Arrhythmia susceptibility and premature death in transgenic mice overexpressing both SUR1 and Kir6.2[ΔN30,K185Q] in the heart

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Flagg TP, Patton B, Masia R, Mansfield C, Lopatin AN, Yamada KA, Nichols CG. Arrhythmia susceptibility and premature death in transgenic mice overexpressing both SUR1 and Kir6.2[ΔN30,K185Q] in the heart. Am J Physiol Heart Circ Physiol 293: H836–H845, 2007. First published April 20, 2007; doi:10.1152/ajpheart.00011.2007.—Sarcoplasmic ATP-sensitive potassium channel; sulfonylurea receptor 2A (SUR2A) or SUR1 (FLAG-SUR1) subunits of the KATP channel, under transcriptional control of the α-myosin heavy chain promoter. In the present study, we generated double transgenic (DTG) mice overexpressing both Kir6.2[ΔN30,K185Q] and FLAG-SUR1 or FLAG-SUR2A and examined the effects on cardiac excitability in vivo. No animals expressing both FLAG-SUR1 and Kir6.2[ΔN30,K185Q] transgenes at a high level were obtained. DTG mice expressing one transgene at a high level and the other at a lower level are born, but they die prematurely. Electrocardiographic analysis of both anesthetized and conscious animals revealed a constellation of arrhythmias in DTG animals, but not in wild-type or single TG littermates. The proarrhythmic effect of the transgene combination is intrinsic to the myocardium, since it persists in isolated hearts. Importantly, this effect is specific for SUR1-expressing DTG animals: DTG animals expressing both Kir6.2[ΔN30,K185Q] and FLAG-SUR2A at high levels exhibit neither impaired survival nor increased arrhythmia frequency, even with both subunits expressed at high levels. In demonstrating the profound arrhythmic consequences of KATP channels comprised of SUR1 and Kir6.2[ΔN30,K185Q] in the myocardium specifically, the results highlight the critical differential activation of SUR1 versus SUR2A, and indicate that expression of hyperactive KATP in the heart is likely to be proarrhythmic.

ATP-sensitive potassium channel; sulfonylurea receptor 2A

ATP-SENSITIVE POTASSIUM (KATP) channels are expressed in a diverse set of tissues, including the heart (30) and the pancreas (8). By responding to physiological changes in the ratio of ATP concentration to ADP concentration, β-cell KATP channels control the membrane potential, entry of Ca2+ through voltage-dependent channels, and hence insulin secretion. In cardiac myocytes, however, the physiological role of the KATP channel is less established. Under normal metabolic conditions, KATP channels are not significantly open and thus do not contribute to action potential repolarization and excitation-contraction coupling (10, 39, 40). When myocytes are exposed to metabolic stress, KATP channels do open, causing action potential shortening and contractile failure (21, 39, 40). However, the precise trigger for KATP activation, the timing of channel opening, and the physiological consequences of activation in vivo remain unclear.

The KATP channel complex consists of at least two principal subunits—a member of the inward rectifying K+ channel (Kir) protein family Kir6.x forms the potassium selective pore, and an ATP-binding cassette family [sulfonylurea receptor (SUR)] member provides a requisite accessory subunit (34). The SUR subunit confers high-affinity sulfonylurea sensitivity to the channel complex and determines the efficacy of potassium channel-opening drugs like diazoxide (SUR1 > SUR2A) and pinacidil (SUR2A > SUR1) (1, 2, 24), and the nucleotide binding folds of SUR are essential for the Mg-nucleotide stimulation of channel activity (15, 29, 36). The coassembly of different SUR isoforms with Kir6.x subunits may well underlie, at least in part, the tissue-specific diversity of KATP function. A number of biochemical observations suggest that Kir6.2 and SUR2A coassemble to form the sarcoplasmal KATP channel (3, 25) and this conclusion is strengthened by the observation that KATP channel activity is absent in genetically altered mice that lack either Kir6.2 (23, 39) or SUR2 (6). Similar studies demonstrate that β-cell KATP channels share the same Kir6.2 subunit but a different accessory subunit (SUR1) completes the complex (7, 32–34).

Genetic ablation of the KATP channel, by disruption of the Kir6.2 gene, has confirmed the essential function of the channel in response to pathological ischemia and stress (23, 40) as well as to ischemic preconditioning (40); the role of the channel when the heart is not exposed to such pathological conditions remains unclear, and the results of aberrant channel activation are unknown. We previously introduced (12, 13, 18) gain-of-function (GOF) KATP channel mutants into the heart by transgene expression. When ATP-insensitive Kir6.2 mutant channels (Kir6.2[ΔN30,K185Q]) are expressed in the heart under the control of the α-myosin heavy chain (MHC) promoter, there is a small increase in KATP channel activity at rest despite a significant reduction in the maximum KATP current density (13, 18). This is consistent with channel activation under basal conditions as a result of the shift in ATP sensitivity [ATP concentration of half-maximal inhibition (Km,ATP) = 51 ± 5 vs. 2,727 ± 12 μM in wild-type and transgenic

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myocytes, respectively] (18). This increase in basal $K_{\text{ATP}}$ is counteracted by an increase in L-type $Ca^{2+}$ current that maintains action potential duration (APD) and increases myocardial contractility (12, 18). Overexpression of either SUR1 or SUR2A in the heart is without significant effect on cardiac function. In neither case is there an effect on the ATP sensitivity of the channel, although there is a decrease in maximal $K_{\text{ATP}}$ current density that depends on the level of transgene expression (13). To address the possibility that both Kir6.2 and SUR genes must be overexpressed to manifest significant consequences, we have now generated double transgenic (DTG) mice that overexpress both SUR1 or SUR2A and Kir6.2[$\Delta N30,K185Q$]. These animals reveal dramatic consequences: specifically, in SUR1-overexpressing DTG animals there is a constellation of arrhythmias leading to premature death.

**MATERIALS AND METHODS**

**Generation of transgenic mice.** The generation of the single FLAG-SUR1, FLAG-SUR2A, and Kir6.2[$\Delta N30,K185Q$] transgenic (TG) mice used in this study was described previously (12, 13, 18). DTG mice overexpressing two $K_{\text{ATP}}$ channel subunits were obtained by breeding single TG mice. All procedures complied with the standards for the care and use of animal subjects as stated in the Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, revised 1996). All studies were approved by the Animal Studies Committee at Washington University.

**Electrocardiogram analysis in anesthetized animals.** Electrocardiograms (ECGs) were obtained in mice (both male and female, $n = 11–40$ per group) anesthetized with Avertin administered intraperitoneally at a dose of 0.25 mg/g body wt. For signal detection 27-gauge needle electrodes were inserted through the skin, following a standard three-lead scheme. ECG signals were amplified with a four-channel differential amplifier (model 1700, A-M Systems), and data were analyzed using custom software. Cumulative survival probability was calculated using the Kaplan-Meier method.

*Fig. 1. Double transgenic (DTG) animals expressing both sulfonylurea receptor (SUR)1 and inward-rectifying $K^+$ channel protein (Kir)6.2[$\Delta N30,K185Q$] die prematurely.*

**A:** breeding scheme used to obtain animals overexpressing sarcolemmal ATP-sensitive potassium ($K_{\text{ATP}}$) channel subunits. **B:** frequency of transgenic (TG) animals weaned from each litter for each of the single TG animals. **C:** frequency of DTG animals weaned from each of the crosses. Breedings of TG animals that express both SUR1 (line 720) and Kir6.2[$\Delta N30,K185Q$] (line 4) at a high level ($\sim$100-fold overexpressed) never produced DTG animals ($n = 14$ litters), suggesting lethality of the gene combination in utero. Crosses using the lower-SUR1 (line 697)- and Kir6.2[$\Delta N30,K185Q$] (line 2)-expressing lines or the high-SUR2A-expressing line (line 494) produced DTG animals at a frequency predicted for Mendelian inheritance. **D:** Kaplan-Meier survival analysis demonstrating the increased mortality of the SUR1 (697 × 4; 720 × 2), but not SUR2A (494 × 4), DTG animals that reached the age of weaning (3 wk).
collected with a Digidata 1200 analog-to-digital converter and Axo-tape 1.0 software (Axon Laboratories). ECG data were analyzed with both commercial (Clampfit 8.0) and custom-built (programmed by A. N. Lopatin) software applications.

Electrocardiogram analysis in conscious animals. Conscious ECG data were obtained from 2- to 4-mo-old mice. Radiofrequency transmitters (TA10EA-F20 or TA10ETA-F20) were implanted in mice anesthetized with ketamine-xylazine. Telemetry was performed in the Mouse Physiology Core of the Center for Cardiovascular Research at Washington University School of Medicine beginning 7 days after transmitter implantation. Each mouse, individually caged, was placed on top of a separate receiver (RPC-1). Lead II ECG recordings were acquired (1 kHz) with commercially available software (Dataquest A.R.T. Gold version 2.0 Acquisition Software, Data Sciences International). Data were recorded at regular time intervals throughout the day for 6–7 wk, or as long as the animals survived in the case of DTG animals (see RESULTS). Heart rates (determined from R-R intervals) were obtained from the Dataquest analysis software. Premature ventricular contractions (PVCs) and instances of atrioventricular (AV) block were manually determined from the entire data set.

Isolated heart recordings. Monophasic action potentials (MAPs) and surface ECGs were measured from freshly isolated hearts. After excision, hearts were retrogradely perfused through the aorta with Krebs-Henseleit (KH) solution (mM: 25 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) at a constant pressure of 75–85 cmH2O and submerged in a glass tissue bath that was continuously bathed with KH solution maintained at 37°C (22). After an initial equilibration period (10–15 min), MAPs were recorded with a DP-304 differential amplifier (Axon Instruments) and digitized at 3.3 kHz with a Digidata 1322A and pCLAMP 8.0. MAP electrodes were constructed from Teflon-coated silver wire (0.01-in. diameter). The exposed tip of the wire was polished with fine sandpaper and plated with AgCl. APD was determined as the time between the action potential peak and 90% of full repolarization.

Cellular electrophysiology. Isolation of ventricular myocytes was performed as described previously (12). Isolated myocytes were transferred into a recording chamber containing either normal Tyrode solution or solution with additions as described below. Macroscopic currents in isolated ventricular myocytes were recorded with standard whole cell voltage-clamp recording techniques. Patch-clamp electrodes (1–3 MΩ when filled with electrode filling solution) were fabricated from soda lime glass microhematocrit tubes (Kimble 73813). Cell capacitance and series resistance were estimated with a 5- to 10-mV hyperpolarizing square pulse from a holding potential (Vhold) of −70 mV following establishment of the whole cell recording configuration. Data were filtered at 5 kHz, pCLAMP 8.2 software and a DigiData 1322 converter were used to generate command pulses
and collect data. Excised patch data were obtained at a membrane potential of \(-50\) mV. In all whole cell experiments current was elicited with a slow voltage ramp protocol from \(-120\) to \(40\) mV over 4 s \((V_{\text{hold}} = -70\) mV during interpulse periods). For analysis of the diazoxide and pinacidil experiments, current was measured at \(-40\) mV. In the metabolic inhibition experiments, total conductance was calculated from the slope in a 10-mV window surrounding the reversal potential of the current and normalized to the cell capacitance. The series resistance was electronically compensated by 80% (to \(2\) M\(\Omega\)) in order to minimize measurement errors. However, given the size of the \(K_{\text{ATP}}\) current, the conductance may be an underestimation of the true magnitude of the conductance, particularly in wild-type cells.

**Data analysis.** All data were analyzed with ClampFit, Microsoft Excel, and Dataquest A.R.T. Gold software, and (except where noted) results are presented as means \(\pm\) SE. Statistical tests and \(P\) values are denoted in the text and figures where appropriate.

**RESULTS**

**Increased mortality of SUR1 \(\times\) Kir6.2\(\Delta N30, K185Q\) DTG mice.** Mice expressing either overactive Kir6.2\(\Delta N30, K185Q\) or SUR1 or SUR2A transgenes in myocardial tissue are viable and grossly normal, even though in the first case \(K_{\text{ATP}}\) channels are ATP insensitive (13, 18). In each of these cases, there is also a suppression of overall \(K_{\text{ATP}}\) density in the ventricular myocardium, which we have speculated results from stoichiometric mismatch of the two \(K_{\text{ATP}}\) subunits (13, 18). In an attempt to restore stoichiometry, we crossed mice from these previously generated (13, 18) independent TG lines to obtain DTG mice that overexpress both these \(K_{\text{ATP}}\) subunits in the myocardium (Fig. 1A). Pups from all single TG lines were born at normal frequency (Fig. 1B), as were mice expressing both Kir6.2\(\Delta N30, K185Q\) and SUR2A at high levels (Fig. 1C).

![Fig. 3. SUR1 DTG, but not SUR2A DTG, animals display arrhythmias. A: averaged ECG records of lead II surface ECG obtained from WT, single TG, and DTG animals. Like the single SUR1 TG animals, SUR1 DTG animals display an increased PR interval. SUR1 DTG animals also exhibit decreased R-wave and increased S-wave amplitudes. *\(P < 0.05\) compared with WT; ANOVA, Fisher’s LSD post hoc test. B: SUR1 DTG animals exhibit a wide range of arrhythmias. Shown are records of sustained 2° atrioventricular (AV) block (top left), 2 instances of ventricular tachycardia (VT; middle left), and atrial tachycardia (bottom left). Arrhythmias were detected in 15 of the 42 DTG animals tested. The most commonly observed arrhythmia was 2° AV block, but there were also multiple observations of atrial and ventricular tachyarrhythmias as well as asystole. AF, atrial flutter. C: importantly, no arrhythmic events were detected in any of the SUR2A DTG \((494 \times 4)\) animals examined \((n = 16)\).](http://ajpheart.physiology.org/)

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Strikingly, however, no DTG (line 720 × line 4) progeny were obtained from multiple matings of single TG animals expressing both SUR1 and Kir6.2[ΔN30,K185Q] at high levels (Fig. 1C). This indicates that this genetic combination is embryonic lethal and is consistent with expression of tonically active K\textsubscript{ATP} current that suppresses cardiac excitability at a critical stage in utero, probably around embryonic day 10 (35, 38).

Although DTG mice expressing SUR1 at lower levels with Kir6.2[ΔN30,K185Q] at high levels (line 697 × line 4) were born at normal frequency, they exhibited a markedly increased mortality rate (Fig. 1D) compared with their single TG counterparts (not shown). Complementary to this, DTG mice expressing SUR1 at high levels together with Kir6.2[ΔN30,K185Q] at low levels also exhibited markedly increased mortality (Fig. 1D). We crossed three other high-SUR1-expressing lines (lines 699, 701, and 723) (13) into the high-Kir6.2[ΔN30,K185Q]-expressing background (line 4), and in each case DTG progeny either were not born or died prematurely (data not shown). In marked contrast to the increased mortality of SUR1 × Kir6.2[ΔN30,K185Q] DTG animals, animals expressing the SUR2A × Kir6.2[ΔN30,K185Q] combination or any single TG subunit exhibited no increase in mortality (Fig. 1D).

Together these results demonstrate that cardiac overexpression of SUR1 specifically, together with Kir6.2[ΔN30,K185Q], increases mortality. It is possible that the overexpression of any two transgenes might cause premature death resulting from non-specific mechanisms associated with protein synthesis or another unrelated mechanism; however, this is unlikely since high expression of the ATP-insensitive Kir6.2 subunit with high expression of the native subunit, SUR2A, is benign. Moreover, the demonstration of the phenotype in crosses of several independent lines indicates that the effect is not dependent on the transgene insertion point but instead reflects the specific effects of the SUR1-Kir6.2[ΔN30,K185Q] gene combination.

Abnormal rhythm and conduction correlate with SUR1 but not SUR2A expression. These results establish a differential consequence of SUR1 versus SUR2A overexpression in vivo.

**Fig. 4.** ECG analysis of conscious WT and SUR1 and Kir6.2[ΔN30,K185Q] single TG mice. A: representative ECG records obtained in conscious animals with an implanted radiotelemetry device. There were few apparent arrhythmias in WT and single TG animals, with the exception of 1 SUR1 TG animal, which displayed premature atrial contractions during 1 stretch of the recording period. Twenty seconds of ECG recording was obtained at regular intervals (ranging from 3 to 60 times per hour) over 6 wk. B: average daily arrhythmia frequency (top) and heart rate (HR; bottom) for each of the animals tested. Importantly, none of the mice died during the course of the study.
and suggest that SUR1 specifically can generate active $K_{ATP}$ that disturbs the electrical activity of the heart. To investigate the changes in cardiac excitability that underlie death in the whole animal, we obtained lead II surface ECG recordings from anesthetized mice expressing either one or both TG $K_{ATP}$ subunits. As shown in Fig. 2, ECGs in mice expressing either the Kir6.2ΔN30,K185Q or SUR2A transgenes separately were not significantly different from those of wild-type animals. SUR1 TG animals displayed a significant delay of AV conduction compared with wild-type animals (1° AV block), but consistent with normal survival of the wild-type and single TG animals no other arrhythmias were detected.

As with the single SUR1 TG mice, SUR1-expressing DTG mice routinely displayed prolongation of the PR interval (Fig. 3A). In addition, ECGs of 19 of the 42 SUR1 DTG mice, tested at random, revealed significant rhythm disturbances.

![Fig. 5](http://ajpheart.physiology.org/) ECG analysis of conscious SUR1 DTG animals reveals an arrhythmic progression. A: representative ECG records obtained from conscious DTG animals at different times after transmitter implantation. Each of the DTG animals displayed a similar progression of arrhythmias beginning with a normal ECG (i) that was followed by a transient appearance of ventricular arrhythmias (ii, a and b). After this initial bout of ventricular arrhythmias, 2° AV block developed (iii) and worsened (iv,a) and was sometimes accompanied by premature ventricular contractions (PVCs; iv,b). Both atrial (v,a) and ventricular (v,b) tacharrhythmias returned and increased in frequency. Ultimately, HR and ECG amplitude declined until the signal ceased to be detected. B: average daily arrhythmia frequency (top) and HR (bottom) for each of the animals tested. Each of the 3 DTG animals examined died during the monitoring period (5, day of death).
2° AV block (Mobitz type I) was the most common arrhythmia, observed in 15 of 42 animals (Fig. 3B). In addition, four SUR1 DTG animals displayed ventricular tachycardia (VT), and atrial tachycardia was observed in three. In two animals there was no detectable electrical activity (asystole) shortly after the anesthetic administration. We also measured the ECG in 16 SUR2A DTG animals. In contrast to the variety of arrhythmias that were apparent in the SUR1 DTG animals, no abnormalities were observed in ECGs from SUR2A DTG animals (Fig. 3C), again consistent with normal mortality.

These results indicate that cardiac overexpression of SUR1, specifically, causes cardiac arrhythmias. When overexpressed alone, SUR1 causes a prolongation of the PR interval. In combination with Kir6.2[ΔN30,K185Q], SUR1 overexpression induces a constellation of ventricular and supraventricular arrhythmias. Again, arrhythmias were observed in SUR1 DTG animals from two different crosses (line 697 × line 4 and line 720 × line 2), but SUR2A DTG animals displayed no arrhythmias, suggesting that the phenotype reflects the specific effect of the transgene combination and, more importantly, the specific effect of SUR1. Collectively, the coincidence of cardiac arrhythmias with premature death suggests a causal relationship. To examine this, we performed long-term monitoring of the ECG in conscious animals.

ECG analysis in conscious animals reveals an arrhythmia progression. We recorded ECGs in conscious animals at regular intervals over 6 wk, using implanted radiotelemetry devices. The results of these studies are summarized in Fig. 4. Only 1% of ~4,800 individually analyzed 20-s records from wild-type or single TG mice displayed any arrhythmia, and there was no evidence of sustained conduction block or ventricular tachyarrhythmia in any of these animals. We did note the appearance of premature atrial contractions in a single SUR1 TG mouse. Furthermore, there was no progressive increase in the overall frequency of arrhythmias or heart rate throughout the 6-wk course of the study, and none of these wild-type or single TG mice died during this period.

In contrast, DTG mice displayed frequent arrhythmias, with nearly 40% of records displaying an arrhythmic event. Each of the three SUR1 DTG animals died during the course of the study, at 111, 115, and 153 days of age, respectively. We examined in detail the development of arrhythmias as a function of time and observed a gradual increase in their frequency in each of the DTG animals (Fig. 5). Initially, the ECGs were unremarkable and heart rates were not significantly different from control animals (Fig. 5A). Within 2 wk, each of the DTG animals displayed episodes of ventricular arrhythmias, manifested as the frequent appearance of PVCs and monomorphic VT (5Ai, a), or polymorphic VT (5Ai, b). This was followed by a brief “recovery phase” that was relatively normal, with occasional episodes of nonsustained AV block (5Aii), but was followed by a prolonged phase of frequent and sustained 2° block that worsened over time (5Aiii, a), sometimes accompanied by an increased frequency of PVCs (5Aiv, b). As a conse-

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Fig. 6. Cardiac electrical activity in vitro. A: representative records of monophasic action potentials recorded from isolated WT and single TG hearts. No action potentials could be recorded in SUR1 DTG hearts, because each of the hearts displayed spontaneous arrhythmias as shown at bottom. APD90, action potential duration at 90% of full repolarization. B: surface ECG recorded during the recovery period following cannulation of the aorta and perfusion with Krebs-Henseleit solution. Each of the DTG hearts tested exhibited arrhythmias during (or shortly after) the 10-min recovery period (n = 5). Shown are records from 1 heart that initially displayed a number of PVC episodes followed by sustained ventricular tachycardia.
quence of conduction block during this phase, the average daily heart rate decreased. After the “AV block phase,” the days immediately before death were characterized by atrial and ventricular tachyarrhythmic episodes that occurred frequently. During this “tachyarrhythmia phase” there were episodes of both atrial tachycardia (5Av,a) and VT (5Av,b). VT episodes, separated by periods of asystole lasting for at least the duration of a single record (20 s), were also observed. Ultimately, the heart rate and ECG signal amplitude gradually decreased until the signal ceased to be detected. The data demonstrate that premature death is preceded by an increased incidence of cardiac arrhythmias, suggesting that aberrant function of the transgenic $K_{\text{ATP}}$ channel results directly in altered cardiac excitability. It is possible, however, that the phenotype is not intrinsic to the heart itself, but instead reflects an “extracardiac” physiological compensation. Accordingly, we tested this by examining the electrical activity of isolated Langendorff perfused hearts.

Increased arrhythmia susceptibility persists in the isolated SURI DTG heart. MAPs and arrhythmia susceptibility were assessed in isolated hearts as shown in Fig. 6. Composed with wild-type controls, the APD in Kir6.2[ΔN30,K185Q] (line 4) hearts tends to be increased, as we showed previously (12), while the APD of the SURI TG (line 697) hearts is not significantly altered (Fig. 6A). We were unable to make reliable measurements of the action potential in isolated SURI DTG (line 697 × line 4) hearts, because each heart tested ($n = 5$) exhibited spontaneous ventricular arrhythmias including both PVCs and VT, illustrated in the surface ECG obtained from the isolated heart (Fig. 6B). Again, this was not the case for the SUR2A DTG (line 494 × line 4) animals ($n = 2$), in which both MAP and surface ECG were normal. Collectively, these data demonstrate the susceptibility of isolated hearts from SURI DTG (line 697 × line 4) but not SUR2A DTG (line 494 × line 4) animals to arrhythmia, indicating that the arrhythmias are intrinsic to the heart and not dependent on other neural factors or circulating hormones.

These results are not a trivial consequence of increased ventricular $K_{\text{ATP}}$ channel activity. To confirm that mutant $K_{\text{ATP}}$ channels are expressed in the myocardium, we measured $K_{\text{ATP}}$ in isolated ventricular myocytes (Fig. 7). $K_{\text{ATP}}$ channels in excised patch-clamp recordings from isolated DTG ventricular myocytes were indeed insensitive to inhibition by intracellular ATP ($K_{IC50} = 551.81 \mu M$ vs. $10.04 \mu M$ in wild type). Contrary to our expectations, however, the coexpression of both subunits did not significantly rescue $K_{\text{ATP}}$ current. As with the single TG animals (13, 18), there was significant suppression of total available $K_{\text{ATP}}$ conductance, assessed by activating $K_{\text{ATP}}$ current with the metabolic inhibitors 2-deoxyglucose and oligomycin. Importantly though, current density was similarly suppressed in both SURI and SUR2A DTG myocytes, suggesting that it is not the suppression of $K_{\text{ATP}}$ current but rather the inclusion of SUR1 in the myocardium that predisposes DTG animals to arrhythmias and premature death (see DISCUSSION).
DISCUSSION

SUR1 expression is detrimental to the heart. The most striking finding of the present study is the premature death of DTG mice that express SUR1, but not SUR2A. In multiple crosses of SUR1 and Kir6.2[ΔN30,K185Q] TG animals, DTG progeny either died prematurely or were not born. If both transgenes were expressed at a high level, DTG animals were never born, suggesting that the combination can be lethal in utero. K_ATP channels are expressed in the developing heart (28), although their role during this period is unknown. The present finding may suggest that K_ATP-dependent modulation of excitability can be important during the normal development of the myocardium.

If either Kir6.2[ΔN30,K185Q] or SUR1 was expressed at a low level while the other was expressed at a high level, DTG mice were born, but they exhibited chronic arrhythmias and died prematurely. That the arrhythmias and premature death reflect nonspecific effects of protein overexpression seems to be ruled out since high-level expression of SUR2A together with Kir6.2[ΔN30,K185Q] does not induce similar pathology. We cannot rule out the possibility that SUR1 has effects that are independent of K_ATP, and in this regard a recent report has suggested that SUR1 regulates a nonselective cation current in neurons (5). Such a current, however, has not been described in cardiac myocytes, and there is no evidence to suggest that another current is activated by metabolic inhibitors. Instead, the increased frequency of arrhythmias both in vivo (conscious and anesthetized animals) and in vitro (isolated hearts) suggests that aberrant activity of channels containing both SUR1 and Kir6.2[ΔN30,K185Q] underlies the observed electrical abnormalities.

Role of SUR1 in specific locations? The expression of SUR1 alone, but not SUR2A, in the heart causes a prolongation of the PR interval that also occurs in SUR1 DTG animals, even though examination of K_ATP currents in ventricular myocytes indicates that there is no major increase in K_ATP currents in these cells. While SUR1 expression has been reported in myocardial tissues (17, 27), specific localization in subregions of the heart has not been documented, and it seems possible that the detrimental consequences of SUR1 expressed in a specific locale other than ventricular myocytes. Several studies of atrial myocytes have suggested that atrial K_ATP channels are not identical to ventricular K_ATP channels and may not share the same molecular architecture: atrial channels may be more sensitive to pharmacological stimulation by diazoxide, which preferentially targets SUR1-containing K_ATP channels (4). Similarly, SUR1 antisense oligonucleotides suppress K_ATP channel activity in atrial myocytes (41). It is possible that by overexpressing SUR1 under the α-MHC promoter, we have altered the expression of K_ATP channels in atrial or AV node cells, resulting in slowed conduction. In studies of Langendorff perfused hearts, hypoxia causes a prolongation of the AH interval that is inhibited by the addition of glibenclamide and exacerbated by the channel openers pinacilid and cromakalim (31). Sinoatrial (SA) node cells also express K_ATP channels (16); however, in the present study there does not appear to be a significant effect on heart rate, suggesting that either SA node cells are not affected by the expression of SUR subunits or there is sufficient compensation to maintain the heart rate in the normal range.

While the structure, and perhaps the existence, of the mitochondrial K_ATP (mitoK_ATP) channel remains unclear, it is conceivable that the observed effects in the present study reflect disruption of the mitoK_ATP channel. There is some evidence of expression of both Kir6.1 and Kir6.2 as well as SUR2A in mitochondria (9, 37), consistent with the idea that these subunits may form mitoK_ATP channels, but we previously demonstrated (18) that the Kir6.2[ΔN30,K185Q] transgene, at least, does not colocalize with MitoTracker Red, suggesting that this subunit is not present in the mitochondria. Finally, the K_ATP channel openers pinacilid and cromakalim increase the risk of ventricular fibrillation in isolated rabbit hearts exposed to hypoxia and reperfusion. Importantly, this effect was inhibited by blockers of the sarcolemmal (HMR-1098) but not mitochondrial (5-hydroxydecanoate) K_ATP channel (11), suggesting that the proarrhythmic effects of aberrant K_ATP channel activation occur through sarcolemmal, and not mitochondrial, channels.

Implications. In the present study, we have generated a novel genetic model of spontaneous arrhythmia and sudden death, and collectively the data support the notion that overexpression of SUR1 in the heart is detrimental. Cardiac expression of both SUR1 mRNA (17) and protein (27) has been reported, although the normal function of the subunit in the heart remains elusive. Since overexpression of SUR1 alone induces AV block, it is intriguing to speculate that SUR1 is important in ensuring normal conduction through the atria, AV node, or His-Purkinje system. Further experiments on cells isolated from subregions of the heart will be needed to test this hypothesis as well as the specific cellular mechanisms that link K_ATP subunit overexpression with the increased incidence of ventricular tachyarrhythmias.

It is now well established that GOF mutations of Kir6.2 cause severe neonatal diabetes (14, 19) due to increased K_ATP channel activity in pancreatic β-cells. It is notable that there are no reports of aberrant cardiac ECG in these patients, even though the cardiac K_ATP is as significant a conductance as the pancreatic K_ATP. We have speculated (26) that this is the result primarily of coassembly of the mutant Kir6.2 subunits with normal SUR2A. The present results dramatically confirm this hypothesis: when SUR1 is expressed with GOF Kir6.2 mutants in the heart, severe ECG disturbances are the result.

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