In MDCK cells, Slo1 channels are mainly expressed at the apical surface. Similarly, transiently transfected MDCK cells target Slo1 channels to their apical surface. To study the role of the microtubules on apical localization of Slo1 channels, we used a viral construct of Slo1 attached to EGFP (Slo1-EGFP) to induce the expression of Slo1. After 72 hrs of infection, cells were either treated with vehicle (control) or with 33 μM nocodazole to disrupt the microtubules. Cells were fixed and immunolabeled with anti α-tubulin to visualize the microtubule network. Using high-resolution confocal microscopy, we found that in control cells the microtubules are localized towards the apical surface, as Slo1. After nocodazole treatment, the microtubule network is completely disrupted and Slo1-EGFP is observed in both apical and basolateral surfaces. To further understand the mechanisms of Slo1 targeting in MDCK cells, we blocked protein synthesis with 1 μg/ml cycloheximide simultaneously with microtubule disruption. In these cells, Slo1-EGFP expression, although still localized at both surfaces, was less prominent at the basolateral surface than without cycloheximide. This result indicates that a part of Slo1-EGFP localized at the basolateral surface after microtubule disruption is redirected from the apical surface and a part of these proteins are newly synthesized. Thus, our results show that in MDCK cells the microtubule network plays an important role in Slo1 apical localization and that normal Slo1 traffic is likely transcytotic first reaching the basolateral surface, then traveling to the apical surface via the microtubule network. Supported by NIH.

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N-Terminal LQT2 Nonsense Mutations Cause a Dominant-Negative Destabilization of WT hERG Subunits

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Familial mutations in *hERG*, the *Human Ether-a-go-go Related Gene*, disrupt cardiac I_{Kr}, leading to Type 2 Long QT Syndrome and potentially fatal ventricular arrhythmias. Of the more than 200 mutations identified to date, eight are nonsense mutations in the hERG 1a amino terminus (NT). How these mutations cause disease is unknown. We found that constructs corresponding to six of these LQT2-linked mutations encoded stable, truncated NT fragments when expressed in HEK cells. We coexpressed these fragments with WT hERG 1a and 1b subunits to mimic heterozygous expression in LQT2 patients and assessed their effects on WT hERG trafficking and expression levels. Western blots revealed that the polypeptides significantly decreased WT 1a and 1b protein expression levels. We hypothesized N-N terminal interactions may mediate the observed reduction and indeed found levels of N-deleted hERG 1a subunit (N-del) were unaffected by the LQT2 polypeptides. Surprisingly, the LQT2 fragments promoted 1a N-del maturation. This indicates the polypeptides are not inherently misfolded and can rescue 1a N-del trafficking, possibly by interacting with downstream regions of the subunit. Reduction of WT protein levels by LQT2 polypeptides therefore requires an intact N-terminus. To determine if the LQT2 polypeptides destabilize WT subunits, we blocked protein synthesis using cycloheximide and tracked WT protein levels over time. Co-expression with LQT2 polypeptides caused a rapid loss of both immature and mature hERG 1a and 1b compared with control indicating accelerated degradation in the ER. In summary, this study demonstrates a novel disease mechanism in which LQT2-linked nonsense mutations in the hERG 1a N-terminus act in a dominant-negative manner by destabilizing ER-resident WT subunits.

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hERG Trafficking is Dependent on a Cytosolic Chaperone Network

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The KCNH2 or human ether-a-go-go related gene (hERG1) encodes the Kv11.1 α-subunit of the hERG potassium channel that underlies the rapidly activating delayed rectifier current IKr. In the heart, mutations in KCNH2 cause a reduction in IKr resulting in the proarrhythmic type 2 long-QT syndrome (LQT2). While multiple factors can cause the loss of the functional phenotype, the dominant mechanism is a trafficking deficiency due to abnormalities in protein folding that result in endoplasmic reticulum (ER) retention. To identify chaperones or co-chaperones potentially involved in the folding and ER retention of hERG we performed a proteomics analysis to identify hERG-interacting proteins. In addition to Hsc70 and Hsp90, key members of the cytosolic chaperone system, we found the co-chaperones Dja1, Bag2, Hop, Tpr2, FKBP38, and the lumenal chaperone calnexin. We recently reported on the putative ER-resident hERG chaperone FKBP38. FKBP38 co-precipitates with hERG both *in vivo* and *in vitro*. Functionally, siRNA knockdown of FKBP38 reduces wild type (WT) hERG trafficking. *In vitro* experiments have also confirmed that translated hERG C-terminus (CT) and cyclic nucleotide binding domain (CNBD) bind to Hsc70 and Hsp90 as well as Dja1 and related family members Dja2 and Dja4. *In vivo* results indicate that overexpression of the Dja proteins result in differential reduction of hERG trafficking. This reduction in hERG trafficking appears to be related to the proteasomal degradation system as inhibiting the proteasome with lactacystin diminishes this effect and re-establishes hERG trafficking to control levels. Another protein involved in degradation, Chip, was also tested and preliminary work indicates that its overexpression leads to decreased hERG expression. Taken together, these data allow us to outline a model of chaperone-mediated hERG maturation and quality control.

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Characterizing Nicotine-Induced α4* nAChR Upregulation with Fluorescence Microscopy

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Nicotine addiction is the world's leading preventable cause of mortality. Smokers also have a much lower incidence of Parkinson's disease. A plausible cellular/molecular mechanism for some responses to chronic nicotine is selective chaperoning of acetylcholine receptor number and stoichiometry (S-CHARNS). To investigate S-CHARNS in a neuronlike environment, we are using single-molecule fluorescence microscopy to monitor localization and trafficking of α4(eGFP)β2 and α4(eGFP)β4 nAChRs expressed in neuroblastoma cells (N2a). As in previous investigations on native neurons and heterologous expression systems, we find large pool(s) of intracellular α4(eGFP)β2 receptors predominantly localized in the endoplasmic reticulum (ER). We also find α4(eGFP)* receptors on vesicles (mobile puncta with diffraction-limited profiles consistent with the point-spread function of our microscope). Time-lapse photobleaching analyses of docked or fused vesicles indicate that vesicles carry 1-2 α4(eGFP)β2 receptor(s). In contrast, cells expressing α4(eGFP)β4 display (a) fewer ER-localized α4(eGFP)* receptors, (b) 5-fold more α4(eGFP)* receptors/vesicle, and (c) ~10-fold more receptors at the plasma membrane (perhaps resulting from (a) and (b)). The β2 and β4 subunits differ most in the M3-M4 intracellular loop, which includes several motifs that may govern the distinct roles of β2 and β4 subunits in α4* receptors. Chronic exposure (24-48 h) to 0.1 μM nicotine results in increased α4(eGFP)β2 or α4(eGFP)β4 receptors at the plasma membrane. We observe many more α4(eGFP)β2 receptors in the filopodia in nicotine-treated cells compared to non-nicotine treated control cells. Thus high-resolution, quantitative imaging of S-CHARNS is providing data required to understand, and eventually to manipulate, changes due to chronic nicotine exposure.

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Protein Kinase C Regulation Of K_{ATP} Channel Recycling

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Pancreatic ATP sensitive potassium (K_{ATP}) channels play an important role in insulin secretion, linking the metabolic state of the beta cell to its excitability. Protein kinase C (PKC) has previously been shown to down regulate the cell surface density of K_{ATP} channels in cardiac, neuronal and recombinant cells, but there are no studies on pancreatic beta cells. Here we show that activation of PKC results in significant down regulation of K_{ATP} channel conductance in the model beta cell line, INS1e. To investigate the underlying mechanism, we expressed a Kir6.2 construct containing an extracellular HA epitope plus SUR1 in INS1e and HEK293 cells and examined the role of PKC in regulation of cell surface density and endosomal trafficking. We found that PKC activation with the phorbol ester drug PMA reduced the surface density of K_{ATP} channels by reducing recycling of endocytosed channels, but had no effect on the rate of endocytosis. Endocytosed channels entered the peripheral and perinuclear compartments when PKC was activated, but remained at a peripheral location when PKC was inhibited with chelerythrine. Since pancreatic beta-cells express 9 different isoforms of PKC, we asked which of these isoforms is responsible for the observed effect. Using the dominant negative approach, our results show that PKC epsilon regulates endosomal trafficking of K_{ATP} channels. We conclude that that activation of PKC epsilon leads to dramatic changes in the distribution of internalised K_{ATP} channels and that the decrease in channel surface density is due to inhibition of channel recycling. The results may have important consequences for the electrical excitability of the pancreatic beta cell and therefore the insulin secretory response.