in thapsigargin (1 μM) or E4031 (5 μM) for 24 h. Fig. 6B shows that the absolute mean peak tail current density increased in all three LQT2 mutations after cell incubation at 27 °C. Fig. 6C shows corresponding Western blots from cells stably expressing each mutation for control conditions; after incubating cells at 27 °C for 24 h; and after cells were exposed to either thapsigargin or E4031 at 37 °C for 24 h. Complex glycosylation (the presence of the 155-kDa protein band) was minimal or absent for control conditions, but was apparent for all three mutations incubated at 27 °C. Complex glycosylation was apparent only in N470D and G601S after incubation in E4031, and in G601S and F805C after incubation in thapsigargin. These data demonstrate that the presence of the 155-kDa band correlates with increased peak tail current densities shown in B for G601S, F805C, or N470D.

Other SERCA Inhibitors Do Not Rescue G601S or F805C—We tested whether a different SERCA inhibitor could also rescue G601S or F805C by using DBHQ (10 μM). We measured peak tail current using the same voltage-clamp protocol in Fig. 2. In control cells ($n = 5$) and for cells incubated with 10 μM DBHQ ($n = 4$) for 8–10 h the mean peak tail $I_{G601S}$ density remained unchanged, 8 ± 2 pA/pF. DBHQ exposure for 8–10 h also failed to increase trafficking of F805C. The mean peak tail $I_{F805C}$ density in control cells was 30 ± 6 ($n = 7$) and 27 ± 3 pA/pF ($n = 3$) after exposure to DBHQ. Similarly, incubating G601S expressing cells in DBHQ (10 μM) or cyclopiazonic acid (1 or 10 μM), another inhibitor of the SERCA, for 10 h failed to cause complex glycosylation of G601S protein (data not shown, $n = 5$ and 2, respectively).
Thapsigargin rescues trafficking of ΔF508 CFTR (12), and to our knowledge our report is the first to show that a low concentration of a single drug can rescue the trafficking of mutant channel proteins found in different diseases. 4-PBA also rescues ΔF508 CFTR after 48 h of exposure (14), therefore we tested if 4-PBA could increase G601S trafficking. 4-PBA (1 or 10 mM) incubation for 48 h did not cause the appearance of the 155-kDa protein band using Western blot analysis (data not shown; n = 2). We conclude that unlike thapsigargin, 4-PBA does not rescue trafficking of the G601S mutation, and this is in agreement with the previous report that 4-PBA did not rescue I$_{NAT0D}$ (9).

**DISCUSSION**

We show that thapsigargin causes pharmacological rescue of the LQT2 mutations G601S and F805C. This is the first study to show that thapsigargin can correct trafficking defective phenotypes in more than one channel type and disease (e.g. HERG and CFTR in LQT2 and cystic fibrosis). This is also the first study to show pharmacological rescue of F805C, a mutation that is not rescued by high affinity HERG channel blockers (5).

Unlike drugs that cause high affinity block of I$_{HERG}$ and pharmacological rescue of some LQT2 mutations, thapsigargin does not block I$_{HERG}$. Recently, fenofibrate (terfenadine carboxylate, see Ref. 9) was shown to rescue I$_{G601S}$ and I$_{NAT0D}$ at drug concentrations below that required for block of I$_{HERG}$. Incubating cells stably expressing F805C in 5 μM fenofibrate for 24 h did not increase I$_{F805C}$ density (data not shown). We conclude that thapsigargin-induced pharmacological rescue is a mechanistically novel approach to increase the surface expression of some LQT2 trafficking defective channels.

The Mechanism of Thapsigargin-induced Rescue of G601S Is Different Than ΔF508 CFTR—The mechanism proposed for thapsigargin-induced pharmacological rescue of the ΔF508 CFTR mutation was SERCA inhibition, and finding that other SERCA inhibitors such as DBHQ and cyclosporin A also rescued the trafficking of ΔF508 CFTR supported this (12). These drugs deplete ER luminal [Ca$^{2+}$] and are thought to alter Ca$^{2+}$-dependent molecular chaperone activity (for review, see Ref. 28). In the present work, we speculated that if similar molecular chaperone proteins regulated LQT2 trafficking defective mutations, then these drugs might also cause the pharmacological rescue in some LQT2 mutations. Our findings suggest that the mechanism for thapsigargin-induced rescue in LQT2 is different from that for ΔF508 in CFTR. The principal difference is that other SERCA inhibitors failed to rescue the G601S or F805C mutation. Thus one possibility is that SERCA inhibition similar to that found with the ΔF508 CFTR mutation is not responsible for the rescue of G601S. Clearly, additional studies are needed to confirm this. Our findings do show that thapsigargin-induced rescue of G601S was evident within 2–3 h of drug exposure. This short time course suggests that thapsigargin acts post-translationally to promote the relocation of intracellular protein to the membrane surface and that changes in gene expression are not essential.

Pharmacological Rescue Requires the Golgi Apparatus for Surface Expression of 601S—Complex glycosylation, or the generation of the 155-kDa protein band on Western blot, is a hallmark of HERG protein maturation and pharmacological rescue (2, 5, 6). The immunocytochemical and confocal imaging results support this; pharmacological rescue redistributed G601S protein from predominantly perinuclear intracellular compartment(s) to the cytoplasm and surface membrane. Furthermore, disrupting the Golgi apparatus using BFA prevented rescue of I$_{G601S}$. We conclude that the G601S mutation is trapped within an intracellular compartment, presumably the ER (1, 6), and that pharmacological rescue of the G601S channel protein with both thapsigargin and E4031 requires an intact Golgi apparatus for complex glycosylation and subsequent transport into the surface membrane. Taken together, our data suggest that thapsigargin-induced and E4031-induced pharmacological rescue of G601 channels follow a similar pathway to the surface membrane. In contrast, brief disruption of the Golgi apparatus by BFA had no effect on WT I$_{HERG}$).

In summary, these findings suggest that thapsigargin selectively rescues the G601S and F805C, but not the N470D LQT2 mutant channels. Thapsigargin-induced rescue does not involve HERG channel block, thus these experiments uncouple HERG channel block from pharmacological rescue. Although thapsigargin also rescues the ΔF508 mutation in CFTR, the mechanisms for pharmacological rescue appear to be different for these two channel types and therefore may not involve common molecular chaperone proteins or processing checkpoints. Furthermore, given the differences in the pharmacological rescue of N470D, G601S, and F805C, we suggest that trafficking of these mutations is differentially regulated and that multiple rescue mechanisms may exist. A more detailed understanding of pharmacological rescue in LQT2 trafficking defective mutations is needed. Although not directly clinically applicable, these findings provide new information about potential mechanisms that may be exploited to develop new therapies for diseases caused by proteins with trafficking defects.

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