Targeted Replacement of Kv1.5 in the Mouse Leads to Loss of the 4-Aminopyridine–Sensitive Component of $I_{K,slow}$ and Resistance to Drug-Induced QT Prolongation

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Abstract—The K$^+$ channel mKv1.5 is thought to encode a 4-aminopyridine (4-AP)–sensitive component of the current $I_{K,slow}$ in the mouse heart. We used gene targeting to replace mKv1.5 with the 4-AP–insensitive channel rKv1.1 (SWAP mice) and directly test the role of Kv1.5 in the mouse ventricle. Kv1.5 RNA and protein were undetectable, rKv2.1 was expressed, and Kv2.1 protein was upregulated in homozygous SWAP hearts. The density of the K$^+$ current $I_{K,slow}$ (depolarizations to +40 mV, pA/pF) was similar in left ventricular myocytes isolated from SWAP homozygotes (17 ± 1, n = 27) and littermate controls (16 ± 2, n = 19). The densities and properties of $I_{peak}$, $I_{fast}$, $I_{slow}$, and $I_a$ were also unchanged. In homozygous SWAP myocytes, the 50-μmol/L 4-AP–sensitive component of $I_{K,slow}$ was absent (n = 6), the density of the 20-mmol/L tetraethylammonium-sensitive component of $I_{K,slow}$ was increased (9 ± 1 versus 5 ± 1, P < 0.05), and no 100- to 200-nmol/L α-dendrotoxin–sensitive current was found (n = 8). APD90 in SWAP myocytes was similar to controls at baseline but did not prolong in response to 30 μmol/L 4-AP. Similarly, QTc (ms) was not prolonged in anesthetized SWAP mice (64 ± 2, homozygotes, n = 9; 62 ± 2, controls, n = 9), and injection with 4-AP prolonged QTc only in controls (63 ± 1, homozygotes; 72 ± 2, controls; P < 0.05). SWAP mice had no increase in arrhythmias during ambulatory telemetry monitoring. Thus, Kv1.5 encodes the 4-AP–sensitive component of $I_{K,slow}$ in the mouse ventricle and confers sensitivity to 4-AP–induced prolongation of APD and QTc. Compensatory upregulation of Kv2.1 may explain the phenotypic differences between SWAP mice and the previously described transgenic mice expressing a truncated dominant-negative Kv1.1 construct. (Circ Res. 2001;88:940-946.)

Key Words: potassium channels • heart • genetically engineered mice • drug-induced long-QT syndrome • arrhythmias

Transgenic and gene-targeting technologies have led to marked advances in the understanding of the molecular basis of cardiac repolarization in the mouse.1–2 Dominant-negative transgenic mice overexpressing mutated Kv1.1, Kv2.x, Kv4.x, and HERG α subunits have less repolarizing K$^+$ current, varying degrees of cardiac action potential duration (APD) and QT prolongation, and arrhythmias.3–7 In these experiments, the transgene may interact with several related K$^+$ channels in the heart, the phenotype may depend on the details of the transgene design,5,6 and the relationship of individual gene products to the phenotype may be unclear. Gene targeting of K$^+$ channels using embryonic stem (ES) cells circumvents several of these difficulties by directly knocking out a single gene product.8–13 However, gene targeting usually leads to loss of the gene in multiple organs and throughout development and is still subject to compensatory upregulation of other genes. Cross-mating lines of mice with different mutations provides one mechanism to sort out these interactions.14

We previously reported dominant-negative transgenic mouse that overexpress in the heart an N-terminal fragment of the rat brain K$^+$ channel rKv1.1, have QT prolongation, and lack a rapidly activating, slowly inactivating, 4-aminopyridine (4-AP)–sensitive K$^+$ current, $I_{K,slow}$, in their ventricular myocytes.3,15 These mice have both spontaneous and inducible ventricular arrhythmias, attributable at least in part to increased dispersion of repolarization and refractoriness between the apex and the base of the heart.16,17 Although these mice have decreased protein levels of Kv1.5, the transgene may disrupt other cardiac K$^+$ channels, including Kv1.4, which has been shown to encode $I_{K,slow}$.14 Thus, the precise role of the loss of Kv1.5 in the pathogenesis of the phenotype is uncertain. In addition, nothing is known about the relationship of Kv1.5 to Kv2.x, the subunits responsible for the 4-AP–resistant component of $I_{K,slow}$.4

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Here we report gene-targeted mice in which mKv1.5 is replaced by the 4-AP–insensitive channel subunit rKv1.1 (SWAP mice). The 4-AP–sensitive component of \( I_{K,slow} \) is absent in ventricular myocytes isolated from these animals, proving definitively that Kv1.5 underlies this current. Of note, total \( I_{K,slow} \) is unchanged at least in part because of the upregulation of the tetraethylammonium (TEA)-sensitive component encoded by Kv2.1. As a result, SWAP mice have normal cellular APDs and QT intervals on baseline electrocardiograms (EKGs) and resistance to drug-induced prolongation of APD and QT intervals after exposure to 4-AP.

Materials and Methods

All animal experiments were approved by the Institutional Animal Care and Use Committees at the University of Pittsburgh or Washington University School of Medicine.

Gene Targeting

The mouse Kv1.5 gene (mKv1.5) was cloned (genomic SV129 library, Stratagene), restriction-mapped, and sequenced. A targeting construct was engineered using a 2-kb 5′ arm consisting of the promoter and 5′-untranslated region (UTR) of mKv1.5, the rat Kv1.1 K+ channel (rKv1.1) tagged with the 9-amino-acid hemagglutinin tag (HA) and cloned into the Smal site located at position −6 of mKv1.5 (relative to the ATG start codon), a neomycin resistance cassette (NeoR), a 3-kb 3′ arm starting at the Xbal site in the 3′-UTR of mKv1.5, and the thymidine kinase gene (TK) for negative selection (Figure 1A). Homologous recombination with this construct should yield rKv1.1 driven by the mKv1.5 promoter, although the effect of the NeoR cassette is unknown, and any 3′ regulatory elements may be lost.

Electroporation of ES cells, identification of ES cell lines heterozygous for the targeted allele, blastocyst injection to obtain chimeras, and mating with C57BL/6 mice to obtain germ-line transmission and mice heterozygous for the targeted allele (SWAP mice) were tested in polymerase chain reaction (PCR) (rKv1.1-HA). Channel properties were tested in Xenopus oocytes using the 2-microelectrode voltage-clamp technique as previously described.19 In vitro transcribed cRNA from mKv1.5 yielded delayed rectifier currents with minimal inactivation (Figure 1B).

Expression of mKv1.5 and rKv1.1-HA In Vitro

In vitro transcribed cRNA from mKv1.5 yielded delayed rectifier currents with minimal inactivation (Figure 1B). Steady-state inactivation curves (generated in 50 mmol/L rubidium chloride from isochronal tail current measurements) gave a total gating valence (\( z = 4.6\pm0.1 \) e−) and a voltage of 10% activation (\( V_{10\%} = -32\pm2 \) mV) (n=6). This gating valence is 23% lower and \( V_{10\%} \) is shifted 20 mV to the right compared with rKv1.1 (\( P<0.01 \)).

Addition of the 9-amino-acid HA epitope to rKv1.1 did not affect the currents when injected into Xenopus oocytes. We subcloned rKv1.1-HA into the prokaryotic/eukaryotic vector pBK-CMV (Stratagene, California) and transfected the construct into COS cells. The HA epitope was easily detected using monoclonal antibodies both on Western blots and by immunofluorescence (data not shown).

Properties of SWAP Mice

Two lines of targeted ES cells were identified (SWAPL77 and SWAPL46; Figure 1C). Additional insertion sites of the rKv1.1 transgene were excluded by genomic Southern blot using a probe within the transgene. The targeted band in the SWAPL46 ES line was reproducibly weaker than the native band, suggesting that the targeted allele was less abundant. This could result from either a mixed population of ES cells or a degree of aneuploidy. Both ES lines underwent germ-line transmission, however, and heterozygotes had one copy of each allele in equal abundance. Experimental results were confirmed on both lines of mice.

SWAP homozygotes from both lines appeared phenotypically normal, and there was no evidence of increased mortality. Hearts were not hypertrophied, and histology of the hearts was normal (data not shown). No overt neurological phenotype was evident in the mice.
Kv1.5 RNA and protein were absent in hearts from SWAP homozygotes (Figures 1D and 1E). Kv1.1 RNA was detected in SWAP homozygotes by Northern blot (Figure 1D), and reverse transcriptase (RT)-PCR was used to confirm the expression of the transgene (Figure 1F). Using antibodies against the HA epitope, we detected a specific band at \( \approx 62 \) kDa in the hearts of SWAP mice that was identical in size to the band produced by transfecting rat neonatal cardiac myocytes with rKv1.1-HA (Figure 1E). We did not detect rKv1.1-HA protein in the brain, although native mKv1.1 protein was quite abundant and runs at \( \approx 72 \) kDa, as previously described (data not shown).

Figure 1. Targeted replacement of mKv1.5 by rKv1.1. A, Schematic representation of the mouse Kv1.5 genomic clone (top) and the targeting construct (bottom). The coding regions of Kv1.5 and Kv1.1 are labeled, and the heavier black line indicates the untranslated regions of Kv1.5 (5'-UTR and 3'-UTR), NEO and TK represent the neomycin resistance cassette and the thymidine kinase cassette, respectively. B, Expression of rKv1.1 and mKv1.5 in Xenopus oocytes; 100-ms depolarizing steps to the voltage indicated were used. C, Genomic Southern blots of BglII-digested DNA from 3 NEO- and TK-resistant cell lines (top left) and from mouse tails (top right). A schematic diagram showing the expected band sizes and location of the probes is shown below. Probe A (A*) was used for the blot shown. Note that only lines L77 and L46 underwent targeting events and that the targeted allele for the L46 cell line appears less abundant than the wild-type allele (see text). Mouse tail DNA for the L77 line comes from an F2 cross of 2 heterozygotes, whereas the tail DNA for the L46 line comes from the F1 and F2 offspring. D, Northern blot analysis with 25 \( \mu \)g/lane of total RNA from the heart and brain of a wild-type mouse (WT) and a SWAP homozygote (SW) probed with cDNA fragments from the N-terminal part of the Kv1.5 coding region (left), the Kv1.1 coding region (middle), and the 3' end of Kv2.1 (right). Arrows point to native Kv1.5 expression and to rKv1.1 transgene expression, whereas the arrowhead points to expression of the native mKv1.1 gene. Note that faint expression of mKv1.1 is present in the whole-heart RNA; this may represent Kv1.1 in cells other than myocytes. Results were repeated using at least 3 mice from each line. To correct for loading during quantitative measurements, Kv2.1 signals were measured on a PhosphorImager and normalized to the signals obtained when the blots were probed with \( \alpha \)-actin. E, Representative Western blot using a rabbit polyclonal anti-Kv1.5 antibody (Upstate Biotechnology, New York, NY), an antibody to the HA epitope (anti-HA, Sigma Immunochemicals, St Louis, Mo), and an anti-Kv2.1 antibody (Alomone Laboratories, Jerusalem, Israel) on crude membrane preparations (100 to 200 \( \mu \)g of protein/lane) from hearts and brains of a wild-type mice, SWAP homozygotes, and cultured rat neonatal cardiac myocytes transfected with either an empty plasmid (−) or a construct containing rKv1.1-HA driven by the \( \alpha \)-myosin heavy chain promoter (−). Identical results were obtained on hearts from both lines of mice. Arrows indicate the bands specific for Kv1.5, Kv1.1-HA, and Kv2.1, whereas asterisks indicate nonspecific bands. Equal protein loading from tissue samples is confirmed by Coomassie blue-stained gels. F, RT-PCR on cDNA from hearts of wild-type mice, SWAP heterozygotes (SW-Het), and SWAP homozygotes (SW-Hom). PCR was performed using a sense primer in the 5'-UTR of Kv1.5 and an antisense primer in the coding region of Kv1.5, followed by Southern transfer of the products and probing with a radiolabeled oligonucleotide in the 5'-UTR of Kv1.5. Parallel reactions were performed in the absence of RT to exclude genomic contamination. Note that only Kv1.1 expression driven by the transgene would be detected by this technique.

K^+ Channel Expression in SWAP Mice

Kv1.5 RNA and protein were absent in hearts from SWAP homozygotes (Figures 1D and 1E). Kv1.1 RNA was detected in SWAP homozygotes by Northern blot (Figure 1D), and reverse transcriptase (RT)-PCR was used to confirm the expression of the transgene (Figure 1F). Using antibodies against the HA epitope, we detected a specific band at \( \approx 62 \) kDa in the hearts of SWAP mice that was identical in size to the band produced by transfecting rat neonatal cardiac myocytes with rKv1.1-HA (Figure 1E). We did not detect rKv1.1-HA protein in the brain, although native mKv1.1 protein was quite abundant and runs at \( \approx 72 \) kDa, as previously described (data not shown).
Kv2.1 RNA expression was not significantly changed in homozygous SWAP compared with control ventricles (0.95±0.14, n=3 each; Figure 1D), but Kv2.1 protein was increased by Western blot analysis (n=3 each; Figure 1E).

**K+ Current Densities Are Unchanged in SWAP Myocytes**

Figure 2 shows representative currents from voltage-clamped, randomly dispersed, left ventricular myocytes isolated from SWAP homozygotes and wild-type controls obtained using 4-second depolarizing steps from a holding potential of −70 mV to potentials between −20 and +40 mV. No differences are apparent in the amplitudes or inactivation rates at either 25°C or 35°C. The mean±SEM peak outward K+ current densities at +40 mV at 25°C were 46±2 pA/pF in cells isolated from SWAP homozygotes (n=27), 45±2 pA/pF in cells from SWAP heterozygotes (n=8), and 47±3 pA/pF in cells from littermate controls (n=19).

The decay phases of the outward currents recorded from these left ventricular myocytes were well-fitted by the sum of 2 exponentials, consistent with the expression of I_{in,t}, I_{K,slow}, and the steady-state noninactivating current I_{ss}. The density of I_{K,slow} (at +40 mV) was similar for cells from SWAP homozygotes (17±1 pA/pF; n=27), SWAP heterozygotes (14±2 pA/pF; n=8), and controls (16±2 pA/pF; n=19). In addition, the decay time constants for I_{K,slow} were indistinguishable for cells isolated from SWAP homozygotes (1259±51 ms; n=27), SWAP heterozygotes (1177±82 ms; n=8), and controls (1270±48 ms; n=19).

Similar experiments were performed on cells isolated separately from either the left ventricular apex or septum of SWAP mice. The mean±SEM peak outward K+ current densities in the left ventricular apex (n=14) and septum (n=8) were 60±6 and 29±3 pA/pF, respectively. These values, as well as the densities of I_{in,t}, I_{K,slow}, and I_{ss}, are indistinguishable from those measured in wild-type apex and septal cells. In addition, analysis of the currents in septal cells isolated from SWAP mice revealed that the mean±SEM I_{ss} density (8.2±0.8 pA/pF) and inactivation time constant (τ_{inact}=199±21 ms) are not significantly different from those determined for I_{ss} in wild-type cells. These results are consistent with previous findings demonstrating that Kv1.4 underlies I_{ss} and does not contribute to I_{K,slow}. They also suggest that Kv1.5 and Kv1.4 do not coassemble to form functional heteromultimeric voltage-gated K+ channels in mouse ventricular myocytes.

**4-AP–Sensitive Component of I_{K,slow} Is Absent in SWAP Myocytes**

After exposure to 50 μmol/L 4-AP, wild-type myocytes showed a marked decrease in I_{K,slow} (Figure 3A), as has been observed in mouse ventricular myocytes. However, application of 4-AP to SWAP myocytes did not significantly affect the outward currents, indicating the absence of the 4-AP–sensitive component of I_{K,slow} in these cells. This finding is consistent with the previously reported absence of Kv1.5 in SWAP mice, suggesting that the residual rapidly inactivating component of I_{K,slow} in wild-type myocytes is not present in SWAP myocytes.

**4-AP–Sensitive Component of I_{K,slow} is Absent and the TEA-sensitive Component of I_{K,slow} is Upregulated in Myocytes from SWAP Heterozygotes**

Figure 3B shows the same recordings from a SWAP heterozygote before and after application of 20 mM TEA. The TEA-sensitive component of I_{K,slow} is increased in the myocytes from SWAP heterozygotes compared to controls, consistent with the upregulation of Kv1.5 in these cells. This upregulation is not observed in SWAP homozygotes, suggesting a specific role for Kv1.5 in the regulation of I_{K,slow} in SWAP myocytes.

**Action Potentials in Myocytes Isolated from SWAP Myocytes**

Figure 4 shows the action potentials in myocytes isolated from SWAP homozygotes obtained using 3-ms suprathreshold current injections at a rate of 1 Hz. In the current-clamp mode, action potentials were evoked from both the left ventricular apex and septum. The QT intervals were determined for I_{K,slow} before and after application of 20 mM TEA. The TEA-sensitive component of I_{K,slow} is increased in the myocytes from SWAP homozygotes, consistent with the upregulation of Kv1.5 in these cells. This upregulation is not observed in SWAP heterozygotes, suggesting a specific role for Kv1.5 in the regulation of I_{K,slow} in SWAP myocytes.
shown in previous studies. This low concentration of 4-AP also blocked \( I_{\text{Kur}} \) by \( \approx 20\% \), an observation also consistent with previous studies. The 50-\( \mu \)mol/L 4-AP–sensitive component of \( I_{\text{K,slow}} \), however, was completely absent in myocytes isolated from SWAP homozygotes (\( n=6 \)). This provides strong evidence that Kv1.5 encodes an \( \alpha \) subunit necessary for this current. In contrast, the component of \( I_{\text{K,slow}} \) sensitive to 20 mmol/L TEA (at +40 mV; Figure 3B) was increased in myocytes from SWAP homozygotes (9\( \pm \)1 pA/pF, \( n=11 \)) compared with controls (5\( \pm \)1 pA/pF, \( n=6 \); \( P<0.05 \)). At this concentration, TEA partially blocks the component of \( I_{\text{K,slow}} \) encoded by Kv2.1 channels. Together with the Western blot data (Figure 1E), these findings suggest that functional Kv2.1 channels are upregulated in response to the loss of Kv1.5.

We were unable to identify any currents in myocytes from either wild-type or SWAP mice that were sensitive to 100 to 200 mmol/L \( \alpha \)-dentrotoxin (DTX, \( n=8 \)). Because Kv1.1 is highly sensitive to DTX, this suggests that functional Kv1.1 channels are not expressed at significant levels in SWAP myocytes.

**Action Potentials in SWAP Myocytes Are Insensitive to 4-AP**

APD\(_{90} \) values determined at 1-Hz stimulation were similar in current-clamped myocytes isolated from SWAP homozygotes (35\( \pm \)2 ms, \( n=8 \), at 25°C; 27\( \pm \)3 ms, \( n=7 \), at 35°C) and wild-type controls (36\( \pm \)3 ms, \( n=16 \), at 25°C; 25\( \pm \)1 ms, \( n=13 \); \( P=\text{NS} \)). As previously reported, wild-type myocytes show marked APD prolongation in response to low concentrations of 4-AP (Figure 4). As predicted from the voltage-clamp studies, myocytes from SWAP mice were insensitive to 30 \( \mu \)mol/L 4-AP (\( n=3 \)).

**QT Interval in SWAP Mice Is Insensitive to 4-AP**

The effects of 4-AP on QT intervals are shown in Figure 5. Baseline QT interval and QTc were not prolonged in anesthetized homozygous SWAP mice compared with age- and strain-matched controls (QTc: 64\( \pm \)2 ms, homozygotes, \( n=9 \); 60\( \pm \)2 ms, heterozygotes, \( n=4 \); 62\( \pm \)2 ms, controls, \( n=9 \)). Injection with 4-AP (10 \( \mu \)mol/kg IP) prolonged QTc in controls but not in SWAP homozygotes (63\( \pm \)1 ms, homozygotes; 66\( \pm \)2 ms, heterozygotes; 72\( \pm \)2 ms, controls; \( P<0.05 \)). The heterozygotes had an intermediate degree of QTc prolongation when injected with 4-AP. Injection with 4-AP did not induce arrhythmias in either control or SWAP mice. Heart rate was not significantly changed in anesthetized SWAP homozygotes compared with controls, and injection with saline had no effect on QTc (data not shown).

**SWAP Mice Have No Increase in Arrhythmias**

Mean heart rate, measured using ambulatory telemetry monitors in unanesthetized untethered mice, was similar in SWAP homozygotes compared with littermate controls (610 versus 622 bpm, \( n=3 \) each). Standard deviation of cycle length over 24 hours, a gross measure of heart rate variability, was also similar in these mice (11\( \pm \)1 versus 10\( \pm \)7 ms). In addition, SWAP homozygotes had no increase compared with littermate controls (\( n=4 \) each) in the frequency of premature atrial complexes (11\( \pm \)3 versus 9\( \pm \)6 per day), premature ventricular complexes (5\( \pm \)1 versus 5\( \pm \)2 per day), or episodes of second-degree atrioventricular block (18\( \pm \)10 versus 22\( \pm \)5 per day). During the 192 hours of telemetry screened, one SWAP homozygote had an atrial couplet and a ventricular triplet, whereas one control mouse had a ventricular couplet.

**Discussion**

Kv1.5 Encodes the 4-AP–Sensitive Component of \( I_{\text{K,slow}} \)

The mouse cardiac K\(^+\) channel subunit mKv1.5 encodes a rapidly activating, very slowly inactivating current when heterologously expressed in *Xenopus* oocytes (Figure 1B). Similar Kv1.5 channel subunits have been cloned from rat and human and are highly sensitive to 4-AP and resistant to TEA. On the basis of these pharmacological and kinetic characteristics, Kv1.5 channels were proposed as the molecular basis for \( I_{\text{Kur}} \) in human atrium and \( I_{\text{K,slow}} \) in mouse ventricle. Antisense experiments in cultured heart cells have provided direct experimental evidence that Kv1.5 contributes to \( I_{\text{Kur}} \) in rat and human.

We previously used a dominant-negative transgenic strategy to disrupt channels of the Kv1.1.x family in the heart, leading to a mouse that lacks the 4-AP–sensitive component of \( I_{\text{K,slow}} \). These mice have decreased protein levels of...
Kv1.5, probably because of increased degradation. The transgene does, however, affect other channels, and these experiments do not completely prove the relationship between mKv1.5 and \(I_{K,\text{slow}}\). In the present study, we have used gene targeting to selectively eliminate mKv1.5 and showed the selective loss of the 4-AP–sensitive portion of \(I_{K,\text{slow}}\). We chose the strategy to replace Kv1.5 with the pharmacologically different channel Kv1.1 with the intention of minimizing other potential changes. The findings presented here definitively link Kv1.5 to a component of \(I_{K,\text{slow}}\) in the mouse ventricle.

Ectopic Expression of Kv1.1 in the Hearts of SWAP Mice

Kv1.1 mRNA and protein are expressed in the hearts of transgenic SWAP mice under the control of the Kv1.5 promoter. (Figures 1D through 1F). We were not able to detect any DTX-sensitive currents, however. This suggests that very few functional Kv1.1 channels are present on the extracellular membranes of mouse ventricular myocytes and that the mouse is functionally acting as a Kv1.5 knockout, although we cannot fully exclude the possibility that heteromeric channels containing Kv1.1 are not DTX-sensitive. The smaller size of the rKv1.1 protein expressed in the mouse heart (62 kDa) compared with native mKv1.1 in the brain (72 kDa) points to tissue-specific differences in posttranslational processing.22 Recent studies have highlighted the importance of helper proteins and \(\beta\) subunits in transporting \(K^+\) channels to the surface membrane.30,31 Kv1.1 is not normally expressed at high levels in the heart (Figure 1D), and the mechanism to process and successfully insert physiological levels of expressed channels into the surface membrane may be absent.

Kv2.1 Upregulation Compensates for the Loss of Kv1.5 in the Hearts of SWAP Mice

Despite the loss of the 50 \(\mu\)mol/L 4-AP component of \(I_{K,\text{slow}}\) in myocytes isolated from SWAP mice, there was no decrease in the overall density of \(I_{K,\text{slow}}\) or of the total outward current compared with controls. SWAP myocytes had an increased density of the 20 mmol/L TEA-sensitive component of \(I_{K,\text{slow}}\) and Western blots showed increased Kv2.1 protein in SWAP hearts. Kv2.1 \(\alpha\) subunits produce slowly activating, slowly inactivating, or noninactivating \(K^+\) currents when expressed heterologously in tissue culture, and antibodies to Kv2.1 block currents of this type in hippocampal neurons.32 Previous studies have shown that the TEA-sensitive component of the rapidly activating, slowly inactivating cardiac current \(I_{K,\text{slow}}\) is selectively eliminated in transgenic mice overexpressing the dominant-negative Kv2.1N216Flag construct in the heart.4 Taken together, these data suggest that upregulation of Kv2.1 is one compensatory mechanism for the loss of Kv1.5 in the ventricles of the SWAP mice. We cannot be certain that the entire compensation in \(I_{K,\text{slow}}\) is attributable to upregulation of Kv2.1. In addition, the differences in the time- and voltage-dependent properties of Kv2.1 between tissue culture cells, hippocampal neurons, and cardiac myocytes likely reflect differences in accessory subunits or posttranslational processing.

Kv2.1 RNA levels are not changed in the SWAP mice, whereas protein levels are increased. The mechanism by which the loss of Kv1.5 leads to posttranscriptional upregulation of Kv2.1 is unknown. The feedback could be based on the action potential shape and ionic currents. Alternatively, proteins that bind to the ion channel subunits could directly mitigate subunit processing, transport to the membrane, or stability.

Difference Between Dominant-Negative Transgenic and Gene-Targeted Mice

Both Kv1.x dominant-negative transgenic and Kv1.5 homozygous SWAP mice lack the 4-AP–sensitive component of \(I_{K,\text{slow}}\).3,15 The Kv1.x dominant-negative transgenic mice have QT prolongation and arrhythmias.3,15–17 In this study, we show that targeted mice lacking mKv1.5 have no QT interval prolongation and no arrhythmias. In fact, these mice are resistant to QT prolongation on exposure to the Kv1.5-blocking agent 4-AP. Likely explanations for the differences include both the effect of the Kv1.x transgene on other cardiac \(K^+\) channels known to be important for repolarization, such as Kv1.4,8,9,14 and compensatory regulation of other \(K^+\) channels, such as Kv2.1, in the SWAP mouse. Clearly, loss of Kv1.5 alone is insufficient to lead to a highly arrhythmogenic phenotype. These findings highlight the fact that transgenic and gene-targeting techniques give different and complementary information on the role of ion channels in cardiac function.

Additional study of both models, along with matting of different strains to form double mutants, should lead to both a better understanding of the role of individual genes in repolarization and susceptibility to arrhythmias and to insights into the mechanisms by which \(K^+\) channel gene expression is regulated in vivo in the heart.

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