Glycosylation Affects the Protein Stability and Cell Surface Expression of Kv1.4 but Not Kv1.1 Potassium Channels

A PORE REGION DETERMINANT DICTATES THE EFFECT OF GLYCOSYLATION ON TRAFFICKING*

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Kv1.1 and Kv1.4 potassium channels are plasma membrane glycoproteins involved in action potential repolarization. We have shown previously that glycosylation affects the gating function of Kv1.1 (Watanabe, I., Wang, H. G., Sutachan, J. J., Zhu, J., Recio-Pinto, E. & Thornhill, W. B. (2003) J. Physiol. (Lond.) 550, 51–66) and that a pore region determinant of Kv1.1 and Kv1.4 affects their surface trafficking negatively or positively, respectively (Zhu, J., Watanabe, I., Gomez, B. & Thornhill, W. B. (2001) J. Biol. Chem. 276, 39419–39427). Here we investigated the role of N-glycosylation of Kv1.1 and Kv1.4 on their protein stability, cellular localization pattern, and trafficking to the cell surface. We found that preventing N-glycosylation of Kv1.4 decreased its protein stability, induced its high partial intracellular retention, and decreased its cell surface protein levels, whereas it had little or no effect on these parameters for Kv1.1. Exchanging a trafficking pore region determinant between Kv1.1 and Kv1.4 reversed these effects of glycosylation on these chimeric channels. Thus it appeared that the Kv1.4 pore region determinant and the sugar tree attached to the S1–S2 linker showed some type of dependence in promoting proper trafficking of the protein to the cell surface, and this dependence can be transferred to chimeric Kv1.1 proteins that contain the Kv1.4 pore. Understanding the different trafficking programs of Kv1 channels, and whether they are altered by glycosylation, will highlight the different posttranslational mechanisms available to cells to modify their cell surface ion channel levels and possibly their signaling characteristics.

Many plasma membrane proteins are N-glycosylated on their extracellular domain in the endoplasmic reticulum (ER) and further glycosylated in the Golgi before transport to the cell surface. A carbohydrate tree on a membrane protein could affect its folding, stability, trafficking to the cell surface, and/or function. The occurrence of carbohydrate-deficient glycoprotein syndrome I–IV disorder in humans, which can be life-threatening, highlights the fact that proper glycosylation of both membrane and secretory glycoproteins is essential for normal development and health (1, 2).

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§ The abbreviations used are: ER, endoplasmic reticulum; CHO, Chinese hamster ovary; PBS, phosphate-buffered saline.

Potassium (K⁺) channels make up a large family of membrane proteins (3, 4) in which many members are glycosylated that play important physiological roles such as action potential repolarization (5). In the Kv1 voltage-gated K⁺ channel subfamily, Kv1.1-Kv1.5 members, which express functionally as homotetramers or heterotetramers, have one N-glycosylation consensus site on the extracellular S1–S2 linker (6). We have presented evidence that N-glycosylation of Kv1.1 K⁺ channels affects the gating function by a combined surface potential and a cooperative subunit interaction mechanism but is not required for cell surface expression (7, 8). We have also used mammalian voltage-gated Kv1 subfamily members expressed in cell lines as a model system to investigate their intracellular trafficking and cell surface expression. In transiently transfected cell lines, homomeric Kv1.1 or Kv1.4 proteins were efficiently N-glycosylated in the ER, but Kv1.1 exhibited inefficient trans-Golgi glycosylation and low cell surface expression levels, whereas Kv1.4 showed efficient trans-Golgi glycosylation and high surface expression (9). We took advantage of these differences to map the determinants on Kv1.1 and Kv1.4 to pore regions that were responsible for these effects because exchanging these regions conferred upon the recipient protein the characteristics of the donor protein (9). We also showed that in a Kv1.1/Kv1.4 heteromer Kv1.1 was dominant negative for surface expression levels (10).

Given the above results, the aims of this report were to determine whether there were any differential effects of N-glycosylation on the intracellular trafficking and cell surface expression levels of Kv1.1 and Kv1.4 channels and to examine whether a trafficking pore region determinant modifies these possible differential effects.

EXPERIMENTAL PROCEDURES

Cell Lines and Molecular Biology—Chinese hamster ovary (CHO) pro5 cells were obtained from American Type Culture Collection (ATCC, Manassas, VA) and maintained in Dulbecco's modified Eagle's medium, or α-MEM, supplemented with 0.35 mM proline, 10% fetal bovine serum at 37 °C under 5% CO₂. COS7 cells were from ATCC. From the first PCR reactions were gel-purified and used in a second PCR reaction to construct Kv1.1N207Q and Kv1.4N354Q. Re-9

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of the gene. LipofectAMINE (Invitrogen) was used with 0.5 μg of plasmid for transfection per 35-mm dish. Cells were transfected for 5 h, rinsed with PBS, washed with then incubated in Sulfo-NHS-SS-biotin (0.5 mg/mL) in PBS with low calcium and magnesium on ice for 20 min. Cells were then washed four times with PBS and then solubilized with radiiodine precipitation buffer with protease inhibitors (120 mM NaCl, 50 mM Tris-Cl, pH 7.4, 0.4% deoxycholate, 1.2% Triton X-100, 0.4 mM phenylmethylsulfonyl fluoride, 0.01 M leupeptin/pepsatin). Samples were centrifuged for 30 min, and supernatants used to precipitate reduced surface proteins with 50 μL of a 1:1 slurry of streptavidin beads (Pierce) by rocking overnight at 5°C. Beads were then washed four times in PBS and bound proteins eluted in SDS sample buffer and run on SDS gels. Total protein or cell surface protein immunobots, following SDS-PAGE proteins were electrotransferred to nitrocellulose or polyvinylidene difluoro filters (Bio-Rad); the filter was blocked with 5% nonfat milk in PBS and then incubated in primary antibody overnight for (Kv1.1, Kv1.1 rabbit polyclonal antibody raised to the N terminus (amino acids 4–27):1000; for Kv1.4, Kv1.4 mouse monoclonal antibody to the N terminus (amino acids 13–37) at 1:2000 (Upstate Biotechnology). After washing, the radiiodine-labeled anti-rabbit secondary or anti-mouse antibodies were added and the bound antibodies detected using enhanced chemiluminescence (Amersham Biosciences, ECL detection kit) and preflashed x-ray film (Kodak XAR5). Aliquots of the original lysate were immunoblotted with actin antibodies (Sigma) to control for possible cell density differences, and Kv1 signals were normalized to actin levels. A MicroTex 8700 scanner was used to capture a film image, and NIH Image software (1.6) that has its own internal calibration was used to perform relative densitometry analysis (9,10). A control signal was taken as 100.0 ± S.E., and a test signal was normalized to it.

Path Clamping—We have described the details of whole cell patch clamp recordings of COS7 cells previously (9, 10). The Axopatch 200B patch clamp and PClamp8 software/Digidata 1200 system were used. Transiently transfected mammalian cells (with green fluorescent protein plasmid) were viewed using Hoffman optics (400× magnification) and a fluorescence microscope to identify transfected cells by their green fluorescence. Our standard recording solution containing (in mM) NaCl 150.0, KCl 5.0, MgCl₂ 1.0, EGTA 10.0, glucose 5.0, and NaHEPES 10.0 (pH 7.3) at room temperature (25°C) was used. Pipette fill solutions were 10x the intracellular solution containing (in mM) KCl 145, MgCl₂ 1.0, EGTA 10.0, glucose 5.0, and NaHEPES 10.0 (pH 7.3). After establishing the whole cell configuration, cells were stepped at –80 mV, and membrane capacitance and series resistance (Rₛ) were estimated using pClamp8 software from a transient capacitance current elicited by a 10-mV hyperpolarizing voltage step from –80 mV. Mean linear membrane leak current, assessed by averaging the current response to 10-mV hyperpolarizations, was subtracted by the P/4 protocol. Membrane capacitance is compensated using the amplifier controls and Rₛ (usually 25–45 μF uncompensated) is compensated by patch clamp circuitry by 85–95%. The general voltage-pulse protocol is to hold at –80 mV and step to 50 mV for 80 ms. The current induced by the voltage step is filtered at 5 kHz (–3 dB cutoff) and digitized at 50-ms intervals. Maximum conductance values (G) are obtained from the mean value of the mean normalized peak current (I/VCₙ) and a predicted Nernst K equilibrium potential (EK) of ~84 mV. Data are displayed as the normalized mean to the control construct as G/Cₙ (maximal conductance/capacitance) ± S.E., n = 10–12.

Protein Half-life Estimations—Kv1.1, Kv1.4, and their N-linked mutant total protein half-lives were estimated by cell labeling with [35S] Methionine/Cysteine (200 Ci/mmol, Tran³²³label, Amersham Biosciences) in medium without methionine or cysteine for 5 h. The labeled medium was removed and cells chased in unlabeled 5 mM methionine and cysteine in complete medium for different times. Solubilized cells (10) were incubated in Kv1.1 antibody (1/5000) or Kv1.4 antibody (1/2000) overnight. Aliquots of lysed cells were acid-precipitated with 1 M trifluoroacetic acid to determine total 35S incorporation into cellular protein for each 35-mm dish. Protein A/G beads (Invitrogen) were added for 2 h with rocking, and the beads were washed to remove nonspecifically bound proteins. Specifically bound Kv1 proteins were eluted in SDS sample buffer, and a volume added to an SDS gel was adjusted using the cpm signal incorporated into total proteins. Kv1 radiolabeled bands were detected by fluorography (Amplify, Amersham Biosciences) with preflashed x-ray film (Kodak XAR-5). Densitometry analysis, as outlined above, was used to estimate signals, and values were normalized to time zero (start of chase), which was taken as 1.0 and plotted as a function of time. Protein half-life values were estimated from a curve fitted to a decaying single exponential time course, mean ± S.E. (ER, Kv1.1, Kv1.4), and their N-linked mutant cell surface protein half-lives were estimated as follows. Intact cells were biotinylated as described above and washed to remove the biotinylation reagent, and a sample for time zero was taken and processed for streptavidin precipitation and immunoblotting analysis as described above. Other biotinylated and washed cells in dishes were returned to control medium and incubated at 37°C for increasing time periods in the absence of biotinylation reagent, before streptavidin precipitation and immunoblotting. Signals were normalized to the value at time zero (start of chase) and plotted as a function of time to obtain the cell surface protein half-lives as described above. A decrease in the cell surface Kv1 signal was indicative that biotinylated Kv1 proteins were internalized and degraded via the endosomal/lysosomal pathway. However, if a fraction of the internalized biotinylated Kv1 proteins were recycled intact back to the surface, then our estimates of surface protein half-life would be overestimated.

Immunofluorescence Localization Pattern—COS7 transfected with Kv1.1, Kv1.4, and their N-linked mutants were incubated on glass coverslips for 20 h posttransfection. The cells were then fixed, permeabilized, blocked, and incubated in primary antibody followed by secondary antibody conjugated with a fluorophore (Alexa dyes, Molecular Probes) and mounting on glass slides (10). Cells were viewed with an Olympus BX50 microscope (with BX-FLA fluorescence attachment and the appropriate filter cube), and photographs were taken using a 100× objective with a camera set for automatic time exposure. Thus photos should be viewed for the overall localization pattern of Kv1 protein and not for their signal intensity. Nontransfected cells showed no fluorescence signal when incubated as described above, and transfected cells showed no signal with only secondary antibody (data not shown). Antibodies to ER proteins (calnexin, Stressgen) and Golgi proteins (GM130 or p230, Transduction Laboratories) were also used to help localize these organelles (data not shown).

RESULTS AND DISCUSSION

Preventing Glycosylation of Kv1.4 Decreased Protein Stability, Induced Partial ER Retention, and Decreased Surface Protein Levels, Whereas It Had Little or No Effect on These Parameters for Kv1.1—Kv1.4 and Kv1.1 have ~86% amino acid identity from their N-terminal T1 domain to the end of their S6 transmembrane domains (6), and thus these are two very closely related proteins. There is only one N-glycosylation consensus site, NX(S/T), on their extracellular protein linkers (Fig. 1, A and B), and this site was N-glycosylated on both channel proteins; for Kv1.1 it is Asn²⁰⁷ and for Kv1.4 it is Asn³⁵⁴ (see below). What is the effect of N-glycosylation of Kv1.1 or Kv1.4 homomers on their protein stability and trafficking to the cell surface? To address these questions, site-directed mutagenesis was used to engineer a glutamine (Gln) at Asn²⁰⁷ and Asn³⁵⁴ on Kv1.1 and Kv1.4, respectively (Fig. 1B). Kv1.1, Kv1.4, and their N-linked mutants were expressed transiently in CHO cells, where they would be expressed as homotetramers in this cell line (9, 10).

Kv1.1 proteins were detected on immunoblots of total protein (ER, Golgi, plasma membrane) as 2–3 bands (Fig. 2A, lane 1) and as 1 band from the cell surface population by surface biotinination and immunoblotting (Fig. 2A, lane 3), as we have shown previously (9). The Kv1.1 bands were high mannose-type glycoproteins because they were sensitive to cleavage by Endo H glycosidase (data not shown). Kv1.1N207Q showed a couple of bands (Fig. 2A, lane 2), but they were not N-glycosylated. The lower band was a nonglycosylated core protein, and the upper band appeared to be phosphorylated (11); both bands were Endo H glycosidase-insensitive (data not shown). In contrast, Kv1.4 monomers detected from total protein (ER, Golgi, plasma membrane) were a mixture of high mannose-type and
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**Fig. 1.** A. conventional schematic of a Kv1 monomer with a carbohydrate tree attached to the S1–S2 linker. B. amino acid sequence of the S1–S2 linker of Kv1.1 and Kv1.4. The dashes for Kv1.4 represent amino acid identities to Kv1.1. C. schematic of Kv1.4, Kv1.1, Kv1.1C1 chimera (with the pore region of Kv1.4), and Kv1.4C2 chimera (with the outer pore region of Kv1.1). D. amino acid sequence of the S1–S2 linker of Kv1.4. The dashes for Kv1.4 represent amino acid identities to Kv1.4.

mature-type glycoproteins (trans-Golgi glycosylated proteins) (Fig. 2D, lane 1), and mature-type glycoproteins were enriched on the cell surface (Fig. 2D, lane 3). Kv1.4N354Q was detected as 1 band in total protein and surface protein (Fig. 2D, lanes 2 and 4), and it was insensitive to Endo H glycosidase (data not shown). Thus, in transiently transfected cell lines the majority of wild type Kv1.1 channels were high mannose-type glycoproteins, whereas the majority of wild type Kv1.4 channels were mature-type glycoproteins.

Kv1.1 and Kv1.1N207Q total protein levels (ER, Golgi, plasma membrane) (Fig. 2A, lanes 1 and 2, and B) and cell surface levels, by estimates of surface protein by biotinylation and immunoblotting and conductance density (Gm/Cm, maximal conductance/capacitance) by patch clamping (Fig. 2A, lanes 3 and 4, and B), were somewhat similar. A similar pattern for surface levels was recorded in transfected COS7 and CAD-like neuronal cells, suggesting that this effect was not cell line-specific (Fig. 2, G and H). A similar total protein level for both Kv1.1 and Kv1.1N207Q suggested that there was no difference in their protein stability. Indeed Kv1.1 and Kv1.1N207Q showed similar total protein half-lives of 4.2 and 4.3 h, respectively (Fig. 3, A and B), and similar surface protein half-lives of 1.1 and 0.8 h, respectively (Fig. 4, A and B). The surface protein level (Fig. 2B) divided by the total protein level (Fig. 2B) gave a surface protein total protein ratio that was >1.0 for Kv1.1N207Q versus Kv1.1; this suggested that Kv1.1N207Q had a similar or somewhat less intracellular retention versus Kv1.1 (Fig. 2C). Similar surface protein total protein ratios suggested that Kv1.1 and Kv1.1N207Q would exhibit similar whole cell immunofluorescence localization patterns in cells. We used transfected COS7 cells because they have consistent ER and Golgi morphology from cell to cell. Kv1.1 and Kv1.1N207Q localization patterns were similar in COS7 cells (Fig. 5, A and B), and signals overlapped extensively with calnexin signals (data not shown), indicating that both proteins exhibited high partial ER retention. Similar results were found in transfected CHO cells (data not shown). In a previous study (8), we tested the effect of glycosylation, particularly sialylation, on the function of Kv1.1 channels expressed stably in CHO cells and found that this modification affected channel gating. We had to further screen numerous cell clones by immunoblotting to find a few that stably expressed Kv1.1 as mostly a mature-type glycoprotein, with sialic acids, on the cell surface. Cell surface half-life estimations were similar for wild type Kv1.1 as mostly a mature-type glycoprotein and Kv1.1N207Q without a sugar tree (data not shown). Thus, in transient transfections Kv1.1 is mostly expressed as a high mannose-type glycoprotein, whereas stable transfectants can be found that do express it mostly as a mature-type glycoprotein on the surface. In either case, cell surface half-lives were similar to Kv1.1N207Q.

In contrast, Kv1.4N354Q total protein (ER, Golgi, plasma membrane) was only ~40% the level of Kv1.4 (Fig. 2D, lanes 1 and 2, and E), whereas for cell surface levels, by estimating surface protein by biotinylation and immunoblotting and conductance density (Gm/Cm, maximal conductance/capacitance) by patch clamping the level was only ~15% the level of control (Fig. 2D, lanes 3 and 4, and E). A similar pattern for surface levels was found in transfected COS7 and CAD-like neuronal cells, which suggested this effect was not cell line-specific (Fig. 2, G and H). Kv1.4N354Q proteins that were expressed on the cell surface exhibited voltage-gated currents consistent with the notion that surface nonglycosylated channels were folded into functional tetramers. A decreased total protein level suggested that the Kv1.4N354Q protein stability was decreased, and this was a possible partial explanation for its lower surface protein level. Indeed, Kv1.4 and Kv1.4N354Q showed different total protein half-lives of 6.3 and 3.8 h, respectively (Fig. 3, C and D), and different cell surface protein half-lives of 5.3 and 1.8 h, respectively (Fig. 4, C and D). Our Kv1.4 surface protein half-life estimation in CHO cells of 5.3 h is longer than the ~1.5 h that was estimated for Kv1.4 in transfected HEK293 (12), a difference that may be cell line-specific. Thus Kv1.4N354Q exhibited a decreased total and surface protein half-life versus Kv1.4. Furthermore, the surface protein total protein ratio here was only ~0.35 for Kv1.4N354Q versus Kv1.4 (Fig. 2F). This finding suggested that, in addition to a decreased protein half-life, the Kv1.4N354Q protein may also be preferentially retained intracellularly versus Kv1.4. Indeed, the immunofluorescence localization pattern in COS7 cells was very different for Kv1.4 versus Kv1.4N354Q. Kv1.4 cells exhibited a localiza-
tion pattern characteristic of high cell surface expression; it showed a diffuse signal on the cell surface, and the perimeter of the cell was clearly visible (Fig. 5C); and its pattern was different from the ER protein calnexin (data not shown). In contrast, Kv1.4N354Q showed a pattern characteristic of a low cell surface expression and high partial ER retention with small intracellular structures similar to aggresomes in some cells (Fig. 5D, arrow); the signal overlapped extensively with calnexin, an ER marker (data not shown). A similar result was seen in transfected CHO cells (data not shown). Thus, it appeared that Kv1.4N354Q showed higher intracellular retention because of an increased ER retention.

We used an additional approach to inhibit the addition of the carbohydrate tree at Asn354 on Kv1.4 without changing asparagine to glutamine. Both Kv1.4 and Kv1.4N354Q were incubated in tunicamycin, which prevented N-glycosylation of Kv1.4 (Fig. 2I, lane 1), and under this condition cell surface protein levels were similar for both proteins (Fig. 2, I and J). Kv1.4N354Q was also incubated with tunicamycin to control for its possible nonspecific effects on protein translation and/or processing. Immunofluorescence of Kv1.4-transfected COS cells in the presence of tunicamycin also showed an increased intracellular localization pattern to the ER and aggresomes, as we recorded in Kv1.4N354Q cells without the inhibitor (data not shown). These findings were consistent with our mutagenesis results that the determinant on Kv1.4 that affected trafficking and protein stability was the carbohydrate tree and not the amino acid to which it was attached.

These results suggested that preventing N-glycosylation of Kv1.4 decreased protein stability, induced high partial ER retention, and decreased the cell surface levels, whereas this had little or no effect on Kv1.1 (whether as a high mannose-
type or a mature-type glycoprotein). It appeared that an ER subpopulation of Kv1.4N354Q proteins that trafficked to the cell surface were functional, and apparently properly folded, but exhibited a lower protein half-life there. Because both total and surface protein half-lives were decreased for Kv1.4N354Q, this suggested that both proteasomal and lysosomal pathways were involved. Another small ER Kv1.4N354Q subpopulation appeared to be shuttled to aggresomes. This subpopulation of Kv1.4N354Q may have been shuttled to aggresomes because these proteins have misfolded; have not folded efficiently in a timely fashion; or have overloaded the ER but are not misfolded proteins (13). Wild type Kv1.1, which exhibited high partial ER retention similar to Kv1.4N354Q, did not induce aggresomes and appeared to be properly folded here (9, 10). These effects on trafficking for Kv1.4N354Q were somewhat similar to those reported for a Shaker K+ channel N-glycosylation mutant in mammalian cell lines (14), except it was suggested that the decreased total protein half-life of the Shaker N-glycosylation mutant was mostly via the proteasomal pathway and not the lysosomal pathway.

Effects of Glycosylation on the Protein Stability, Intracellular Localization, and Cell Surface Expression Levels of Kv1.1 or Kv1.4 Were Reversed by Exchanging Their Outer Pore Trafficking Determinant Regions—We have shown that trans-Golgi glycosylation and cell surface expression levels of Kv1.1 and Kv1.4 are a function of an outer pore region, because ex-
changing these determinants conferred upon the recipient channel the pore characteristics of the donor for these two parameters (9). We hypothesized that these different pore regions may interact with unknown chaperone-like proteins associated with the ER and Golgi, which may act positively or negatively on the above parameters (9). What is the effect of N-glycosylation on the cell biology of chimeric Kv1.1 and Kv1.4 channels with their pore regions exchanged? We used Kv1.1 C1 (Kv1.1 with the pore region of Kv1.4) and Kv1.4 C2 (Kv1.4 with the outer pore region of Kv1.1) (Fig. 1C). There are only nine amino acid differences in the whole S5-pore-S6 region between these two wild-type channels, and eight of them are in the outer pore region (Fig. 1D). Kv1.1 C1N207Q cell surface levels were only 50% of the level of control Kv1.1 C1 by both protein and Gm/Cm estimations (Fig. 6, A, lanes 1 and 2, and B), whereas Kv1.4C2N354Q cell surface levels were similar to Kv1.4C2 levels by protein estimations (Fig. 6, C, lanes 1 and 2, and D). However, it appeared that Kv1.4C2N354Q was relatively insensitive to depolarization and thus exhibited a low Gm/Cm versus Kv1.4C2 control, although the protein level was similar (Fig. 6, C and D). The explanation for this reduced functionality of Kv1.4C2N354Q is currently unknown. Immunofluorescence localization patterns for these chimeras in COS cells were also reversed; Kv1.1 C1N207Q showed an intracellular retention pattern, and Kv1.1 C1 did not, whereas Kv1.4 C2N354Q and Kv1.4 C2 both exhibited a similar cellular localization pattern indicative of high partial ER retention (Fig. 5, E–H). These results suggested that the Kv1.1 and Kv1.4 pore region trafficking determinants dictated the N-glycosylation effect on the cell biology of the channel.

Possible Mechanism and Significance of the Effects of Glycosylation in K+ Channel Trafficking—Glycosylation of membrane proteins is a common posttranslational modification, but the role that this process plays in the function of glycoproteins appears to be variable (see Ref. 8). In addition, the role of glycosylation in the protein stability and trafficking of membrane proteins also appears to be variable, although glycosylation of secretory and membrane glycoproteins is generally thought to promote protein stability (15).

Our study suggests that two K+ channels with high amino acid identity exhibited very different responses to N-glycosylation in regard to protein stability and trafficking. We speculate that Kv1.4 N-glycosylation plays an important role in promoting proper folding or timely protein folding in the ER and/or acting as an ER export signal for trafficking to the cell surface, as well as increasing protein stability, whereas this modification had little or no effect on these parameters for Kv1.1. Some Kv1.4N354Q ER proteins were apparently shuttled to aggresomes, whereas another population was transported to the cell surface and appeared to be functional, although its protein half-life was decreased there. It appears that Kv1.4 cell surface levels can be altered by multiple positive determinants such as a sugar tree addition (current study), a pore region determinant (9), and a C-terminal motif (discussed in Refs. 9, 10, and 16). Kv1.1 surface levels appear to be controlled by a mostly negative determinant in the pore region that induces high ER retention (9, 10), and the sugar tree addition has little or no effect on protein stability or surface levels. Interestingly, this glycosylation effect on surface levels was dependent on the wild type trafficking pore region determinant that we identified previously (9), because exchanging the pore region determinant between Kv1.4 and Kv1.1 reversed these effects. We speculate that the Kv1.4 pore and sugar tree show some type of dependence in promoting proper ER folding and/or ER export as well as trafficking to the cell surface, and this dependence can be transferred to chimeric Kv1.1 proteins that contain the pore of Kv1.4. In this regard, we have also shown for Kv1.4 that a cytoplasmic C-terminal trafficking determinant required the presence of the Kv1.4 pore region determinant to exert its effect (16). Thus, the Kv1.4 pore determinant appears to be required for both the glycosylation effect on trafficking in our current study and the C-terminal determinant effect on trafficking in our previous study (16).

Kv1.1/Kv1.4 heteromers and Kv1.4 homomers are found in the brain (17), and we have found previously that Kv1.1/Kv1.4 heteromers in cell lines show low cell surface levels versus Kv1.4 homomers, because Kv1.1 promoted high ER retention (10). Lack of N-glycosylation could be a limiting factor for surface expression of Kv1.4 homomers in the brain if they are required at high surface expression levels in different neuronal populations or at distinct locations on a neuron. It is unknown whether differential glycosylation of Kv1.4, in different neuronal populations, might also affect surface expression levels. In any event, dysfunction in glycosylation potential, either by a mutation in a N-glycosylation site or by alterations in the glycoprotein processing machinery, would be predicted to affect the trafficking of Kv1.4 proteins, but it would have little effect on these parameters for Kv1.1 proteins.

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