Characterization of mice with a combined suppression of $I_{to}$ and $I_{K,slow}$

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Congenital and acquired long Q-T syndromes are characterized by marked Q-T prolongation, polymorphic ventricular tachycardias (VTs), and sudden death (6, 21). Recently, voltage-gated K⁺ channel subunit genes have been identified as loci of mutations underlying familial long Q-T syndromes (8, 22). Typically, these mutations lead to marked increases in action potential duration (APD) as well as to increased spatiotemporal dispersion of repolarization and reentrant arrhythmias. At present, there is considerable interest in determining the relative importance of these changes (in APD, Q-T intervals, and dispersion) in arrhythmia generation and maintenance as well as in delineating the underlying cellular and molecular mechanisms involved. Several studies have shown that marked differences exist in the relative expression levels of voltage-gated K⁺ channels both between species and in different regions of the heart. However, there are marked electrophysiological and pharmacological similarities between specific voltage-gated outward K⁺ currents in different species, suggesting that the molecular correlates of the underlying K⁺ channels are the same (20). As a result, genetically engineered mice are increasingly being used to probe the roles of specific ion channels and other regulatory molecules in the repolarization of action potentials, the maintenance of normal rhythms, and in the pathophysiology of cardiac arrhythmias (3, 7, 10, 14, 16, 17).

Several lines of mice with a long Q-T phenotype have been generated recently using targeted deletion and dominant negative approaches (3, 10, 14, 23). These models are characterized by differing degrees of Q-T prolongation as well as susceptibility to spontaneous and inducible arrhythmias. With the use of a dominant negative construct (KvL1.1N206Tag) that targets the Kv1.x-subfamily of Kv-α subunits, for example, London et al. (17) reported that the slow inactivating outward K⁺ current ($I_{K,slow}$) is selectively attenuated and that APD and Q-T intervals are prolonged in mice expressing the transgene Kv1DN (17). In addition, spontaneous and inducible arrhythmias are observed in Kv1DN mice, and optical mapping studies (2) suggest that an increase in the spatial dispersion of repolarization underlies the reentrant arrhythmias observed in the Kv1DN animals.

Recently, the molecular correlates of both components of the transient outward current ($I_{to}$), the fast ($I_{to,f}$) and slow components of $I_{to}$ ($I_{to,s}$), in mouse ventricular myocytes have been identified (3, 12, 13). Expression of a dominant negative Kv4-α-subunit,
 Kv4.2W362F (Kv4DN), for example, results in the elimination of I_{to}, whereas the targeted deletion of the Kv1.4 (Kv1.4→−) gene (18) eliminates mouse ventricular I_{to,s} (13). In ventricular myocytes from Kv4DN animals, I_{to,s} is upregulated, an effect that was abolished when the Kv4DN transgene was expressed in the Kv1.4→− background (12). Interestingly, although action potentials and Q-T intervals are markedly prolonged in Kv4DN and Kv4DN × Kv1.4→− animals, spontaneous arrhythmias are very rare and the spatial dispersion of repolarization is reduced (not increased) in these animals. Taken together with the results in Kv1DN animals (2, 17), these results suggest that increased dispersion of repolarization rather than the extent of Q-T prolongation is the primary determinant of arrhythmia susceptibility. To test this hypothesis directly, Kv4DN and Kv1DN mice were crossed to produce animals expressing both transgenes in an identical genetic background. Whole cell voltage-clamp experiments reveal that I_{to,s}, I_{to,f}, and the 4-amino-pyridine (4-AP)-sensitive component of I_{k,slow} are eliminated in ventricular myocytes from Kv1/Kv4DN animals. In addition, action potentials and Q-T intervals are prolonged significantly, although spontaneous arrhythmias are markedly suppressed in Kv1/Kv4DN animals.

MATERIALS AND METHODS

Generation of Kv1.1/Kv4.2DN mice. Adult female (8–12 wk old) FVB mice homozygous for the dominant negative Kv1.1N206 transgene (11, 17) were crossbred with male C57/BL6 mice homozygous for the dominant negative transgene Kv4.2W362F (3). The resulting animals were screened for the presence of one or both of the transgenes by PCR analysis of tail DNA. Four genotypes were identified among the offspring: wild-type (WT), Kv1.1N206Tag expressing (Kv1DN), Kv4.2W362F expressing (Kv4DN), and Kv1/ Kv4DN mice expressing both constructs. All animal studies were performed in accordance with the guidelines of the Harvard Medical Area Standing Committee on Animals and the Animal Care and Use Committee at Washington University Medical School after approval by the Institutional Animal Care and Use Committee.

Single cell electrophysiological recordings. Ventricular myocytes were isolated from the left ventricular apex of adult WT, Kv1DN, Kv4DN, and Kv1/Kv4DN animals as described previously (12, 13, 23). Briefly, animals were anesthetized with 3% halothane (97% oxygen), and, once deep anesthesia was confirmed, the hearts were rapidly removed, cannulated, and perfused (12, 13, 23). Whole cell voltage- and current-clamp recordings were obtained at room temperature (23°C) or at 35°C within 48 h of cell isolation. Experiments were performed using an Axopatch 1B patch-clamp amplifier (Axon Instruments) interfaced to a 350-MHz Pentium computer connected to the recording equipment with a Digidata 1200 analog-to-digital (A/D) interface and pCLAMP 7 software package (Axon Instruments).

For voltage-clamp experiments, the bath solution contained (in mM) 136 NaCl, 4 KCl, 1 CaCl2, 2 MgCl2, 2 CoCl2, 10 HEPES, 10 glucose, and 0.02 tetrodotoxin (TTX) (pH 7.4, 295–300 mosM); TTX and Co2+ were eliminated when action potentials were recorded. For both current- and voltage-clamp experiments, the recording pipette solution contained (in mM) 135 KCl, 10 EGTA, 10 HEPES, and 5 glucose (pH 7.2, 295–300 mosM). Recording electrodes were fabricated from soda lime glass (Kimble), coated with Sylgard (Dow Corning), and fire polished; tip resistances were 1.5–2.5 MΩ. Series resistances were in the range of 3–4 MΩ and were compensated electronically by 80–90%; voltage errors resulting from the uncompensated series resistance were always <5 mV and were not corrected. Outward K+ currents were routinely evoked during 500-ms or 4-s depolarizing voltage steps to test potentials between −40 and +60 mV from a holding potential (HP) of −70 mV after a 20-ms prepulse to −20 mV to eliminate the residual (20 μM TTX insensitive) component of the voltage-gated Na+ current. Inwardly rectifying K+ currents were recorded in response to hyperpolarizing voltage steps to test potentials between −90 and −120 mV from a HP of −70 mV

Voltage- and current-clamp data were compiled and analyzed using CLAMPfit (Axon Instruments) and Excel (Microsoft). For each cell, the spatial control of the membrane voltage was assessed by analyzing the decays of the capacitative transients evoked during subthreshold (10 mV voltages steps from the HP (−70 mV); only cells with capacitative transients well described by single exponentials were analyzed further. Whole cell ventricular myocyte membrane capacitances were determined by integration of the capacitative transients evoked during brief (25 ms) subthreshold (±10 mV) voltage steps from a HP of −70 mV. The whole cell membrane capacitances (means ± SD) of ventricular cells isolated from WT, Kv1DN, Kv4DN, and Kv1/Kv4DN animals were 123 ± 24 pF (n = 35), 113 ± 18 pF (n = 20), 117 ± 27 pF (n = 20), and 120 ± 31 pF (n = 15), respectively. Leak currents were always <100 pA and were not corrected. The plateau outward K+ current in each cell was defined as the current remaining 3 s after the onset of the depolarizing voltage steps, and the peak outward current was defined as the maximum value of the outward K+ current during 500-ms voltage steps. Current amplitudes measured in individual cells were normalized to cell size (whole cell membrane capacitance) and current densities (in pA/pF) reported. Inactivation time constants were determined from (single or double) exponential fits to the decay phases of the outward K+ currents recorded during 4-s depolarizing voltage steps (12, 13, 23).

Electrocardiogram (ECG) recordings. Electrocardiogram (ECG) recordings were obtained from awake free-moving mice as previously described (19). In brief, adult (3–6 mo old) animals were sedated with pentobarbital sodium (50 mg/kg ip), and transmitters (model EA-F20, DataSciences) were implanted. The positive lead was tunneled to the left anterior chest wall above the apex of the heart and the negative lead to the right shoulder. This configuration approximates to the right shoulder. This configuration approximates to lead II on the surface ECG. Twenty-four hours after surgery, ECGs were recorded continuously for 24 h using a 500-Hz A/D converter, acquired data were stored on a Pentium PC using custom-made software, and offline analysis of the whole recording was performed using an algorithm with automatic R wave and premature beat detection (19). Premature ventricular beats were identified when at least two of the following three criteria were met: 1) atrial or QRS complex with alteration or inversion of the T wave, 2) postextrasystolic pause, and 3) or atrioventricular (AV) dissociation. For bradycardia, a pause of ≥300 ms was considered significant. All ECGs were analyzed by a blinded operator.

Measurement of heart rate and P-R/Q-T intervals. Heart rate and P-R and Q-T intervals were measured every 20 min, resulting in 72 measurements per animal. Intervals were measured on screen with calipers on an averaged and over-
laid signal derived from all beats during a 4-s period. This has been shown to reduce artifacts without significantly affecting measurements (19). In case of noise during the 4 s, the measurement was taken 1 min later. Q-T intervals were corrected for heart rate using an established formula (19).

Right ventricular electrophysiological stimulation in sedated mice. Electrophysiological studies were performed by transvenous right ventricular stimulations in anesthetized mice (50 mg/kg ip pentobarbital sodium) (4, 14). Right ventricular refractory periods were assessed with 2-ms decrements after a 10-beat stimulation train of 100-ms cycle length. The stimulation protocol consisted of 20-beat trains with up to three extrastimuli; each extrastimulus was initially coupled with the basic cycle length (BCL) and, subsequently, the coupling interval was decreased in 10-ms steps until the shortest possible interval was reached. Ventricular stimulation was performed at BCLs of 100 ms and either 70 or 130 ms depending on ventricular refractory periods. After the ventricular stimulations and the assessment of refractory times, isoproterenol (10 μg) was injected intraperitoneally to increase the heart rate by at least 30%, with additional doses of up to a total of 20 μg in case of insufficient increases in heart rate. After a minimum of 2 min for the stabilization of the heart rate, the ventricular stimulation was repeated. Per definition, an induced VT had to last more than five beats; shorter runs of induced ventricular premature beats (VPBs) were counted as premature complexes but not classified as VT. All induced VTs had to be confirmed by a second stimulation to be counted. During the procedure, heart rate and respiration were continuously monitored, and the animals were placed under a heating lamp to maintain body temperature. For all stimulations, ECG leads I, II, and III were recorded on a PC for offline analysis. Only mice surviving at least 24 h after the stimulation were included. All electrophysiological studies were performed and analyzed by a blinded operator.

Statistical analysis. Statistical analysis was performed using ANOVA for continuous variables, with Bonferroni’s test for post hoc analysis. The Kruskal-Walluis test was used for non-Gaussian distributed values, and χ²-tests were performed for categorical values. Analysis was performed with Prism 2.01 for Windows (Graphpad; San Diego, CA) and SPSS 10.0.1 (SPSS; Chicago, IL). All data are presented as means ± SD except where otherwise noted, and a P value <0.05 was considered significant.

RESULTS

Outward K⁺ currents are significantly attenuated in Kv1/Kv4DN mouse ventricular myocytes. To assess the functional consequences of the expression of Kv1DN and Kv4DN, electrophysiological experiments were completed at both room temperature (25°C) and physiological temperature (35°C) on myocytes isolated from the left ventricular apex of adult Kv1/Kv4DN animals. Outward K⁺ currents in these cells were then compared with similar recordings obtained from left ventricular apex cells isolated from WT, Kv1DN, and Kv4DN animals. As shown in Fig. 1A, waveforms of the depolarization-activated outward K⁺ currents in WT, Kv1DN, Kv4DN, and Kv1/Kv4DN cells are distinct. Peak outward K⁺ current densities (at +40 mV; means ± SE) obtained at 25°C, for example, are significantly lower in Kv1/Kv4DN cells (P < 0.001) than in WT, Kv1DN, or Kv4DN cells (Table 1). In contrast with the low peak outward K⁺ current densities in Kv1/Kv4DN cells, the densities of the steady-state outward K⁺ currents (Iˢᵒ), determined as the currents remaining at the end of 4-s voltage steps, in these cells are indistinguishable (Table 1). Consistent with a previous report (24), analyses of the decay phases of the outward K⁺ currents evoked during 4-s depolarizations reveal that the currents in Kv1DN cells are well described by the sum of two exponentials. These analyses also revealed that Iˢᵒ is selectively attenuated in Kv1DN cells and that Iᵗ₀,ˢ is eliminated and Iᵗ₀,اصة is upregulated in Kv4DN cells (Table 1). Iᵗ₀,اصة, for example, was detected in all Kv4DN cells (n = 20) with current density of 9.4 ± 1.7 pA/pF. In Kv1/Kv4DN cells, however, the decay phases of the outward K⁺ currents were slowed significantly (compared with WT, Kv1DN, and/or Kv4DN cells) and, in addition, are well described by single exponentials, reflecting the presence of Iˢᵒ and Iᵗ₀,اصة. Similar results were obtained at physiological temperature. As illustrated in Fig. 1B, increasing the temperature to 35°C resulted in marked changes in current kinetics. Nevertheless, kinetic analyses of the decay phases of the outward K⁺ currents at 35°C also revealed that only Iˢᵒ and Iᵗ₀,اصة remained in left ventricular myocytes from Kv1/Kv4DN mice. The component of Iˢᵒ remaining in these cells was insensitive to 50 μM 4-AP (Fig. 2A) and was blocked by 20 mM TEA, which also partially blocks Iˢᵒ (Fig. 2B). In addition, Iᵗ₀,اصة, which is not evident in WT left ventricular apex cells but is upregulated in Kv4DN cells (12, 13), was undetectable in Kv1/Kv4DN cells, consistent with a dominant negative effect of Kv1.1N206 on Iᵗ₀,اصة, which has been previously shown to be encoded by Kv1.4 (12, 13, 17). Table 1 summarizes the analysis of voltage-gated outward K⁺ currents in left ventricular apex cells.

Action potentials are prolonged in Kv1/Kv4DN mouse ventricular myocytes. The voltage-clamp experiments described above revealed that Iᵗ₀,ˢ, Iᵗ₀,اصة, and the (50 μM) 4-AP-sensitive component of Iˢᵒ are eliminated in left ventricular apex cells isolated from Kv1/Kv4DN animals. To determine the effects of elimination of these K⁺ channel currents on repolarization in Kv1/Kv4DN cells, action potential waveforms in left ventricular apex cells were examined at physiological temperature and compared with those recorded from WT, Kv1DN, and Kv4DN cells. As illustrated in Fig. 3A, current-clamp experiments revealed that action potentials recorded at 1 Hz from Kv1/Kv4DN cells are broader than action potentials recorded from WT, Kv1DN, and Kv4DN cells. The action potential prolongation in Kv1/Kv4DN cells is more prominent when recorded at 10 Hz at 35°C, a frequency that is comparable with the heart rate (~600–700 beats/min) in conscious mice. Analyses of APDs revealed that APD at 90% repolarization values (means ± SE) were increased significantly (P < 0.01) in Kv1/Kv4DN cells (Fig. 3B). No differences in resting membrane potentials or in action potential amplitudes, however, were observed in these cells compared with WT, Kv1DN, or Kv4DN cells (data not shown).
P-R and Q-T intervals are prolonged in a stepwise fashion. Heart rate and P-R and Q-T intervals during the 24-h period were assessed from 10 animals in each group as a repeated measure with 72 levels. To simplify the presentation, Fig. 4 shows 24-h levels. The mean heart rates of Kv1DN (658 ± 66 beats/min) were significantly higher than those of WT, Kv4DN, and Kv1/Kv4DN mice [626 ± 68, 633 ± 61, and 628 ± 60 beats/min, respectively, P < 0.001, Fig. 4A (top)]. Overall, the mean P-R intervals showed a significant stepwise prolongation, with the shortest P-R intervals in WT mice and the longest P-R intervals in Kv1/Kv4DN mice [WT, 31.0 ± 3.8 ms; Kv1DN, 31.2 ± 2.7 ms; Kv4DN, 32.7 ± 2.9 ms; Kv1/Kv4DN, 33.5 ± 3.0 ms (overall P < 0.001; P < 0.001 between all groups except WT vs. Kv1DN; Fig. 4B)]. Similarly, the Q-Tc intervals showed a significant stepwise increase from WT to Kv1/Kv4DN [WT, 53.0 ± 4.8 ms; Kv1DN, 55.9 ± 4.6 ms; Kv4DN, 59.3 ± 4.4 ms; Kv1/Kv4DN, 61.4 ± 6.3 ms (overall P < 0.001; P < 0.001 between all groups)].

Table 1. Comparison of voltage-gated outward K⁺ currents in adult left ventricular apex cells

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>I_peak</th>
<th>I_to,f</th>
<th>I_to,s</th>
<th>I_K,slow</th>
<th>I ss</th>
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<tr>
<td>WT</td>
<td>35</td>
<td>55.4 ± 2.5</td>
<td>32.7 ± 3.8</td>
<td>ND</td>
<td>17.2 ± 1.1</td>
<td>5.5 ± 0.5</td>
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<tr>
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<td>45.1 ± 3.0†</td>
<td>28.3 ± 3.2</td>
<td>ND</td>
<td>11.9 ± 1.1</td>
<td>4.9 ± 0.4</td>
</tr>
<tr>
<td>Kv4DN</td>
<td>20</td>
<td>31.7 ± 1.6‡</td>
<td>9.4 ± 1.7</td>
<td>ND</td>
<td>16.4 ± 0.9‡</td>
<td>5.9 ± 0.6</td>
</tr>
<tr>
<td>Kv1/Kv4DN</td>
<td>15</td>
<td>13.5 ± 0.7∥†</td>
<td>ND</td>
<td>ND</td>
<td>8.8 ± 1.0∥§</td>
<td>4.7 ± 0.5</td>
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</table>

Values are means ± SE; n = no. of cells. Data (in pA/pF) were analyzed from recordings obtained at +40-mV voltage steps; cells were studied at 25°C. Outward K⁺ currents were studied in the following groups: wild-type (WT), Kv1.1 dominant negative (Kv1DN), Kv4.2W362F dominant negative (Kv4DN), and mice expressing both dominant negative constructs (Kv1/Kv4DN). I_peak, peak outward current; I_to,f and I_to,s, fast and slow components, respectively, of the transient outward current (I_to); I_K,slow, slow inactivating outward K⁺ current; I ss, steady-state outward K⁺ current; ND, currents not detectable in any of the analyzed cells. *P < 0.05 vs. WT; †P < 0.05 vs. Kv1DN and Kv4DN; ‡P < 0.05 vs. Kv1DN; §P < 0.05 vs. Kv4DN.
These differences were maintained throughout the day, as shown in Fig. 4C.

With the use of two-way repeated-measures ANOVA for both time of day and genotype, we found a significant circadian variability in heart rate in all groups (ANOVA, $P < 0.001$) [Fig. 4A (bottom)]. The main effect of time of day on P-R interval [Fig. 4B (bottom)] and Q-T interval (data not shown) was significant ($P < 0.001$), confirming that both P-R and Q-T intervals are subject to circadian variability. This variability contrasts with the relatively constrained range of values for the Q-Tc interval.

Spontaneous tachyarrhythmias occur only in mice expressing the Kv1DN transgene. A blinded analysis was performed in 10–11 animals per group. A histogram depicting the total number of arrhythmic events including VPBs and all other arrhythmias is depicted in Fig. 5. More than five events per 24-h period were detected in 1 of 10 WT mice, 6 of 10 Kv1DN mice, 2 of 10 Kv4DN, and 1 of 11 Kv1/Kv4DN mice. The differences were significant between the groups regarding the number of animals with more than five events during the 24-h recording (overall $P = 0.023$) and between WT and Kv1DN mice ($P < 0.02$) and Kv1/ Kv4DN and Kv1DN mice ($P < 0.02$) and showed a trend between Kv4DN and Kv1DN mice ($P < 0.07$). The mean number of VPBs was not statistically significant between the groups (WT, $2.6 \pm 4.3$; Kv1DN, $8.7 \pm 10$; Kv4DN, $3.9 \pm 4.4$; Kv1/Kv4DN, $2.7 \pm 6.5$, $P = 0.069$), due in part to the wide variance within groups. Couplets, triplets, and quadruplets were seen in 4 of 10 Kv1DN mice and in 1 of 11 Kv1/Kv4DN mice. In contrast, none of the WT and Kv4DN animals had complex arrhythmias (overall $P < 0.02$; $P < 0.05$, WT vs. Kv1DN; $P < 0.05$, Kv1DN vs. Kv4DN; $P = 0.09$, Kv1DN vs. Kv1/Kv4DN). However, the incidence of these complex arrhythmias was low, with a total number of nine couplets in four Kv1DN mice and two

**Fig. 2.** Effects of 4-aminopyrimidine (4-AP; A) and TEA (B) on the outward K$^+$ currents in Kv1/Kv4DN mouse left ventricular apex cells. Outward K$^+$ currents were recorded at 25°C, as described in Fig. 1. Recordings were completed before and after exposure to 50 μM 4-AP or 20 mM TEA, and the drug-sensitive currents were obtained by offline digital subtraction of the records in the presence of 4-AP or TEA from the controls. As is evident, the 4-AP-sensitive component of $I_{K,slow}$ is undetectable, whereas the TEA-sensitive component of $I_{K,slow}$ is present in Kv1/Kv4DN cells.

**Fig. 3.** Action potentials are prolonged in Kv1/Kv4DN ventricular cells. In the current-clamp mode, action potentials evoked by 3-ms suprathreshold current injections were recorded at 35°C at 1 Hz. To record action potentials at higher stimulation rates (10 Hz), a train (30) of conditioning pulses was applied before recordings were obtained. A: representative action potential waveforms recorded at 1 and 10 Hz from left ventricular apex cells isolated from WT, Kv1DN, Kv4DN, and Kv1/ Kv4DN animals. B: action potentials in Kv1/Kv4DN cells are prolonged significantly at 1 and 10 Hz. *$P < 0.05$ vs. WT; †$P < 0.05$ vs. WT; ‡$P < 0.05$ vs. WT and Kv1DN; §$P < 0.005$, 1 vs. 10 Hz. APD$_{90}$, action potential duration at 90% repolarization.

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**Table 1.** Effects of 4-aminopyrimidine (4-AP; A) and TEA (B) on the outward K$^+$ currents in Kv1/Kv4DN mouse left ventricular apex cells. Outward K$^+$ currents were recorded at 25°C, as described in Fig. 1. Recordings were completed before and after exposure to 50 μM 4-AP or 20 mM TEA, and the drug-sensitive currents were obtained by offline digital subtraction of the records in the presence of 4-AP or TEA from the controls. As is evident, the 4-AP-sensitive component of $I_{K,slow}$ is undetectable, whereas the TEA-sensitive component of $I_{K,slow}$ is present in Kv1/Kv4DN cells.
couplets in one Kv1/Kv4DN mouse. In the Kv1DN group, three of four mice with couplets also displayed more than five isolated VPBs per 24-h period, and, in one of these mice, an episode of a spontaneous polymorphic VT lasting for more than five beats was detected (Fig. 6).

There were no significant differences in the incidence of bradyarrhythmias. Both pauses ≥300 ms and Mo-
bitz type II and III AV block were observed in all groups: second-degree AV block was seen in one or more instances in four WT, four Kv1DN, five Kv4DN, and three Kv1/Kv4DN animals ($P$ = not significant (NS)). Complete AV block was detected in one WT, three Kv1DN, two Kv4DN, and one Kv1/Kv4DN animal, and ventricular pauses (of any kind) >300 ms were seen in four WT, four Kv1DN, five Kv4DN, and five Kv2/Kv4DN animals ($P$ = NS). One animal from each of the WT, Kv1DN, and Kv1/Kv4DN groups showed a prolonged (>5 min) episode of accelerated AV junctional rhythm.

Kv4DN mice are less inducible than WT and Kv1DN mice during programmed stimulation. Electrophysiological studies were completed in 15 WT, 14 Kv1DN, 17 Kv4DN, and 14 Kv1/Kv4DN mice with an average age of 146 ± 27, 167 ± 26, 138 ± 27, and 159 ± 32 days, respectively ($P$ = NS). Similarly, the weight at stimulation did not differ significantly. Ventricular arrhythmias were not inducible by supraventricular stimulations in any of the animals. Mean ventricular threshold with a 1.0-ms pulse was 0.49 ± 0.1, 0.55 ± 0.2, 0.62 ± 0.1, and 0.63 ± 0.2 V in WT, Kv1DN, Kv4DN, and Kv1/Kv4DN mice, respectively ($P$ = NS). When right ventricular refractory periods were assessed with 2-ms steps, significant prolongation was observed only in the Kv4DN group (WT, 44.1 ± 12.9; Kv1DN, 44.0 ± 10.4; Kv4DN, 62.1 ± 11.3; Kv1Kv4DN, 53.6 ± 13.1, $P < 0.05$, WT vs. Kv4DN).

VT lasting more than five beats was induced in one animal in the Kv1DN group but in none of the animals in the other groups. Isoproterenol was administered intraperitoneally at doses of 11.8 ± 4 μg ($P$ = NS between groups) to increase the heart rate from 435.3 ± 95.2 to 578.8 ± 80.3 beats/min ($P$ = NS between groups). During the subsequent stimulation, 10 of 15 WT mice (66%), 6 of 14 Kv1DN mice (42%), 2 of 17 Kv4DN mice (12%), and 2 of 14 Kv1/Kv4DN mice (14%) were inducible for VTs [overall $P = 0.003$; $P < 0.002$, WT vs. Kv4DN; $P < 0.005$, WT vs. Kv1/Kv4DN; $P < 0.05$, Kv1DN vs. Kv4DN; $P = 0.09$ (NS), Kv1DN vs. Kv1/Kv4DN]. In inducible mice, both polymorphic and alternating VTs were seen, with some mice changing morphology during the tachycardia. All induced arrhythmias ended spontaneously; none of the mice died as a consequence of the arrhythmia.

**DISCUSSION**

In this study, we investigated the hypothesis that prolongation of APD and Q-T intervals is not per se arrhythmogenic. To that end, we crossbred Kv1DN mice with Kv4DN mice to generate Kv1/Kv4DN mice in an identical genetic background (1–3, 14). The effect of the elimination of Kv1.x- and Kv4.x-encoded currents was evaluated both in vitro and in vivo.

Single cell patch-clamping experiments showed that peak outward $K^+$ current densities decreased in a stepwise fashion from WT to Kv1/Kv4DN mice. Analysis of the currents revealed, as expected, that overexpression of Kv1.1N206 and Kv4.2W362F in the heart resulted in the elimination of $I_{to,f}$, $I_{to,s}$, and the 4-AP-sensitive component of $I_{K,slow}$, whereas $I_{ss}$ remained unchanged. In these cells, this left only the (50 μM) 4-AP-insensitive component of $I_{K,slow}$ and $I_{ss}$ as the remaining repolarizing $K^+$ currents. Thus Kv1.1N206 abolished the upregulation of $I_{to,s}$, which is encoded by Kv1.4 channels (18), probably by trapping and degradation of Kv1.4 polypeptides in the endoplasmic reticulum (11).

As anticipated, both Kv1DN and Kv4DN transgenes prolonged APD. However, the effect seen in Kv4DN...
mice was more pronounced than that observed in Kv1DN mice, confirming that \( I_{to} \) plays the predominant role in shaping mouse APD. The most pronounced Q-T interval prolongation was seen in Kv1/Kv4DN mice, which also exhibited a frequency-dependent two-to threefold increase in APD at 90% repolarization: in Kv1/Kv4DN cardiocytes (and to a lesser extent also in Kv1DN and Kv4DN cells), the 4-AP-insensitive TEA-sensitive component of \( I_{K_{slow}} \) is the predominant outward current and is likely coded by Kv2.1. Other groups have shown that Kv2.1 \( K^+ \) channels exhibit a cumulative inactivation after a train of depletions due to slow recovery from inactivation (15). Indeed, 10 300-ms test voltage steps to +50 mV at a cycle length of 500 ms (2 Hz) resulted in a >70% suppression of the outward \( K^+ \) currents evoked in Kv1/Kv4DN cells at the tenth compared with the first pulse.

The unexpected finding that the heart rate of Kv1DN mice is significantly higher than that in the other groups needs clarification. A recent study (9) has demonstrated the expression of Kv1.5 polypeptide in the sinoatrial node of the guinea pig and ferret, but the functional role of these channels in the sinus node remains unclear. Indeed, if \( I_{K_{slow}} \) participates directly or indirectly in determining the resting potential of the sinus node cells, then attenuation of this current may cause depolarization of pacemaker cells and acceleration of heart rate. We also observed a small stepwise increase in P-R intervals from WT to Kv1/Kv4DN mice, which suggests a role of both Kv1.x and Kv4.x channels in atrial or AV conduction. The lack of a statistically significant difference in P-R intervals between WT and Kv1DN mice is likely due to the markedly higher heart rate in Kv1DN mice, which itself can shorten the P-R interval. Significantly, the prolongation of the P-R interval was not associated with an increase in the incidence of bradyarrhythmias or AV block.

Our in vivo data showed that, in awake free-moving mice, APD prolongation was reflected in a highly significant increase in the Q-T/Q-Tc intervals, whereas the magnitude of Q-Tc prolongation was less pronounced than the increase in APD. Notably, the extent of Q-Tc inerval prolongation did not correlate with the incidence or severity of spontaneous arrhythmias: only mice expressing the Kv1DN transgene showed both frequent and high-degree (couplets, triplets, and salvos) spontaneous ventricular arrhythmias, including a run of polymorphic VT. In contrast, significant spontaneous arrhythmias were not detected in either WT or Kv4DN mice. Thus the disruption of a specific component of the repolarizing \( K^- \) current produced an arrhythmogenic substrate, which is likely due to an enhanced dispersion of repolarization (2). On the other hand, we observed a similar incidence of bradyarrhythmias across all genotypes of mice, confirming our earlier (unpublished) observations in WT FVB mice. However, this is in contrast to a previous report (12) that indicated a higher degree of AV block only seen in Kv4DN mice. It is likely that the continuous monitoring for 24 h and the use of our custom-made software increased the capability for detecting bradyarrhythmias. Because ECG artifacts are not uncommon in telemetric recordings in free-moving mice, we used very strict criteria for the classification of ventricular tachyarrhythmias. This implies, however, that we might have increased specificity at the cost of sensitivity.

The in vivo electrophysiological studies revealed a surprisingly high inducibility of WT crossbred mice after isoproterenol. This contrasts with previous studies from our and other laboratories (5, 14), which showed that FVB and other WT mice were not inducible. Here, we report that more than one-half of the crossbred FVB × C57/B6 mice were inducible after administration of isoproterenol. Thus the genetic background in mice may have important implications for arrhythmia induction. Remarkably, expression of the Kv4DN transgene in the heart of WT and Kv1DN mice was associated with a marked suppression of arrhythmia induction after isoproterenol.

The “protective” effect of the Kv4DN transgene on spontaneous and inducible arrhythmias is likely due to pronounced homogenous prolongation of the refractory period throughout the heart. Indeed, Baker et al. (1) showed that Kv4DN mice had no dispersion of repolarization and were not inducible by ventricular stimulation, whereas WT mice showed a small (~10 ms) dispersion between apex and base. In contrast, mice expressing the Kv1DN transgene showed a marked increase in the dispersion of repolarization, a prerequisite for the long-lasting VTs that were inducible in vitro in all the Kv1DN mice (2). It is therefore likely that the Kv4DN transgene abolishes dispersion and consequently reduces spontaneous arrhythmias and arrhythmias induced after isoproterenol.

In conclusion, we showed that dominant negative constructs eliminated both components of \( I_{to} \) and the 4-AP-sensitive component of \( I_{K_{slow}} \) in mouse ventricular myocytes, resulting in the prolongation of APD in vitro. In vivo, this was reflected by a significant stepwise increase of Q-T intervals and also, to a lesser extent, of P-R intervals. The more marked prolongation of APDs and Q-T intervals in mice expressing the Kv4DN transgene is associated with a significant reduction of spontaneous ventricular arrhythmias.

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