Identification and Pharmacological Correction of a Membrane Trafficking Defect Associated with a Mutation in the Sulfonylurea Receptor Causing Familial Hyperinsulinism*

Christopher J. Partridge, David J. Beech, and Asipu Sivaprasadarao‡

From the School of Biomedical Sciences, University of Leeds, Leeds LS2 9JT, United Kingdom

This paper is available on line at http://www.jbc.org

Persistent hyperinsulinemic hypoglycemia of infancy (PHHI) is a genetic disorder characterized by excess secretion of insulin and hypoglycemia. In most patients, the disease is caused by mutations in sulfonylurea receptor-1 (SUR1), which, in association with Kir6.2, constitutes the functional ATP-sensitive potassium (K\textsubscript{ATP}) channel of the pancreatic \(\beta\)-cell. Previous studies reported that coexpression of the PHHI mutant R1394H-SUR1 with Kir6.2 in COS cells produces no functional channels. To investigate if the loss of function could be due to impaired trafficking of mutant channels to the cell membrane, we have cotransfected wild-type and mutant SUR1 subunits with Kir6.2 into HEK293 cells and examined their cellular localization by immunofluorescent staining. Our results show that unlike the wild-type subunits, which showed fluorescence at the cell surface, the mutant subunits displayed fluorescence in punctate structures. Co-immunostaining with antibodies against organelle-specific marker proteins identified these structures as the trans-Golgi network. Limited localization in clathrin-positive, but transferrin receptor-negative vesicles was also observed. The post-endoplasmic reticulum localization suggests that the mutation does not impair the folding and assembly of the channels so as to cause its retention by the endoplasmic reticulum. Diazoxide, a K\textsubscript{ATP} channel opener drug that is used in the treatment of PHHI, restored the surface expression in a manner that could be prevented by the channel blocker glibenclamide. When expressed in Xenopus oocytes, R1394H-SUR1 formed functional channels with Kir6.2, indicating that the primary consequence of the mutation is impairment of trafficking rather than function. Thus, our data uncover a novel mechanism underlying the therapeutic action of diazoxide in the treatment of PHHI, i.e. its ability to recruit channels to the membrane. Furthermore, this is the first report to describe a trafficking disorder effecting retention of mutant proteins in the trans-Golgi network.

* The work was funded by the Medical Research Council, United Kingdom. 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.
‡ To whom correspondence should be addressed: School of Biomedical Sciences, Worsley Bldg., University of Leeds, Leeds LS2 9JT, UK. Tel.: 44-113-2344326; Fax: 44-113-2344331; E-mail: a.sivaprasadarao@leeds.ac.uk.

1 The abbreviations used are: PHHI, persistent hyperinsulinemic hypoglycemia of infancy; SUR1, sulfonylurea receptor-1; ABC, ATP-binding cassette; NBF, nucleotide-binding fold; ER, endoplasmic reticulum; TGN, trans-Golgi network; FITC, fluorescein isothiocyanate; HMK, heavy chain myosin kinase; PBS, phosphate-buffered saline; CFTR, cystic fibrosis transmembrane regulator.

© 2001 by The American Society for Biochemistry and Molecular Biology, Inc.

Persistence hyperinsulinemic hypoglycemia of infancy (PHHI), also known as familial hyperinsulinism, is an autosomal recessive disorder characterized by excess secretion of insulin, leading to hypoglycemia (1, 2). In the majority of cases, the disease is caused by mutations in the ATP-sensitive potassium (K\textsubscript{ATP}) channel that impair the ability of the insulin-secreting pancreatic \(\beta\)-cells to respond to changes in blood glucose levels (2–6). In normal individuals, a rise in the blood glucose levels is accompanied by an increase in the uptake and metabolism of glucose by the \(\beta\)-cells, leading to increased production of ATP. The resultant elevation of intracellular [ATP]/[ADP] closes K\textsubscript{ATP} channels, leading to membrane depolarization and activation of voltage-gated calcium channels. As a result, the intracellular calcium levels increase, triggering exocytotic secretion of insulin (7).

Our understanding of the mechanism by which nucleotides regulate the K\textsubscript{ATP} channels has improved with the cloning and heterologous expression of these channels (8). The pancreatic \(\beta\)-cell K\textsubscript{ATP} channel is an octamer consisting of four Kir6.2 and four sulfonylurea receptor-1 (SUR1) subunits (9–11). Kir6.2 is a member of the inwardly rectifying potassium (Kir) channel family, which forms the potassium-conducting pore of the channel (12). SUR1 is a member of the ATP-binding cassette (ABC) superfamily (13); it contains two nucleotide-binding folds, NBF1 and NBF2, with which it senses changes in intracellular [ATP]/[ADP] and transmits the signal to the pore. NBF1 appears to be the principal site for ATP binding, whereas NBF2 binds Mg-ADP. These binding domains cooperate with each other in mediating the nucleotide regulation of the pore function (14–16). SUR1 also contains the high affinity binding sites for two classes of therapeutic drugs. The sulfonylurea drugs (glibenclamide and tolbutamide) inhibit the channels and are used in the treatment of non-insulin-dependent (type II) diabetes mellitus. The other class of drugs, known as potassium channel opener drugs (e.g. diazoxide), activate the channel and are used to suppress insulin secretion.

Kir6.2, unlike many other Kir channels, cannot form functional channels at the plasma membrane when expressed in the absence of SUR1 (8). This was shown to be due to the presence of a novel endoplasmic reticulum (ER) retention signal (RXR) in the C-terminal end of the subunit (17). SUR1, like Kir6.2, also contains an ER retention signal and fails to traffic to the cell surface when expressed alone. When coexpressed, however, the subunits are thought to mask each other’s ER retention signals, resulting in the release of the complex from the ER and the appearance of the channel at the cell surface. Removal of the C-terminal 36 amino acids containing the ER retention signal allows Kir6.2 to form functional channels at
the cell surface (12). Using this construct, it has been demonstrated that an ATP inhibitory site resides at the intracellular end of Kir6.2 (12, 18).

A significant proportion of PHHI mutations are located within the NBF domains of SUR1 (2–6, 19). Functional analyses of these mutations in COS cells revealed two types of phenotypic change: some mutations abolish channel function, and others reduce the response to changes in the intracellular [ATP]/[ADP] ratio (4, 5). More recently, it was shown by Cartier et al. (20) that the ΔF1388 mutation in SUR1 causes retention of the mutant channel in the ER. In that work, the authors showed that removal of the RKR retention signal partially overcomes the effect of the mutation. Here, we have investigated the effect of one nonfunctional PHHI mutation, R1394H, on trafficking of the channel. We show that HEK293 cells stably transfected with R1394H-SUR1 and Kir6.2 present no surface expression for either of the subunits; instead, they are retained mainly in the trans-Golgi network (TGN). In addition, pretreatment with dioxazide alone, but not combined with glibenclamide, reverses the defect, thereby rescuing the cell-surface expression. And finally, the Kir6.2-R1394H-SUR1 mutant channel complex is functional in Xenopus oocytes. Taken together, our data demonstrate that the R1394H mutation does not impair the channel function, but prevents its surface expression through retention in the TGN.

EXPERIMENTAL PROCEDURES

Materials—LipofectAMINE™ and G418 were obtained from Life Technologies, Inc. Blasticidin was purchased from Invitrogen. Diazoxide, glibenclamide, and insulin were purchased from Sigma. Antibodies directed against either SUR1 and Kir6.2 were fixed, permeabilized, and then probed with antibodies directed against either SUR1 (upper panels) or the FLAG epitope on Kir6.2 (lower panels). The cells were stained with FITC-conjugated secondary antibodies. Representative confocal images taken using identical settings are shown.

FIG. 1. Coexpression of R1394H-SUR1 with Kir6.2 in HEK293 cells results in defective K<sub>ATP</sub> channel trafficking. Stable HEK293 cells coexpressing either wild-type SUR1 (WT) or R1394H-SUR1 with Kir6.2-FLAG cells were transfected with pcDNA3 harboring the Kir6.2-HMK-FLAG sequence and pcDNA6 containing wild-type or mutant His<sub>6</sub>-SUR1 using LipofectAMINE™. 24 h post-transfection, the medium was supplemented with 800 μg/ml G418 and 5 μg/ml blasticidin to select and propagate stably transfected cells. The clones were amplified and maintained in medium supplemented with the two antibiotics. Electrophysiology—Xenopus oocytes were co-injected with in vitro generated cRNA encoding either His<sub>6</sub>-tagged wild-type SUR1 or R1394H-SUR1 (80 ng) and HMK-FLAG-tagged wild-type Kir6.2 (20 ng) and incubated in Barth’s solution. 48–72 h post-injection, whole cell currents were recorded in high potassium Ringer’s solution containing 90 mM KCl, 1 mM MgCl<sub>2</sub>, 1.8 mM CaCl<sub>2</sub>, and 5 mM HEPES (pH 7.4) at 21–24 °C using a two-electrode voltage clamp. All electrodes were back-filled with 3 M KCl and had resistances between 2 and 3 megohms. A ramp voltage protocol from −120 to +50 mV was used with the cells being held at −30 mV. Currents were filtered at 1 kHz and sampled at 4 kHz. The details are as described previously (22).

Immunocytochemistry—The stable cell lines were seeded on polylysine-coated coverslips and grown at 37 °C overnight. Cells were washed in phosphate-buffered saline (PBS), fixed for 15 min in 10% Formalin, and permeabilized with 0.1% Triton X-100 in PBS for 15 min, followed by extensive washes in PBS. Background fluorescence was blocked by three 5-min incubations in 1 mg/ml sodium borohydride. Following extensive washing in PBS, the cells were blocked in 5% goat serum in PBS containing 1 μM sodium azide for 3 h and then incubated for 2 h at room temperature with a primary antibody. The antibodies used were either mouse monoclonal (anti-FLAG (1:200 dilution), anti-transferrin receptor (1:2 dilution), anti-calreticulin (1:200 dilution), or anti-clathrin (1:200 dilution)) or rabbit polyclonal (anti-Kir6.2 (1:200 dilution) or anti-SUR1 (1:200 dilution)). For TGN, sheep anti-TGN46 antibodies (1:50 dilution) were used. Following extensive washes in PBS, the cells were incubated for 1 h at room temperature with appropriate secondary antibodies. The secondary antibodies used were FITC-conjugated goat anti-mouse or anti-rabbit IgG, Cy3-conjugated goat anti-mouse antibody, or Texas Red conjugated anti-sheep IgG. Following eight washes in PBS, the cells were mounted onto a microscope slide with VECTASHIELD (Vector Labs, Inc.) and air-sealed with nail varnish. Cells were viewed on a Leica TCS NT laser scanning confocal microscope equipped with a 488-nm laser excitation filter for FITC and a 564-nm filter for Cy3. Fluorescent images were visualized under an oil-immersed ×40 objective obtained using the appropriate laser. Immunocytochemistry on Xenopus oocyte sections was performed as described previously (22).

RESULTS

Expression and Subcellular Distribution of Wild-type and Mutant K<sub>ATP</sub> Channels in HEK293 Cells—Previous studies have shown that when Kir6.2 is cotransfected with R1394H-SUR1 into COS cells, no functional expression can be detected (4, 5). To investigate if this is due to the failure of the mutant channel to traffic to the plasma membrane, we stably cotransfected HEK293 cells with Kir6.2 (tagged at the C terminus with
the FLAG epitope) and either wild-type SUR1 or its mutant, R1394H. Cells were permeabilized and probed for Kir6.2 (using anti-FLAG antibody) and then with mouse monoclonal antibodies against an organelle-specific protein: calreticulin (ER), clathrin (coated vesicles), or the transferrin receptor (early endosome). Sheep polyclonal anti-TGN46 antibody was used to label the TGN. After extensive washing, the cells were stained with FITC-conjugated anti-rabbit IgG (to detect SUR1) and Cy3-conjugated anti-mouse antibodies or Texas Red-conjugated anti-sheep antibodies (to detect marker proteins). Representative confocal images are shown.

To identify the nature of the punctate structures, cells were co-immunolabeled for SUR1 with rabbit anti-SUR1 antibody and then with mouse monoclonal antibodies against an organelle-specific protein: calreticulin (ER), clathrin (coated vesicles), or the transferrin receptor (early endosome). Sheep polyclonal anti-TGN46 antibody was used to label the TGN. After extensive washing, the cells were stained with FITC-conjugated anti-rabbit IgG (to detect SUR1) and Cy3-conjugated anti-mouse antibodies or Texas Red-conjugated anti-sheep antibodies (to detect marker proteins). Representative confocal images are shown.

To identify the nature of the punctate structures, cells were co-immunolabeled for SUR1 with rabbit anti-SUR1 antibody and then with mouse monoclonal antibodies against an organelle-specific protein: calreticulin (ER), clathrin (coated vesicles), or the transferrin receptor (early endosome). Sheep polyclonal anti-TGN46 antibody was used to label the TGN. After extensive washing, the cells were stained with FITC-conjugated anti-rabbit IgG (to detect SUR1) and Cy3-conjugated anti-mouse antibodies or Texas Red-conjugated anti-sheep antibodies (to detect marker proteins). Representative confocal images are shown.

To identify the nature of the punctate structures, cells were co-immunolabeled for SUR1 with rabbit anti-SUR1 antibody and then with mouse monoclonal antibodies against an organelle-specific protein: calreticulin (ER), clathrin (coated vesicles), or the transferrin receptor (early endosome). Sheep polyclonal anti-TGN46 antibody was used to label the TGN. After extensive washing, the cells were stained with FITC-conjugated anti-rabbit IgG (to detect SUR1) and Cy3-conjugated anti-mouse antibodies or Texas Red-conjugated anti-sheep antibodies (to detect marker proteins). Representative confocal images are shown.

To identify the nature of the punctate structures, cells were co-immunolabeled for SUR1 with rabbit anti-SUR1 antibody and then with mouse monoclonal antibodies against an organelle-specific protein: calreticulin (ER), clathrin (coated vesicles), or the transferrin receptor (early endosome). Sheep polyclonal anti-TGN46 antibody was used to label the TGN. After extensive washing, the cells were stained with FITC-conjugated anti-rabbit IgG (to detect SUR1) and Cy3-conjugated anti-mouse antibodies or Texas Red-conjugated anti-sheep antibodies (to detect marker proteins). Representative confocal images are shown.

To identify the nature of the punctate structures, cells were co-immunolabeled for SUR1 with rabbit anti-SUR1 antibody and then with mouse monoclonal antibodies against an organelle-specific protein: calreticulin (ER), clathrin (coated vesicles), or the transferrin receptor (early endosome). Sheep polyclonal anti-TGN46 antibody was used to label the TGN. After extensive washing, the cells were stained with FITC-conjugated anti-rabbit IgG (to detect SUR1) and Cy3-conjugated anti-mouse antibodies or Texas Red-conjugated anti-sheep antibodies (to detect marker proteins). Representative confocal images are shown.

To identify the nature of the punctate structures, cells were co-immunolabeled for SUR1 with rabbit anti-SUR1 antibody and then with mouse monoclonal antibodies against an organelle-specific protein: calreticulin (ER), clathrin (coated vesicles), or the transferrin receptor (early endosome). Sheep polyclonal anti-TGN46 antibody was used to label the TGN. After extensive washing, the cells were stained with FITC-conjugated anti-rabbit IgG (to detect SUR1) and Cy3-conjugated anti-mouse antibodies or Texas Red-conjugated anti-sheep antibodies (to detect marker proteins). Representative confocal images are shown.

To identify the nature of the punctate structures, cells were co-immunolabeled for SUR1 with rabbit anti-SUR1 antibody and then with mouse monoclonal antibodies against an organelle-specific protein: calreticulin (ER), clathrin (coated vesicles), or the transferrin receptor (early endosome). Sheep polyclonal anti-TGN46 antibody was used to label the TGN. After extensive washing, the cells were stained with FITC-conjugated anti-rabbit IgG (to detect SUR1) and Cy3-conjugated anti-mouse antibodies or Texas Red-conjugated anti-sheep antibodies (to detect marker proteins). Representative confocal images are shown.

To identify the nature of the punctate structures, cells were co-immunolabeled for SUR1 with rabbit anti-SUR1 antibody and then with mouse monoclonal antibodies against an organelle-specific protein: calreticulin (ER), clathrin (coated vesicles), or the transferrin receptor (early endosome). Sheep polyclonal anti-TGN46 antibody was used to label the TGN. After extensive washing, the cells were stained with FITC-conjugated anti-rabbit IgG (to detect SUR1) and Cy3-conjugated anti-mouse antibodies or Texas Red-conjugated anti-sheep antibodies (to detect marker proteins). Representative confocal images are shown.

To identify the nature of the punctate structures, cells were co-immunolabeled for SUR1 with rabbit anti-SUR1 antibody and then with mouse monoclonal antibodies against an organelle-specific protein: calreticulin (ER), clathrin (coated vesicles), or the transferrin receptor (early endosome). Sheep polyclonal anti-TGN46 antibody was used to label the TGN. After extensive washing, the cells were stained with FITC-conjugated anti-rabbit IgG (to detect SUR1) and Cy3-conjugated anti-mouse antibodies or Texas Red-conjugated anti-sheep antibodies (to detect marker proteins). Representative confocal images are shown.
of the pancreatic $K_{ATP}$ channel, can promote the surface expression of the Kir6.2-R1394H-SUR1 mutant channel complexes. We incubated cells in medium containing diazoxide for 24–48 h prior to immunofluorescence analysis. Fig. 4 shows that diazoxide (100 $\mu$M) restored the surface expression of the mutant channel. Glibenclamide (1 $\mu$M), an antagonist of $K_{ATP}$ channels, completely prevented the diazoxide-stimulated surface expression of the mutant channel. The staining pattern of glibenclamide-treated cells was similar to that of the untreated cells (Fig. 4).

Electrophysiological and Immunocytochemical Analysis of Wild-type and Mutant $K_{ATP}$ Channels Expressed in Xenopus Oocytes—Trafficking studies have shown that $\Delta$F508 (31) and G408C (32) mutant CFTR channels can be functionally expressed in Xenopus oocytes, but not in mammalian cells, indicating that oocytes can support cell-surface expression of some mutant channels. We used this premise to test if oocytes can support cell-surface expression of Kir6.2-R1364H-SUR1. For this, we co-injected Xenopus oocytes with cRNA encoding Kir6.2 and wild-type SUR1 or R1394H-SUR1. After incubation for 48–72 h to allow expression of the channels, the whole cell K+ currents were recorded in 90 mM extracellular potassium using a two-electrode voltage clamp (Fig. 5). There were no detectable basal currents. However, application of the metabolic poison sodium azide and diazoxide caused a rapid increase in the inward currents (Fig. 5B). The currents were inhibited when glibenclamide (1 $\mu$M) was included in the activation mixture. The pharmacology of the mutant channel thus appears to be qualitatively similar to that of the wild-type channel (Fig. 5A) (8, 33). Likewise, the current-voltage relationships and the rectification properties of the mutant channel showed little difference from those of the wild-type channel (Fig. 5D). To eliminate the possibility that the observed increase in currents (Fig. 5B) was due to diazoxide-induced recruitment of the mutant channel into the membrane, we examined the effect of azide alone; Fig. 5C shows that metabolic poisoning alone was enough to induce currents. Consistent with this, immunostaining of sections of Xenopus oocytes, co-injected with R1394H-SUR1 and Kir6.2, but not treated with diazoxide or sodium azide, showed fluorescence at the plasma membrane for both subunits (Fig. 5E). These data illustrate the importance of using both mammalian and oocyte expression systems as complementary approaches to study the function and trafficking of mutant channels.

FIG. 3. Decreased temperature does not reverse the trafficking defect of R1394H-SUR1. Stable cells coexpressing R1394H-SUR1 and Kir6.2 were incubated at 37 or 27°C for 48 h and then immunostained for SUR1 as described in the legend to Fig. 1.

DISCUSSION

Previous studies have shown that the PHHI mutant R1394H-SUR1, when coexpressed with Kir6.2 in mammalian cell lines, does not produce functional channels (4, 5). In this study, we found that this is because the subunits fail to reach the cell surface (Fig. 1), as they are retained by the TGN (Fig. 2). This contrasts with the previous studies, which showed that mutations that cause trafficking defects, in almost all cases, lead to misfolding and retention of misfolded proteins in the ER (27). Examples include $\Delta$F508 CFTR (34), the N470D HERG potassium channel (29), the vasopressin V2 receptor (30), and the more recently reported Kir6.2-$\Delta$F1388-SUR1 channel complex (20). In these situations, the quality control machinery of the ER ensures that the misfolded proteins are degraded by diverting them to proteasomes or lysosomes (27, 35). The R1394H mutation of SUR1 thus appears to be different from all other cases reported so far and is, to our knowledge, the first genetic mutation to cause retention of membrane protein in a post-ER compartment.

The R1394H mutation does not appear to impair the folding and assembly of the channel to the extent that it would necessitate retention by the quality control mechanism of the ER. Zerangue et al. (17) showed that the wild-type SUR1 and Kir6.2 subunits, when expressed independently, are retained in the ER because they both contain the retention signal RKR. When coexpressed, however, the subunits assemble and mask each other’s retention signals, as a result of which the channel is released by the quality control system of the ER. The finding that R1394H-SUR1 and Kir6.2 subunits were not retained in the ER suggests that the mutation does not impair the ability of SUR1 to assemble with Kir6.2. This is further supported by the fact that when coexpressed in Xenopus oocytes, the mutant SUR1 forms functional channels with Kir6.2 at the plasma membrane of the cell (Fig. 5).

The mechanisms by which proteins are sorted into specific membrane compartments are not fully understood (27). Properly folded and assembled proteins exit the ER and enter the TGN, where they are sorted into appropriate transport vesicles, which direct them to their normal destination. Proteins destined for the plasma membrane, for example, segregate into...
secretory vesicles, which carry them to the plasma membrane via the secretory pathway (26). However, some membrane proteins appear to use a nonsecretory pathway to reach the cell surface (36, 37). These proteins are packaged into AP-1 adapter-containing clathrin-coated vesicles at the TGN. Although the major function of these vesicles is to direct proteins to lysosomes via late endosomes, some vesicles, after shedding their clathrin coats, appear to carry the cargo proteins to the plasma membrane. Some plasma membrane proteins such as the transferrin receptor and the low density lipoprotein receptor, which recycle between the plasma membrane and intracellular compartments, also employ clathrin-coated vesicles, but use a different adapter protein, AP-2 (26). To identify at which point during these transport events the Kir6.2-R1394H-SUR1 mutant channel complex is retained, we used antibodies specific to individual compartments. The resulting data (Fig. 2) showed that the mutant channel is mainly retained in the TGN. Limited, but notable amounts of the mutant protein were also found in clathrin-positive vesicles. These findings suggest that mutant channels are stranded in the TGN. However, a small proportion of these appear to leave the TGN in the form of clathrin-coated vesicles; these vesicles may direct the mutant protein either to endosomes/lysosomes or to the plasma membrane. However, the absence of colocalization with the transferrin receptor (Fig. 2) suggests that, after leaving the TGN, the mutant channels are unlikely to reach the plasma membrane and undergo endocytosis. Instead, the clathrin-coated vesicles may deliver the mutant protein to lysosomes.

Arg<sup>1394</sup> in native SUR1 is just downstream of the Walker A motif of NBF2. An examination of the amino acid sequences of ABC subfamilies (A–G) revealed that this arginine is conserved in NBF2 of most members of the ABC-B (MDR) and ABC-C (MRP) subfamilies but not in any of the other subfamilies. Key members of these two subfamilies include P-glycoprotein, CFTR, and SUR1. Although NBF1 shows significant sequence identity to NBF2, the residue corresponding to position 1394 is not an arginine in NBF1. Commensurate with this, homology modeling of NBF2 with the crystal structures of the ATP-binding subunit of histidine permease (38) and RAD50c, a protein involved in DNA repair (39), revealed that residues at this position do not participate in ATP binding. The fact that Arg<sup>1394</sup> is conserved among the members of only some subfamilies, including SUR1, raises the possibility that this residue may play a role that is unique to these members; one possibility is that it regulates membrane trafficking.

A variety of approaches have been employed to correct trafficking defects. These include reduction of growth temperature (28, 29), the use of chemical chaperones such as glycerol (28, 29), the use of protein-specific ligands (29, 30), and inactivation of ER retention signals (20, 40). Reduction of temperature, which has been successfully used to improve folding in the ER and hence surface expression of ΔF508 CFTR (28) and the N470D HERG potassium channel (29), has no effect on Kir6.2-R1394H-SUR1 (Fig. 3). This was not unexpected because, as noted above, the R1394H mutation does not appear to affect the folding and assembly of K<sub>ATP</sub> channels to the extent that it would necessitate retention by the quality control machinery of the ER.

There are several examples in the literature where trafficking defects have been corrected by treating the cells with ligands specific to the protein (30). Application of diazoxide, a K<sub>ATP</sub> channel opener drug and a drug used in the treatment of PHHI, has fully restored the surface expression of Kir6.2-R1394H-SUR1; glibenclamide prevented the rescuing effect of diazoxide (Fig. 4). However, the mechanism by which diazoxide restores membrane expression of the mutant is unclear. Since the function of diazoxide seems to be to stabilize the activated state of the channel (presumably the Mg<sup>2+</sup>-ADP-bound form), we speculate that it is in the activated conformation that K<sub>ATP</sub> channels traffic to the membrane. The ability of glibenclamide, a drug that stabilizes the inactive form of the channel, to antagonize the action of diazoxide is consistent with this argument.

The therapeutic basis for the treatment of PHHI patients with diazoxide has thus far been thought to be associated with its ability to activate K<sub>ATP</sub> channels already present in the plasma membrane. However, the present finding that diazoxide can promote membrane trafficking of PHHI mutant channels uncovers a new molecular basis for the therapeutic action of diazoxide, i.e. its ability to recruit channels to the plasma membrane.

In summary, we have shown that the R1394H mutation does not abolish channel function once at the plasma membrane, but
causes retention of the channel complex in the TGN. The demonstration that diazoxide, a drug used to treat PHHI, is able to mobilize the mutant channel complex to the membrane provides a new molecular basis for the therapeutic action of this drug. Finally, the finding that the Kir6.2 SUR1 mutant channel complex is retained in a post-ER compartment represents a new cellular mechanism by which genetic mutations can cause trafficking defects.

Acknowledgment—We thank Dr. Ponnambalam for providing anti-transferrin receptor and anti-TGN46 antibodies and helpful discussions.

REFERENCES

1. Permutt, M. A., Nestorowicz, A., and Glaser, B. (1996) *Diabetes* Re 4, 347–355.
2. Dunne, M. J., Kane, C., Shepherd, R. M., Sanchez, J. A., James, R. F., Johnson, P. R., Aynaley-Green, A., Lu, S., Clement, J. P., Lindley, K. J., Seino, S., and Aguilar-Bryan, L. (1997) *Nature* 386, 703–706.
3. Thomas, P. M., Cote, G. J., Wohllk, N., Haddad, B., Mathew, P. M., Rabl, W., Aguilar-Bryan, L., Gagel, R. F., and Bryan, J. (1995) *Science* 268, 426–429.
4. Nichols, C. G., Shyng, S. L., Nestorowicz, A., Glaser, B., Clement, J. P., Gonzalez, G., Aguilar-Bryan, L., Permutt, M. A., and Bryan, J. (1996) *Science* 272, 1765–1787.
5. Shyng, S. L., Ferrigni, T., Shepard, J. B., Nestorowicz, A., Glaser, B., Permutt, M. A., and Nichols, C. G. (1998) *Diabetes* 47, 1145–1151.
6. Onokoski, T., Ammala, C., Huoigio, H., Cote, G. J., Chapman, J., Cosgrove, K., Ashfield, R., Huang, E., Komulainen, J., Ashcroft, F. M., Dunne, M. J., Kere, J., and Thomas, P. M. (1999) *Diabetes* 48, 408–415.
7. Ashcroft, F. M., and Gribble, F. M. (1999) *Diabetologia* 42, 903–919.
8. Inagaki, N., Goni, T., Shepler, J. P., Namba, N., Inazawa, J., Gonzalez, G., Aguilar-Bryan, L., Seino, S., and Bryan, J. (1995) *Science* 270, 1166–1170.
9. Clement, J. P., Kurtilkar, K., Gonzalez, G., Schwanstecher, M., Pante, U., Aguilar-Bryan, L., and Bryan, J. (1997) *Nature* 18, 827–838.
10. Shyng, S., and Nichols, C. G. (1997) *J. Gen. Physiol.* 110, 655–664.
11. Seino, S. (1999) *Annu. Rev. Physiol.* 61, 337–362.
12. Tucker, S. J., Gribble, F. M., Zhao, C., Trapp, S., and Ashcroft, F. M. (1997) *Nature* 387, 179–183.
13. Schneider, E., and Hanke, S. (1998) *FEBS Microbiol. Rev.* 22, 1–20.
14. Matsuo, M., Kikura, N., Amachi, T., and Ueda, K. (1999) *J. Biol. Chem.* 274, 37479–37482.
15. Ueda, K., Komine, J., Matsuo, M., Seino, S., and Amachi, T. (1999) *Proc. Natl. Acad. Sci. U.S.A.* 96, 1268–1272.
16. Matsuo, M., Trapp, S., Tanizawa, Y., Kikura, N., Amachi, T., Oka, Y., Ashcroft, F. M., and Ueda, K. (2000) *J. Biol. Chem.* 275, 11184–11191.
17. Zerangue, N., Schwappach, B., Jan, Y. N., and Jan, L. Y. (1999) *Neuron* 22, 527–548.
18. Tanabe, K., Tucker, S. J., Matsuo, M., Proks, P., Ashcroft, F. M., Seino, S., Amachi, T., and Ueda, K. (1999) *J. Biol. Chem.* 274, 3931–3933.
19. Nestorowicz, A., Wilson, B. A., Schoor, K. P., Inoue, H., Glaser, B., Landau, H., Stanley, C. A., Thornton, P. S., Clement, J. P., Bryan, J., Aguilar-Bryan, L., and Permutt, M. A. (1996) *Hum. Mol. Genet.* 5, 1813–1822.
20. Cartier, E. A., Conti, L. R., Vandenberg, C. A., and Shyng, S. L. (2001) *Proc. Natl. Acad. Sci. U.S.A.* 98, 2882–2887.
21. Towler, M. C., Prescott, A. R., James, J., Lucoq, J. M., and Ponnambalam, S. (2000) *Exp. Cell Res.* 259, 167–179.
22. Hough, E., Beech, D. J., and Sivapradasaran, A. (2000) *Pflugers Arch.* 449, 1–17.
23. Kunkel, T. A. (1985) *Proc. Natl. Acad. Sci. U.S.A.* 82, 488–492.
24. Jethmalani, S. M., Henle, K. J., and Kaufhal, G. P. (1994) *J. Biol. Chem.* 269, 23603–23609.
25. Trowbridge, I. S., Collawn, J. F., and Hopkins, C. R. (1995) *Annu. Rev. Cell Biol.* 9, 129–161.
26. Kirchhausen, T. (2000) *Annu. Rev. Biochem.* 69, 699–727.
27. Sanders, C. R., and Nagy, J. K. (2000) *Curr. Opin. Struct. Biol.* 10, 438–442.
28. Aizawa, T., Komatsu, M., Asanuma, N., Sato, Y., and Sharp, G. W. (1998) *Trends Pharmacol. Sci.* 19, 496–499.
29. Morello, J. P., Petaj-Rapo, U. E., Bichet, D. G., and Bovisier, M. (2000) *Trends Pharmacol. Sci.* 21, 466–469.
30. Druzin, M. L., Wilkinson, D. J., Smit, L. S., Worrell, R. T., Strong, T. V., Frizzell, R. A., Dawson, D. C., and Collins, F. S. (1991) *Science* 254, 1797–1799.
31. Smit, L. S., Strong, T. V., Wilkinson, D. J., Macel, M. J., Mansoura, M. K., Wood, D. L., Cole, J. L., Cutting, G. R., Cohn, J. A., Dawson, D. C., and Collins, F. S. (1995) *Hum. Mol. Genet.* 4, 269–273.
32. Gribble, F. M., Ashfield, R., Ammala, C., and Ashcroft, F. M. (1997) *J. Physiol. Lond.* 498, 87–98.
33. Cheng, S. H., Gregory, R. J., Marshall, J., Paul, S., Souza, D. W., White, G. A., O’Riordan, C. R., and Smith, A. E. (1990) *Cell* 63, 827–834.
34. Machan, G. C., Patterson, C., Zhang, W., Younger, J. M., and Cyr, D. M. (2001) *Nat. Cell Biol.* 3, 100–105.
35. Leitinger, B., Hille-Rehfeld, A., and Spiess, M. (1995) *Proc. Natl. Acad. Sci. U.S.A.* 92, 10109–10113.
36. Futter, C. E., Connolly, C. N., Cutler, D. F., and Hopkins, C. R. (1995) *J. Biol. Chem.* 270, 11099–11103.
37. Hung, I. W., Wang, I. X., Nikaido, K., Liu, P. Q., Ames, G. F., and Kim, S. H. (1999) *Nature* 396, 703–707.
38. Hopfner, K. P., Karcher, A., Shin, D. S., Craig, L., Arthur, L. M., Carney, J. P., and Tainer, J. A. (2000) *Cell* 101, 788–800.
39. Chang, X. B., Cui, L., Hou, Y. X., Jensen, T. J., Aleksandrov, A. A., Menges, A., and Riordan, J. R. (1999) *Mol. Cell* 4, 137–142.