Selective Golgi export of Kir2.1 controls the stoichiometry of functional Kir2.x channel heteromers

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Summary
Surface expression of ion channels and receptors often depends on intrinsic sequence motifs that control their intracellular transport along the secretory pathway. Although members of the Kir2.x subfamily share two such motifs – a diacidic ER export motif and a positively charged Golgi export motif – they strongly differ in their surface expression. Whereas Kir2.1 shows prominent plasma membrane localization, Kir2.4 channels accumulate within the Golgi complex. By constructing chimeras between Kir2.1 and Kir2.4 subunits, a stretch of 20 amino acids was identified in the Kir2.1 C-terminus that is both necessary and sufficient to promote anterograde transport of Kir channel subunits at the level of trafficking from the Golgi to the plasma membrane. The core element of the identified sequence bears a tyrosine-dependent YXXΦ consensus motif for adaptin binding, with the flanking residues determining its functional efficiency. As the signal is dominant in promoting surface transport of Kir2.1/Kir2.4 channel heteromers and is recognized by both the epithelial and neuronal intracellular sorting machinery, the preferential Golgi export of Kir2.1 will control the stoichiometry of Kir2.x heteromers expressed on the cell surface.

Introduction
Inwardly rectifying potassium (Kir) channels exert diverse cellular functions, such as controlling membrane excitability, heart rate, hormone release and neuronal signal transduction (Hille, 1992). Seven subfamilies of Kir channels have been identified based on sequence similarity and functional properties (Douplnik et al., 1995; Fakler and Ruppersberg, 1996; Nichols and Lopatin, 1997; Krapivinsky et al., 1998; Döring et al., 1998). Members of the Kir2.x subfamily share the biophysical characteristic of strong inward rectification that is due to a highly voltage-dependent block of the channel pore by intracellular polyamines and Mg$^{2+}$ (Lopatin et al., 1994; Fakler et al., 1995). Voltage-dependent inward rectification enables Kir2.x channels to stabilize the resting membrane potential near the $K^+$ equilibrium potential and prevents them from shunting action potentials. The four members of the Kir2.x subfamily (Kir2.1-2.4) are expressed in both muscle and endothelial cells (Nichols and Lopatin, 1997; Töpert et al., 1998). The expression patterns and the functional diversity of native inward-rectifier $K^+$ currents suggest that channels may form heteromers. Indeed, a recent study has conclusively demonstrated that Kir2.1 can co-assemble with all other members of its subfamily (Preisig-Müller et al., 2002). Therefore, mutations in Kir2.1 may impair the function of both homomeric Kir2.1 and heteromeric channels formed with other Kir2.x subunits. Heteromultimerization may in fact contribute to the heterogeneous phenotype of Andersen’s syndrome, a Kir2.1 channelopathy with cardiac arrhythmias, periodic paralysis and developmental dysmorphisms (Plaster et al., 2001; Jongsma and Wilders, 2001).

Cellular excitability is not only determined by the type of ion channels but also by the number present in the plasma membrane. Surface expression of ion channels and other integral membrane proteins might be controlled beyond the level of protein biosynthesis by intrinsic signal motifs that promote anterograde or retrograde transport along the secretory pathway (Griffith, 2001; Ma and Jan, 2002). These sequence motifs work by recruiting specific coat protein complexes that induce the formation of transport vesicles not only destined for certain subcellular localizations but also preferentially loaded with the respective cargo protein (Bonifacino and Lippincott-Schwartz, 2003). Whereas biochemical and structural work has shed light on the molecular mechanisms of selective transport along the early secretory pathway (Bonifacino and Glick, 2004; Lee et al., 2004), the mechanisms of transport from the Golgi to the plasma membrane are far less understood. We and others have recently identified two anterograde trafficking signals in Kir2.1 channels that control surface expression of the channel by promoting its export from the endoplasmic reticulum (ER) and from the Golgi complex (Ma et al., 2001; Stockklausner et al., 2001; Stockklausner and Klöcker, 2003). Despite both sequence motifs being highly conserved among all members of the Kir2.x subfamily, their plasma membrane expression differs strongly. In the present study, we have characterized an...
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grade trafficking signal in Kir2.1 that is both necessary for efficient transport of the channel to the cell surface and sufficient to increase surface transport of Kir2.4 at the level of Golgi-to-plasma membrane traffic. Since it also controls surface expression of heteromeric Kir2.1/Kir2.4 complexes, we propose that the motif governs the stoichiometry of functional Kir2.x channel heteromers.

Materials and Methods

Gene construction
N-terminal fusion constructs of Kir channel subunits with enhanced green fluorescent protein (EGFP) were designed by inserting the respective cDNA in-frame into the EGFP-C1 eukaryotic expression plasmid (BD Biosciences). Mutagenesis was done as described elsewhere (Stockklausner et al., 2001) and verified by sequencing. The hemagglutinin (HA) epitope was introduced into the extracellular domains of mKir2.1 and gpKir2.4 at amino acid positions 116 and 122, respectively. A dominant-negative dynamin-1 mutant (K44A) was kindly provided by S. L. Schmid (Scripps Institute, La Jolla, CA).

Cell culture and transfection
Opossum kidney (OK) cells (American Type Culture Collection) were grown in DMEM-F12 supplemented with 10% fetal calf serum (FCS) and 1% penicillin/streptomycin (P/S; Invitrogen). They were transfected on DIV7 with Lipofectamine Opti-MEM and 0.7 µl of each cDNA in 25 µl Opti-MEM and 0.7 µl Opti-MEM were mixed and added to coverslips. Two days after transfection, cells were processed for immunocytochemistry as described below.

Immunocytochemistry
For immunocytochemical detection of the HA epitope, transfected cells were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 15 minutes at 4°C and pretreated with 10% normal goat serum (NGS) in PBS with 0.05% Triton X-100 (PBS-T) for 1 hour at room temperature (RT) to block unspecific antibody binding. Then they were incubated with a monoclonal mouse anti-HA antibody (Santa Cruz Biotechnology) diluted 1:200 in 2% NGS/PBS-T for 1 hour at RT. Immunoreactivity was visualized by a goat anti-mouse IgG secondary antibody conjugated to Cy3 (1:1000 in 10% NGS/PBS-T). To detect selectively the population of HA-tagged channels expressed on the cell surface or for endocytosis assays, antibody staining was performed in vivo without use of detergents. Unless otherwise stated, antibody incubation was carried out before fixation in serum-free medium for 15 minutes at 37°C for the primary and secondary antiserum, respectively. The in vivo labeling approach often led to a clustered surface distribution of the respective constructs that was artificially induced by antibody crosslinking (Delling et al., 2002).

Immunoblot analysis
36 hours after transfection, cells were lysed in TEEN-T (50 mM Tris pH 7.6, 1 mM EDTA, 1 mM EGTA, 150 mM NaCl, 1% Triton X-100, protease inhibitors) for 30 minutes on ice. Nuclei and cell debris were pelleted by low-speed centrifugation at 1000 g for 5 minutes at 4°C. The supernatants were separated by reducing SDS-PAGE on a 10% polyacrylamide gel, transferred onto PVDF membranes and incubated with a mouse monoclonal anti-GFP antibody (1:5000; BD Biosciences). Following incubation with a secondary HRP-conjugated anti-mouse IgG antiserum (1:2500; Santa Cruz Biotechnology), labeled proteins were detected using ECL-plus reagent (Amersham Biosciences). Density analysis of labeled protein bands was carried out using Scion Image 4.02.

Quantification of surface expression
All mutant Kir channels were tagged with an extracellular HA epitope and surface expression was quantified by fluorescence intensity measurements of anti-HA immunocytochemistry without use of detergents, as described before (Stockklausner and Klöcker, 2003). To exclude differences in antibody binding affinities, only surface expression levels of constructs with the same extracellular domain structure were compared with each other (Kir2.1 versus intracellular Kir2.1 mutants or Kir2.4 versus intracellular Kir2.4 mutants, respectively). For each construct, pixel intensity values of stainings from at least two independent transfections were corrected for background and integrated over 10 areas of 0.2116 mm² each (Scion Image 4.02). Wild-type (control) and mutant (test) constructs were always processed in parallel. Surface fluorescence intensity values were related to the translation efficiencies of the respective constructs determined by immunoblot analysis. We preferred immunoblot analysis to determine total protein levels over an approach employing GFP fluorescence intensity, because several channel constructs accumulated in the Golgi complex leading to local saturation of the GFP fluorescence signal when imaging all subcellular regions of a given cell. Statistically significant differences were assessed using the unpaired Student’s t-test. Data are given as means±e.m. expressed as relative surface expression levels of the respective control.

Imaging
Cells were imaged with a confocal laser scanning microscope (LSM510, Zeiss) using the following excitation wavelengths and filter settings: EGFP, excitation 488 nm Ar laser/emission BP505-530 nm; cy3, excitation 543 nm He laser/emission LP560 nm.

Results
Kir2.1 and Kir2.4 differ in their degree of surface expression
When heterologously expressed in opossum kidney (OK) cells, the inward-rectifier K⁺ channels Kir2.1 and Kir2.4 exhibit different levels of cell-surface expression (Fig. 1A). Thus, GFP-fused Kir2.1 (gfpKir2.1) was predominantly found in the Golgi complex and the plasma membrane (Stockklausner et al., 2001), whereas GFP-fused Kir2.4 (gfpKir2.4) was predominantly concentrated in a juxtanuclear compartment. As judged from plasma membrane fluorescence, the amount of gfpKir2.4 present on the cell surface was significantly lower
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that of GFPKir2.1. The observation with GFP-fused Kir subunits was corroborated by experiments that selectively detected channel proteins in the plasma membrane by means of an extracellular epitope tag (Fig. 1B). These findings were somewhat surprising given that both Kir2 subunits contain the two previously identified anterograde trafficking signals (Fig. 1C,D), a diacidic sequence motif in the C-terminus promoting ER export (Ma et al., 2001; Stockklausner et al., 2001) and a positively charged cluster motif in the N-terminus conferring efficient Golgi export (Stockklausner and Klöcker, 2003). Consequently, there must exist further intrinsic sequence information controlling the subcellular distribution of these Kir channel subunits.

Golgi-to-plasma membrane transport of Kir2.4 is less efficient

First, we investigated the subcellular trafficking of GFPKir2.4 in more detail. As shown in Fig. 2A, GFPKir2.4 channels accumulated within the Golgi complex as indicated by colocalization with the red fluorescent protein DsRed fused to the targeting sequence of the Golgi-resident enzyme galactosyltransferase I and by reversible redistribution into the ER after incubation with the fungal Golgi toxin BFA. Either a slow anterograde Golgi-to-plasma membrane transport or a high rate of endocytosis and retrograde transport to the trans-Golgi network (TGN) could account for the accumulation of GFPKir2.4 protein in the Golgi complex. To distinguish between these alternatives, we performed surface labeling of GFPKir2.4 in vivo to detect retrograde transport of endocytosed GFPKir2.4 to the TGN (Fig. 2B). Cells expressing extracellularly HA-tagged GFPKir2.4 were incubated with α-HA antibody for different periods of time (3-16 hours) before labeled protein complexes were visualized by incubation with a fluorescent secondary antiserum after fixation and permeabilization of the cells. As antibody labeling could interfere with retrograde transport of the channel and lead to mistargeting into lysosomes, experiments were performed in the presence of the membrane-permeable leupeptin analog ALLN (100 µM) to prevent degradation of internalized antibody in lysosomes. No accumulation of α-HA immunoreactivity was observed within the Golgi complex or within any other intracellular compartment even after 16 hours. Only weak plasma membrane staining was seen, indicating no substantial internalization within 16 hours. In good agreement with these data, inhibition of clathrin-dependent endocytosis by co-expressing a dominant-negative dynamin-1 mutant (dn-Dyn) (Damke et al., 1994) did not change the subcellular distribution of GFPKir2.4 (Fig. 2B), whereas endocytic transferrin uptake was inhibited (Fig. S1 in supplementary material). These results strongly suggest that the different levels of surface expression of GFPKir2.1 and GFPKir2.4 are due to less efficient anterograde transport of GFPKir2.4 to the plasma membrane.

Sequence information in the Kir2.1 C-terminus is both necessary and sufficient for efficient surface targeting

To identify the difference in sequence responsible for the distinct trafficking patterns, we designed chimeras of Kir2.1 and Kir2.4 by swapping their cytoplasmic N- and C-terminal domains (Fig. 3A). In a first set of experiments, the C-terminal domain of Kir2.1 was found to contain the critical targeting information for efficient plasma membrane expression (Fig. 3B). A more detailed analysis of the surface expression of further C-terminal chimeras revealed that a stretch of 20 amino acids within the Kir2.1 C-terminus was necessary for efficient plasma membrane targeting and was sufficient to increase surface expression of GFPKir2.4 by approximately 4-fold (Fig. 3C,D). These findings were independent of the epithelial cell type used; similar results were obtained in COS-7 cells [GFPKir2.4, 1.03±0.33; GFPKir2.4-int(2.1), 4.19±0.7; P<0.01]. The experiments described so far do not distinguish between a forward trafficking signal in Kir2.1 that actively promotes anterograde Golgi-to-plasma membrane transport or a Golgi retention signal in Kir2.4 that might have been abolished in the C-terminal GFPKir2.4/Kir2.1 chimeras. We therefore
exchanged the identified stretch of amino acids for a flexible glycine/serine-linker (GFPKir2.1-GS, GFPKir2.4-GS). Whereas surface expression of GFPKir2.4 did not change after inserting the GS linker, surface expression of GFPKir2.1-GS was significantly reduced compared with the wild-type channel (Fig. 3E). These data argue in favor of an active forward trafficking signal in the Kir2.1 C-terminus.

The alignment of the identified C-terminal segments exhibits a high degree of sequence homology between the two channels with very few non-conservative residue exchanges (Fig. 4A). A central element of the sequence stretch consists of the residues YIPL, which closely resemble the consensus motif YXXΦ known to confer binding to adaptins (with X for any amino acid and Φ for a bulky, hydrophobic residue). Mutation of all four amino acids to alanine (YIPL-AAAA) significantly reduced surface expression of Kir2.1, implicating this sequence motif in the surface transport of the channel (Fig. 4B). Moreover, the sole exchange of the tyrosine residue for alanine (Y242A) was sufficient to decrease surface expression of Kir2.1. However, as this consensus motif is conserved between Kir2.1 and Kir2.4, it cannot explain the different rates in their Golgi-to-plasma membrane transport. We thus focused on residues surrounding the YIPL motif for further sequence characterization of the identified trafficking determinant (Fig. 4C). Homologous exchange of both proline residues P242 and P246 upstream of YIPL in the Kir2.4 C-terminal sequence for serine residues significantly increased surface expression compared with wild-type (GFPKir2.4-P242S,P246S). The increase in surface expression was even more evident if all residues upstream of the YIPL motif were adapted to the Kir2.1 sequence. Still, this mutation did not completely reproduce the effects of the whole 20 residue motif identified in the Kir2.1 C-terminus in promoting surface expression of Kir2.4 (GFPKir2.1-KSRITS, 2.6±0.2; GFPKir2.4-int(2.1), 4.72±0.5), which indicates that residues downstream of the YIPL motif must also be important for its efficiency.

Finally, we tested whether the function of the trafficking determinant depended upon its intramolecular position. Instead of inserting it at the homologous position, the stretch of 20 amino acids containing the Golgi-to-plasma membrane signal was fused to the very N- and C-termini of Kir2.4, respectively. Whereas the C-terminal fusion construct (GFPKir2.4-signal-C) was retained in the ER, the N-terminal fusion construct (GFPKir2.4-signal-N) was readily exported from the ER and reached the Golgi complex. However, surface expression of GFPKir2.4-signal-N was not increased over wild-type Kir2.4, suggesting that the sequence motif was non-functional at this position (GFPKir2.4, 1±0.15; GFPKir2.4-signal-N, 1.16±0.38; not significant).

The identified trafficking determinant controls surface transport of channel heteromers

Since Kir2.1 and Kir2.4 are able to form channel heteromers (Preisig-Müller et al., 2002; Schram et al., 2002), we addressed the question of whether the identified signal governs Golgi-to-plasma membrane traffic of heteromeric channels. Equal amounts of cDNAs coding for extracellularly HA-tagged Kir2.4 (Kir2.4-HA) were either expressed alone or co-expressed with GFPKir2.1 (without HA-tag) and surface expression of Kir2.4-HA was quantified. As shown in Fig. 5A, co-expression of GFPKir2.1 significantly increased the amount of Kir2.4-HA expressed on the cell surface. In a control experiment, we compared surface expression of Kir2.4-HA co-expressed either with GFPKir2.1 or with GFPKir2.1-int(2.4) lacking the identified trafficking determinant. Supporting a role for surface transport of channel heteromers, we observed that removing the critical sequence stretch from co-expressed Kir2.1 (GFPKir2.1-int(2.4)) led to a significant decrease in the amount of Kir2.4-HA on the cell surface when compared with the heteromeric assembly of wild-type GFPKir2.1 and Kir2.4-HA (Fig. 5B).

Recognition of the identified transport motif is conserved in neurons

As the recognition of trafficking signals might vary with cell
type and native co-expression of Kir2.1 and Kir2.4 is restricted to neurons (Töpert et al., 1998), we tested whether the identified trafficking determinant promotes surface transport of Kir channels in neurons. Primary hippocampal cultures were transfected with extracellularly HA-tagged GFPKir2.4 and GFPKir2.4-int(2.1), respectively, and processed for anti-HA immunocytochemistry without membrane permeabilization. Similar to what had been observed in epithelial cells, the identified signal motif was sufficient to increase surface expression of Kir2.4 significantly, by a factor of ~4. This indicates that the identified trafficking motif participates in a general sorting mechanism conserved across cell types (Fig. 6).

Discussion
Here, we characterized an anterograde trafficking motif in the cytoplasmic C-terminus of Kir2.1 channels that is not only necessary to promote its Golgi-to-plasma membrane transport in both epithelial cells and neurons, but is also sufficient to increase surface expression of Kir2.4 when inserted at the homologous position. As it also controls surface transport of heteromeric Kir2.1/Kir2.4 channels, we propose that the identified motif governs the stoichiometry of Kir2.x heteromers on the cell surface.

The transport of cargo protein between the various intracellular compartments of the secretory pathway is accomplished by transport carriers that form at sites at which
compartment-specific coat protein complexes have been recruited. These coat proteins not only participate in the formation of coated vesicular carriers, but also confer cargo selection by interacting with specific sorting signals contained in the cytoplasmic domains of cargo proteins (Bonifacino and Traub, 2003; Bonifacino and Lippincott-Schwartz, 2003).

Mapping the identified Golgi-to-plasma membrane sorting signal onto the crystal structures of the bacterial K+ inward-rectifier channel KirBac1.1 or the eukaryotic G-protein-gated Kir3.1 subunit (Nishida and MacKinnon, 2002; Kuo et al., 2003), we assume an intramolecular position exposed to the cytoplasm. Such accessibility of the trafficking determinant from the cytoplasm is consistent with it serving as a recognition domain for coat protein complexes. Which coat protein complexes should be considered as potential interaction partners, being able to bind to the Kir2.1 C-terminal sequence but failing to do so in Kir2.4? The signal sequence bears the motif YIPL, which closely resembles the consensus sequence YXXΦ known to interact with adaptor protein (AP) complexes (Bonifacino and Traub, 2003). These are involved in vesicle formation for selective protein transport between different intracellular compartments: the AP-2 complex mediates rapid endocytosis from the plasma membrane; AP-1, AP-3 and AP-4 mediate sorting steps at the trans-Golgi network (TGN) and/or endosomes (Boehm and Bonifacino, 2001; Robinson and Bonifacino, 2001). Each of these complexes is composed of four adaptin subunits. In most cases, recognition of the YXXΦ motif relies on the medium (μ) chain adaptin, but exceptions from this rule have been described (Ohno et al., 1995; Ohno et al., 1996; Hirst et al., 1999; Nishimura et al., 2002). Both functional and structural work shows that the tyrosine and hydrophobic Φ residue are essential for adaptin binding, whereas the X residues and sequence information flanking the tetrapeptide contribute to the affinity and specificity of AP binding (Ohno et al., 1995; Boll et al., 1996; Owen and Evans, 1998; Owen et al., 2001). Phosphorylation of the critical tyrosine residue has been shown to prevent
adaptin binding both in vitro and vivo (Boll et al., 1996; Ohno et al., 1996; Schaefer et al., 2002). In line with the hypothesis that the identified trafficking determinant serves as a recognition domain for an AP complex, we observed that not only mutation of YIPL to alanine but also the sole exchange of the tyrosine residue was sufficient to reduce surface expression of Kir2.1 significantly. Moreover, as YIPL is conserved between the two channels, we found the flanking residues upstream of YIPL to be crucial for explaining the different efficiencies between surface transport rates of Kir2.1 and Kir2.4. Until now, only the AP-1B, AP-3 and AP-4 complexes have been assigned a function in Golgi-to-plasma membrane traffic (Fölsch et al., 2001; Gan et al., 2002; Nishimura et al., 2002; Simmen et al., 2002). AP-1B and AP-4 are rather unlikely to be the interacting coats of the identified signal, because µ1B, the YXXΦ-recognizing subunit of the AP-1B complex, is exclusively expressed in polarized epithelia, whereas we found that the newly identified signal is functional across cell types; moreover, binding of µ4, the YXXΦ-recognizing subunit of AP-4, shows a preference for aromatic residues near the critical tyrosine, which are not found in Kir2.1 (Ohno et al., 1999; Aguilar et al., 2001). The AP-3 complex is mainly involved in transport between the TGN and lysosome-related organelles (Robinson and Bonifacino, 2001), but has also been implicated in surface transport of the vesicular stomatitis virus glycoprotein VSV-G (Nishimura et al., 2002). Signal recognition by AP-3 depends on acidic residues in the proximity of the critical tyrosine, and the signal-binding affinity of µ3A is regulated by upstream CK2 phosphorylation (Ohno et al., 1998; Madrid et al., 2001). Although the signal identified in Kir2.1 shares both of these sequence characteristics, preliminary experiments did not show the recruitment of µ3A to sites of Kir2.1 expression, which would indicate an interaction of the two proteins. In addition, the surface transport of Kir2.1 was not affected upon co-expression of a dominant-negative δ adaptin that had previously been shown to interfere with the AP-3-dependent VSV-G traffic to the cell surface (our unpublished work) (Nishimura et al., 2002).

Besides primary sequence characteristics, the intramolecular position of sorting signals with respect to their spatial distance from the plasma membrane can be critical for their recognition by coat protein complexes (Bonifacino and Traub, 2003; Shikano and Li, 2003). To test for a positional dependence, the signal identified in Kir2.1 was not only inserted at the homologous position into the Kir2.4 C-terminus but was also fused to its very N- and C-terminal ends, assuming this would increase the signal-to-membrane distance without limiting the accessibility of the signal. Indeed, increasing linear spacing of the signal with respect to the closest transmembrane domain of the channel resulted in a loss of its function. It has been shown that components of various coat protein complexes, including AP complexes, can interact with phospholipids in the plasma membrane, which enhances signal recognition (Gaidarov et al., 1996; Collins et al., 2002; Rohde et al., 2002). Positional dependence of signal recognition could thus reflect the need for stabilizing plasma membrane interactions of the signal–coat-protein complex. This view is supported by a previous study demonstrating an important role of positively charged amino acids for selective Golgi export of Kir channels (Stockklausner and Klöcker, 2003). By electrostatic interaction, these positive charges could increase the local concentration of phosphoinositides in the vicinity of the channel and thereby favor the formation of a stable signal–coat-protein complex for its Golgi-to-plasma membrane traffic.

An alignment of the respective C-terminal segments shows complete conservation of the YXXΦ motif between all members of the Kir2.x subfamily. However, the flanking residues differ; strikingly, serine 234 in Kir2.1 is replaced by proline in all other Kir2.x subunits. Since our experiments predict this residue to be critical for signal function, with a serine at this position promoting signal recognition and a proline preventing it, we propose Kir2.1 to be preferentially transported to the cell surface not only over Kir2.4 as shown here, but over all other members of its subfamily as well. What are the functional consequences of such differential trafficking? Assuming free assembly of channel heteromers at the level of the ER, our study suggests that Kir2.1 will govern the stoichiometry of functional Kir2.x heteromers (i.e. the heteromers expressed on the cell surface) by controlling the efficiency of their Golgi export. As demonstrated in the study, plasma membrane expression of Kir2.1 homomers and Kir2.1-

![Fig. 6. The identified Golgi-to-plasma membrane trafficking motif is recognized by the neuronal sorting machinery. (A) Representative confocal images of hippocampal neurons expressing extracellularly HA-tagged GFPKir2.4 and GFPKir2.4-int(2.1), respectively. Surface expression of channel constructs was determined by α-HA immunocytochemistry without membrane permeabilization (TX, Triton X-100). The clustered distribution of GFPKir2.4-int(2.1) is artificially induced by the in vivo surface-labeling procedure. (B) Quantification of surface expression (n=10 neurons). Cy-3 fluorescence intensity values of neuronal surface stainings were corrected for background and related to total protein expression measured by GFP fluorescence. Data are given as relative intensity values per µm². *Statistically significant when compared with respective control (P<0.01; unpaired Student’s t-test; see Materials and Methods).](Image 227x538 to 568x719)
containing heteromers is preferred over surface expression of homomeric Kir2.4. Though the definite physiological consequences of this bias in surface stoichiometry of Kir2.x channels towards Kir2.1 subunits must remain speculative at this point, the present data further elucidate the dominantly-negative effects of Kir2.1 mutations on Kir2.x currents, as seen in Andersen’s syndrome.

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