Transgenic upregulation of $I_{K1}$ in the mouse heart leads to multiple abnormalities of cardiac excitability.

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Transgenic upregulation of $I_{K1}$ in the mouse heart leads to multiple abnormalities of cardiac excitability. Am J Physiol Heart Circ Physiol 287: H2790–H2802, 2004. First published July 22, 2004; doi:10.1152/ajpheart.00114.2004.—To assess the functional significance of upregulation of the cardiac current ($I_{K1}$), we have produced and characterized the first transgenic (TG) mouse model of $I_{K1}$ upregulation. To increase $I_{K1}$ density, a pore-forming subunit of the Kir2.1 (green fluorescent protein-tagged) channel was expressed in the heart under control of the $\alpha$-myosin heavy chain promoter. Two lines of TG animals were established with a high level of TG expression in all major parts of the heart: line 1 mice were characterized by 14% heart hypertrophy and a normal life span; line 2 mice displayed an increased mortality rate, and in mice ≥1 mo old, heart weight-to-body weight ratio was increased by >100%. In adult ventricular myocytes expressing the Kir2.1-GFP subunit, $I_{K1}$ conductance at the reversal potential was increased ~9- and ~10-fold in lines 1 and 2, respectively. Expression of the Kir2.1 transgene in line 2 ventricular myocytes was heterogeneous when assayed by single-cell analysis of GFP fluorescence. Surface ECG recordings in line 2 mice revealed numerous abnormalities of excitability, including slowed heart rate, premature ventricular contractions, atrioventricular block, and atrial fibrillation. Line 1 mice displayed a less severe phenotype. In both TG lines, action potential duration at 90% repolarization and monophasic action potential at 75–90% repolarization were significantly reduced, leading to neuronlike action potentials, and the slow phase of the T wave was abolished, leading to a short Q–T interval. This study provides a new TG model of $I_{K1}$ upregulation, confirms the significant role of $I_{K1}$ in cardiac excitability, and is consistent with adverse effects of $I_{K1}$ upregulation on cardiac electrical activity.

The cardiac $K^+$ current ($I_{K1}$) (14) has been under intense scrutiny for several decades in an effort to understand its role in cardiac excitability and to find potent drugs for use in clinical conditions. Unfortunately, only a few potent and specific agents have been found. Terikalant (RP-62719) (8) is probably the only promising drug, but several studies have shown that it also inhibits the rapidly activating $K^+$ current, and its effects are not consistent with the block of $I_{K1}$ alone (15).

This situation has changed significantly in recent years as the role of $I_{K1}$ has been assessed in several studies using advanced genetic tools. 1) Knock out of Kir2.1 and Kir2.2 genes (39, 40) in mice has led to results undoubtedly suggesting that the products of these genes underlie $I_{K1}$, with Kir2.1 contributing more than Kir2.2. 2) We recently produced and characterized transgenic (TG) mice with suppressed $I_{K1}$ due to the expression of a dominant-negative nonfunctional subunit of the Kir2.1 channel (Kir2.1-AAA-GFP) driven by the $\alpha$-myosin heavy chain ($\alpha$-MHC) promoter (25). Dominant-negative suppression of $I_{K1}$ led to prolongation of the action potential (AP) and major ECG intervals but had no effect on mice viability and, thus, provided a useful tool for functional studies of $I_{K1}$ (21). Miake et al. (26, 27) used adenoviral technology to deliver a Kir2.1 transgene into a guinea pig heart. In their study, $I_{K1}$ was downregulated with a dominant-negative construct and, for the first time, upregulated by overexpression of the wild-type Kir2.1 subunits.

Although most of the principal results of the previous studies could be predicted with some certainty, the quantitative part of $I_{K1}$ function, as well as species-related differences, requires further investigation. Here, we have addressed the role and the significance of $I_{K1}$ in cardiac excitation by producing the first TG mouse model of $I_{K1}$ upregulation using overexpression of wild-type Kir2.1 subunits in the heart (22). In contrast to the adenoviral gene transfer, which can be applied only to adult animals and produces TG expression only in a limited localized area of the heart, TG technology provides an opportunity to study the effects of widespread $I_{K1}$ upregulation during the entire life span of the animals. Wild-type Kir2.1 channel subunits were expressed as a green fluorescent protein (GFP) fusion protein that significantly aids the analysis of TG expression but does not affect the channel function in any measurable way.

This study shows that overexpression of Kir2.1 subunits and subsequent upregulation of $I_{K1}$ in the mouse heart lead to multiple abnormalities of excitability, including 1) pronounced shortening of APs and effective refractory period (ERP) and 2) nearly complete elimination of the slow T wave, leading to a short Q–T interval. Increased density of $I_{K1}$ was frequently associated with severe abnormalities in cardiac excitability, such as junctional escape, atrioventricular (AV) block, atrial fibrillation, heart hypertrophy, and sometimes animal death. The results of this study, as well as the previously published data on $I_{K1}$ suppression (25, 38), also demonstrate an amazing tolerance of the mouse heart to large-scale manipulations of this current.

MATERIALS AND METHODS

Expression of Kir2.1 subunits in mouse heart. A PCR-based approach with overlapping primers was used to fuse wild-type Kir2.1 cDNA to GFP at the COOH terminus without any linkage. The Kir2.1-GFP construct was then subcloned into a pcDNA3 mammalian expression plasmid for in vitro testing and also downstream of the...
α-MHC promoter (29) region subcloned into PBS2 SK(+) plasmid. The complete transgene was cut out using NcoI restriction enzyme and sent to the TG Core at the University of Michigan to produce TG mice via cDNA microinjection of fertilized C57BL/6SJL oocytes according to established techniques (12). Primers corresponding to the sequence of GFP protein were used to identify GFP-positive mice (founders) by PCR analysis of genomic tail DNA. Two TG lines, designated lines 1 and 2, were established by breeding the founders into C57BL/6 wild-type background, thus creating heterozygous mice. Mice from F1 to F4 generations were used in experiments. Control and TG mice were matched by age, weight, and gender wherever it was deemed necessary.

Sectioning and imaging. Hearts were first perfused on a Langendorff apparatus with PBS for removal of blood. They were then perfused for 5 min with PBS containing 3% paraformaldehyde for 5 min. All procedures described above were carried out at room temperature. Hearts were then disconnected from the apparatus and further fixed with 3% paraformaldehyde in PBS overnight at 4°C. After fixation, hearts were reconnected to the Langendorff apparatus and perfused with 5×, 10×, and 20× sucrose in PBS for ~5 min, soaked for 0.5 h at each concentration, and stored at 4°C until they were sectioned. No longer than 2 days after the procedure, atrial and ventricular tissues were separated, transferred into 70% ethanol, and sent to the School of Dentistry Histology Core at the University of Michigan for paraffin embedding, 6-μm sectioning, and staining.

Whole heart images were taken at the Microscopy and Image Analysis Laboratory (University of Michigan) using a Leica stereo microscope equipped with a GFP fluorescence imaging system.

Solutions. Krebs-Henseleit bicarbonate solution consisting of (mM) 25.0 NaHCO3, 118 NaCl, 4.7 KCl, 1.2 MgSO4, 1.2 NaH2PO4, 2.5 CaCl2, 0.5 Na-EDTA, and 15 glucose, oxygenated with 95% O2-5% CO2, with pH adjusted to 7.3–7.4 (37°C) with NaOH, was used for Langendorff perfusion.

Modified Tyrode solution consisted of (mM) 137 NaCl, 5.4 KCl, 0.5 MgCl2, 0.16 NaH2PO4, 3 NaHCO3, 5 HEPES, and 5 glucose; pH was adjusted to 7.35 (room temperature) with NaOH. Modified Tyrode solution was oxygenated with 100% O2 for 10–20 min before use. BSA (2%) and 300 μM Ca2+ were added during IκB and K+ outward current recordings; extracellular solution was supplemented with 2% BSA and 1 mM Ca2+ during AP recordings.

Intracellular solution for Ca2+ current (Ica) recordings consisted of (mM) 111 CsCl, 20 tetraethylammonium chloride, 10 glucose, 14 EGTA, 10 HEPES, and 5 Mg-ATP, with pH adjusted to 7.2 with CsOH. Extracellular solution consisted of (mM) 137 tetraethylammonium chloride, 1 MgCl2, 10 glucose, 2 CaCl2, and 10 HEPES, with pH adjusted to 7.4 with CsOH.

For IκB and AP recordings, intracellular solution (KIT) consisted of (mM) 140 KCl, 10 K-EGTA, 10 K-HEPES, 5 K2ATP, and 1 MgCl2, with pH adjusted to 7.35 with KOH. Simplified KINT solution consisting of (mM) 140 KCl, 10 K-EGTA, and 10 K-HEPES was used for flow cytometry experiments.

Isolated heart perfusion. Mice were anesthetized with Avertin [10 g of tribromoethanol alcohol (Aldrich) + 10 ml of tert-amyl alcohol (Aldrich), stored at 4°C as a stock solution]. A 2.5% working solution supplemented with 0.3% CaCl2 solution (ice cold or at room temperature), and immediately perfused with the same solution for 1–2 min and repositioned at approximately the same location when subsequent recordings were necessary.

ERP recordings. After sinus node ablation, bipolar pacing electrodes were placed on the apex of the left ventricle, and the hearts were paced at a basic cycle length of 150 ms. Programmed stimulation was performed by delivering a train of 10 pulses at a basic cycle length of 150 ms (S1) followed by a single premature pulse (S2). S2 was decremented by 2 ms in multiple runs to determine the ERP.

Surface ECG recordings. Mice were anesthetized with Avertin (20 μl/g; see above), and, after complete induction of anesthesia (<2 min), 32-gauge needle electrodes were inserted subcutaneously according to a standard three-lead (left foreleg, right foreleg, and left rear leg) ECG scheme. For long-lasting (~20-min) ECG recordings, body temperature control at 36–37°C was achieved using a homeothermic blanket control unit (Harvard Apparatus). There was no temperature control for short-term (<2-min) recordings. The data were collected as described above (see Monophasic AP recordings).

To reduce signal drift, breathing-related, and other artifacts, up to 40 individual ECG events were aligned at the peak of the Q or R wave and averaged using a homemade application (Delphi 5, Borland International).

Telemetric ECG recordings. Telemetric ECG recordings were performed at the Center for Integrative Genomics at the University of Michigan. ECG transmitters (model ETA-F20, Data Science International) were implanted in mice anesthetized with ketamine-xylazine. Telemetry was performed 4–20 days after transmitter implantation to allow for appropriate recovery. Quasi-lead II ECG recordings were acquired at a 1-kHz sampling rate with commercially available software (Data Sciences International) according to various time schedules. Heart rates and other parameters were determined from R-R intervals and individual ECG events using homemade software (see above).

All survival surgeries were performed under aseptic conditions with protocols approved by the veterinary staff of the University Committee on Use and Care of Animals at the University of Michigan.

Preparation of isolated cardiomyocytes. Two- to 6-mo-old (generations F1–F4) mice were anesthetized with Avertin, hearts were removed, and cardiomyocytes were isolated from the lower two-thirds of the heart by collagenase treatment, as described elsewhere (25). Cells were used in experiments within 1–5 h. Neither 2,3-butanedione monoxime nor EGTA (see below) was used for cell isolations designated for electrophysiological experiments.

Flow cytometry. Flow cytometry of isolated ventricular myocytes was performed as described elsewhere (25). Briefly, myocytes were isolated in the presence of 10 mM 2,3-butanedione monoxime (ACROS Organics) and 10 μM Na-EGTA to better preserve the rod shape of the myocyte. After two to three steps of spin-resuspend purification in simplified KINT solution at ~500 rpm on an Eppendorf 5810 centrifuge, myocyte suspensions were filtered through a 200-μm metal mesh and analyzed on a Coulter Epics XL cytometer at the University of Michigan Flow Cytometry Core Facility. Data were analyzed using WinMDI (version 2.8) software (http://facs.scripps.edu/software.html) and Microsoft Excel as described elsewhere (25).

Confocal imaging. Hearts were isolated as described above, blood was washed out by perfusion with Ca2+-free modified Tyrode solution, and hearts were immersed in 1:2 OCT-20% sucrose in PBS medium and immediately frozen at −80°C for long-term storage. Sections (14 μm) were then obtained using a cryostat (model HM
500m, Microm), rinsed with PBS supplemented with 200 mM glycine, and then immediately used for imaging. Neither whole hearts nor individual cryosections were fixed before analysis, because fixation with paraformaldehyde (or similar fixative) causes a significant increase in background fluorescence, preventing precise analysis in low-level-expressing TG lines (line 1 mice; see RESULTS).

Confocal imaging was performed using an Olympus FV-500 confocal microscope at the University of Michigan Microscopy and Image Analysis Laboratory. The pin-hole aperture setting corresponded to 0.72 μm z-resolution. All images were taken under identical conditions to allow for semiquantitative comparison of fluorescence intensity.

Patch-clamp recordings. Whole cell ionic currents and APs from single isolated cardiomyocytes were essentially recorded as described elsewhere (25). Specifically, tip resistance was 1–2.5 MΩ when pipettes were filled with KINT or similar solution, and series resistance compensation was routinely set at >80% in all experiments. All recordings were obtained at room temperature (22–26°C).

The essential difference in measuring $I_{K1}$ density in this study is the timing for current recordings, which is different from that used in our previous publication (25). Although in the previous study, $I_{K1}$ values were measured immediately (within <1 min) after the whole cell configuration was established, in the present study, $I_{K1}$ amplitude was assessed at 1–4 min of a stabilization period, and the data were averaged. It has been reported that cell dialysis, even within a short time, leads to a significant increase of inward currents (23). The latter effect also depends on the shape and access resistance of the pipettes. Thus pipette parameters, potentially different from those used previously, were kept consistent throughout the study. The result of the described changes in the protocol is the higher density (that previously reported) of $I_{K1}$.

Outward K$^+$ currents were recorded in response to an 800-ms voltage step from a holding potential of −75 to +80 mV and analyzed essentially as described previously (23) using the following expression: $I(t) = I_{0}\exp(-t/\tau) + I_{K,slow1}\exp(-t/\tau_{K,slow1}) + I_C$, where $C$ is amplitude of the steady-state component (23). The short pulse duration (800 ms) does not allow for an accurate estimation of the slowest component of the outward K$^+$ current ($I_{K,slow2}$; ~1.2–1.8 s) (20, 42) and the steady-state current ($I_{KSS}$). Thus the slowest relaxation kinetics in this study better represents the $I_{K,slow1}$ component of the outward current: ~490 ms (Table 1), similar to ~400 ms reported by Zhou et al. (42). The use of the term $I_{K,slow}$ vs. $I_{KSS}$ seems inappropriate, because the relaxation time of $I_{KSS}$ was estimated to be ~1.2 s (20, 36). Because $I_{K,slow1}$ and $I_{KSS}$ cannot be estimated with certainty from our data, the term $I_C$ is used for the remaining current.

$I_C$ were recorded in the presence of 1 mM extracellular Ca$^{2+}$ (see Solutions). Membrane potential was first stepped from a holding potential of −50 mV to −75 mV for 100 ms, returned to the holding potential for 20 ms (to inactivate Na$^+$ channels), and then stepped to a series of membrane potentials between −50 and +50 mV.

Data analysis. Data analysis was performed using a Microsoft Excel Application and homemade software (see above). Values are means ± SE unless stated otherwise. Statistical significance was estimated using a two-tailed t-test with equal variances.

Definitions. Some definitions of the major ECG intervals used here are different from those we used in previously (25) (see Fig. 8). Originally, ECG signals were inverted to make the slow phase of T wave positive (25), which affected determination of some major peaks such as Q, R, and S. A standard approach, where the slow phase of the T wave signal is negative and T0 stands for the amplitude of this phase, is now used. Thus the major P wave signal is positive. Q stands for the beginning of the QRS complex, and the R wave is the major positive peak followed by the major negative S wave; r represents the secondary positive peak after the R wave (see Fig. 8A). Tr is the initial rapid component of the T wave, which sometimes is seen as a deviation of the signal from the tangent to the steepest slope of that region (19). The Tr wave is easily identifiable in recordings from TG mice (see Fig. 8). To represents the amplitude of the negative peak of the slow phase of the T wave, which follows the positive Tr wave. The P-R interval is the time between the beginning of the P wave and the beginning of the QRS complex (different from Ref. 25). The extended QRS complex duration was determined to be the duration of the QRS complex plus the duration from S to r.

RESULTS

Effects of Kir2.1-GFP overexpression in TG mice. Of 13 founder mice, 2 died prematurely, 9 did not express any measurable level of GFP fluorescence or did not transmit the TG, and 2 led to the establishment of viable lines (1 and 2) with high levels of GFP fluorescence in all major parts of the heart. The green fluorescence of GFP-tagged channel subunits greatly aided the characterization of TG animals by providing a direct visual estimation of the level and pattern (see below) of TG expression even before any functional measurements on isolated myocytes or whole hearts were performed.

Line 2 mice displayed a highly increased mortality rate, whereas line 1 mice had a life span indistinguishable from normal (wild-type) mice (Fig. 1A). Detailed analysis of mortality rate in line 2 showed an increase in death rate at ~day 12–15, with half lifetime of ~20–25 days, in ~60–70% of mice, whereas the remaining mice were viable for >2 mo and were followed for up to 1 yr before they were may be still “sacrificed”? With few exceptions (see below), most of the data from line 2 mice were derived from adult (>1 mo old) surviving animals.

Analysis of the heart weight-to-body weight ratio ($\times 10^3$) revealed significant heart hypertrophy in line 2 mice. Heart weight-to-body weight ratios were increased from 5.5 ± 0.2 (n = 22) in control mice to 11.8 ± 0.6 (n = 21, P < 0.01) in mice that died prematurely (<1 mo; group 1) and to 6.9 ± 0.4 (P < 0.01) in the older surviving littermates (group 2). On average, an ~113% increase in heart weight-to-body weight
myocytes from both TG lines did not reveal statistically significant changes in mean membrane capacitance of isolated clamp experiments (see below) did not reveal statistically significant. In contrast, patch-line 1

There was no difference between male and female survival, so all data were pooled. B: Kir2.1-GFP subunits are highly expressed throughout the heart of TG mice. Hearts isolated from age-matched control (Ctr) and TG mice of line 2 were photographed using normal reflective or green fluorescent protein (GFP) fluorescence imaging. Images A and C and images B and D were obtained under identical conditions. Note significant ventricular hypertrophy as well as profound enlargement of the right atria.

AJP-Heart Circ Physiol • VOL 287 • DECEMBER 2004 • www.ajpheart.org

The level and low-resolution pattern of TG expression along the heart can be easily estimated by GFP fluorescence. Figure 1B shows representative images of isolated hearts taken from control and line 2 mice under normal illumination as well as after excitation of GFP. Kir2.1-GFP subunits were expressed in left and right ventricles as well as in both atria. The GFP fluorescence in line 1 mice was less intense (not quantified) but was also observed throughout the heart.

Visual inspection of atrial tissue in line 2 TG mice revealed significant deviations from normal. Both atria were enlarged in all age groups, with right atrium-to-body weight ratio increased by 141% (P = 0.004) and left atrium-to-body weight ratio increased by 65% (P = 0.008, n = 32 TG and 20 control mice). In contrast, there was no measurable change in atrial weight from line 1 mice.

Additional analysis of paraffin sections taken from ventricular and atrial tissues of adult animals, stained with hematoxylin and eosin (counterstaining) or trichrome (to demonstrate the presence of collagen), did not reveal any significant deviations from normal.

Flow cytometry analysis of isolated cardiomyocytes. It is important to know for the functional analysis of the whole heart experiments the anatomic and regional distribution of Kir2.1-GFP expression in the mouse heart. It follows from published work (18, 25) that the level of α-MHC promoter activation in individual cells is highly variable, leading to a heterogeneous expression of the transgenes. Although GFP fluorescence was detected in all major compartments of the heart of both TG lines, the number of cells expressing the transgene as well as the level of expression in individual ventricular myocytes varied significantly between the two TG lines (Fig. 2). About 70% of ventricular myocytes in line 2 displayed expression levels comparable to, yet smaller than, that of previously described mice expressing a dominant-negative Kir2.1-AAA subunit (25), translating to an ~10-fold increase in GFP fluorescence above background level. Line 1 myocytes displayed a somewhat lower level of GFP fluorescence, about threefold above background; therefore, the percentage of expressing cells could not be determined with certainty because of a significant overlap with background fluorescence. The best estimate from the two independent experiments gives >90% of cells expressing Kir2.1-GFP in this line. Although isolated atrial myocytes were not analyzed in this study, the data in Fig. 1B suggest that the same pattern of expression may also apply to atria.

Heterogeneous expression of Kir2.1-GFP subunits in TG hearts. To further investigate the patterns of Kir2.1-GFP expression, cryosections of ventricular tissue obtained from the hearts of control and TG mice were analyzed using fluorescent confocal imaging. Consistent with the nearly uniform GFP fluorescence throughout the heart seen on macroimages (Fig. 1B), there was no significant difference in the pattern and intensity of GFP fluorescence between left and right ventricular cryosections of the same TG line (not shown). Nevertheless, detailed inspection of confocal images revealed a highly heterogeneous expression of the Kir2.1-GFP transgene in individual myocytes (Fig. 3). In line 2 cryosections, GFP fluorescence can be clearly observed not only in the sarcolemmal membrane but also inside the cells. Detailed inspection of images shows that although some part of GFP fluorescence correlates with that originating from T-tubule membranes (characteristic reg-
Cryosections from line 1 hearts (Fig. 3C) displayed a reduced GFP fluorescence compared with line 2 preparations, again consistent with the single-cell data. The speckled pattern of GFP fluorescence was also missing in line 1 preparations. 

I_{K1} is significantly increased in Kir2.1-GFP-overexpressing mice. Whole cell patch-clamp recordings from isolated cardiomyocytes revealed a manifold upregulation of inward and outward I_{K1} in both lines (Fig. 4, Table 1). Only cells displaying GFP fluorescence clearly above background (visual inspection; not quantified) were used in the analysis. In line 1, inward I_{K1} density measured at −100 mV was increased 6-fold and outward density at −60 mV was increased >12-fold. Similar results, with nearly the same level of I_{K1} upregulation, were also observed in line 2 mice. Although most patch-clamp experiments were carried out in >2-mo-old mice, which represents a population of “surviving” mice (Fig. 1A), on one occasion we analyzed I_{K1} in ventricular myocytes isolated from a young mouse in line 2 (mouse 2a) that displayed signs of severe illness (weakness and slow motion) and would probably have died soon. In this case, I_{K1} upregulation was enormous: ∼16- and ∼88-fold increase of inward and outward current, respectively. Line 2 mice of group 1 (<1 mo old) were not studied further because of their high mortality rate.

The following single-cell data refer to line 1 and surviving line 2 mice (group 2). Although the differential level of upregulation of I_{K1} at hyperpolarized (−100 mV) and depolarized (−60 mV) potentials can be explained by changes in the rectification properties of I_{K1}, it was more reasonable to suggest that imperfections in voltage clamping of such large currents (sometimes reaching 10–20 nA) were the major reason for this discrepancy. Although voltage drops across access resistance of the patch pipettes were reduced to a practical minimum with use of series resistance compensation, accumulation/depletion of K⁺ in narrow T-tubular space (4) could not be avoided. Thus larger inward currents would be affected, most leading to apparently smaller magnitudes of upregulation than for smaller outward currents. To achieve a better quantitative description, we have analyzed the slope conductance of I_{K1} at the reversal potential. The results show that I_{K1} upregulation is intermediate between that obtained for increases in inward and outward current densities: 9-, 10-, and 46-fold increase in line 1 and line 2 mice and in mouse 2a, respectively. I_{K1} in myocytes of both TG lines rectify strongly, but rigorous quantitative analysis of the steepness of rectification was not possible because of a high level of I_{K1} density.

Consistent with a highly increased I_{K1} density in TG myocytes, their resting potential measured in the current-clamp mode was significantly more negative than that in control myocytes: −70.0 ± 0.9 mV (P < 0.01), −77 ± 1.3 mV (P < 0.01), and −78.3 ± 0.7 mV in control, line 1, and line 2 myocytes, respectively.

Effects of I_{K1} upregulation on other K⁺ currents. The amplitudes of other components of the outward K⁺ current were also measured (Fig. 5). Surprisingly, we found that the amplitudes of transient outward current (I_{to}) (37) and the slowly inactivating component of I_{K,slow} (I_{K,slow1}) (41, 42) and the steady-state current amplitude (I_{c}) all measured at +80 mV (Table 1), were not changed in a way comparable to changes in I_{K1} conductance, suggesting little or no re-

Fig. 2. Variable expression of Kir2.1-GFP subunits in TG myocytes. Left: density plots for control, line 1, line 2 (group 2; surviving mice), and Kir2.1-AAA-GFP (25) myocytes. FS, forward scatter; F_{GFP}, intensity of GFP fluorescence. Clusters with low intensity and small FS (arrows) correspond to cell debris and other impurities. Ellipses indicate regions used for calculation of fluorescence corresponding to myocytes. Right: histograms of fluorescence for control, line 1, line 2, and Kir2.1-AAA-GFP (25) myocytes. ▽ Peak of fluorescence from control myocytes and nonexpressing myocytes from TG lines; ▼ myocytes expressing Kir2.1-GFP subunits.

ularly spaced fluorescence with periodicity corresponding to sarcomere length; not shown), most of it probably arises from other intracellular structures and particles such as Golgi apparatus, endoplasmic reticulum, or inclusion bodies, producing a speckled pattern of fluorescence (Fig. 3, A and B). More importantly, and consistent with the data on isolated myocytes (see above), the level of Kir2.1-GFP expression varied significantly on a microscale. Figure 3, A and B, shows examples where adjacent cells display significantly different levels of intracellular fluorescence. It is difficult to judge the potentially different level of Kir2.1-GFP expression in sarcolemmal membranes of these cells because of their close proximity.
modeling (1, 6, 35) of these conductances. Specifically, statistically significant changes were observed for $I_{K,\text{slow1}}$ (H11011 20% decrease in line 2 mice) and 2) the steady-state component, i.e., $I_{C}$ (H11011 36% increase in line 1 mice). It has been shown that the amplitudes of different components of outward $K^+$ current vary significantly across the heart tissue (37), which potentially may present a problem in estimating their densities using a mixed population of myocytes isolated from the lower two-thirds of the heart. Experimentally, however, most of the cells were probably derived from the left ventricle because of its relative contribution to the overall tissue mass. In all myocytes, the $I_{\text{to,f}}$ component was present, whereas the $I_{\text{to,s}}$ component was absent, suggesting that the cells were not derived from the septum (37). Furthermore, to achieve statistically sound results, a large number of myocytes (up to $\sim$50) were analyzed.

Additionally, kinetic parameters of $I_{\text{to,f}}$ and $I_{K,\text{slow1}}$ components of outward currents were not affected (Table 1).

$I_{Ca}$ is affected by $I_{K1}$ upregulation. It was reasonable to suggest that upregulation of a $K^+$ current may lead to a shortening of AP (see below) and, consequently, to a compensatory increase of an inward current, e.g., $I_{Ca}$ (Fig. 5). Indeed, we found that the density of $I_{Ca}$ was increased in TG mice. For example, at 0 mV, $I_{Ca}$ was increased from 8.4 $\pm$ 0.5 pA/pF ($n = 15$) in control mice to 9.1 $\pm$ 0.5 pA/pF ($n = 15$) in line 1 and to 10.5 $\pm$ 0.8 pA/pF ($n = 15, P < 0.05$) in line 2 mice, an $\sim$10% (not statistically significant) and $\sim$20% increase, respectively. Neither the membrane potential for peak current amplitude nor the kinetics of inactivation were affected in any significant way (not shown).

APs are significantly abbreviated by $I_{K1}$ upregulation. Consistent with significantly increased $I_{K1}$ density, AP duration (APD) measured in TG ventricular myocytes (room temperature) was substantially decreased in both TG lines. APD at 90% repolarization (APD$_{90}$) was decreased by $\sim$60% and APD at 75% repolarization (APD$_{75}$) was reduced by 25–30% (not statistically significant), but APD at 50% repolarization (APD$_{50}$) was not affected (Fig. 6, Table 2).

A similar abbreviation of AP, leading to a “neuronlike” AP, was also observed in MAP recordings (37°C). MAP at 90% repolarization (MAP$_{90}$) was decreased by $\sim$77% and $\sim$82%, MAP at 75% repolarization (MAP$_{75}$) was decreased by $\sim$71% and $\sim$76%, and MAP at 50% repolarization (MAP$_{50}$) was not significantly affected in line 1 and line 2 mice, respectively (Fig. 6).
Consistent with shortening of the AP, the ERP was reduced from 36.2 ± 1.5 ms \((n = 9)\) in control mice to 25.1 ± 3.0 ms \((n = 7; P < 0.004)\) and 20.8 ± 2.3 ms \((n = 5; P < 0.0001)\) in line 1 and line 2 TG mice, respectively (~31 and 43% reduction).

Severe abnormalities of excitability in Kir2.1-GFP TG mice. Severe abnormalities of cardiac excitability were observed in anesthetized line 2 mice (Fig. 7). Surface ECG recordings lasting ~10 min revealed multiple abnormalities of excitability, including slowed heart rate, AV block, atrial fibrillation, and premature ventricular contractions (Fig. 7).

Figure 7A shows an ECG recording from a line 2 mouse with an abnormal cardiac rhythm, with R-R intervals ranging from 100 to 400 ms (Fig. 7B1). In line 2, several mice displayed sustained episodes of AV block, as shown by the R-R interval graph in Fig. 7B2. Figure 7B4 shows an example of atrial tachycardia. Most line 2 mice displayed one type or multiple types of severe arrhythmias similar to those shown in Fig. 7, whereas many line 1 mice were characterized by a stable heart rhythm, and control mice did not display any arrhythmias. Heart rates in anesthetized TG mice were 8.2% and 9.0% slower in line 1 and line 2 mice, respectively (Table 3). In summary, ECG analysis of 22 control, 20 line 1, and 18 line 2 mice revealed the following abnormalities: atrial fibrillation in 0 control, 4 line 1, and 5 line 2 mice; atrial tachycardia + AV block in 0 control, 0 line 1, and 1 line 2 mouse; atrial arrest in 0 control, 0 line 1, and 3 line 2 mice; junctional escape + junctional rhythm in 0 control, 3 line 1, and 3 line 2 mice; AV block in 0 control, 0 line 1, and 7 line 2 mice; premature ventricular contractions in 0 control, 2 line 1, and 2 line 2 mice; and ventricular tachycardia in 0 control, 0 line 1, and 1 line 2 mouse.

ECG analysis of >20 h of continuous recordings in line 1 mice implanted with telemetric radio transmitters (not shown) did not reveal any significant deviations from normal compared with the scale of abnormalities observed in line 2 mice.

ECG episode parameters are significantly affected in TG mice. Detailed analysis of ECG episodes revealed significant changes in the shape and/or duration of major intervals. The effect most relevant to this study was the nearly complete elimination of the slow phase of the T wave in TG mice, so that it was even difficult to decide what represented the T wave in the first place. The slow phase of the T wave was always prominent in control mice, with the peak amplitude of downward deflection equal to 36.7 ± 5.1 μV, but it was undetectable in all TG mice, such that its amplitude could not be distinguished from the “isoelectric” level, which was 4.2 ± 0.9 and 2.8 ± 1.0 μV in line 1 and line 2 mice, respectively (Table 3).

We strongly believe that in TG mice the T wave is nearly complete when the signal returns to the isoelectric level after the r wave (Fig. 8A). Consistent with this notion, we have observed in some recordings what can be called the “beginning of a T wave,” which can be seen as a secondary r wave, designated Tr, quickly returning to an isoelectric level (Fig. 8, B–D). For practical measurements of Q-T intervals in TG mice, the end of the T wave was defined as the point of highest curvature of the Tr wave on reaching the isoelectric level. Consistent with the qualitative examination of ECG traces, Q-T and Q-Tc intervals were significantly shorter in TG than in control mice, whether they were measured in anesthetized mice (Table 3; ~60% shortening) or in isolated heart preparations (Table 4; ~45% shortening). Q-T intervals were ~26% shorter in anesthetized control mice than in isolated hearts. In contrast, Q-T intervals in TG mice were nearly identical, whether they were measured in anesthetized mice or in isolated hearts.

Another prominent feature observed in all TG mice was the significantly increased amplitude of the negative wavelet of the P wave \((P2)\) (Fig. 8). Although positive deflection \((P1)\) was increased by only 31% and 20% in line 1 and line 2 mice, respectively, the amplitude of negative wavelet \((P2)\) was increased nearly threefold in both TG lines.

Durations of the P-R and Q-S intervals were affected differentially in line 1 and line 2 mice. There was virtually no change in the P-R interval in line 1 mice, whereas it was increased by ~32% in line 2 animals. The QRS complex was
not significantly increased in line 1 mice but was prolonged by ~10% in line 2 animals. The extended QRS complex was prolonged by ~18% (P = 0.06) and ~38% in line 1 and line 2 mice, respectively.

Fig. 6. Action potentials are significantly abbreviated in hearts of TG mice. A: in isolated ventricular myocytes of both TG lines, action potential duration (APD) at 90% repolarization (APD$_{90}$) was significantly reduced, there was little change in APD at 75% repolarization (APD$_{75}$), and there was no change in APD at 50% repolarization (APD$_{50}$). **$P < 0.01$. Inset: representative single-cell action potentials ($V_m$). B: monophasic action potentials (MAP) at 90 and 75% repolarization (MAP$_{90}$ and MAP$_{75}$) were significantly abbreviated, and MAP at 50% repolarization (MAP$_{50}$) was not affected in both TG lines. Inset: representative MAPs.

Fig. 5. Outward K$^+$ and Ca$^{2+}$ currents in ventricular myocytes of TG mice. A and B: representative recordings of outward K$^+$ currents in control and line 1 TG myocytes. Currents were recorded in response to a series of voltage steps from $-75$ mV holding potential to between $-60$ and $+80$ mV. Na$^+$ and Ca$^{2+}$ currents were not blocked (23). Extracellular Ca$^{2+}$ concentration was reduced to 300 μM. Currents at the most depolarized potential were not affected by $I_{K1}$ upregulation. Insets: currents at less depolarized potentials ($-60$ to $0$ mV) shown in greater detail. "Crossover" effect (B, inset) can be observed in TG myocytes because of significant upregulation of $I_{K1}$ and the presence of "negative slope conductance": currents at $-60$ mV are larger than those at $-40$ or $-20$ mV. C: current-voltage relations for peak amplitude of L-type Ca$^{2+}$ current ($I_{Ca,L}$) in control and TG myocytes.
DISCUSSION

Understanding the role of \( I_{K1} \) is one of the challenging tasks in cardiac electrophysiology, and the quantitative aspects of its involvement in cardiac excitability remain under investigation. To advance further in this direction, we have complemented our previous TG mouse model of \( I_{K1} \) suppression \((25)\) with a TG mouse model of \( I_{K1} \) upregulation \((22)\).

**How much is too much?** Probably the most striking result from this and previous studies \((25, 38)\) is the shear magnitude of \( I_{K1} \) variation that can be tolerated by the mouse heart. Mice are viable even when \( I_{K1} \) is downregulated \(\sim20\) fold \((25, 38);\) yet they can also tolerate a \(\sim10\)-fold increase in \( I_{K1} \) density. The scale of the \( I_{K1} \) regulation in TG mice surpasses that obtained in guinea pig hearts, where a nearly fivefold suppression and a nearly twofold increase in \( I_{K1} \) were achieved by adenoviral transfection \((27).\) Yet the inherent differences between two models (mouse vs. guinea pig) do not allow for a quantitative comparison.

The results show that there is, nonetheless, a critical level of \( I_{K1} \) overexpression beyond which the survival of TG mice is hampered. Line 2 mice present an example of in-line variability of transgene expression; even though all mice of this line were

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**Table 2. Duration of single-cell AP and MAP in control and TG mice**

<table>
<thead>
<tr>
<th></th>
<th>Control ((n = 25))</th>
<th>Line 1 ((n = 10))</th>
<th>Line 2 ((n = 14))</th>
</tr>
</thead>
<tbody>
<tr>
<td>APD(_{90})</td>
<td>21.8±2.7</td>
<td>7.9±0.4*</td>
<td>7.9±0.5*</td>
</tr>
<tr>
<td>APD(_{75})</td>
<td>8.5±1.0</td>
<td>6.0±0.4</td>
<td>6.4±0.5</td>
</tr>
<tr>
<td>APD(_{50})</td>
<td>3.3±0.3</td>
<td>3.1±0.3</td>
<td>3.0±0.3</td>
</tr>
</tbody>
</table>

**Table 3. Surface ECG parameters in control and TG mice**

<table>
<thead>
<tr>
<th></th>
<th>Line 1</th>
<th>Line 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>476±5</td>
<td>437±9*</td>
</tr>
<tr>
<td>T wave, μV ((T_n))</td>
<td>36.7±5.1</td>
<td>42.2±0.9†</td>
</tr>
<tr>
<td>P1, μV</td>
<td>76.7±6.9</td>
<td>100.8±7.2</td>
</tr>
<tr>
<td>P2, μV</td>
<td>12.5±1.4</td>
<td>38±4.4†</td>
</tr>
<tr>
<td>P-R, ms</td>
<td>43.8±0.9</td>
<td>42.6±1.4</td>
</tr>
<tr>
<td>QRS, ms</td>
<td>8.5±0.1</td>
<td>8.9±0.3</td>
</tr>
<tr>
<td>QRS(_1), ms</td>
<td>13.1±0.4</td>
<td>15.4±1</td>
</tr>
<tr>
<td>Q-T, ms</td>
<td>51.1±2.3</td>
<td>29.9±0.6†</td>
</tr>
<tr>
<td>Q-T(_c), ms</td>
<td>44.6±2.0</td>
<td>25.7±0.7†</td>
</tr>
</tbody>
</table>

*Values are means ± SE; \(n = \) number of mice. AP, action potential; APD, AP duration; APD\(_{90}\), APD\(_{75}\), and APD\(_{50}\), AP at 90, 75, and 50% repolarization; MAP, monophasic AP; MAP\(_{90}\), MAP\(_{75}\), and MAP\(_{50}\), MAP at 90, 75, and 50% repolarization.

*\(P < 0.01\) vs. control.

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**Fig. 7.** Multiple abnormalities of cardiac excitability in TG mice. A: representative lead II ECG recording from a line 2 mouse displaying a highly irregular heart rhythm and atrial fibrillation \((A\text{F}; \bigcirc).\) *Breathing artifacts. B\(_1\): R-R interval graphs produced from ECG recording in A display highly irregular rhythm but no premature beats \((B\text{F}).\) While some line 2 mice display stable cardiac rhythm \((B\text{2}).\) B\(_2\): R-R interval graph obtained from an ECG recording of a line 2 mouse display stable cardiac rhythm along with multiple incidents of atrioventricular block. B\(_3\): atrial tachycardia \((AT; \bigcirc)\) in a line 2 mouse.
descendants from the same founder mouse, at least two distinct populations can be identified: surviving and dying mice (Fig. 1). Clearly, the $I_{K1}$ amplitude, as well as the level of GFP fluorescence (not quantified), is significantly higher in dying line 2 myocytes. The latter suggests that copy number of the transgene is reduced or other transgene DNA rearrangements are taking place in individual surviving mice. Consistent with this, we find that later generations of this line display a nearly normal life span, with the level of $I_{K1}$ expression and corresponding GFP fluorescence reduced to that of line 1 mice (unpublished observations). We can speculate that higher levels of $I_{K1}$ expression in young dying line 2 mice lead to extreme shortening of the AP and, consequently, a significant increase in $I_{Ca}$ and, ultimately, life-threatening hypertrophy.

If we abstract from an extreme case of young dying mice of line 2, we would wonder if $I_{K1}$ is indeed one of the major conductances, why do such large-scale variations not lead to catastrophic consequences, at least in some cases? An ~10-fold increase in conductance at the reversal potential in TG mice translates to a 10-fold decrease in cell input resistance, which would render myocytes significantly less excitable. Nevertheless, the level of reduction in excitability is obviously not large enough to stop conduction. Somewhat prolonged duration of QRS complexes (Table 3) in line 1 mice and significant prolongation of the QRS1 complex in line 2 mice are probably at least partially due to reduced cellular excitability.

We believe that numerous redundancies in ion channel expression might be the underlying reason for the stability of the heart. Overexpression of $I_{K1}$ destabilizes the heart, and, for example, survival under pathological conditions or drug administrations is affected. In support of this point, we recently found that the tolerance of TG hearts to specific drug application is significantly influenced (21). We also expect that the effects of other challenges to the heart would be strongly impacted by the level of $I_{K1}$ expression.

Significant ventricular and atrial hypertrophy (in line 2 mice) in TG hearts may or may not be directly related to $I_{K1}$ overexpression but, rather, explained by overexpression of a foreign gene, an integral Kir2.1-GFP fusion protein in our case. For example, Huang et al. (13) showed that overexpression of cytosolic GFP alone may lead to dilated cardiomyopathy. Overexpressing a “control” transgene (XXX-GFP or Kir2.1-NO GFP) cannot solve the general problem of side effects in TG overexpression models, because other control TG mice will only be another, probably different, source of artifacts. Nevertheless, most of the effects reported here, including hypertrophy, are consistent with the electrical phenotype (also see Discussion in Ref. 25).

For example, it is reasonable to suggest that increased outward $I_{K1}$ and, thus, significant shortening of the AP in TG hearts lead to a compensatory increase in $I_{Ca}$ density and,
ultimately, to hypertrophy. Most of the data are consistent with this hypothesis. The density of $I_{Ca}$ is indeed upregulated to a greater extent in line 2 than in line 1 TG mice. Outward $I_{K1}$ density is ~18% higher and MAP_{90} and MAP_{75} are ~27% and 23% shorter, respectively, in line 2 than in line 1 mice (Tables 1 and 2). Although $I_{ca}$ and APs were not measured in group 1 of line 2 mice (dying), $I_{K1}$ was even more upregulated; thus APs were probably shortened and $I_{ca}$ increased even more in this group of mice (mouse 2a), and a hypertrophy of ~113% was enormous by all means. Because heart hypertrophy may be associated with downregulation (31) and upregulation (30) of $I_{vo}$, the “calcium hypothesis” becomes the most probable theory (2), regardless of the original source of Ca^{2+} mishandling.

Additionally, although the effect of a GFP tag on heart hypertrophy cannot be excluded, it may not be the major underlying reason, because in mice with a dominant-negative $I_{K1}$ suppression (Kir2.1-AAA), the level of GFP expression is significantly higher (Fig. 2; log scale) in terms of single-cell expression and the total number of cells expressing the transgene; yet Kir2.1-AAA mice are characterized by only modest (~17%) hypertrophy (25).

**TG expression is widespread and heterogeneous in both TG lines.** The α-MHC-driven expression of Kir2.1 subunits is widespread (Fig. 1B). Nearly uniform green fluorescence can be observed in left and right ventricles as well as in both atria. We have not yet conducted a more detailed investigation as to whether the transgene is expressed in specialized cells such as Purkinje fibers or the SA node, but we can at least make some reasonable suggestions. For example, we suggest that SA nodal cells may not express the TG at a high level, inasmuch as it would have led to a much greater change in the heart rate than was observed in this study. Analysis of single-cell GFP fluorescence in line 2 mice supports this view, in that there is a significant population of myocytes with a background level of fluorescence (Fig. 2), suggesting that there are isolated cells or probably small regions of the heart tissue devoid of TG expression.

The data also suggest that fluorescence intensities, from individual cells or from low-resolution spatial images, cannot be easily translated to electrophysiological profiles, because, despite higher levels of fluorescence in individual ventricular myocytes in line 2 mice (Fig. 2), the slope conductance (at equilibrium potential) is nearly the same in both TG lines. Thus more detailed functional mapping of transgene expression will be necessary in every case.

**Little ion channel remodeling in mice overexpressing $I_{K1}$.** Ion channel remodeling in response to changes of specific conductance in TG animals has been observed in many studies (1, 6, 35) and is considered a common phenomenon. The data show that the density of $I_{vo}$ was not changed in myocytes from both TG lines. This is somewhat unexpected, because both lines display appreciable hypertrophy (~14% and ~25% in lines 1 and 2, respectively; only surviving mice of line 2 were studied), and downregulation of several K^{+} currents, including $I_{vo}$ (17), was shown to be a common hypertrophic response. However, hypertrophy is not always associated with a change in $I_{vo}$. For example, Wang et al. (33) showed that $I_{vo}$ density was not affected in the mouse model of pressure-overload hypertrophy. It seems that the present data fall in the latter category.

Nevertheless, several other components of outward K^{+} current were indeed affected in a statistically significant way: $I_{K1,slow}$ was downregulated by ~20% in line 2 mice, and the steady-state component, $I_{ca}$, was upregulated by ~36% in line 1 mice. However, the magnitude of the latter changes was not comparable to the level of upregulation of $I_{K1}$.

Surprisingly, we have also found only a modest statistically significant increase of ~20% in the amplitude of $I_{ca}$ in line 2 mice, less than one would expect from the scale of $I_{K1}$ upregulation and the corresponding dramatic shortening of AP. Upregulation of $I_{K1}$ leads to significant abnormalities in cardiac excitability. In TG mice that survive an enormous $I_{K1}$ increase, we have observed multiple potentially life-threatening conditions, more severe than those observed in Kir2.1-AAA mice (25). Nevertheless, it is not only the relative scale of $I_{K1}$ up- and downregulation (~10 and ~20 times, respectively) that is important, but the absolute changes in $I_{K1}$ conductance: ~100 and ~1,000 pS/pF in downward and upward directions, respectively.

Although results indicate that $I_{K1}$ density in individual transgene-expressing ventricular myocytes is upregulated nearly to the same extent in both TG lines, line 2 mice are characterized by a much more severe electrical phenotype than line 1 mice. Although the above experimental finding may initially appear rather contradictory, several important issues should be taken into consideration.

First, detailed analysis of GFP fluorescence in confocal images from cryopreserved slices (Fig. 3) as well as visual inspection of isolated myocytes using conventional fluorescent microscopy (not shown) revealed significant intracellular accumulation of the Kir2.1-GFP protein. This may suggest that, despite a higher total expression of the transgene, the level of expression in the sarcolemmal membrane may be similar in both TG lines.

Second, changes in several parameters of excitability representing ventricular mass, such as shortening of the Q-T interval, are very similar, reflecting a similar significant and global increase in $I_{K1}$ density.

Third, higher density of $I_{ca}$ in line 2 than in line 1 isolated myocytes may be a reflection of heterogeneous (cell-to-cell variability) overexpression of $I_{K1}$ in line 2 mice.

Fourth, and probably most important, heterogeneous and differential expression of Kir2.1 subunits in special tissues of the heart, such as the conduction system, may underlie the differences between global parameters of excitability in two TG lines, even though the subsets of individual ventricular myocytes have similar increases in $I_{K1}$ density.

Beyond a much shorter average life span for ~60–70% of line 2 mice, ECG recordings from surviving animals displayed numerous abnormalities ranging from AV block to atrial fibrillation. These data suggest that the most significant difference between the two TG lines is in the expression pattern of Kir2.1 channels in atrial tissue and the conduction system. The P-R interval and duration of the QRS and QRS1 complexes were significantly longer in line 2 mice, suggesting a slowing of the conduction rate. The amplitudes and the shape of the P_{1} wave were not statistically different between the two TG lines, but both displayed a characteristic and significant increase in the amplitude of the P_{2} phase of the P wave (Fig. 8). The latter is commonly related to atrial hypertrophy (10) in humans and was confirmed by visual inspection of the isolated hearts and...
atrial weights in line 2 mice. However, atrial hypertrophy may not be the major reason underlying changes in P wave shape, because line 1 mice do not display any significant atrial hypertrophy; yet the amplitude of the P2 wavelet is increased nearly to the same extent as in line 2 mice. Thus probably pure electrical phenotype (conduction pattern) underlies P wave changes in both TG lines.

**IK1 in arrhythmogenesis.** The changes in the Q-T interval and T wave amplitude were similar in both TG lines and correlated strongly with shortening of the MAP. Accordingly, ventricular ERP was significantly reduced in both TG lines, thus providing a potential substrate for arrhythmogenesis.

Although it is well established that a long Q-T interval is associated with cardiac electrical instability and sudden cardiac death, little is known about the relation between short Q-T interval and cardiac excitability. Increasing evidence shows that the short Q-T interval is indeed associated with life-threatening arrhythmias in humans (9, 11), which our data strongly support. Thus the TG mouse model of IK1 upregulation may be considered the first mouse model of short Q-T syndrome.

Consistent with the arrhythmogenic potential of IK1, it has been firmly established that this current is significantly upregulated during atrial fibrillation (3, 7, 32). Accordingly, suppression of IK1 by barium has been shown to terminate ventricular fibrillation in guinea pig hearts (34).

**Where is the T Wave in the mouse ECG recordings?** The results of this study help resolve the long-standing question in the electrophysiology of rodents: which phase of the surface ECG recordings represents correctly the repolarization phase of AP? Although some researchers believe that the end of the T wave can be mapped to the point before the slow phase of the T wave (19) and others, including us, rely on the slow phase itself (25, 28), there is a view that none of the above correlates well with AP repolarization (5). The data in this study demonstrate without a doubt that the slow phase of the T wave indeed represents AP repolarization (Fig. 8), because it is completely abolished in IK1-overexpressing mice. This effect is specific to IK1, because none of the other major conductances is significantly affected. The data also strongly argue against the view that the T wave is finished before the slow phase is in effect. Figure 8, B and D, clearly indicates that deviations from the steepest slope of the ECG signal returning to the isoelectric point actually indicate the beginning of the T wave.

In conclusion, we have produced for the first time the TG mouse model of IK1 upregulation and have demonstrated significant deviations of major parameters of cardiac excitability from normal. The results suggest that upregulation of this current may have serious adverse effects on cardiac excitability. We strongly believe that the TG mouse model of IK1 upregulation will become a useful tool for further delineating the role of IK1 in normal and pathological conditions as well as under drug administrations.

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