A Hyperprostaglandin E Syndrome Mutation in Kir1.1 (Renal Outer Medullary Potassium) Channels Reveals a Crucial Residue for Channel Function in Kir1.3 Channels*

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Loss of function mutations in kidney Kir1.1 (renal outer medullary potassium channel, KCNJ1) inwardly rectifying potassium channels can be found in patients suffering from hyperprostaglandin E syndrome (HPS), the antenatal form of Bartter syndrome. A novel mutation found in a sporadic case substitutes an asparagine by a positively charged lysine residue at amino acid position 124 in the extracellular M1-I5 linker region. When heterologously expressed in Xenopus oocytes and mammalian cells, current amplitudes from mutant Kir1.1a[N124K] macroscopic channels were reduced by a factor of ~12 as compared with wild type. A lysine at the equivalent position is present in only one of the known Kir subunits, the newly identified Kir1.3, which is also poorly expressed in the recombinant system. When the lysine residue in guinea pig Kir1.3 (gpKir1.3) isolated from a genomic library was changed to an asparagine (reverse HPS mutation), mutant channels yielded macroscopic currents with amplitudes increased 6-fold. From single channel analysis it became apparent that the decrease in mutant Kir1.1 channels and the increase in mutant gpKir1.3 macroscopic currents were mainly due to the number of expressed functional channels. Coexpression experiments revealed a dominant-negative effect of Kir1.1a[N124K] and gpKir1.3 on macroscopic current amplitudes when coexpressed with wild type Kir1.1a and gpKir[K110N], respectively. Thus we postulate that in Kir1.3 channels the extracellular positively charged lysine is of crucial functional importance. The HPS phenotype in man can be explained by the lower expression of functional channels by the Kir1.1a[N124K] mutant.

In inwardly rectifying potassium (Kir) channels, four iden-

tical or different subunits assemble into functional proteins, as is the case in most other K+ channels (1). All subunits share a unique overall tertiary structure with a single pore region (H5) flanked by two transmembrane regions (M1 and M2) (Ref. 2, for review see Ref. 3). From the primary subunit structure but also from the functional characteristics of the homomeric channels, a classification scheme of four main subfamilies emerged: strongly rectifying Kir2 channels (4–6), G protein-gated Kir3 channels (8–10), and Kir6 subunits that associate with sulfonylurea receptors into ATP-sensitive K_ATP channels (11, 12). Structural and functional evidence now argues that Kir1.1 (ROMK, KAB-1) and the channels previously termed Kir4 (BIRK1, BIR10, Kir4.1, KAB-2, Kir4.2) may be grouped into one subfamily termed Kir1 (2, 13, 14).

Kir1.1 (ROMK) channels are moderately expressed in many tissues including brain and heart but are present predominantly in kidney (15). Several splice variants of Kir1.1 cDNA have been isolated that give rise to at least three protein isoforms termed Kir1.1a, b, and c (ROMK1–3). They differ in their N-terminal sequence (15) and are differentially expressed along the nephron in the rat kidney (16). Kir1.1 subunits have been reported to associate with other Kir subunits (13) and proteins such as the cystic fibrosis transmembrane regulator, a member of the superfamily of ABC transporters (17). In heterologous expression systems, Kir1.1 yields mildly inwardly rectifying potassium channels that are subject to regulation by phosphorylation (18), intracellular ATP (2), and pH (19).

Kir1.1 channels are of particular functional importance in the kidney. The hyperprostaglandin E syndrome, a renal disorder resulting from impairment of tubular reabsorption, can be caused by either mutations in the furosemide-sensitive Na-K-2Cl cotransporter (NKCC2) or by mutations in Kir1.1 (20–23). The two types of mutations lead to an impairment of transepithelial ion transport in the thick ascending limb of Henle’s loop and have similar but not identical pathophysiological consequences. This renal disorder mimics long term furosemide treatment (24) and is characterized in the fetus by excessive saluresis and polyuria leading to polyhydranmios and prematurity birth. After birth, affected infants also suffer from the typical patterns of impaired tubular reabsorption in the thick ascending limb of Henle’s loop (24, 25). Characteristically, the strong stimulation of prostaglandin E2 release re-

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1 The abbreviations used are: Kir, inwardly rectifying potassium channel; gpKir1.3, guinea pig Kir1.3; hKir1.3, human Kir1.3; ROMK, renal outer medullary potassium channel; PCR, polymerase chain re-

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results in further aggravation of saluretic polyuria, secretory diarrhea, vomiting, mediation of fever, osteolysis, and failure to thrive (24). This renal disorder is inherited in an autosomal recessive manner and affects 1 in 50,000–100,000 newborns (26).

Expression studies of several mutations in KCNJ1 revealed an almost complete loss of Kir1.1 channel function (23). In the thick ascending loop, this defect prevents luminal potassium recycling with secondary inhibition of the furosemide-sensitive Na-K-2Cl cotransporter, thereby disrupting electrogenic chloride reabsorption (27). The molecular mechanisms leading to nonfunctional Kir1.1 channels have not been defined in detail but may include incorrect targeting, abnormal pH regulation, misfolding, or occlusions of the permeant pathway. In the present report, we describe the clinical and functional analysis of a novel HPS mutation, N124K,3 that affects an extracellularly localized residue of Kir1.1. A positively charged lysine at the equivalent position is present in none of the known Kir subunits except Kir1.3. Like Kir1.1, Kir1.3 is expressed predominantly in kidney but also in lung and pancreas (13). Under heterologous expression and functional characterization of Kir1.1a and Kir1.3 channels, we demonstrate that these lysine residues are responsible for impaired channel function in mutant Kir1.1a and for low macroscopic currents in Kir1.3 channels.

**EXPERIMENTAL PROCEDURES**

**Mutation Analysis by Single-strand Conformation Polymorphism Analysis and DNA Sequencing—**Genomic DNA was extracted from peripheral leukocytes. Aberrant band patterns for the KCNJ1 gene were sought by means of single-strand conformation polymorphism analysis (28) using the same primers as in a previous study (21). PCR was performed in a 20-μl volume containing 50 ng of genomic DNA, 1.5 mM MgCl2, 5 mM Tris, pH 8.3, 50 mM KCl, 10 pmol of each primer, and 2 U of Taq polymerase. After an initial step at 94 °C for 5 min, PCR was conducted for 30 cycles with denaturation at 94 °C for 45 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s. The reaction was completed with a final elongation step at 72 °C for 10 min. Amplified products were separated using the CleanGel DNA analysis kit (Amersham Pharmacia Biotech) with the Multiphor II electrophoresis system (Amersham Pharmacia Biotech). Migration was performed at 18 watts constant power at 15 °C for 1 h. The band patterns were visualized by the silver-staining method. Direct sequencing was performed after reamplification of the remaining PCR product using 5'-Cy5-label-3'-dC (Life Technologies, Inc.) for expression in COS-7 cells. For expression in Xenopus laevis oocytes, cDNAs were subcloned into the polyadenylating transcription vector pSGEM (a gift from Dr. M. H. Grosveld, Nijmegen, The Netherlands). Oligonucleotides and antisense primers were complementary to uRNA sequences (sequences are available upon request). For construction of vectors containing the Kir cDNA using LipofectAMINE and Opti-MEM I (Life Technologies, Inc.) following the manufacturer’s protocol. Whole-cell recordings were performed at room temperature 48–72 h posttransfection in a bath solution consisting of 135 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl2, 10 mM glucose, 5 mM HEPES, pH 7.4. Patch pipettes were pulled from borosilicate glass capillaries (Kimble Products, Sussex, UK), Sylgard-coated (Dow Corning, Corning, NY) and heat-polished to give input resistances of 4–6 MΩ. The pipette recording solution contained 140 mM KCl, 2 mM MgCl2, 1 mM CaCl2, 5 mM HEPES, pH 7.3. Currents were recorded with an EPC9 patch clamp amplifier (Heka) and low-pass filtered at 2.9 kHz. Stimulation and data acquisition were also controlled by PULSE/PULSEFIT software. Series resistance of cells was routinely compensated by >80%, resulting in a maximum voltage error of 1–2 mV. Data are presented as mean ± S.D. (number of cells).

**RESULTS**

**Case Report and Mutational Analysis in KCNJ1—**Replacement of an asparagine by a lysine at position 124 (N124K) in Kir1.1a (KCNJ1) was found in a heterozygous state in a sporadic case of hyperprostaglandin E syndrome (HPS). The pregnancy of the affected infant’s mother was complicated by polyhydramnios and premature birth after 28 weeks of gestation. The child developed hypokalemic alkalosis, hypostenuria, and...
from a holding potential of 0 mV. Injected oocytes to 500-ms voltage steps between B graphs on the right. The Errors are mean lysine residues in the extracellular M1-H5 linker of each subunit. A amplitudes in oocytes averaged 50.6 μA (n = 5) under the same recording conditions (Fig. 1A). Expression of Kir1.1a[N124K] in COS-7 cells resulted in a similar reduction in amplitude as compared with WT Kir1.1a (135 ± 24 pA, n = 3 versus 1.12 ± 1.1 nA, n = 33). To estimate possible effects in heterozygous individuals, oocytes were injected with equal cRNA amounts of Kir1.1a and Kir1.1a-[N124K] (total amount equal to that used before). Surprisingly, cojected oocytes averaged only 1.75 ± 0.6 μA (n = 5), i.e. current amplitudes were also only a fraction of WT currents. This indicated a dominant-negative effect of the mutant Kir1.1a[N124K] subunits, and in heterozygous individuals, renders a total rescue of channel function by a simple heteromerization of WT and mutant subunits quite unlikely (see “Discussion”).

**Cloning and Sequence Analysis of a Guinea Pig Kir1.3 Subunit**—It is also conceivable that in analogy to other Kir channels, kidney Kir1.1 subunits coassemble with other subfamily members expressed in the same cells. A second subfamily member, Kir1.2 (K_Av-2; Kir4.1), is also expressed in the kidney and is functionally similar to Kir1.1a (13, 14). In addition, using an uncharacterized Expressed Sequence Tag sequence (IMAGp998j11108), we isolated a novel full-length clone from a guinea pig genomic library. A sequence analysis of the 2.2-kilobase BamHI fragment from one λ-phage clone, transferred in pBlue-script SK+, showed a complete intronless coding region of 1128 bp flanked by 357 bp of genomic sequence upstream and 703 bp downstream. The main open reading frame encoded a protein of 375 amino acids that shared 95% identity to a recently published human Kir channel sequence termed Kir1.3 (13), Kir1.2 (29), or IRKK (30), respectively (Fig. 2). As phylogenetic analysis shows, both human and guinea pig sequences are more closely related to Kir1.1/Kir1.2 than to any other Kir subunit. Thus, we consider the novel sequence to represent a species ortholog of the human Kir1.3 (hKir1.3) and term it gpKir1.3 following a new emerging nomenclature for Kir channels. The main sequence variations between the two species were located in the first 20 amino acids in the N-terminal region, which were only ~45% similar. Sequence comparison showed 62.7% identity to Kir1.2 and 47.0% identity to Kir1.1 subunits (Fig. 2). Identity scores to members of other Kir subfamilies were significantly lower (<40%). Further analysis of gpKir1.3 recognized a C-terminal SNV motif also found in Kir1.2 subunits, indicating a putative interaction site with PDZ-domain proteins (31). An ATP binding site (Walker A motif), present in Kir1.1 and Kir1.2 (2, 14), was absent from gpKir1.3.

Northern analysis of tissue total RNA identified strong expression of gpKir1.3 transcripts primarily in guinea pig kidney (data not shown). Thus gpKir1.3 was a likely candidate to associate with Kir1.1 subunits in renal Kir channels. hKir1.3 subunits isolated from human kidney remained uncharacterized since individually they failed to express in Xenopus oocytes (13). We noticed that in contrast to any other known Kir subunit, both hKir1.3 and gpKir1.3 carried the equivalent of the Kir1.1a[N124K] mutation, a positively charged lysine residue at position 110 (Lys-110) in the less conserved extracellular M1-H5 linker. Therefore the contribution to channel function of this residue was investigated in WT and mutant gpKir1.3.

**Heterologous Expression of gpKir1.3 and gpKir1.3[K110N]**—Macroscopic current evaluation of expressed WT and mutant Kir1.3[K110N] channels revealed that this lysine residue in fact was crucially important for the integrity of Kir1.3 channel function. Contrary to a recent report on hKir1.3 (13), gpKir1.3 cRNA injections (equal amounts and same recording conditions as before) did not fail to express currents but gave rise to a moderate current amplitude of 4.5 ± 1.6 μA (n = 7) at a membrane potential of −100 mV. Moreover, exchange of the HPS lysine for an asparagine (K110N), as in WT Kir1.1a channels, potentiated macroscopic currents to an average 27 ± 6.7

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**Fig. 1. Quantitative analysis of Kir1.1a/Kir1.1a[N124K] (A) and gpKir1.3/Kir1.3[K110N] (B) channels in Xenopus oocytes.** A and B, original recordings on the left depict current responses of cRNA-injected oocytes to 500-ms voltage steps between +80 mV and −140 mV from a holding potential of 0 mV. [K]_o = 96 ms. Steady-state current amplitudes derived from WT and mutant channels and a combination of both measured at a potential of −100 mV are summarized in the bar graphs on the right. The inset diagrams illustrate the position of the lysine residues in the extracellular M1-H5 linker of each subunit. Errors are mean ±S.D.

hypercalcemia with subsequent nephrocalcinosis. Single-strand conformation polymorphism analysis revealed two aberrant bands in the KCNJ1 gene of the patient. Direct sequencing demonstrated (i) a heterozygous base exchange of a thymine to base position 372 resulting in a missense mutation (N124K) and (ii) the insertion of an additional cytosine at base position 1055–58, causing a frameshift mutation starting at His-354 and a premature stop codon at amino acid position 362 in the mutated protein. Cosegregation analysis identified N124K as the maternal, and the frameshift, as the paternal allele. Thus, the clinical diagnosis of hyperprostaglandin E syndrome was confirmed genetically.

**Heterologous Expression of Wild Type Kir1.1a and Mutant Kir1.1a[N124K] Channels**—To investigate the functional consequences of the novel HPS mutation N124K, WT, and mutant Kir1.1a channels were heterologously expressed in both Xenopus oocytes and mammalian COS-7 cells. As demonstrated earlier (23), WT Kir1.1a cRNA/cDNA in both systems gave rise to robust inwardly rectifying K^- currents with properties typical of weakly rectifying Kir channels (2). With the extracellular K^- concentration ([K]_o) raised to 96 mM, Kir1.1a current amplitudes in oocytes averaged 50.6 ± 8.0 μA (n = 9) at −100 mV membrane potential (Fig. 1A). When mutant Kir1.1a-[N124K] channels were expressed in oocytes, the kinetics of the macroscopic current were indistinguishable from WT Kir1.1a, but amplitudes were dramatically reduced to −8% (3.9 ± 1.2 μA; n = 5) under the same recording conditions (Fig. 1A). Expression of Kir1.1a[N124K] in COS-7 cells resulted in a similar reduction in amplitude as compared with WT Kir1.1a (135 ± 24 pA, n = 3 versus 1.12 ± 1.1 nA, n = 33).
μA (n = 7), i.e. by a factor of ~6 (Fig. 1B), without noticeably changing other biophysical properties of macroscopic currents (see below). Robust whole-cell currents were also obtained after transfection of COS-7 cells with mutant Kir1.3[K110N] cDNA (968 ± 60 pA, n = 12), but only minor currents (100–150 pA) were observed in few cells transfected with WT Kir1.3.

As shown from ramp and voltage-jump responses in varying concentrations of [K+]o, both WT and mutant Kir1.3[K110N] channels were highly selective for K+ ions with large amplitudes negative to the K+ Nernst potential ENR. Measured Nernst (zero current) potentials were 283 mV for 5 mM, 267 mV for 10 mM, 243 mM for 25 mM, and 28 mV for 100 mM [K+]o (Fig. 3A), which was in perfect agreement with ENR as predicted from the Nernst equation and followed [K+]o with a slope of approximately 54 mV per decade (Fig. 3B). These data indicated that the conductance was predominantly carried by K+ ions as expected for Kir channels. Other functional characteristics of gpKir1.3 were more similar to Kir1.2 channels. Unlike weakly rectifying Kir1.1, but not unexpected from the presence of a crucial negatively charged residue (E157) in the second transmembrane segment (Fig. 2) that is directly involved in the binding of Mg2+ or polyamines (32), Kir1.3 rectified more strongly and displayed only moderate outward currents (Fig. 3C). Interestingly, both in Kir1.1 and in Kir1.3 channels, the HPS mutation and reverse HPS mutation, respectively, did not change the rectification properties of the channels. Rectification of the Kir1.3 I-V relation remained identical with either 2 mM Mg2+ or 0 mM Mg2+, 100 μM EGTA in the internal solution (data not shown) as has been shown for Kir2.1 channels (33).

As a glutamate at position 157 may not suffice for total rectification as is the case in Kir2 channels (36), additional residues are likely involved in the rectification mechanism. When analyzing the block by extracellular Ba2+, we found that in some aspects the two subfamily members Kir1.1 and Kir1.3 were quite distinct. The open channel block of Kir1.3 channels by Ba2+ was strongly time- and voltage-dependent (Fig. 4A) and differed considerably from Kir1.1 channels (34) with respect to kinetics of block and Ba2+ sensitivity. Measured in COS-7 cells with a fast microperfusion assay, Kir1.3 and Kir1.3[K110N] channels demonstrated the characteristically slow unbinding of Ba2+ from the pore (τOFF = 4.96 ± 0.7 s; n = 6), which is typical of all Kir2 channels (Refs. 33 and 34; Fig. 4C).
tration-response analysis of macroscopic steady-state currents plotted was found in Kir1.3[K110N] channels (Fig. 6A). These values were only slightly smaller than the unitary conductances of Kir1.1 (30 pS (2)) and the high conductance state of Kir1.2 (4.1) channels (36 pS (14)). As reflected in the macroscopic currents (Fig. 3C), the current-voltage relationship of single Kir1.3 channels was strongly rectifying. Further quantitative analysis revealed an open probability of WT Kir1.3 channels of $p_o = 0.52 \pm 0.05$ ($n = 6$) at a membrane potential of $-100$ mV and a value in the same range for mutant Kir1.3[K110N] channels ($p_o = 0.48 \pm 0.04$; $n = 6$; Fig. 6B). Thus the product $p_o \cdot \gamma$ as a measure of average current flux through single channels was virtually identical between WT and mutant Kir1.3 channels. The ratio of macroscopic currents, however, was $-1:6$, suggesting that current potentiation in Kir1.3[K110N] channels was mainly due to elevated expression of functional channels (Fig. 6C). The equivalent analysis performed for WT and mutant Kir1.1a channels under our recording conditions yielded identical values for both the unitary conductance ($\gamma = 34$ pS; $n = 4$) and open channel probability ($p_o = 0.68 \pm 0.14$ for Kir1.1a and $p_o = 0.66 \pm 0.11$ for Kir1.1a[N124K]).

**Heteromerization of Kir1.1a and Kir1.3 Channels**—With respect to a potential heteromerization between Kir1.3 and Kir1.1 subunits in the kidney, Shuck et al (13) reported that hKir1.3 inhibited expression of both Kir1.1 and Kir1.2 channels by $\sim 50\%$, which was interpreted as being caused by the formation of unviable heteromeric channel complexes. Analysis of current amplitudes and using the above-mentioned criteria of current rectification and Ba$^{2+}$ block oocyte coinjection experiments with WT and mutant Kir1.1a and gpKir1.3 subunits revealed different results for gpKir1.3 in our experiments. When Kir1.1a cRNA was injected together with Kir1.3 cRNA (1:10 dilution to titrate amplitudes), macroscopic current amplitudes were not significantly different from those induced by Kir1.1a cRNA injection alone (Fig. 7A). However, in contrast to channels solely composed of Kir1.1 subunits, current properties after coinjections were dominated by Kir1.3, i.e., they were uniformly strongly rectifying and showed a Ba$^{2+}$ block with a slow OFF rate and high Ba$^{2+}$ affinity ($K_i = 14.2 \pm 0.5\mu M$). When mutant Kir1.3[K110N] and Kir1.1 subunits were coinjected (Fig. 7B), composite currents also demonstrated strong rectification and slow Ba$^{2+}$ OFF rates, i.e. Kir1.3-like properties. However, this time, amplitude values exceeded the sum of the individual components, and currents had a lower Ba$^{2+}$ sensitivity ($K_i = 53.8 \pm \mu M$). In comparison, current amplitudes were reduced by more than half when mutant Kir1.3[K110N] subunits were coinjected with the HPS mutant Kir1.1[N124K]. The weakly rectifying currents resulting from this combination showed Kir1.1-like features with low Ba$^{2+}$ sensitivity ($K_i = 72.6 \pm \mu M$) and fast OFF rates. Taken together, these data implied that (i) WT and/or mutant Kir1.1a/Kir1.3 channels were likely to interact in the formation of heteromeric Kir channels with specific properties, that (ii) heteromeric Kir1.3[K110N]/Kir1.1a channels in which lysines are absent yield largest current flux, and (iii) that the impaired function of mutant Kir1.1[K110N] channels remained unrescued by coexpressed Kir1.3 subunits.

**DISCUSSION**

Patients suffering from HPS are characterized by an impaired renal salt reabsorption. Recent findings identified several mutations as the primary cause of HPS, which result in loss of function of the inwardly rectifying potassium channel Kir1.1 by yet unknown mechanisms. The HPS mutation described here, Kir1.1a[N124K], replaces an uncharged for a charged extracellular residue in the linker region between the M1 transmembrane segment and the pore region and has attracted our interest for two reasons. First, the homologous
Extracellular Lysine Residues in Kir1.1 and Kir1.3 Channels

Fig. 4. Voltage- and time-dependent block by extracellular Ba\(^{2+}\) of gpKir1.3 channels. A, current responses of Kir1.3[K110N] channels in COS-7 cells to voltage steps between −70 mV and −130 mV from a holding potential of −80 mV in the maintained presence of 1 mM Ba\(^{2+}\) demonstrate voltage dependence of Ba\(^{2+}\) open-channel block. B, bar graphs summarizing the time constants of block (τ\(_{\text{ON}}\)) and relaxation (τ\(_{\text{OFF}}\)) for WT Kir1.1a channels (124 ± 74 ms and 230 ± 71 ms; n = 7) and Kir1.3[K110N] channels (98 ± 46 ms and 4.96 ± 0.68 s, n = 7) expressed in COS-7 cells. Original traces in the inset show inhibition of Kir1 currents by extracellularly applied Ba\(^{2+}\) (1 mM) in COS-7 cells at a membrane potential of −120 mV. Note the different time course of the unblocking reaction of Ba\(^{2+}\), which is slow for gpKir1.3[K110N] (τ\(_{\text{OFF}}\) = 4.96) and fast for Kir1.1a (τ\(_{\text{OFF}}\) = 183 ms). Scale bars represent 2 μA and 20 s; black bars indicate application of Ba\(^{2+}\).

Fig. 5. Concentration-response relationship of the Ba\(^{2+}\) block of gpKir1.3 and Kir1.1a currents. Current inhibition relative to block by a saturating concentration of Ba\(^{2+}\) is plotted versus the concentration of the blocking cation at a holding potential of \(V_h = -80\) mV. Data from WT gpKir1.3 (●) and mutant Kir1.3[K110N] (○) as well as WT Kir1.1a (●) and mutant Kir1.1a[N124K] (○) expressed in oocytes are shown for comparison. Curves are least squares fits of data points to a Michaelis-Menten equation (1/1 + [A/K]m) revealing \(K_{i, \text{Ba}}\) of 16.8 μM (gpKir1.3), 25.1 μM (gpKir1.3[K110N]), 84.8 μM (Kir1.1a), and 282 μM (Kir1.1a[N124K]). A and n are variables. Insets show continuous recordings of currents at −80 mV upon application of different Ba\(^{2+}\) concentrations (indicated by black bars).

position in Kir2.1 subunits was implicated in being responsible for functional differences between the human and the chicken ortholog of the subunit (35). The exchange of a negatively charged residue (Glu-125 in human Kir2.1) to an uncharged residue (Gln-125 in chicken Kir2.1) reduced the single channel conductance and lowered the sensitivity to Ba\(^{2+}\) block (35), suggesting that this extracellular site might be in close vicinity to the conduction pathway in the channel. Second, in the novel Kir1.3 channels, a positively charged lysine (Lys-110) aligns with Asn-124 in Kir1.1a subunits. Since initial attempts failed to elicit currents from individually expressed hKir1.3 subunits (13), we suspected a similar mechanism to underlie the loss of function HPS mutation Kir1.1a[N124K].

Indeed Kir1.1a[N124K] currents were strongly decreased and Ba\(^{2+}\) sensitivity lowered >3-fold compared with WT channels. By analogy, the reverse HPS mutation K110N introduced in gpKir1.3 gave rise to a strong macroscopic current amplitude ~6-fold larger than for WT gpKir1.3 channels. Thus, exchange of a positively charged by an uncharged residue near the extracellular pore region of both Kir1.1a and Kir1.3 channels was accompanied by a strong rise of macroscopic current amplitudes. With both single channel conductance and open channel probability of Kir1.1a[N124K] and gpKir1.3 WT channels virtually unchanged by the mutation, the underlying molecular mechanism for this impaired function was found to be primarily due to altered expression of functional channels. We suspect that the extracellular Lys-110 residue in Kir1.3 channels (sporadically mutated in HPS-Kir1.1) may be of crucial importance for channel assembly.

Furthermore the Lys-110 residue may be important in determining the functional properties of the pore. The exposed extracellular localization of these residues open the possibility for a direct influence of permeant and nonpermeant ions or pH on the regulation of channel open probability. The HPS and reversed HPS mutation in Kir1.1a and gpKir1.3 subunits, respectively, did neither noticeably affect single channel conductance nor open channel probability. Mutant and WT gpKir1.3 channels had a higher sensitivity to Ba\(^{2+}\) compared with Kir1.1 channels, and the typically slow unbinding of Ba\(^{2+}\) may result from the blocking cation being trapped in the pore. In contrast, as reflected by the fast Ba\(^{2+}\) unblocking kinetics in Kir1.1a[N124K] mutant channels, the negatively charged lysine substantially lowered the sensitivity to Ba\(^{2+}\). This indicates that although the exact function of these residues slightly...
formation of heteromeric channels. To date the functional role of Kir1.3 channels is still elusive because heterologous expression of Kir1.3 alone yields only small currents. On the other hand, coexpression of Kir1.3 and Kir1.1a results in large currents and generates K⁺ channels with distinct properties that clearly differ from channels obtained by expression of Kir1.3 alone. Because Kir1.1 and Kir1.3 are both strongly expressed in the kidney, it is tempting to speculate that the functional form of Kir1.3 may be a heteromultimer with Kir1.1a and/or possibly other channels of the Kir family. It should be noted that Kir1.3 mRNA has also been found in pancreas and lung in the adult and in several fetal tissues including brain and may thus, in connection with other subunits, also play a role in the developing central nervous system.

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