RNA Editing in the Central Cavity as a Mechanism to Regulate Surface Expression of the Voltage-Gated Potassium Channel Kv1.1

Anne K. Streit#,1, Lina A. Matschke#,1, Amalia M. Dolga2, Susanne Rinné1 and Niels Decher1,*

1Institut für Physiologie und Pathophysiologie, Fachbereich Medizin, Philipps-Universität Marburg, D-35037 Marburg, Germany;
2Institut für Pharmakologie und Klinische Pharmazie, Fachbereich Pharmazie, Philipps-Universität Marburg, D-35032 Marburg, Germany

*Running title: RNA Editing of Kv1.1 and Channel Trafficking

#To whom correspondence should be addressed: Niels Decher, Philipps University of Marburg, Institute for Physiology and Pathophysiology, Vegetative Physiology, Deutschhausstrasse 1-2, D-35037 Marburg, Germany, Tel.: (+49) 6421-28-62148, E-mail: decher@staff.uni-marburg.de

#These authors contributed equally to this work

Keywords: RNA editing; Kv channel; Trafficking; Surface expression; Kv1.1

Background: Kv1.1 channels are regulators of neuronal activity, undergoing RNA editing.

Results: RNA editing leads to reduced Kv1.1 channel expression at the cell surface, while heteromeric channels with Kv1.4 are not affected.

Conclusion: RNA editing is a mechanism to regulate homomeric Kv1.1 channel trafficking.

Significance: Fine tuning of Kv1.1 surface expression by RNA editing contributes to the complexity of neuronal Kv channel regulation.

ABSTRACT

Kv1.1 channels undergo a specific enzymatic RNA deamination, generating a channel with a single amino acid exchange located in the inner pore cavity (Kv1.1I400V). We studied I400V edited Kv1.1 channels in more detail and found that Kv1.1I400V gave rise to much smaller whole-cell currents than Kv1.1. In order to elucidate the mechanism behind this current reduction, we conducted electrophysiological recordings on whole-cell and single-channel level and did not find any differences. Next, we examined channel surface expression in Xenopus oocytes and HeLa cells using a chemiluminescence assay and found the edited channels to be less readily expressed at the surface membrane. This reduction in surface expression was verified by fluorescence imaging experiments. Western blot analysis - for comparison of protein abundances and glycosylation pattern - did not show any difference between Kv1.1 and Kv1.1I400V, further indicating that a changed trafficking of Kv1.1I400V is causing the current reduction. Block of endocytosis by dynasore or AP180C did not abolish the differences in current amplitudes between Kv1.1 and Kv1.1I400V, suggesting that backward trafficking is not affected. Thus, our data suggest that I400V RNA editing of Kv1.1 leads to a reduced current size by a decreased forward trafficking of the channel to the surface membrane. This effect is specific for Kv1.1 since co-expression of Kv1.4 channel subunits with Kv1.1I400V abolishes these trafficking effects. Taken together, we identified RNA editing as a novel mechanism to regulate homomeric Kv1.1 channel trafficking. Fine tuning of Kv1.1 surface expression by RNA editing might contribute to the complexity of neuronal Kv channel regulation.

Voltage-gated potassium (Kv) channels form a large family of ion channels with diverse functional properties. The channels are tetramers consisting of four subunits, each containing six transmembrane segments. Kv1.1 channels which are in the focus of this current study are widely expressed in neurons (1). They are key players of repolarization, but also regulate the threshold for action potential initiation and thus have a crucial role in the determination of action potential duration and frequency (2). The importance of this particular channel becomes evident in several hyperexcitability disorders caused by mutations in the corresponding KCNA1 gene. Kv1.1-associated disorders include episodic ataxia, myokymia and certain types of epilepsy (3-5). Recently, Wykes and colleagues used a rat model
of focal epilepsy to show that lentiviral over-expression of Kv1.1 can suppress epileptic activity (6), strongly suggesting an anti-epileptic role of this channel.

It is noteworthy that Kv1.1 is the only Kv channel member for which it is known that the mRNA is target of enzymatic RNA deamination. The adenosine deamination enzyme ADAR2 converts one single adenosine nucleoside to inosine. This leads to a change of codon interpretation during translation and thus to a change of the amino acid isoleucine 400 in the pore forming S6 segment of the channel to a valine (I400V) (7). The first functional change reported for the I400V editing was that the Kv1.1I400V subunits have decreased affinity to the β2 ATP (8). In mice and humans the ratio between edited (Kv1.1I400V) and the non-edited (Kv1.1) channel abundance varies depending on the cell type (7,9). We have previously shown that both channel versions form functional channel tetramers and co-assemble to form ‘heteromeric’ channels of Kv1.1 and Kv1.1I400V. Several consequences of the Kv1.1I400V editing have been described so far: Channel tetramers containing edited Kv1.1I400V subunits have an altered pharmacology and a reduced sensitivity to block by highly unsaturated fatty acids, like arachidonic acid or anandamide (9). Further, using the kainic acid rat epilepsy model, we found that Kv1.1 editing levels are increased in the entorhinal cortex of chronic epileptic rats when compared to healthy control animals (10). However, it is not yet clear, if seizures influence the editing rates or vice versa.

In light of the relevance of Kv1.1 for brain physiology and pathophysiology, it is of great interest to learn more about the regulation of this channel by RNA editing. Here, we describe for the first time that Kv1.1 RNA editing at the I to V site in the central cavity influences channel trafficking leading to reduced abundance of channels at the cell surface and thus reduced current densities. These findings contribute to the already complex picture of Kv1.1 channel regulation by RNA editing.

**EXPERIMENTAL PROCEDURES**

**Cell culture and transfection-** HEK293, HeLa and CHO-K1 cells were cultivated at 37 °C and 5 % CO2 in DMEM medium (Invitrogen) or in case of CHO-K1 Ham’s F-12 Nutrient Mixture (Gibco), supplemented with 10 % FCS and 1 % Penicillin/Streptomycin solution (Invitrogen). For electrophysiological recordings or imaging experiments cells were grown either on plastic (NUNC) or glass bottom (WillCo) 35 mm petri dishes, respectively. At a confluence of 60-70 % cells were transfected with FuGENE6 (Promega) or JetPRIME (Peqlab). For patch-clamp and fluorescence imaging experiments, a total amount of 1 µg of cDNA per 35 mm dish was used for transfection.

**Expression of ion channels in Xenopus oocytes-** Ovarian lobes were obtained from anesthetized Xenopus toads, mechanically separated with forceps and then incubated for 60 - 120 min in an OR2 solution (in mM: 82.5 NaCl, 2 KCl, 1 MgCl2, 5 HEPES) containing 2 mg/ml collagenase. Thus separated oocytes were washed in ND96 solution (see below) supplemented with Na-pyruvate (275 mg/l), theophylline (90 mg/l) and gentamicin (50 mg/l) and stored in the same solution at 18 °C before and after injection of cRNA. A volume of 50 nl cRNA solution per oocyte was injected with a NanojectII microinjector (Drummond Scientific).

**Electrophysiology-** All electrophysiological recordings where performed at room temperature (20 – 22 °C). Whole-cell recordings were performed with an EPC10 amplifier (HEKA) 24 - 30 h after transfecting a total amount of 1 µg cDNA per 35 mm dish. Series resistance was compensated by 50 %. The extracellular solution contained in mM: 135 NaCl, 5 KCl, 10 Glucose, 1 MgCl2, 0.33 NaH2PO4, 1 CaCl2, 2 Na-Pyruvate, 10 HEPES (pH 7.4). Intracellular solution was used as bath solution.

**RNA Editing of Kv1.1 and Channel Trafficking**

RNA Editing of Kv1.1 and Channel Trafficking
KCl, 1.8 CaCl₂, 1 MgCl₂, 5 HEPES (pH 7.5). 3M KCl was used as intracellular solution. Pipette resistances were between 0.5 and 1.5 MΩ.

Chemiluminescence assay in Xenopus oocytes- Surface expression of Kv1.1 channel constructs was studied using a chemiluminescence based assay. For wild-type Kv1.1 and Kv1.1I400V we introduced after the residue G200 an hemagglutinin-epitope (HA) with the amino acid sequence “YPYDVPDYA” into the first extracellular loop of the channel (see Fig. 3B, inset). cRNA of HA-tagged channels was injected into Xenopus oocytes and surface expression was analyzed after 48 h. First, oocytes were incubated in ND96 plus 1 % BSA on ice for 30 min to reduce unspecific antibody binding, followed by incubation with primary antibody (rat anti-HA (Roche), 1:100) for 1 h, extensive washing and incubation with secondary antibody (goat anti-rat-IgG, HRP-coupled (Dianova), 1:500) for 1 h. Oocytes were again extensively washed, before they were individually placed into a vial with 20 µl of luminescence substrate (SuperSignal Femto (Thermo Scientific)). Light emission was detected with a GloMax luminometer (Promega).

Chemiluminescence assay in HeLa cells- HeLa cells were transfected with the indicated constructs using jetPRIME (Peqlab). After 48 h, cells were fixed with 4 % PFA (in PBS), washed three times with PBS and blocked with 10 % normal goat serum (in PBS). Cells were stained with a monoclonal anti-HA primary antibody (HA-probe (F-7), Santa Cruz) and washed intensively with PBS. As a secondary antibody a horseradish-peroxidase (HRP)-conjugated antibody (goat anti-mouse IgG, HRP-coupled (Dianova), 1:500) for 1 h. Oocytes were again extensively washed, before they were individually placed into a vial with 20 µl of luminescence substrate (SuperSignal Femto (Thermo Scientific)). Light emission was detected with a GloMax luminometer (Promega).

Western blot for protein quantification- To quantify the functional expression of Kv1.1, Kv1.1I400V and Kv1.4 channel proteins, whole oocytes were lysed and protein extraction was carried out as described previously (11). Briefly, 20 oocytes were used for each lysis reaction. The oocytes were homogenized in 400 µl lysis buffer (NaCl, 150 mM; Tris/HCl, 20 mM; Triton X-100, 1 %; Protease Inhibitor Cocktail (Roche), 10 µl; pH 7.5). Insoluble material was separated by centrifugation (13000 rpm) for 15 min at 4 °C. 15 µl of the supernatant were mixed with 5x SDS sample buffer, heat denatured at 95 °C for 5 minutes, separated on 10 % SDS-polyacrylamide gels and visualized by immunoblotting. For the detection of untagged channels, anti Kv1.1 (Alamone Labs) and anti Kv1.4 primary antibodies (provided by J. Trimmer) were used. The binding of the primary antibodies was detected using peroxidase-conjugated goat anti-rabbit IgG (Pierce) or peroxidase-conjugated goat anti-mouse IgG (Pierce), respectively and a chemiluminescent extended-duration substrate (SuperSignal WEST Dura, Pierce). Uninjected oocytes were used as negative controls. Band intensities were analyzed with ImageJ software (NIH). For western blot analysis in HeLa cells, 48 h after transfection cells were washed with PBS, scratched from the dish bottom and centrifuged at 2000 rpm for 5 min at 4 °C. The supernatant was discarded and 200 µl of RIPA buffer (TRIS-Base, 50 mM; NaCl, 150 mM; NP40, 1 %; sodium-desoxycholate, 0.25 %; EDTA, 1 mM; Protease Inhibitor Cocktail (Sigma), 10 µl; pH 7.4) were added. After 30 min of incubation on ice the lysates were centrifuged at 13000 rpm for 15 min at 4 °C. 16 µl of the protein containing supernatant were mixed with 5x SDS sample buffer, denatured 5 min at 95 °C, centrifuged at 5000 rpm for 3 min and transferred to 10 % SDS-polyacrylamide gels. For all western blots Bradford assays were performed to ensure loading equal amounts of total protein. Ponceau S staining after blotting was used to verify correct protein transfer and ensure equal lane loading of western blots.

Fluorescence imaging- HeLa-cells were transfected with 1 µg cDNA per dish and 24 h later fixed for 10 min in 4 % paraformaldehyde and blocked for 1 h with 1 % BSA in PBS. The short fixation time was selected to prevent permeabilization of cell membranes. Primary antibody (rat anti-HA (Roche)) and secondary antibody (goat anti-rat IgG Alexa Fluor, 488 (Life technologies)) were diluted 1:100 or 1:500 in PBS, respectively and incubation time for each antibody was 1 h. All washing steps were performed with PBS. For live cell imaging CHO-K1 cells at a confluence of 60 % were transfected using FuGENE6 (Promega). For co-transfections with trans-Golgi or ER-markers 1 µg of EGFP-tagged Kv1.1 or Kv1.1I400V cDNA and 0.5 µg of the DsRed-tagged marker cDNA were used per dish. Both plasmids, pDsRed-Monomer transmedial Golgi marker and pDsRed2-ER marker, were obtained from Clontech. For co-transfection of the edited and the non-edited channel, 1 µg of EGFP-tagged Kv1.1I400V cDNA and DsRed-tagged Kv1.1 cDNA were used. To label lysosomes the culture medium was replaced 1 h
RESULTS

**I400V edited Kv1.1 channels** (**Kv1.1**I400V) have regular single-channel conductance and kinetics-

Single-channel recordings were performed in the on-cell configuration from HEK293 cells transiently transfected with cDNA of either Kv1.1 or Kv1.1I400V (Fig. 2A). Using whole-cell current-clamp measurements, we determined an average membrane potential of -47 ± 1 mV. Thus, we could calculate the required holding potential in on-cell recordings to obtain distinct transmembrane potentials of the patch. Upon depolarization from -77 mV to +53 mV, some patches contained Kv1.1 channels, showing typical single channel activities (Fig. 2A). In accordance with previously reported Kv1.1 single channel recordings (12-13), we observed Kv1.1 channel openings occurring in bursts (Fig. 2A). The overall appearance of Kv1.1I400V single-channel openings was very similar to that of Kv1.1 (Fig. 2A). We carried out a detailed analysis of the single-channel behavior (for further description see (9)), starting with a comparison of the mean burst durations. Bursts were analyzed from the last 700 ms of 1 s pulses at +53 mV. To allow consistent analysis, single bursts were defined to be separated by closures >10 ms. Brief closures, usually <2 ms, occurred within bursts. The mean burst durations of Kv1.1 (298 ± 54 ms) and Kv1.1I400V (375 ± 92 ms) were not significantly different (Fig. 2B). From the same recordings, we obtained the general open probability (Pₒ) of the channels. There was no significant difference in Pₒ between Kv1.1 (0.65 ± 0.07) and Kv1.1I400V (0.51 ± 0.13) (Fig. 2C). When single-channel amplitudes are plotted versus the transmembrane potential the single-channel conductance can be determined from the slope of the best-fit line (examples given in Fig. 2D). Single-channel conductances obtained this way were 11.5 ± 1.3 pS for Kv1.1 and 11.8 ± 2.5 pS for Kv1.1I400V. Thus, the single-channel conductance was also not significantly different for the edited and unedited Kv1.1 channels (Fig. 2E). Summarizing, the single-channel characteristics of Kv1.1 are not influenced by the I400V editing and changes in single-channel conductivity cannot explain the strongly reduced current amplitudes of Kv1.1I400V. Therefore, we set out to analyze if the I400V editing alters surface expression of the channels.

Kv1.1I400V has a reduced surface expression while protein translation and stability are not affected- First, we wanted to verify the effect on current size in *Xenopus* oocytes, to see if this expression system was suitable to study surface expression. Therefore, *Xenopus* oocytes were

before imaging by HBSS (Gibco) containing 50 nM LysoTracker (Molecular Probes). Fluorescence microscopy was performed with an Axio Observer.Z1 microscope (Zeiss), equipped with a Zeiss Plan-Apochromat 60×/1.40 Oil DIC objective. During live cell imaging, cells were maintained at 37 °C using an objective heater (Biotoptechs). For EGFP-tagged constructs a standard Zeiss filter set (38HE) and for DsRed-tagged constructs, a filter set of AHF Analysentechnik AG (Tübingen, Germany) (F46-005) was used. Images were taken with a Zeiss 12bit ‘AxioCam MRm’ camera, and digital images were processed using Zeiss AxioVision Software.

Statistical analyses- Data are reported as means ± S.E.M. Statistical significance was calculated using unpaired Student’s t-test. *, indicates P < 0.05; **, P < 0.01; ***, P < 0.001. “n.s.” indicates no significant change.

**RESULTS**

Reduced current amplitudes of Kv1.1I400V in whole-cell patch clamp recordings- To compare electrophysiological characteristics of Kv1.1 and Kv1.1I400V we individually transfected the two channel variants in HEK cells and performed voltage-clamp recordings in the whole-cell configuration. From a resting membrane potential of -80 mV voltage steps between -70 and +50 mV were applied to activate the channels (Fig. 1A). In terms of kinetics, the current responses of Kv1.1 and Kv1.1I400V were indistinguishable. Plotting of the current-voltage relationship (I/V), analyzed from the current at the end of each activating voltage step (Fig. 1A, see arrow), revealed strong differences between Kv1.1 and Kv1.1I400V (Fig. 1B). At +50 mV, we found a more than 50% reduction of the peak current densities in cells that were expressing Kv1.1I400V (25 ± 6 pA/pF), compared to Kv1.1 expressing cells (54 ± 7 pA/pF) (Fig. 1C). The conductance-voltage relationship (G/V) was, as previously described (8), not significantly changed (Fig. 1D) and thus, an altered voltage dependence cannot explain the reduced current amplitudes of Kv1.1I400V. Therefore, the reduced current density can result from (i) differences in single-channel behavior, (ii) changes in synthesis or stability of the channel protein or (iii) altered intracellular channel transport. With the following set of experiments we investigated each of these possibilities to identify the mechanism underlying the reduced current density observed for the edited channels.

...
RNA Editing of Kv1.1 and Channel Trafficking

We investigated whether RNA editing affects channel trafficking in mammalian cells. To this end, we transfected HeLa cells with the Kv1.1-HA and Kv1.1 I400V-HA constructs into the dsRed-Monomer vector. In this way, we obtained channels that were double-tagged with a dsRed at their intracellular N-terminus and an HA-epitope in the extracellular S1-S2 linker of Kv1.1 and Kv1.1 I400V. Thus, obtained HA-tagged channels (Fig. 3B, inset) allow quantification of their surface expression in a luminometric assay. As a control, we performed voltage-clamp recordings of the HA-tagged channels, to ensure that the introduced epitope does not interfere with the channel function (Fig. 3B). The difference in relative current amplitude between Kv1.1-HA and Kv1.1 I400V-HA is very similar to that observed using the untagged channels (Fig. 3A vs. 3B). In the subsequent surface expression assay Kv1.1 I400V-HA channels showed only 45 ± 6 % of the relative light units (RLU) measured for Kv1.1-HA channels (Fig. 3C). Strikingly, the ratios (Kv1.1 vs. Kv1.1 I400V) of RLU reduction in the surface expression assay and the current reduction recorded in TEVC are very similar (compare Fig. 3A-B vs. C). These data propose a reduced surface expression of edited channels as a reason for the reduction in whole-cell current amplitude. However, it was unclear how the RNA editing of Kv1.1 leads to reduced surface expression of channels. There are basically two mechanisms that could account for this reduction in surface expression: (i) Reduced abundance of channel protein or (ii) altered intracellular channel trafficking to/from the membrane. We performed western blot experiments of whole cell protein lysates from oocytes expressing Kv1.1 or Kv1.1 I400V using an anti-HA antibody to detect the global channel protein expression (Fig. 3D). Both channels are detected as double-bands at ~60 and 65 kDa, due to different degrees of glycosylation (Fig. 3D), as was already previously described (14). Thus, I400V editing does not alter the glycosylation pattern of Kv1.1 channels. Analysis of band intensities from four different western blots revealed that there is also no significant difference in the protein amount between Kv1.1 and Kv1.1 I400V (Fig. 3E). These data show that neither protein synthesis nor stability is affected by Kv1.1 editing, thus suggesting that channel trafficking is altered. Next, we addressed the question whether altered forward or backward trafficking is causing the reduced surface expression. Therefore, we incubated the injected oocytes for 24 h with dynasore, a dynamin inhibitor that blocks endocytosis and thus 'backward' trafficking of channels from the membrane. TEVC recordings revealed that dynasore incubation (40 µM) did not antagonize the reduction in current amplitudes observed for the I400V edited channels (Fig. 4A). This suggests that reduced surface expression of Kv1.1 I400V is not due to increased backward trafficking or endocytosis. We have also performed experiments in *Xenopus* oocytes and HeLa cells (Fig. 4B-E), blocking endocytosis by over-expression of the C-terminal part of the clathrin adaptor protein AP180 (AP180C) (15-17). The experiments performed in *Xenopus* oocytes using AP180C, indicate that the Kv1.1 I400V has no change in clathrin-mediated endocytosis (Fig. 4B). The results are virtually the same, as obtained with 40 µM dynasore (Fig. 4A vs. 4B). Next, we performed experiments with AP180C using HeLa cells, as endocytosis and the effects of AP180C were previously primarily studied in mammalian cell lines. Here, we studied Kv1.1 with an extracellular HA-epitope and performed a luminometric assay detecting for the whole cell population only those Kv1.1 channels present at the plasma membrane. As AP180C increases Kir2.1 surface expression by blocking channel endocytosis (18), we used a Kir2.1 channel with an extracellular HA-epitope as a positive control. As expected, the luminometric surface expression assay showed that AP180C increases the surface expression of Kir2.1 (Fig. 4C), while surface expression of Kv1.1 and Kv1.1 I400V was not affected (Fig. 4D-E). Thus, all sets of experiments: using dynasore and AP180C in oocytes (Fig. 4A-B) and the experiments using AP180C in HeLa cells (Fig. 4C-E), indicate that Kv1.1 I400V channels do not underlie an increased clathrin-mediated endocytosis.

**Fluorescence imaging and chemiluminescence assays in HeLa cells confirm reduced surface expression of Kv1.1 I400V.** To study the Kv1.1 I400V surface expression in mammalian cells, we subcloned the Kv1.1-HA and Kv1.1 I400V-HA constructs into the dsRed-Monomer vector. In this way, we obtained channels that were double-tagged with a dsRed at their intracellular N-terminus and an HA-epitope in the extracellular S1-S2 linker. We transfected HeLa cells with equal amounts of either channel construct and after 48 h we fixed the cells without...
permeabilization using PFA. The magenta fluorescence provides an overview of the channel localization including intracellular signals by the dsRed-fused to the N-terminus of the channel. The surface expression of the channels was detected with the help of a rat HA-primary antibody targeting the extracellular HA-epitope of Kv1.1 and a green fluorescent secondary antibody. As shown in representative images, Kv1.1 (Fig. 5A) and Kv1.1\textsubscript{I400V} (Fig. 5B) could be detected intracellularly (magenta) and at the surface membrane (green). Next, we counted membrane-stained versus non membrane-stained cells to quantify the changes in surface expression. Membrane stained cells were counted in blinded experiments. Percentages of membrane stained cells were obtained by counting all cells versus membrane stained cells in each field of view. The percentage of cells that showed membrane staining was significantly higher for the Kv1.1-HA channel (35 ± 4 %) than for the Kv1.1\textsubscript{I400V}-HA channel (12 ± 2 %) (Fig. 5C), indicating that the non-edited channel is more readily trafficked to the surface membrane than the edited one. Next, we performed western blot analysis of Kv1.1-HA and Kv1.1\textsubscript{I400V}-HA transfected cells which show that the overall protein abundance is unchanged (Fig. 5D). As there was no difference detected, we could rule out changes in protein abundance as a cause for the reduced surface expression of Kv1.1\textsubscript{I400V} channels.

To give a more quantitative measure, we repeated the experiments shown in Fig. 5A-C using a luminometric assay, detecting only the extracellular HA-epitope of Kv1.1 channels (fixed, non-permeabilized cells). Thus, the luminometric assay detects for the whole population of cells all Kv1.1 channels present at the plasma membrane. This chemiluminescence assay showed that the surface expression of Kv1.1\textsubscript{I400V} was reduced by about half (Fig. 5E) which is in good agreement with the surface expression data obtained using oocytes (Fig. 3C).

Co-expression with Kv1.4 channels “rescues” surface expression of edited channels in HeLa cells and Xenopus oocytes- In neurons, Kv1.1 channel subunits are often co-expressed with other Kv1 channel subunits, e.g. Kv1.4. Previous studies have shown that different Kv1 channel subunits show pronounced differences in trafficking behavior, with Kv1.4 usually showing stronger surface expression than Kv1.1 (19). However, Kv1.1 expression can be boosted by heteromerization with Kv1.4 subunits. We repeated the imaging experiments described above, this time co-expressing Kv1.1-HA or Kv1.1\textsubscript{I400V}-HA with Kv1.4 (untagged). Indeed, after co-transfection with Kv1.4, the intensity of the Kv1.1-HA signal at the plasma membrane was markedly increased (Fig. 5F vs. Fig. 5I). However, also for Kv1.1\textsubscript{I400V}-HA the signal at the plasma membrane was strongly increased when Kv1.4 was co-transfected (Fig. 5G) and no apparent differences remained between heteromeric channels containing edited or non-edited Kv1.1 subunits (Fig. 5F-G). In fact, the percentage of membrane stained cells was not significantly different for Kv1.1 (43 ± 5 %) and Kv1.1\textsubscript{I400V} (51 ± 3 %), when both channel variants were co-expressed with Kv1.4 (Fig. 5H). This shows that heteromerization with Kv1.4 channel subunits can abolish the differences in surface expression observed with homomeric Kv1.1 or Kv1.1\textsubscript{I400V} channels. Western blot experiments show that also when co-expressed with Kv1.4, the Kv1.1 and Kv1.1\textsubscript{I400V} channels showed similar amounts of overall protein abundance (Fig. 5I).

To more quantitatively confirm that Kv1.4 co-expression can counteract the influence of Kv1.1 and Kv1.1\textsubscript{I400V} surface expression, we performed a chemiluminescence assay with HeLa cells expressing HA-tagged Kv1.1 or Kv1.1\textsubscript{I400V} channels co-transfected with Kv1.4. The luminometric quantification of Kv1.1 surface expression in the presence of Kv1.4 again revealed that the effects of Kv1.1\textsubscript{I400V} on surface expression are abolished in the presence of Kv1.4 (Fig. 5J). Here, no significant differences in relative light units were detected, confirming equal surface expression levels of Kv1.1 and Kv1.1\textsubscript{I400V} (Fig. 5J).

This effect could also be confirmed in TEVC recordings of oocytes co-expressing Kv1.4 with either Kv1.1 or Kv1.1\textsubscript{I400V} (Fig. 6). As a control, we injected same amounts of either Kv1.1 or Kv1.1\textsubscript{I400V} alone; again Kv1.1\textsubscript{I400V} channels elicited approximately one third (2.4 ± 0.2 µA) of the current magnitude of Kv1.1 channels (6.3 ± 0.6 µA) (Fig. 6A). Also here, using untagged channels, western blot analysis with whole cell lysates showed that protein abundance was not different between Kv1.1 and Kv1.1\textsubscript{I400V} (Fig. 6A, inset). In contrast, co-injection of a 1:1 cRNA mixture of either Kv1.1 or Kv1.1\textsubscript{I400V} with Kv1.4 (Fig. 6B) led to current amplitudes that were not significantly different from each other (5.7 ± 0.3 µA and 5.0 ± 0.7 µA, respectively), supporting the abovementioned compensatory effect of Kv1.4 subunits on trafficking of edited Kv1.1 channel subunits. To rule out that the observed
compensating effect of Kv1.4 on Kv1.1\(^{1400V}\) current size is caused by changes in protein expression levels, we performed western blot analysis using anti-Kv1.1 and anti-Kv1.4 antibodies (Fig. 6C). Kv1.1 and Kv1.1\(^{1400V}\) channels have also in the presence of Kv1.4 a similar protein abundance (α-Kv1.1 in Fig. 6C).

In addition, the protein abundance of Kv1.4 is similar for the co-expression with either Kv1.1 or Kv1.1\(^{1400V}\) (α-Kv1.4 in Fig. 6C).

As I400V RNA editing leads to reduced Kv1.1 channel expression at the cell surface, while Kv1.4 channels are not affected, Kv1.1 RNA editing is a mechanism to regulate the trafficking of homomeric Kv1.1 channels.

Kv1.1\(^{1400V}\) channels are most likely retained in the ER, where most of the Kv1.1 channels reside. Next we performed additional experiments using ER, Golgi and lysosomal markers to pursue the question were Kv1.1\(^{1400V}\) is retained. In our initial imaging experiments (Fig. 5), we used fixed cells to detect the minor fraction of Kv1.1 and Kv1.1\(^{1400V}\) channels present at the plasma membrane. As we were now more closely focusing on the intracellular distribution pattern, we used live cell imaging experiments using CHO-K1 cells. As previously described (19-20), Kv1.1 is primarily retained in the ER and plasma membrane expression can be hardly detected (Fig. 7). Note that for detecting Kv1.1 at the plasma membrane one has to choose an efficient method of fixing cells and detecting channels with an antibody targeting an extracellular epitope (Fig. 5). Also using live cell imaging there is no apparent difference in the intracellular fluorescence pattern of Kv1.1 and Kv1.1\(^{1400V}\).

Both, Kv1.1 and Kv1.1\(^{1400V}\), show only a minor co-localization (merge to white) with markers of the trans-Golgi and lysosomes (Fig. 7A-B), and thus, there is no sign of a retention of the edited channel in these compartments. Both channels, Kv1.1 and Kv1.1\(^{1400V}\), primarily co-localize with an ER-marker (Fig. 7C). Therefore, we conclude that Kv1.1\(^{1400V}\) channels are not retained in a new compartment. Co-transfection of Kv1.1 tagged with DsRed and Kv1.1\(^{1400V}\) tagged with EGFP (Fig. 7D) also revealed a strong co-localization (in white), again with an ER-like fluorescence pattern (Fig. 7D). Note that there is no new or separate compartment for the edited channels.

In summary, the live cell imaging data suggest that the Kv1.1\(^{1400V}\) is not retained in a new compartment and thus retention most likely occurs in the ER, where most of the Kv1.1 channels reside.

**DISCUSSION**

In the present study we provide evidence that RNA editing reduces the surface expression of Kv1.1 channels. We could exclude reduced abundance of the channel protein as well as increased internalization as possible reasons for the reduced surface expression and thus conclude that forward trafficking must be impaired. Kv1.1 is, as previously reported, primarily located in intracellular compartments, especially the endoplasmic reticulum (19-20). Since the intracellular fluorescence patterns of Kv1.1 and Kv1.1\(^{1400V}\) are indistinguishable (Fig. 5A and B), it is most likely that the Kv1.1\(^{1400V}\) channels are retained in their ‘favorite’ compartments, without causing a significant change in the intracellular fluorescence intensity. Here, we have to consider that even if only half of the amount of Kv1.1 channels reaches the plasma membrane, one would not expect a major change in the fluorescence at the endoplasmic reticulum, considering that only a small fraction of Kv1.1 channels reach the plasma membrane. Therefore, we can currently only speculate that Kv1.1\(^{1400V}\) channel proteins are likely to be retained in the endoplasmic reticulum.

On protein level, the ADAR-mediated RNA editing changes one single amino acid residue in the inner cavity of the Kv1.1 channel pore. We were surprised to find out that an amino acid exchange in the inner pore can influence trafficking, as it is an unusual location for a trafficking signal. The question arises, how this amino acid position can influence channel trafficking. This question is not trivial and we can currently only speculate on that topic. The limiting step in forward trafficking of most membrane-proteins is ER export (21-22). Classical transport signals within membrane-proteins are usually located in the N- and/or C-terminus (23). For Kv1 channels three non-consecutive amino acid positions in the outer pore loop have been shown to regulate ER export (24-25). How the outer pore loop influences trafficking is still under debate and the hypothesis of a yet unknown ER associated protein that can interact with those amino acids and act as a mediator in trafficking has arisen (25). Amino acid exchanges in the drosophila Shaker channel at the site homologous to the human 1400 editing site, have shown that this reside in the inner cavity can allosterically influence the conformation of the outer pore (26). We speculate that the abovementioned ER associated protein might bind to the outer pore and decide about the fate of the channel based on the outer pore conformation.
RNA Editing of Kv1.1 and Channel Trafficking

which in turn depends on the editing status. Alternatively, the same or a similar protein might directly make contact to the inner cavity and identify the residue at the editing site. Another common reason for the retention of proteins in the ER is misfolding. One might speculate that the edited version of the Kv1.1 channel protein is not as effectively folded as the non-edited version and misfolded channel proteins would be retained in the ER. This hypothesis however is less plausible, given the fact that the currents arising from homomeric Kv1.1I400V channels are kinetically nearly identical to those of Kv1.1 and no obvious ER-stress was observed. Most importantly, our western blot analyses clearly show that protein translation efficiency, stability and glycosylation are not affected.

Considering the results presented in our current study, the I400V editing of Kv1.1 channels appears to lead to a clear loss-of-function, as fewer channel proteins reach the plasma membrane. However, we have also learned from our previous studies that channels containing edited Kv1.1I400V subunits are resistant to block by endogenous lipids like highly unsaturated fatty acids (9). This effect would result in a gain-of-function by the I400V editing. Currently, it is hard to speculate which of the effects has the major physiological relevance. We have shown that Kv1.4 channel subunits by forming heteromeric channels abolish the differences in trafficking between Kv1.1 and Kv1.1I400V. This adds another level of complexity to the prediction whether the I400V editing results in a loss- or gain-of-function under physiological conditions. It might be the case that depending on the circumstances and cellular environment either a loss-of-function or a gain-of-function prevails.

The Kv1.1I400V editing is another example where amino acids not directly facing the cytosol regulate channel surface expression. Unfortunately, the puzzling question how the amino acid sequence or structure in the pore region of ion channels regulate channel trafficking remains unanswered. Nevertheless, the new insights into the consequences on Kv1.1 channel trafficking presented in this study might help to further our understanding of the physiological role of the I400V RNA editing of Kv1.1. Together with the previous findings of altered pharmacology (9), Kvβ-induced inactivation (8) and lipid-induced inactivation (9), our data further highlight the complexity achieved by this single RNA editing event.

REFERENCES

1. Veh, R. W., Lichtinghagen, R., Sewing, S., Wunder, F., Grumbach, I. M., and Pongs, O. (1995) Immunohistochemical localization of five members of the Kv1 channel subunits: contrasting subcellular locations and neuron-specific co-localizations in rat brain. Eur J Neurosci 7, 2189-2205
2. Goldberg, E. M., Clark, B. D., Zagha, E., Nahmani, M., Erisir, A., and Rudy, B. (2008) K+ channels at the axon initial segment dampen near-threshold excitability of neocortical fast-spiking GABAergic interneurons. Neuron 58, 387-400
3. Browne, D. L., Gancher, S. T., Nutt, J. G., Brunt, E. R., Smith, E. A., Kramer, P., and Litt, M. (1994) Episodic ataxia/myokymia syndrome is associated with point mutations in the human potassium channel gene, KCNA1. Nat Genet 8, 136-140
4. Rajakulendran, S., Schorge, S., Kullmann, D. M., and Hanna, M. G. (2007) Episodic ataxia type 1: a neuronal potassium channelopathy. Neurotherapeutics 4, 258-266
5. Smart, S. L., Lopantsev, V., Zhang, C. L., Robbins, C. A., Wang, H., Chiu, S. Y., Schwartzkroin, P. A., Messing, A., and Tempel, B. L. (1998) Deletion of the Kv1.1 potassium channel causes epilepsy in mice. Neuron 20, 809-819
6. Wykes, R. C., Heeroma, J. H., Mantoan, L., Zheng, K., MacDonald, D. C., Deisseroth, K., Hashemi, K. S., Walker, M. C., Schorge, S., and Kullmann, D. M. (2012) Optogenetic and potassium channel gene therapy in a rodent model of focal neocortical epilepsy. Sci Transl Med 4, 161ra152
7. Hoopengardner, B., Bhalla, T., Staber, C., and Reenan, R. (2003) Nervous system targets of RNA editing identified by comparative genomics. Science 301, 832-836
8. Bhalla, T., Rosenthal, J. J., Holmgren, M., and Reenan, R. (2004) Control of human potassium channel inactivation by editing of a small mRNA hairpin. Nat Struct Mol Biol 11, 950-956
9. Decher, N., Streit, A. K., Rapiedius, M., Netter, M. F., Marzian, S., Ehling, P., Schlichthörl, G., Craan, T., Renigunta, V., Köhler, A., Dodel, R. C., Navarro-Polanco, R. A., Preisig-Müller, R., Klebe, G., Budde, T., Baukrowitz, T., and Daut, J. (2010) RNA editing modulates the binding of drugs and highly unsaturated fatty acids to the open pore of Kv potassium channels. *EMBO J* **29**, 2101-2113

10. Streit, A. K., Derst, C., Wegner, S., Heinemann, U., Zahn, R. K., and Decher, N. (2011) RNA editing of Kv1.1 channels may account for reduced ictogenic potential of 4-aminopyridine in chronic epileptic rats. *Epilepsia* **52**, 645-648

11. Rinné, S., Renigunta, V., Schlichthörl, G., Zuzarte, M., Bittner, S., Meuth, S. G., Decher, N., Daut, J., and Preisig-Müller, R. (2013) A splice variant of the two-pore domain potassium channel TREK-1 with only one pore domain reduces the surface expression of full-length TREK-1 channels. *Pflügers Arch* **466**, 1559-1570

12. Koren, G., Liman, E. R., Logothetis, D. E., Nadal-Ginard, B., and Hess, P. (1990) Gating mechanism of a cloned potassium channel expressed in frog oocytes and mammalian cells. *Neuron* **4**, 39-51

13. Stühmer, W., Stocker, M., Sakmann, B., Seeburg, P., Baumann, A., Grupe, A., and Pongs, O. (1988) Potassium channels expressed from rat brain cDNA have delayed rectifier properties. *FEBS Lett* **242**, 199-206

14. Pineda, R. H., Knoeckel, C. S., Taylor, A. D., Estrada-Bernal, A., and Ribera, A. B. (2008) Kv1 potassium channel complexes in vivo require Kvbeta2 subunits in dorsal spinal neurons. *J Neurophysiol* **100**, 2125-2136

15. Ford, M. G., Pearse, B. M., Higgins, M. K., Vallis, Y., Owen, D. J., Gibson, A., Hopkins, C. R., Evans, P. R., and McMahon, H. T. (2001) Simultaneous binding of PtdIns(4,5)P2 and clathrin by AP180 in the nucleation of clathrin lattices on membranes. *Science* **291**, 1051-1055

16. Renigunta, V., Fischer, T., Zuzarte, M., Kling, S., Zou, X., Siebert, K., Limberg, M. M., Rinné, S., Decher, N., Schlichthörl, G., and Daut, J. (2014) Co-operative endocytosis of the endosomal SNARE protein syntaxin-8 and the potassium channel TASK-1. *Mol Biol Cell* **25**, 1877-1891

17. Ye, W., and Lafer, E. M. (1995) Bacterially expressed F1-20/AP-3 assembles clathrin into cages with a narrow size distribution: implications for the regulation of quantal size during neurotransmission. *J Neurosci Res* **41**, 15-26

18. Groves, B., Gong, Q., Xu, Z., Huntsman, C., Nguyen, C., Li, D., and Ma, D. (2007) A specific role of AGS3 in the surface expression of plasma membrane proteins. *Proc Natl Acad Sci U S A* **104**, 18103-18108

19. Manganas, L. N., and Trimmer, J. S. (2000) Subunit composition determines Kv1 potassium channel surface expression. *J Biol Chem* **275**, 29685-29693

20. Zhu, J., Watanabe, I., Gomez, B., and Thornhill, W. B. (2003) Heteromeric Kv1 potassium channel expression: amino acid determinants involved in processing and trafficking to the cell surface. *J Biol Chem* **278**, 25558-25567

21. Jarvis, S. E., and Zamponi, G. W. (2007) Trafficking and regulation of neuronal voltage-gated calcium channels. *Curr Opin Cell Biol* **19**, 474-482

22. Ma, D., Zerangue, N., Lin, Y. F., Collins, A., Yu, M., Jan, Y. N., and Jan, L. Y. (2001) Role of ER export signals in controlling surface potassium channel numbers. *Science* **291**, 316-319

23. Deutsch, C. (2002) Potassium channel ontogeny. *Annu Rev Physiol* **64**, 19-46

24. Utsunomiya, I., Tanabe, S., Terashi, T., Ikeno, S., Miyatake, T., Hoshi, K., and Taguchi, K. (2010) Identification of amino acids in the pore region of Kv1.2 potassium channel that regulate its glycosylation and cell surface expression. *J Neurochem* **112**, 913-923

25. Zhu, J., Gomez, B., Watanabe, I., and Thornhill, W. B. (2005) Amino acids in the pore region of Kv1 potassium channels dictate cell-surface protein levels: a possible trafficking code in the Kv1 subfamily. *Biochem J* **388**, 355-362

26. Peters, C. J., Fedida, D., and Accili, E. A. (2013) Allosteric coupling of the inner activation gate to the outer pore of a potassium channel. *Sci Rep* **3**, 3025
Acknowledgements- We thank Oxana Nowak for a helping hand with the molecular biology and Günther Schlichthörl for his support with the single-channel analyses. We would like to thank Nikolay Klöcker for providing us information for the method of the luminometric assay.

FOOTNOTES
*This work was supported by grants of the Deutsche Forschungsgemeinschaft DE1432-3/2 to N.D. and the P.E. Kempkes Foundation to A.K.S. We are grateful to Oxana Nowak and Vanessa Huhn for excellent technical support and to Prof. James Trimmer for providing us Kv1.1 and Kv1.4 antibodies.

FIGURE LEGENDS

FIGURE 1. Whole-cell voltage-clamp recordings of transiently transfected HeLa cells. A, representative current recordings of Kv1.1 and Kv1.1 I400V, the inset depicts the voltage protocol. B, current-voltage relationships (I/V) for Kv1.1 (n = 17) and Kv1.1 I400V (n = 13) derived from the end of the test potentials (see arrow in A). C, peak current density analyzed at + 50 mV for Kv1.1 (n = 17) and Kv1.1 I400V (n = 13). D, the G/V relationships derived from the tail currents at -40 mV.

FIGURE 2. I400V RNA editing of Kv1.1 channels does not alter single-channel conductance and kinetics. A, representative single-channel recordings of Kv1.1 and Kv1.1 I400V after transient expression in HEK cells, recorded in the on-cell configuration. The membrane patch was depolarized to approximately +53 mV. (C) and (O) mark the open and the closed state of the channel. B, the mean burst durations of Kv1.1 (n = 13) and Kv1.1 I400V (n = 6) are not significantly different. C, the open probabilities (Po) of Kv1.1 (n = 14) and Kv1.1 I400V (n = 6) are also not significantly different. D, single-channel amplitudes of Kv1.1 and Kv1.1 I400V plotted against the transmembrane voltage of the patch. The slope conductances illustrated in (E) were derived from such linear fits. E, single-channel slope conductance of Kv1.1 (n = 7) and Kv1.1 I400V (n = 7) are not significantly different (n.s.).

FIGURE 3. Kv1.1 I400V has a reduced surface expression while protein translation and stability are not affected. A, Kv1.1 and Kv1.1 I400V current amplitudes were analyzed at +40 mV, 48 h after injection of equal amounts of either cRNA into Xenopus oocytes. Kv1.1 I400V (n = 28) had strongly reduced current amplitudes compared to Kv1.1 (n = 29). B, HA-tagged Kv1.1 and Kv1.1 I400V channel subunits (see inset) showed a similar difference in relative current as the non-tagged versions (compare to A). C, HA-tagged channel subunits were expressed in Xenopus oocytes and surface expression was analyzed with a chemiluminescence assay. The count of RLUs was significantly lower for Kv1.1 I400V-HA (n = 64) than for Kv1.1-HA (n = 70). D, western blot analysis of protein lysates from Kv1.1 I400V-HA or Kv1.1-HA expressing oocytes. Both channel versions, Kv1.1 and Kv1.1 I400V, are detected as double-bands with similar intensities. n.c., negative control. E, quantification of band intensities showed no significant difference between Kv1.1 and Kv1.1 I400V (n = 4 blots).

FIGURE 4. Reduced surface expression is not caused by enhanced endocytosis of Kv1.1 I400V. A, Kv1.1- or Kv1.1 I400V-cRNA injected Xenopus oocytes were incubated for 24 h with 40 µM of the dynamin inhibitor dynasore. TEVC recordings of non-incubated control oocytes and dynasore incubated oocytes were carried out and current magnitudes at +40 mV were compared. Neither Kv1.1 nor Kv1.1 I400V channels were significantly influenced by the dynasore incubation. B, Kv1.1 or Kv1.1 I400V cRNA were injected alone (0.05 ng/oocytes) or together with the same amount of AP180C cRNA and current magnitudes at +40 mV were recorded after 24 h. When expressed alone, Kv1.1 (n = 8) and Kv1.1 I400V (n = 9) current sizes differed significantly, as shown above. Neither Kv1.1 (n = 8) nor Kv1.1 I400V (n = 10) current sizes were influenced by co-expression of AP180C. C, HA-tagged Kir2.1 channels were expressed alone (0.5 µg per dish) or together with AP180C (0.25 µg per dish) in HeLa cells and surface expression was measured after 48 h using a chemiluminescence assay. Kir2.1 surface expression was significantly increased by co-expression of AP180C, D, a chemiluminescence assay of HA-tagged Kv1.1 and E, Kv1.1 I400V channels (0.5 µg per dish, respectively) expressed in HeLa cells alone or together with AP180C (0.25 µg per dish) was performed 48 h after transfection. No significant influence of AP180C on the surface expression of either channel was detected.
FIGURE 5. Fluorescence imaging reveals reduced surface expression of Kv1.1\(^{1400V}\) channels in HeLa cells and ‘rescue’ of surface expression by Kv1.4. A, HeLa cells were transfected with either Kv1.1 or B, Kv1.1\(^{1400V}\) constructs that are intracellularly tagged with dsRed-Monomer (magenta) and extracellularly tagged with an HA-epitope which was detected via an HA-specific antibody (green). Cells were PFA fixed and not permeabilized. C, counting of membrane stained cells revealed a significantly lower percentage of surface expression for Kv1.1\(^{1400V}\). D, western blots of whole cell protein lysates with anti-HA antibodies show no difference between Kv1.1 and Kv1.1\(^{1400V}\) protein abundance; n.c., negative control. E, HA-tagged Kv1.1 and Kv1.1\(^{1400V}\) channels were expressed in HeLa cells and surface expression was measured in a chemiluminescence assay. Kv1.1\(^{1400V}\) showed significantly lower surface expression. F, Kv1.4 was co-transfected with either Kv1.1 or G, Kv1.1\(^{1400V}\) (DNA ratio of 10:1). H, counting of membrane stained cells, when co-expressed with Kv1.4, no significant difference in membrane expression between Kv1.1 and Kv1.1\(^{1400V}\) was detected. I, western blots of whole cell protein lysates with anti-HA antibodies show no difference between Kv1.1 and Kv1.1\(^{1400V}\) abundance when co-expressed with Kv1.4; n.c., negative control. J, HA-tagged Kv1.1 or Kv1.1\(^{1400V}\) channels were co-expressed with Kv1.4 channel subunits in HeLa cells and surface expression was measured in a chemiluminescence assay. No significant difference between Kv1.1-HA and Kv1.1\(^{1400V}\)-HA surface expression level was detected in the presence of Kv1.4.

FIGURE 6. Co-expression with Kv1.4 channels ‘rescues’ current amplitudes of I400V edited channels. A, oocytes were injected with either Kv1.1 or Kv1.1\(^{1400V}\) and current amplitudes were measured in TEVC recordings by voltage steps to 0 mV. The current amplitudes of Kv1.1 (n = 32) and Kv1.1\(^{1400V}\) (n = 32) were significantly different, while western blot analysis of whole cell lysates using Kv1.1 antibodies revealed no difference in protein abundance (inset). B, when Kv1.1 and Kv1.1\(^{1400V}\) were co-expressed together with Kv1.4 (in a 1:1 ratio), the current amplitudes of the heteromeric Kv1.1/Kv1.4 channels were not significantly different (n = 35 for Kv1.1/Kv1.4 and n = 33 for Kv1.1\(^{1400V}$/Kv1.4). The total amount of cRNA injected per oocyte was always 0.05 ng (50 nl of a 1 ng/µl cRNA solution). C, protein abundance of Kv1.1 or Kv1.1\(^{1400V}\) (upper panel) and Kv1.4 (lower panel) was tested in western blot analysis of whole cell lysates from oocytes co-expressing Kv1.1 or Kv1.1\(^{1400V}\) and Kv1.4. No differences between Kv1.1 and Kv1.1\(^{1400V}\) expression levels were observed when co-expressed with Kv1.4. Vice versa, Kv1.4 expression levels were the same for co-expression with Kv1.1 or Kv1.1\(^{1400V}\).

FIGURE 7. Kv1.1\(^{1400V}\) channels are most likely retained in the ER, where most of the Kv1.1 channels reside. CHO-K1 cells were transfected with either Kv1.1 or Kv1.1\(^{1400V}\), N-terminally tagged with EGFP. A, co-transfection of Kv1.1 or Kv1.1\(^{1400V}\) with a DsRed-tagged marker for the trans-Golgi compartment. Note that co-localization merges to white. B, co-staining of Kv1.1 or Kv1.1\(^{1400V}\) transfected cells with a fluorescent acidotropic probe (LysoTracker) to probe for co-localization with lysosomes. C, co-transfection of Kv1.1 or Kv1.1\(^{1400V}\) with a DsRed-tagged marker for the endoplasmic reticulum. D, co-expression of an EGFP-tagged Kv1.1\(^{1400V}\) and a DsRed-tagged Kv1.1 construct.
Figure 1

A

B

C

D

[Diagrams and graphs showing current density, peak current density, and conductance in response to voltage changes for Kv1.1 and 400V].
Figure 2
Figure 3

A  
B  
C  
D  
E  

Relative band intensity

Kv1.1-1400V-HA

Kv1.1-HA 1400V-HA

Relative Light Units (RLU)

Kv1.1-HA 1400V-HA

Relative current

Kv1.1 1400V

Kv1.1-HA 1400V-HA

Relative current

Kv1.1-HA 1400V-HA

Relative current

Kv1.1-HA 1400V-HA

Relative current

Kv1.1-HA 1400V-HA

Relative current

Kv1.1-HA 1400V-HA

Relative current

Kv1.1-HA 1400V-HA

Relative current

Kv1.1-HA 1400V-HA
Figure 4

A

B

C

D

E

Relative current

Relative Light Units (RLU)

Relative Light Units (RLU)

Relative Light Units (RLU)

Relative Light Units (RLU)

Kv1 control

Kv1 + AP100C

Kv2 control

Kv2 + AP100C

Kv1 control

Kv1 + AP100C

Kv2 control

Kv2 + AP100C

Kv1 control

Kv1 + AP100C

Kv2 control

Kv2 + AP100C

Kv1 control

Kv1 + AP100C

Kv2 control

Kv2 + AP100C

n.s.

n.s.

n.s.

n.s.

n.s.

n.s.

n.s.

n.s.

n.s.

n.s.

n.s.

n.s.

n.s.
Figure 5

A) Kv1.1

B) I400V

C) Membrane stained cells (%)

D) Western blot

E) Relative Light Units (RLU)

F) Kv1.1 + Kv1.4

G) I400V + Kv1.4

H) Membrane stained cells (%)

I) Western blot

J) Relative Light Units (RLU)
Figure 6

A

B

C

Figure 6
Figure 7

A

Kv1.1 trans-Golgi co-loc.

I400V trans-Golgi co-loc.

B

Kv1.1 lysosomes co-loc.

I400V lysosomes co-loc.

C

Kv1.1 ER co-loc.

I400V ER co-loc.

D

I400V Kv1.1 co-loc.
RNA Editing in the Central Cavity as a Mechanism to Regulate Surface Expression of the Voltage-Gated Potassium Channel Kv1.1
Anne K. Streit, Lina A. Matschke, Amalia M. Dolga, Susanne Rinné and Niels Decher
J. Biol. Chem. published online August 6, 2014

Access the most updated version of this article at doi: 10.1074/jbc.M113.545731

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts