Regional upregulation of Kv2.1-encoded current, $I_{K,\text{slow2}}$, in Kv1DN mice is abolished by crossbreeding with Kv2DN mice

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Recent advances in mice genetically modified at various potassium channels have fostered a better understanding of the electrophysiological roles of ionic currents in this species. Functional knockout of the rapidly activating ($I_K$) or slowly activating ($I_{K,\text{slow}}$) components of the delayed rectifier potassium current, the two important repolarization currents responsible for the congenital long QT syndrome, did not lead to either QT prolongation or arrhythmogenic substrate (3, 13). In contrast, attenuation of Kv1.5-, Kv4-, or Kv2-encoded currents in mice with a dominant-negative (DN) approach resulted in prolongation of the action potential duration (APD) in vitro and the QT interval of the electrocardiogram in vivo (5, 14, 16). These results confirmed the substantial difference in ventricular ion channel expression in adult mice from that in humans. Previous studies examined the electrophysiological properties of Kv1.5- and Kv4-like currents in ventricular myocytes isolated from the adult mouse heart, which demonstrated two distinct outward potassium currents, i.e., a rapidly activating, slowly inactivating current ($I_{K,\text{slow1}}$) and a fast component transient outward current ($I_{\text{tof}}$) (5, 10, 18). However, the properties of the Kv2-like current have not been illustrated.

The transgenic mouse model (Kv1DN) of long QT syndrome was created by overexpression of the NH2 terminus and the first transmembrane segment of Kv1.1 in the heart (Kv1.1N206Tag) (14). This truncated channel forms homo- and heteromultimeric complexes in vitro and coassembles with wild-type Kv1.x channels to form nonfunctional complexes that are trapped in the endoplasmic reticulum (9). Mice overexpressing Kv1.1N206Tag in the heart are characterized by a prolonged QT interval and a spontaneous monomorphic ventricular tachycardia (VT) (14). Transvenous programmed electrical stimulation induced polymorphic VT in 50% of these mice (12). Cardiac myocytes derived from these mice had a prolonged APD caused by the loss of a rapidly activating, slowly inactivating current, $I_{K,\text{slow1}}$, which correlated with a marked decrease in the level of Kv1.5 polypeptide (14).

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Programmed electrical stimulation at the apex, but not at the base, of Kv1DN hearts perfused on a Langendorff system resulted in a long-lasting VT (4). Optical mapping experiments revealed substantially longer effective refractory periods (ERPs) at the base of Kv1DN left ventricles, which resulted in increased spatial dispersion of repolarization and reentrant arrhythmia (4). Interestingly, none of the other mouse models, in which the $I_{Ks}$ (3), $I_{Ks}$ (13), Kv4 (5), or Kv2 (16) channel was functionally knocked out, was reported to have proarhythmic activity. Therefore, uncovering the ionic basis of these discrepancies may be very helpful for a better understanding of how these channels are functionally integrated to maintain the electrical stability of the heart.

In the present study, we characterize an electrical remodeling response of the cardiocytes derived from the Kv1DN mouse hearts. A current component ($I_{Ks,slow}$) is selectively upregulated in the myocytes isolated from the apex of the left ventricle but not in those from the base. This spatially restricted electrical remodeling may underlie the enhanced dispersion of repolarization observed in Kv1DN hearts and the spontaneous and inducible arrhythmias in these animals. The upregulation of Kv2.1 transcripts in Kv1DN mouse hearts revealed by Northern blot and RT-PCR and the elimination of transcripts in Kv1DN hearts and the spontaneous and inducible arrhythmia similar to that in a previous study (18) was made. Recordings were started after 5 min of membrane rupture at room temperature (22–24°C). Linear “leakage” currents were corrected off-line only when the input resistances were ≤1 (but ≥0.3) GΩ ($n = 5$). Junction potentials (∼10 mV) resulting from the low-chloride pipette solution were corrected off-line.

**Solutions.** Solution A contained (in mM) 137 NaCl, 4.7 KCl, 1.2 MgCl$_2$, 1.0 KH$_2$PO$_4$, 11 glucose, 10 HEPES, and 0.125 Na$_2$EDTA, pH 7.35. Solution B was made from solution A with 0.5 mg/ml collagenase (type I; Worthington), 0.2 mg/ml hyaluronidase (type II; Sigma), and 1% FCS added. Tyrode solution contained (in mM) 137 NaCl, 5.4 KCl, 1.2 MgCl$_2$, 1 CaCl$_2$, 10 glucose, and 10 HEPES, pH 7.35 with NaOH. CoCl$_2$ was added (2 mM) when potassium currents were measured. The appeared [K+]o involved tetraethylammonium (TEA), extracellular KCl was substituted with equimolar TEA-Cl when the concentration of TEA was ≥2.5 mM to maintain a constant ionic strength. Other blocking reagents were added directly into Tyrode solution. Standard pipette solution was composed of (in mM) 110 K-aspartate, 20 KCl, 0.5 CaCl$_2$, 10 HEPES, 5 EGTA, 5 Mg$_2$ATP, 5 Na-creatine phosphate, and 0.5 GTP-Tris, pH 7.2 with KOH (pCa 7.6). For calcium current ($I_{Ca}$), recording, potassium in both the Tyrode and pipette solutions was substituted with equimolar cesium.

**Data analysis.** Electrophysiological data were analyzed with Clampfit 6.0, Microsoft Excel, and Microcal Origin 5.0. The current inactivation kinetics was described by a sum of exponentials with the following function: $y(t) = A_{1} \exp(-t/\tau_{1}) + C$, where $A_{1}$ and $\tau_{1}$ represent the amplitude(s) and time constant(s), respectively, for the inactivating current component(s), and $C$ is the offset, which includes the remaining portion of those components, other noninactivating currents, and the leakage current. Goodness of fit was judged by visual inspection and the correlation coefficients ($R$), which were normally ≥0.975 in this study. The number of exponentials was determined by F-test (18), and $P < 0.05$ was taken to indicate a better fit. Concentration effects were quantified by fitting the data with a Hill equation: $I_{current}/I_{control} = 1/[1 + (D/CIC_{50})^{nH}]$, where $D$ is the drug concentration, $I_{control}$ is the concentration for 50% inhibition, and $nH$ is the Hill coefficient. Data are expressed as means ± SD unless indicated. ANOVA was applied for analyzing the multi-group data. Student’s t-test was used to compare unpaired data between two groups, and a two-tailed $P < 0.05$ was taken to indicate statistical significance.

**Generation of Kv1/Kv2DN mice.** Adult female FVB mice heterozygous for the DN transgene Kv1.1N206 were crossbred with male C57/B6 mice carrying the DN transgene Kv2.1N216 (16). The resulting animals were screened for the presence of one or two transgenes by PCR analyses of tail DNA. Kv1/Kv2DN mice expressed both transgenes.

**RESULTS**

$I_{Ks,slow}$ exists in both control and Kv1DN mouse ventricular cardiocytes. We previously reported (18) that the outward current of adult mouse ventricular cardiocytes was composed of at least three components, each of which displayed distinct inactivation kinetics at 37°C. Here we confirm that, at room temperature, the decay
of the outward currents elicited in control myocytes by 5-s pulses could be best fitted by three exponentials with the following time constants: \( \tau_1 \approx 40 \text{ ms} \), \( \tau_2 \approx 350 \text{ ms} \), and \( \tau_3 = 1.6 \text{–} 2.0 \text{ s} \) (Fig. 1, A and C). These components represent \( I_{to} \), \( I_{K,slow1} \) (18), and \( I_{K,slow2} \), respectively. By contrast, the outward currents in almost all the myocytes (86 of 89 cells in this study) derived from Kv1DN mouse hearts had only two exponential components (Fig. 1, B and C). The time constants were indistinguishable from those of \( I_{to} \) and \( I_{K,slow2} \) in the control myocytes, confirming that \( I_{K,slow1} \) was completely eliminated. Only at \(-20 \text{ mV}\) did the time constants of the fastest components (\( I_{to} \)) differ between the two groups. However, it is unlikely that \( I_{to} \) inactivates any faster at this particular membrane potential in the Kv1DN myocytes than in control myocytes. In fact, \( I_{to} \) occupies a relatively small segment (\(-200 \text{ ms}\)) during the whole recording course (5 s) because of its fast inactivation kinetics, and its amplitude was relatively small at \(-20 \text{ mV}\), making clear separation of this current from the overlapping \( I_{K,slow1} \) in control myocytes somewhat difficult. In Fig. 1D, the relative amplitudes obtained from the exponential fittings showed a larger proportion of \( I_{to} \) in the total outward current in Kv1DN myocytes than in that of the controls (e.g., \( 53.4 \pm 14.3\% \) vs. \( 27.1 \pm 8.2\% \) at 20 mV; \( P < 0.01 \)). However, the absolute values of the amplitudes of \( I_{to} \), after normalization to individual cell capacitances, did not significantly differ between the two groups (e.g., \( 16.4 \pm 9.4 \) vs. \( 11.6 \pm 5.6 \text{ pA/pF} \) at 20 mV; \( P > 0.05 \)).

**TEA-sensitive** \( I_{K,slow2} \) **is upregulated in Kv1DN myocytes.** Although the exponential fitting permits the assessment of the amplitude values of each component, the accuracy of this method is limited. We therefore applied TEA to better evaluate \( I_{K,slow2} \). Our results showed that 5 mM TEA inhibited most of the \( I_{K,slow2} \) without significantly affecting the other two components (Fig. 2A). The time constants of the inactivation kinetics (obtained by single-exponential fittings to the current decays) of the TEA-sensitive current were indistinguishable from those of \( I_{K,slow2} \) before the administration of TEA (data not shown). The TEA-sensitive current started to activate at about \(-30 \text{ mV}\), and the activation process was relatively slow. The current-voltage relationship (Fig. 2B) showed an outward rectification. Interestingly, the current density of the TEA-sensitive component in the Kv1DN myocytes was increased by \(-90\%\) at potentials positive to 30 mV compared with the control myocytes (\( P < 0.05 \)). Thus cardiocytes derived from Kv1DN hearts underwent electrical remodeling with a significant enhancement of the expression of a TEA-sensitive current, \( I_{K,slow2} \), to compensate for the loss of \( I_{K,slow1} \).

To determine whether the electrical remodeling involved additional currents, we examined the current densities of other ion channels in the control and Kv1DN myocytes. First, the density of the steady-state current (\( I_{ss} \)) determined as the current level at the end of 5-s pulses was compared between the two groups. No significant difference was found, although the current density was slightly larger in Kv1DN myocytes than in the controls (4.9 \pm 1.7 vs. 3.9 \pm 1.7 pA/pF at 30 mV, \( n = 11 \) for each group; \( P > 0.05 \)). This small difference could be accounted for by the incomplete inactivation of \( I_{K,slow2} \) during the 5-s depolarization. The inward rectifier potassium current (\( I_{K1} \)) evoked by a series of 200-ms pulses from a holding potential of \(-40 \text{ mV}\) in Kv1DN myocytes also displayed densities at all test potentials (ranging from \(-110 \text{ to} -20 \text{ mV}\)) indistinguishable from those of the controls (data not shown). Similarly, the current density, activation and inactivation kinetics and their voltage dependence, and the
Regional remodeling: enhancement of $I_{K,slow2}$ at apex of Kv1DN hearts. A number of studies have emphasized the electrical heterogeneity that exists within the heart, pointing to regional differences both in electrical properties of ventricular myocardium and in the response of distinct regions to pharmacological agents and pathological states (2). The current density of $I_{K,slow2}$ in randomly studied Kv1DN myocytes (Fig. 2) displayed marked variation, suggesting varying densities of this current in the Kv1DN mouse cardiocytes. Moreover, previous optical mapping studies revealed markedly longer refractory periods at the base than at the apex of Kv1DN hearts (4). A premature impulse applied to the apex of Kv1DN hearts induced reentry after encountering a functional line of conduction block (4). To further evaluate the molecular basis of the spatial dispersion of the repolarization, we compared the three major outward current components expressed in the myocytes isolated from the apical segments of the left ventricle free wall with those from the base segments of either control or Kv1DN mouse hearts. Here, $I_{K,slow1}$ and $I_{K,slow2}$ were determined by measuring the 25 M 4-aminopyridine (4-AP)-sensitive (14) and 500 M TEA-sensitive currents, respectively, in response to a 5-s pulse from HP of −80 mV to 40 mV. $I_{to}$ was determined by fitting the current traces in the presence of 25 M 4-AP by two exponentials. The current was elicited by a 1-s pulse from −80 to 40 mV. These studies revealed that cardiac myocytes derived from the apex of Kv1DN hearts expressed a twofold higher density of $I_{K,slow2}$ current compared with cardiocytes derived from the base (P < 0.05; Fig. 3C). By contrast, $I_{to}$ density did not differ in cardiocytes derived from either the apex or base. Moreover, in control cardiocytes, no significant difference was found between the current densities of $I_{to}$, $I_{K,slow1}$, or $I_{K,slow2}$ expressed in cardiocytes derived from the apex and those from the base. However, the current density of $I_{K,slow2}$ in the Kv1DN apical cells was significantly greater than that in the control apical myocytes (P < 0.01), whereas in the basal myocytes it remained similar to the level of $I_{K,slow2}$ in the control basal cells (P > 0.05). Together,

Fig. 2. Upregulation of $I_{K,slow2}$ in Kv1DN myocytes. A: current traces from a representative control and a Kv1DN myocyte before (a) and after (b) the administration of 5 mM tetraethylammonium (TEA). The voltage protocol was the same as described in Fig. 1. Digital subtraction (a − b) was performed off-line to demonstrate the TEA-sensitive current (c). B: current-voltage (I-V) relationship of the TEA-sensitive current in control and Kv1DN myocytes. Current amplitudes measured at the peak of the TEA-sensitive current were normalized to individual cell capacitances. Values are means ± SE (n = 14 for control; n = 9 for Kv1DN). *P < 0.05 vs. control.

Fig. 3. Current densities of $I_{to}$ (A), $I_{K,slow1}$ (B), and $I_{K,slow2}$ (C) in apical and basal myocytes isolated from control and Kv1DN mouse ventricles. $I_{to}$ was obtained by fitting the current trace in response to a 1,000-ms depolarization pulse from −80 to 40 mV in the presence of 25 M 4-aminopyridine (4-AP) by 2 exponentials. $I_{K,slow1}$ and $I_{K,slow2}$ were determined as the 25 M 4-AP and 500 M TEA-sensitive currents, respectively. Sample numbers of each group are shown in the bars. Ap and Bs, data from apical and basal myocytes, respectively. Values are means ± SE. *P < 0.05, **P < 0.01 vs. Kv1DN apex.
these data indicate a selective upregulation of $I_{K,slow2}$ in the apical segments of Kv1DN hearts.

**Biophysical properties of $I_{K,slow2}$.** Because the TEA-sensitive current provided us with a fairly pure $I_{K,slow2}$, a single-exponential fit to the rising phases of the current was performed to determine the activation kinetics of $I_{K,slow2}$. The resulting time constants were then plotted as a function of the membrane potential. These data showed no significant differences in activation time constants between the control and Kv1DN groups (Fig. 4A), indicating that the activation kinetics of $I_{K,slow2}$ was not altered in the transgenic mouse myocytes. The activation rate of $I_{K,slow2}$ was much slower than that of the other two major components of the outward current, $I_{Na}$ and $I_{K,slow1}$, which were described previously (18).

The activation time constants ($\tau_{act}$) of $I_{K,slow2}$ are voltage dependent (Fig. 4A). When the test potentials ($V_m$) were increased, the activation process was faster. This relationship is well described by a single-exponential function: $\tau_{act} = A \cdot \exp(-V_m/k) + \tau_0$, where $k$ defines the steepness of the voltage dependence and $\tau_0$ reflects the “steady-state” time constants, which are normally obtained at the most positive membrane potential. In the control group, the fitting yielded $k = 2.47 \pm 0.78$ mV ($A = 0.08 \pm 0.18$ ms and $\tau_0 = 18.86 \pm 2.64$ ms), whereas in the Kv1DN group, the values of the three parameters were $k = 2.44 \pm 0.66$ mV, $A = 0.03 \pm 0.04$ ms, and $\tau_0 = 21.95 \pm 6.76$ ms. No statistically significant differences were found between these two groups.

The elimination of the contaminating $I_{K,slow1}$ in the Kv1DN myocytes enabled us to better describe the electrophysiological properties of $I_{K,slow2}$. In fact, we did not observe any significant difference in the biophysical and pharmacological properties of $I_{K,slow2}$ be-
between control and Kv1DN groups, thus validating the approach of further characterizing this current in the Kv1DN mouse cardiocytes. We next determined the steady-state activation of $I_{K,\text{slow}2}$. A prepulse of 200 ms from the holding potential (HP) of −50 mV to 50 mV was applied 3 ms before the test steps to inactivate $I_{K}$.

The 100-ms test pulses ranging from −50 to 40 mV were followed by a repolarization step of 100 ms at −40 mV to evoke the tail currents (Fig. 4B, inset). The tail currents were measured as the difference between the peak current and that at the end of the pulse, normalized to the maximal current ($I_{\text{max}}$), and plotted as a function of the membrane potentials (Fig. 4B). The steady-state activation is well described by the Boltzmann function:

$$I_{\text{max}} = 1 / (1 + \exp[(V_{1/2} - V_{m})/S]),$$

where $V_{m}$ is the membrane potential, $V_{1/2}$ is the half-maximal activation voltage, and $S$ is the slope factor that reflects the steepness of the voltage dependence.

The resulting $V_{1/2}$ and $S$ from seven observations were −13.5 ± 5.8 and 7.9 ± 2.1 mV, respectively.

As described above, $I_{K,\text{slow}2}$ inactivated very slowly, with a time constant of ~1.6 s at room temperature. The inactivation kinetics, similar to those of $I_{Ko}$ and $I_{K,\text{slow}1}$, did not have an appreciable voltage dependence (Fig. 1C). To evaluate the steady-state inactivation of $I_{K,\text{slow}2}$, a standard double-pulse protocol was applied in the Kv1DN myocytes. Test prepulses of 10 s from −80 to 40 mV were applied to reach a steady state of inactivation, followed by a 10-s depolarization pulse at 40 mV to evoke the current (Fig. 4C, inset). $I_{K,\text{slow}2}$ was determined as the difference between the current at 200 ms after depolarization and that at the end of the pulse. The normalized current values were then plotted against the test membrane potentials (Fig. 4C) and fitted by a single Boltzmann function. The averaged half-inactivation voltage ($V_{1/2}$) and the slope factor ($S'$) from eight observations were $−31.0 ± 5.8$ and $−6.7 ± 2.2$ mV, respectively. Fitting the mean data shown in Fig. 5B resulted in a $V_{1/2}$ of $−32.0$ mV and an $S'$ of $−6.5$ mV.

The recovery kinetics from the steady-state inactivation of $I_{K,\text{slow}2}$ was also assessed in the Kv1DN myocytes. Two 8-s depolarization pulses (from HP of −70 to 40 mV) of different intervals were applied, and the currents were measured. The current elicited by the second pulse was then normalized to that elicited by the first pulse and is plotted in Fig. 4D. Two exponents were needed to describe the recovery kinetics of $I_{K,\text{slow}2}$ with an initial time constant of 149.0 ± 55.3 ms for ~35% recovery and 2,079.6 ± 946.8 ms for the remaining $I_{K,\text{slow}2}$ to recover ($n = 5$).

Pharmacological profile of $I_{K,\text{slow}2}$. To study the sensitivity of $I_{K,\text{slow}2}$ to the blockade of TEA, cells were superfused with Tyrode solution containing different concentrations of this agent (Fig. 5A). The amplitude of currents, measured as the difference between the level at 200-ms depolarization and that at the end of the pulse, were normalized to the respective control values (before drug application) and plotted as a function of the concentration (Fig. 5B). The concentration-dependent effect was evaluated by fitting the data to the Hill equation. The IC$_{50}$ of TEA necessary to block $I_{K,\text{slow}2}$ was $638 ± 231 \mu$M (mean ± SE; $n = 7$), and the $n_H$ of 1.0 indicates that TEA binds to a single site of the

Fig. 5. Effects of TEA and 4-AP on $I_{K,\text{slow}2}$. Experiments were conducted in Kv1DN myocytes exclusively. A: current traces before (a) and after administration of 0.5 (b) and 5 (c) mM TEA. Currents were elicited by an 8-s step pulse from −80 mV (HP) to 40 mV at 0.03 Hz. B: summary of the effect of TEA. Currents were measured as the difference between the level at 200-ms depolarization and that at the end of pulse. Data were fitted by the Hill equation ($n = 6$). C: current recordings in the absence (a) and presence of 4-AP (b = 1, c = 5 mM). D: concentration dependence of block by 4-AP on $I_{K,\text{slow}2}$; $n = 5$. 

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These data suggest that $I_{K,slow2}$ is much more sensitive to TEA than the two major current components of the outward current, $I_{to}$ and $I_{K,slow1}$, which are relatively insensitive to TEA (up to 10 mM) (18).

Another potassium channel blocker, 4-AP, also inhibits $I_{K,slow2}$ (Fig. 5C). The IC$_{50}$ of 4-AP to block this current was 892 ± 156 µM (mean ± SE; n = 4) with n_H of ~1.0 (Fig. 5D). It is noteworthy that both $I_{to}$ and $I_{K,slow1}$ are more vulnerable to the blockade of 4-AP than $I_{K,slow2}$ (IC$_{50}$ of ~300 and ~30 µM, respectively) (8, 14). In contrast, E-4031 at 2 µM had no significant effect on $I_{K,slow2}$ (data not shown), indicating that the rapidly activating, delayed rectifying potassium channel ($I_{Kr}$) did not contribute to the $I_{K,slow2}$. Similarly, this current was not sensitive to 28.6 nM $\alpha$-dendrotoxin (DTX; data not shown). At this concentration, DTX effectively inhibited the Kv1.2-encoded currents (7).

Functional role of $I_{K,slow2}$. To evaluate the functional importance of the upregulation of $I_{K,slow2}$, we applied 5 mM TEA to control and Kv1DN myocytes and investigated its effect on their action potentials. As shown in Fig. 6A, the APD of a representative Kv1DN cardiac cell was significantly prolonged by the application of 5 mM TEA, whereas only a mild effect was seen in a control myocyte. The prolongation was more profound in the late phase of repolarization. Similar effects were observed in several other Kv1DN and control myocytes. At this concentration, TEA prolonged the APD by 70.4 ± 51.6% (from 61.1 ± 1.1 to 105.0 ± 3.9 ms; n = 7) in Kv1DN cardiocytes and by 17.4 ± 16.7% (from 48.3 ± 39.4 to 56.2 ± 45.7 ms; n = 10) in control myocytes ($P < 0.01$), whereas the prolongation of APD at 30%, 50%, and 70% repolarization in both groups did not differ significantly (Fig. 6B). The prolongation of the APD by TEA in Kv1DN myocytes showed a large variation, reflecting the uneven upregulation of the TEA-sensitive current, $I_{K,slow2}$, in the whole ventricle and its different functional contributions in myocytes that have different lengths of repolarization. These results indicate that $I_{K,slow2}$ participates in the last stage of repolarization in the formation of the action potential, which is supported by the relatively slow activation kinetics of this current. Upregulated $I_{K,slow2}$ contributes more to the repolarization of the action potential in Kv1DN myocytes.

$I_{K,slow2}$ is eliminated by crossbreeding with Kv2DN mice. Our data indicate that the biophysical and pharmacological properties of $I_{K,slow2}$ are similar to those of the Kv2 family of voltage-gated potassium channels (see DISCUSSION). To show the correlation between the expression of the Kv2 gene and this current, we first checked the steady-state levels of Kv2.1 transcript in either control or Kv1DN hearts. The results revealed that there was a twofold increase in the steady-state level of Kv2.1 transcript (Fig. 7A). Real-time PCR also showed a twofold increase in Kv2.1 transcript (data not shown). To further determine the gene that codes for the upregulated $I_{K,slow2}$, we crossbred Kv1DN mice with mice expressing a truncated Kv2 polypeptide in the heart (Kv2DN). Kv1/Kv2DN cardiocytes exhibited outward currents (Fig. 7B) that lacked both the 4-AP-sensitive currents (Fig. 7E) and the TEA current (Fig. 7F) sensitive components of $I_{K,slow}$.

Thus, overexpression of Kv2N216 eliminated $I_{K,slow2}$ in Kv1DN mice.

DISCUSSION

$I_{K,slow2}$ is encoded by Kv2.1. Our recent studies (14, 18) suggest that, besides $I_{to}$, a rapidly activating, slowly inactivating 4-AP-sensitive delayed rectifier current plays an important role in the cardiac repolarization of murine hearts. This current, first termed $I_{slow}$ (now referred to as $I_{K,slow1}$), has an inactivation constant of ~400 ms at room temperature. The continued decline of the outward current of mouse ventricular myocytes after 3–4 s of depolarization, when $I_{K,slow1}$ is already fully inactivated, pointed toward the existence of another component ($I_{K,slow2}$) with slower inactivation kinetics than $I_{K,slow1}$. In the present paper, we have described the electrophysiological features and functional significance of $I_{K,slow2}$. To separate the existing three components in the total outward current of the control (wild-type) myocytes, we applied three exponentials to best describe the current decay. Although...
the curve-fitting method to determine the number of current components is relatively crude and may introduce bias, we have found that the utility of three-exponential fitting in control myocytes is not only statistically valid (examined by F-test, which indicated that goodness of fit by 3 exponentials is superior to the 2-exponential model at potentials more positive than 10 mV when all current components are well activated; Ref. 18) but also well supported by the functional data: 1) The 4-AP-sensitive current ($I_{K,slow1}$) had only one inactivation component with time constants (400–500 ms) similar to $\tau_2$ obtained from the control outward current, and it was completely inactivated within 3–4 min, as could be calculated from $\tau_2$; 2) the TEA-sensitive (at <1 mM) current ($I_{K,slow2}$) also had single-exponential inactivation kinetics with time constants of 1.6–2 s indistinguishable from $\tau_3$; 3) in Kv1DN mouse myocytes, $I_{K,slow1}$ was selectively eliminated, leaving $I_{to}$ and $I_{K,slow2}$ with unchanged time constants compared with $\tau_1$ and $\tau_3$ in the control current. Obviously, the use of two exponentials to fit the current decay of control myocytes will give different kinetic parameters, and therefore the time constants of $\approx$80 ms for inactivation of $I_{to}$ and $>1$ s for “$I_{K,slow}$” reported by Xu et al. (17) actually reflect an incomplete separation of $I_{to}$, $I_{K,slow1}$, and $I_{K,slow2}$. In fact, in the studies on Kv2DN mice (16), these authors described an “accelerated” inactivation kinetics of $I_{K,slow}$, so they suspected that it might have two components and that the slower component was likely encoded by the Kv2 $\alpha$-subunits. The present study refines and extends these observations and fully characterizes $I_{K,slow2}$.

The two Shab subfamily members (Kv2.1 and Kv2.2) encode typical delayed rectifying currents (7) that have biophysical and pharmacological features similar to those of $I_{K,slow2}$. We found that the steady-state levels of Kv2.1 transcript were upregulated in Kv1DN mouse hearts, which correlated with the higher levels of $I_{K,slow2}$, whereas the transcript of Kv2.2 was not detectable by Northern blot analysis or RT-PCR. Importantly, overexpression of Kv2.1N216 in the heart eliminated $I_{K,slow2}$. Together, the results confirm that $I_{K,slow2}$ is encoded by Kv2.1 (16), which was selectively increased and showed a spatially restricted response to the elimination of $I_{K,slow1}$ in the Kv1DN mice.

Prolongation of APD and QT interval-induced electrical remodeling. We created a mouse with a marked reduction in $I_{K,slow1}$, a significant prolongation of the APD, and a prolonged QT interval (14). The mouse heart exhibited an enhanced spatial dispersion of repolarization and a highly arrhythmogenic substrate (4, 12). Here we showed that the suppression of $I_{K,slow1}$ was associated with an upregulation of $I_{K,slow2}$. The gating properties and pharmacological features of $I_{K,slow2}$ in the transgenic mice were not altered. Therefore, it is likely that the increase of $I_{K,slow2}$ was due to either an increase in the number of the functional channels or an increase of open probability, although single-channel recordings were not conducted to rule out the possibility of altered single-channel conductance. Thus the prolongation of APD and QT interval likely triggered the induction of $I_{K,slow2}$ to partially compensate for the loss of $I_{K,slow1}$. Interestingly, a similar electrical remodeling phenomenon was also described in transgenic mice (Kv4DN) overexpressing Kv4.2W362F (5), a nonconducting mutant Kv4.2 $\alpha$-subunit, in the heart. The selective suppression of $I_{to}$ in these mice resulted in the induction of $I_{to,s}$, a slowly inactivating transient outward current (10). Together, these observations suggest that the electrical
remodeling induced by the inhibition of a repolarizing current is gene specific and triggers a compensatory response of a current most similar to the suppressed current.

Is the compensatory response beneficial or detrimental to the electrical stability of the heart? Obviously, the increased \( I_{\text{K,slow2}} \) in the transgenic mouse cardiocytes plays a more important role in shortening the APD, as evidenced by the enhanced TEA-induced prolongation of the APD in Kv1DN cardiocytes. Thus the induction of \( I_{\text{K,slow2}} \) leads to a partial compensation for the loss of \( I_{\text{K,slow1}} \). However, this adaptation is spatially restricted to the apex of the heart for an unknown reason. Thus the lack of a full compensatory response in the whole heart may result in an increased spatial dispersion of repolarization and a highly arrhythmogenic substrate. Indeed, the gradients of APD and ERP from apex (shorter) to base (longer) showed a twofold increase in the Kv1DN mouse hearts compared with controls (4). Therefore, it is likely that the loss of \( I_{\text{K,slow1}} \) and the spatially restricted expression of \( I_{\text{K,slow2}} \) underlie the increased dispersion of the APD and ERP and consequently render the myocardium more susceptible to spontaneous and inducible reentrant arrhythmias. In contrast, the electrical remodeling in the heart of Kv4DN mice (upregulation of \( I_{\text{to,ap}} \)) was associated with a protective effect against ventricular arrhythmias (5). Thus the prolongation of the QT interval may lead to specific compensatory responses that are protective in one case but detrimental in others.

**Limitations of this study.** The outward current of mammalian cardiac myocytes is normally composed of several different depolarization-activated ionic current components. At least five repolarization currents (10, 14, 16) have been described in adult murine ventricular myocytes: \( I_{\text{to}}, I_{\text{to,s}}, I_{\text{K,slow1}}, I_{\text{K,slow2}}, \) and sustained current (\( I_{\text{sus}} \)). Although these currents have distinct gating and pharmacological properties, it is still difficult to efficiently separate and accurately assess each component. In the present paper, we have used low concentrations of 4-AP or TEA to investigate \( I_{\text{K,slow1}} \) or \( I_{\text{K,slow2}} \) because, at these concentrations, the two blockers had little effect on the other current. However, the 4-AP- or TEA-sensitive currents represent only part of the \( I_{\text{K,slow1}} \) or \( I_{\text{K,slow2}} \) (tested at \( \sim IC_{50} \) concentrations). Thus the densities of these currents may have been underestimated in our studies. For the measurement of \( I_{\text{to}} \), a curve fitting to a sum of two exponentials was used to resolve the contamination problems and the current traces were elicited by a short (1-s) depolarization pulse in the presence of 25 \( \mu \)M 4-AP to suppress \( I_{\text{K,slow1}} \).

It is well known that divalent cations can shield the membrane surface charge and thus shift the voltage dependence of steady-state activation and inactivation (1). An average of 15- or 7-mV shift in the half-activated or -inactivated voltages, respectively, was observed in our previous study (18) for \( I_{\text{K,slow1}} \) by 2 mM cobalt without changes in the slope factors and kinetics. Because of the concern that organic calcium antagonists may affect the Kv1.5-like current \( I_{\text{K,slow1}} \) (18), which may complicate the analysis in wild-type mouse cardiocytes, we continued to use cobalt to block the calcium channel in this study. It is highly possible that cobalt exerts a similar effect on \( I_{\text{K,slow2}} \) and, therefore, the voltage dependence of the current gating reported here may have been positively shifted.

In a previous optical mapping study examining the action potentials on the epicardial surface of the left ventricle (4), we demonstrated a shorter APD in the apex than in the base. In the present study, we used myocytes isolated from the apex and base of the left ventricles. Apart from an enhanced expression of \( I_{\text{K,slow2}} \) in the apical myocytes, we also observed that the densities of this current (and \( I_{\text{to}} \) and \( I_{\text{K,slow1}} \), as well) varied widely within each group. This variability may represent transmural heterogeneity of the expression of outward potassium currents. Thus the actual regional differences in the whole heart may also have been underestimated by simply comparing apical and basal myocytes.

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