ONLINE SUPPLEMENT

EXPERIMENTAL METHODS

Experimental animals:

Details on the production of transgenic (TG) mice overexpressing a WT (WT) Kir2.1 subunit and a green fluorescent protein (GFP) fusion protein are presented by Li et al.[1] All animals used in this study were from line 1, 3rd and 4th generation, and included a total of 21 transgenic (TG) and 12 of the wild-type littermates (WT). The mice were used as follows:

WT: 4 for analysis of arrhythmia duration and frequency, 6 for hypertrophy assessment (3 of which were used for the slot blot) and 2 for western blotting.

TG: 8 for analysis of arrhythmia duration and frequency, 6 for hypertrophy assessment (3 of which were used for the slot blot), 5 for the Ba^{2+} experiments and 2 for western blotting.

Western Blot:

For western blotting of Kir2.1, membrane fractions from hearts of 2 TG and 2 WT mice were prepared as described elsewhere.[2] Equal amounts of total protein were subjected to western blot analysis. The expression of the endogenous Kir2.1 and the Kir2.1-GFP fusion protein was probed using anti-Kir2.1 (Alomone Labs) antibody.

Time space plots (TSP) and measurement of the core diameter:

For 2 rotation of the spiral wave, a vertical TSP was constructed and the core diameter measured as detailed earlier (see also description of Figure 16 below).[3,4]. Time is on the X axis; the Y direction represents the vertical distance. For simulations, the vertical space aspect of the TSP represents 2 cm, and for experiments it represents 7mm.

SUPPLEMENTAL EXPERIMENTAL RESULTS

I_{K1} is functionally upregulated in TG mice hearts.

Li et al[1] observed by immunofluorescence localization of GFP that the α-MHC-driven expression of Kir2.1 subunits was widespread and nearly uniform in both left and right ventricles in the TG mouse used in this study. However, they did not study the Kir2.1 protein levels in the WT vs TG hearts. In figure 1, we present results of our western blot analysis in such hearts. Membrane fractions from 2 WT and 2 TG hearts were prepared and probed with anti-Kir2.1 antibodies. In both, WT and TG lanes, a band at around 47 kDa is evident in the WT and TG cases. This corresponds to the native Kir2.1 protein. The equal intensity of the bands demonstrates equal loading of the samples. However, a higher band at about 70 kDa is prominent only in the TG lane, corresponding to the molecular weight of the Kir2.1-GFP chimera. This indicates that the protein product of the Kir2.1-GFP transgene is expressed in the hearts of TG mice.

COMPUTATIONAL METHODS

Development of the Single Cell Mouse Ventricular Model at 37 °C

Our goal in this study was twofold: 1. To develop a new, simplified, single-cell model of the mouse ventricular action potential at physiological temperatures (37 °C), with only Hodgkin-Huxley-type descriptors for the various ionic currents, and keep the intracellular ionic concentrations constant. 2. To incorporate this single cell model into an isotropic, uniform, two-dimensional (2-D) sheet of cardiac cells to simulate the fast rotor activity observed on the mouse epicardial surface.

The mouse ventricular cell model was developed based on semi-quantitative experimental data in mouse ventricular myocytes, and equations were adapted from earlier murine models.[5,6] The mouse model includes descriptors for the following ionic mechanisms that have been deemed to contribute to the genesis of the cardiac action potential: a fast inward Na^+ current (I_{Na}); the L-type Ca^{2+} current (I_{CaL}); the Ca^{2+}-independent transient outward K^+ current (I_{to}); the slowly inactivating, 4-AP sensitive delayed rectifier K^+ current (I_{Kslow}); the steady-state outward K^+ current (I_{ss}); an inwardly rectifying K^+ current (I_{K1}); a background current (I_B) consisting of three components [a Na^+ (I_{BNa}), a Ca^{2+} (I_{BCa}) and a K^+ (I_{BK}) background current]; a Na^+-K^+ pump current (I_{NaK}); a Ca^{2+} pump current (I_{CaP}); and a Na^+-Ca^{2+} exchanger current (I_{NaCa}). A more detailed description for each ionic mechanism is provided below.

Na^+ Current (I_{Na})

The equation for I_{Na} consists of a fast activation variable (m^3), a fast inactivation variable (h), and a slow inactivation variable (j), similar to earlier ventricular models.[5,7] The steady-state activation and inactivation characteristics of I_{Na} were based on an earlier description in the rat model,[5] whereas the kinetics of the sodium current were adapted from the guinea-pig ventricular cell model.[7] The maximum conductance value of I_{Na} was chosen such that the CV of the cardiac wavefront in the two dimensional sheet of cardiac cells was ≈ 55.6 cm/sec, which is in accordance with our experimental measurements in WT mice, viz. 61 $\pm$ 9 cm/sec.

L-type Ca^{2+} Current (I_{CaL})

The formulation for I_{CaL} is identical to an earlier equation in a rat model,[5] except that the activation/inactivation kinetics were scaled from room temperature to physiological temperature, assuming a Q_{10} value of 2.1, based on a recent study.[8] In the new mouse model, for purposes of simplicity, there is no formulation of a sub-space calcium concentration, and even the "global" intracellular calcium concentration is clamped to a fixed value of 150 nM. Thus, there is no Ca^{2+}-dependent inactivation of I_{CaL} in this version of the model.

Ca^{2+}-independent Transient Outward K^+ Current (I_{to})

We first formulated model equations for I_{to} at room temperature, which included a rapid activation and an inactivation gates. The half-maximum voltages of the steady-state activation ($V_{1/2,act}$) and inactivation ($V_{1/2,inact}$) for I_{to} in the mouse model were –25.0 mV and –55.8 mV respectively, based on experimental measurements.[9,10] Note that the $V_{1/2,act}$ value was based on measurements made for I_{Kslow} in mouse ventricular myocytes.[9] This is because (i) there are no independent measurements for the steady-state activation characteristics of I_{to} in mouse, and (ii) based on their experimental current-voltage (I-V) characteristics, I_{to} and I_{Kslow} are deemed to display similar, but not identical voltage dependence of activation.[10,11] The experimental $V_{1/2,act}$ value was -12.5 ± 2.6 mV,[9] but was shifted by –12.5 mV as it was measured in the presence of 2 mM $CoCl_2$,[9] since it is now known that divalent cations shift the steady-state activation/inactivation characteristics of I_{to}.[12] In contrast, the $V_{1/2,inact}$ value was based on experimentally obtained values of -55.8 ± 1.3 mV, recorded in the absence of divalent cations.[10] The time constant for activation of I_{to} was based on an equation in the recently published mouse model;[6] its magnitude was reduced by 25% so that its values were similar to those

seen experimentally.[10] The equation describing the time constant for inactivation was chosen such that it was independent of membrane voltage at depolarized potentials, in accordance with recent experiments,[11] and its value was ≈ 30 msec, as seen experimentally.[10] In addition, this time constant had a bell-shaped curve at hyperpolarized potentials, so that recovery from inactivation was progressively faster at more hyperpolarized potentials, in accordance with experimental results.[10] Initially, the maximum conductance value of I_{to} was chosen such that its peak current density at a clamp potential of +30 mV (from a holding potential of –80 mV) was 34.68 pA/pF, which is comparable to experimentally recorded values of 34.5 $\pm$ 2.6 pA/pF at 22 °C.[10] In Figure 2, we present the steady-state activation/inactivation characteristics (A), the time constants for activation/inactivation (B), the time-dependent currents activated in response to voltage-clamp pulses (C), and the peak I-V relationship for I_{to} at room temperature (D).

Next, we modified the equations describing I_{to} for 37 °C in the following manner: The steady-state activation/inactivation characteristics were assumed to be similar at 22 °C and 37 °C. The time constants for activation and inactivation were scaled by a factor of 2.66, which was obtained by calculating Q_{10} values, by comparing reactivation kinetics of I_{to} at –80 mV for 22 °C and 32 °C.[10] The current density of I_{to} was reported to be ≈ 45 pA/pF at 32 °C, compared to ≈ 35 pA/pF at 22 °C;[10] this represents an increase of ≈ 1 pA/pF per °C. Therefore, we assumed the current density of I_{to} at 37 °C to be ≈ 50 pA/pF at +30 mV, and the maximum conductance value of I_{to} was adjusted accordingly. In Panels E and F of Figure 2 are shown the simulated time-dependent currents activated in

response to voltage-clamp pulses from a holding potential of –80 mV (E), and the I-V relationship for I_{to} at 37 °C (F).

Delayed rectifier K^+ current (I_{Kslow})

The 4-AP sensitive delayed rectifier K^+ current I_{Kslow} has also been referred to as I_{sus},[13] I_{Kur},[14] and I_{slow}.[9] I_{Kslow} activates rapidly, but declines much more slowly or ≈ 20 times more slowly than I_{to} in response to depolarized voltage-clamp pulses. Furthermore, recent reports indicate that I_{Kslow} can be further classified into two components, I_{Kslow1} and I_{kslow2}, based on their biophysical properties/molecular correlates.[15,16] However, we have formulated equations for I_{Kslow} only, in this version of our mouse model. The values of $V_{1/2,act}$, and $V_{1/2,inact}$ were –12.5 mV and –44.7 mV respectively (Figure 3A). These were based on experimental measurements of –12.5 ± 2.6 mV for activation,[9] and –44.7 ± 1.7 mV for inactivation.[10] The time constant for activation of I_{Kslow} was formulated to be a bell-shaped curve (Figure 3B), so that its values were similar to those observed experimentally at depolarized potentials.[10] For comparison, we also show the activation time constant from a recently published mouse ventricular cell model;[6] the values are similar to those used in our model at depolarized potentials. However, they tend toward infinity at more hyperpolarized potentials; hence the equation from the published mouse model was not used. The time constant for inactivation of I_{Kslow} shows a characteristic similar to that of I_{to}, that of being relatively independent of membrane voltage at depolarized potentials, and having a bell-shaped curve at more hyperpolarized potentials. However, the absolute values are much higher than that of I_{to}. For example, the time constant for inactivation was ≈ 1.1 seconds at depolarized potentials (compared to 30 msec for I_{to}), and the recovery at –80 mV was ≈ 740 msec (compared to ≈ 35 msec for

I_{to}). The inactivation curve and corresponding experimental data[10] are shown in Figure 3B. The maximum conductance value of I_{Kslow} at 22 °C was chosen such that the peak density of the depolarization activated I_{Kslow} at a clamp potential of +30 mV (from a holding potential of –80 mV) was 34.3 pA/pF, which is comparable to experimentally recorded values of 34.2±3.5 pA/pF.[10] The time-dependent currents activated in response to voltage-clamp pulses from a holding potential of –80 mV, and the peak I-V relationship for I_{Kslow} at 22 °C are shown in Figure 3C and 3D respectively.

We then modified the equations representing I_{Kslow} for 37 °C in a manner similar to that previously shown for I_{to}. The steady-state activation/inactivation characteristics were assumed to be similar at 22 °C and 37 °C. The time constants for activation and inactivation were scaled by a factor of 2.18, which was obtained by calculating Q_{10} values, by comparing reactivation kinetics of I_{Kslow} at –80 mV for 22 °C and 32 °C.[10] The current density of I_{Kslow} was reported to be ≈ 45 pA/pF at 32 °C, compared to ≈ 35 pA/pF at 22 °C;[10] this again represents an increase of ≈ 1 pA/pF per °C. Therefore, we assumed the current density of I_{Kslow} at 37 °C to be ≈ 50 pA/pF at +30 mV, and the maximum conductance value of I_{Kslow} was adjusted accordingly. Panels E and F of Figure 3 show the simulated time-dependent currents activated in response to voltage-clamp pulses from a holding potential of –80 mV, and the I-V relationship for I_{Kslow} at 37 °C.

Steady-state Outward K^+ current (I_{ss})

I_{ss} in mouse ventricular myocytes has been modeled to activate slowly, but does not decay or inactivate. The value of $V_{1/2,act}$ (Figure 4A) was chosen to be 0 mV, which was ≈ 12 mV depolarized from the corresponding value of I_{Kslow}, based on the

experimental observation that the activation of I-V curve for I_{ss} occurs at more depolarized potentials compared to I_{Kslow}.[10] The time constant for activation of I_{ss} was again formulated to be a bell-shaped curve (Figure 4B), and its values were similar to those observed experimentally at depolarized potentials.[10] The activation time constant from a recently published mouse ventricular cell model[6] is also shown for comparison in red; the values are similar to those used in our model at depolarized potentials. However, again as observed previously for I_{Kslow}, they tend toward infinity at more hyperpolarized potentials; hence the equation from the published mouse model was not used. The maximum conductance value of I_{ss} at 22 °C was chosen such that the peak density of the depolarization activated I_{ss} at a clamp potential of +30 mV (from a holding potential of –80 mV) was 15.34 pA/pF, which is comparable to experimentally recorded values of 15.6 ± 1.6 pA/pF.[10] Figure 4C and D show, respectively, the time-dependent currents activated in response to voltage-clamp pulses from a holding potential of –80 mV, and the peak I-V relationship for I_{ss} at 22 °C.

Next, we modified the I_{ss} equations for 37 °C in a manner similar to that previously described for I_{to} and I_{Kslow}. The steady-state activation characteristic was assumed to be similar at 22 °C and 37 °C. The time constant for activation was scaled by a factor of 2.18, and assumed to be similar to that of I_{Kslow}, since no corresponding experimental values are available for I_{ss}. The current density of I_{ss} was reported to be ≈ 25 pA/pF at 32 °C, compared to ≈ 15 pA/pF at 22 °C;[10] this again represents an increase of ≈ 1 pA/pF per °C. Therefore, we assumed the current density of I_{ss} at 37 °C to be ≈ 30 pA/pF, and the maximum conductance value of I_{ss} was adjusted accordingly. In Figure 4, Panels A and F show, respectively, the simulated time-dependent currents activated in

response to voltage-clamp pulses from a holding potential of –80 mV, and the peak I-V relationship for I_{ss} at 37 °C.

Inward Rectifier K^+ Current (I_{K1})

The equation for I_{K1} was based upon a recent update in the formulation for the rat ventricular model.[17] This equation incorporates the characteristic square-root dependence of the maximum conductance value of I_{K1} on external K^+ ions ($[K^+]_o$), which was missing in the original rat model.[5] The maximum conductance value of I_{K1} was adjusted so that the peak value of outward I_{K1} current at –60 mV was 0.68 pA/pF, which is comparable to the experimental values of 0.68 ± 0.1 pA/pF recorded in mouse ventricular myocytes.[1] To simulate the I_{K1} current in TG mice overexpressing Kir2.1, the maximum conductance value was multiplied by 12.0, since the ratio of the maximum outward currents at –60 mV between TG and wild-type mice was 12.23.[1] Figure 5 displays the I-V characteristics for I_{K1} in the WT (WT) and TG (TG) mice.

Other Currents and Model Ionic Concentrations

The formulations for the other currents including the background current (I_B), Na^+-K^+ pump current (I_{NaK}), the Ca^{2+} pump current (I_{CaP}), and the Na^+-Ca^{2+} exchanger current (I_{NaCa}), were similar to earlier formulations in the rat ventricular model.[5] As stated earlier, the intracellular Ca^{2+} concentration was fixed to 150 nM, and the internal K^+ concentration was fixed to 140 mM. The internal sodium concentration was fixed at 16.0 mM, based on experimental measurements of 16.9 ± 1.4 mM intracellular sodium ions in rats at 6.0 Hz stimulation frequency.[18] The external concentrations of Na^+, K^+, and Ca^{2+}

ions were fixed at 156.0 mM, 4.0 mM, and 1.8 mM, respectively, based on Tyrode's solution used during experiments in the present study.

Computational Methods for the Single Cell Model

The mathematical model of the mouse ventricular myocyte was implemented in "C" language on a 32 processor, parallel computer (MicroArray cluster machine). The differential equations in this model were solved using a fixed time step (Δt = 0.005 msec), modified Euler method, as in an earlier study.[19] The steady-state cardiac action potentials were obtained by pacing the ionic model for 10 seconds at 1 Hz (after making a parameter change).

Computational Methods for Two-dimensional (2D) Model

The propagation of the cardiac impulse was simulated in a two-dimensional (2D), homogeneous, isotropic tissue of 2×2 cm^2, consisting of 200×200 nodes (ventricular cells), and no-flux boundary conditions at the edges (We could induce sustained reentry in a smaller sheet of 1×1 cm^2 only in the TG case, and the frequency characteristics in both areas were identical). We used the Euler method to integrate the voltage at each node, which was governed by the conventional reaction-diffusion equation, assuming uniform, isotropic tissue, as in a recent study.[19] The 2D simulations were coded in "C", and solved on a 32-processor, parallel computer (MicroArray cluster machine). Rotors were initiated by the standard cross-field stimulation protocol. The results for the rotor dynamics are discussed in the Main manuscript.

SUPPLEMENTAL SIMULATION RESULTS

Single Cell Model

In Figure 6 we present the simulated mouse ventricular action potential under control conditions (37 °C) elicited by a stimulus current of 1.4 nA applied for 3 msec at a frequency of 1Hz. It was qualitatively similar to an experimentally recorded action potential at 1 Hz and at 35 °C,[16] shown in the inset. Both action potentials show a rapid repolarization initially, followed by a late, slower phase of repolarization. It is important to note that the slower late phase of repolarization is seen despite the absence of a Ca^{2+} transient in the model, as well as in the experiments, where EGTA was used in the recording pipette.[16] The rapid repolarization or the triangular nature of the action potential is mainly seen to be due to the interaction of I_{CaL}, I_{to} and I_{Kslow}, whereas I_{ss} plays a relatively minor role (see Figure 5A in the main manuscript for more detailed description). The main repolarizing current in the late phase of repolarization is I_{K1}, which counteracts other ionic currents such as the electrogenic Na^{+}-Ca^{2+} exchanger and the background current (I_B), to bring about the action potential back to its resting potential (see Figure 7, which depicts currents I_{K1}, I_B, I_{NaCa}, and I_{NaK} underlying the action potential. The Ca^{2+} pump current I_{CaP} was ≈ 1 pA, and hence is not shown).

To validate the new mouse model, we investigated various aspects of its repolarization properties, and compared them to experimental results whenever possible. In Figure 8, the simulated action potentials at 1 and 10 Hz are seen to be very similar, and thus show little or no rate-dependence; this is qualitatively similar to experimental results,[20] digitized in the inset of Figure 8. The simulated action potential when I_{Kslow} was reduced to 20% of its original value is shown in Figure 9; it is qualitatively similar to

that seen experimentally for the mouse cardiac action potential [9]in the presence of 50 μM 4-AP (shown in inset at 35 °C), which has been shown to ≈ block 80% of I_{Kslow}.[20] Next, we investigated the effect of completely blocking I_{to} on the simulated action potential, as shown in the topmost panel of Figure 10. The action potential was more prolonged at the early phase, compared to the later one. We compared this to experimental results, which are digitized and shown in the middle panel in Figure 10. The recordings in these experiments were made at room temperature, and show action potentials and corresponding currents recorded from the same respective myocytes from either wild-type or TG mice that were generated to express a dominant-negative N-terminal fragment of the K(v)4.2 pore-forming potassium channel subunit (that encodes for I_{to}) under the control of the alpha-myosin heavy chain promoter.[21] The action potential from the Kv4.2N TG mouse is prolonged at both early and late repolarization phases. Experimentally, I_{to} is absent in Kv4.2N mice, although there was no statistical difference in the currents measured at the end of a 500 msec depolarizing voltage clamp pulse. Even then, the mean current density was ≈ 20% smaller in Kv4.2N compared to controls, and this can also be seen clearly in the middle panel in Figure 10, where the magnitude of the currents in the two experimental recordings is different. The currents that remain at the end of a 500 msec depolarizing pulse include I_{Kslow} and I_{ss}.[10] Therefore, we simulated action potentials by blocking I_{to} completely, and reducing both I_{Kslow} and I_{ss} densities by 25%; now the simulated action potential prolongation was qualitatively more similar (bottom panel, Figure 10) to that observed experimentally (Also, again note that there is a temperature difference; the simulations were carried out at 37 °C, whereas experiments were done at 22 °C). Figure 11, shows the simulated action potentials in WT and Kir2.1

overexpressing mice (where I_{K1} conductance was increased 12.0 fold, and I_{ss} density was multiplied by 1.36, based on experiments[1]), which are qualitatively similar to experimentally recorded monophasic action potentials.[1] On blocking I_{ss}, the action potential in our model showed a prolongation, mainly at the terminal phase of repolarization (Figure 12). We could not find any comparison to experimental results, since a direct pharmacological blocker of I_{ss} is not available at this time.[10] Finally, we simulated the effect of 5 mM 4-aminopyridine (4-AP), by completely blocking I_{to} and I_{Kslow}, and by 40% block of the maximum conductance value of I_{ss}, as has been suggested experimentally.[11] As shown in Figure 13, our simulated action potential is again qualitatively very similar to experimentally recorded action potentials in presence of 5 mM 4-AP at room temperature.[22]

In summary, our minimal model of the mouse cardiac action potential is able to qualitatively reproduce many of the repolarization dynamics seen experimentally, based on descriptors for the major repolarizing K^+ currents deemed to be present in mouse ventricular myocytes. Clearly, further improvements in the model are possible, and will be attempted in future studies. These will include incorporating a more rigorous description for I_{CaL} (including Ca^{2+}-dependent inactivation), as well as intracellular Ca^{2+} ($[Ca^{2+}]_i$) regulation. Additional improvements in K^+ current formulation such as including descriptors for I_{Kslow1} and I_{Kslow2}, instead of just I_{Kslow} as in this model, and the activation/reactivation kinetics of I_{to} based on more detailed experimental data, will help simulate a qualitatively better mouse action potential.

Heart Size versus Reentry Stability

Previously,[23] we demonstrated that stable rotors are possible in the mouse heart with a ventricular surface area of ≈1 cm^2. However, success was quite variable and instability was the norm. In fact, it was necessary to repeat the pacing protocols many times to obtain reentry. In the present study we used much less aggressive protocols and therefore, unlike the TG heart, the reentrant activity in the WT was short-lived.

The simulations of reentry presented in Figures 5 and 6 of the main manuscript were conducted using 2 x 2 cm^2 sheets. The rotors were stable regardless of whether they were induced in TG or WT sheets. In an effort to better understand the role of size, we carried out additional simulations using much smaller sheets. When the simulations were conducted using 2-D sheets of 1 x 1 cm^2, the TG rotor was stable and maintained the frequency of ≈ 45 Hz (data not shown). The example of Figure 14 shows 20 msec snapshots of reentry in a sheet of 1 × 1 cm^2 with WT action potential properties. The rotation period was ≈ 50 ms. After 2 rotations, the rotor interacted with the sheet boundary and stopped. Thus while reentry was sustained at ≈ 18.5 Hz in a WT 2 x 2 cm^2 sheet, it was not-sustained in a WT 1 x 1 cm^2 sheet. These results support the notion that by increasing the diffusion coefficient D (i.e., accelerating the conduction velocity) and also abbreviating action potential duration, I_{K1} overexpression reduces the rotor diameter and allows for stable rotation in the small mouse heart.

Conduction velocity (CV) distribution in simulated WT and TG rotors.

In Figure 15, a snapshot of the CV distribution in a 0.5 x 0.5 cm^2 area during sustained reentry shows that CV is greatly reduced near the center of rotation (the core) and increases with distance from the core. In the WT sheet (left), very low CV's can be

found at relatively large distances away from the center of rotation compared to TG. However, because of I_{K1} upregulation in the TG sheet, both the core size and therefore the spatial extension of its electrotonic influence on the reentrant activity of the surrounding tissues are greatly reduced, allowing a faster growth of CV with increasing distances from the core. This is depicted by the tighter clustering of low CV's near the core in the TG case.

I_{K1} upregulation reduces the core size.

We have postulated that the steeper increase in CV from the core to the periphery in the TG rotors compared to WT is due the changes in passive membrane properties of the cardiac tissue brought about by the increase in I_{K1} (decrease in input resistance with increasing I_{K1}) which causes the core to diminish in size. To verify this, we measured the diameter of the rotor's core in simulated and experimental WT and TG setting. In Figure 16, we display time-space plots (TSP) of TG and WT experimental and simulated reentry. The characteristic shape of the TSPs is that of spiral wave activation.[3,4] Each TSP displays 2 rotations. The diameter of the core is calculated by measuring the projection of the distance between 2 arms of the TSP[3,4] (the green line on the scale at the left of the TSP). In the simulated WT (panel A) and TG (panel B) reentry, the core diameter measured during 2 rotations is, respectively, 3.9±0.3mm and 1.9±0.1mm ($p<0.001$). In panels C and D, examples of 2 rotations from a WT and a TG rotor are respectively shown. The diameter of the core is 0.94±0.03mm for WT and 0.52±0.19mm for TG ($p<0.01$). The average core diameter for 3 WT and 3 TG rotors is 1.06±0.16mm and 0.6mm±0.15, respectively, $p<0.001$.

I_{K1} upregulation reduces the space constant.

To obtain an estimate of the space constant, we took an array of 200 by 10 cells (length and width respectively), and applied subthreshold stimuli (for 3 msec) at one end of the 2D array. The distribution of voltage at 3 msec along the length of the cable was used to obtain an estimate of the space constant (λ) by fitting the voltage normalized to the voltage at the site of injection to a simple exponential decay function of the form $V=V_0e^{-x/\lambda}$ (Figure 17 B). As expected, in the TG case where I_{K1} was increased by 12 fold, the model displayed a smaller space constant than the WT case (λ= 0.43 mm vs λ= 0.66 mm). This can be seen in the colored voltage distribution maps (Figure 17 A) where the voltage decreased faster with distance in the TG sheet compared to the WT.

FIGURE LEGENDS FOR ONLINE SUPPLEMENT

Figure 1. Western blot. Membrane fractions from 2 WT and 2 TG hearts probed with anti Kir2.1 antibody. Left lane WT, right lane TG.

Figure 2. Characteristics of I_{to}. **(A.)** Steady-state activation and inactivation characteristics at 22 °C. **(B.)** Time constants for activation/inactivation as a function of voltage at 22 °C. Experimental data for activation (∗) and inactivation (o) for I_{to} are plotted.[10] **(C.)** Simulated time-dependent I_{to} current in response to voltage-clamp pulses, (from –50 to +40 mV, in steps of +10 mV, holding potential of –80 mV) at 22 °C. **(D.)** Peak Current-Voltage (I-V) relationship for I_{to} at 22 °C, along with experimental data (o).[10] (**E.**) Simulated time-dependent I_{to} current at 37 °C, and **(F.)** Peak I-V relationship for I_{to} at 37 °C.

Figure 3. Characteristics of I_{Kslow} **(A.)** Steady-state activation and inactivation characteristics at 22 °C. **(B.)** Time constants for activation/inactivation as a function of voltage at 22 °C. Experimental data for activation (∗) and inactivation (o) for I_{Kslow} are plotted.[10] Red curve is representative of activation time constant for I_{Kslow} from a recently published mouse model.[6] **(C.)** Simulated time-dependent I_{Kslow} current in response to voltage-clamp pulses, (from –50 to +40 mV, in steps of +10 mV, holding potential of –80 mV) at 22 °C, along with experimental data (o).[10] **(D.)** Peak Current-Voltage (I-V) relationship for I_{Kslow} at 22 °C. (**E.**) Simulated time-dependent I_{Kslow} current at 37 °C, and **(F.)** Peak I-V relationship for I_{Kslow} at 37 °C.

Figure 4. Characteristics of I_{ss} **(A.)** Steady-state activation characteristic at 22 °C. **(B.)** Time constant for activation as a function of membrane voltage at 22 °C. Experimental data for activation (o) is plotted.[10] Red curve is representative of activation time constant for I_{ss} from a recently published mouse model.[6] **(C.)** Simulated time-dependent I_{ss} current in response to voltage-clamp pulses, (from –50 to +40 mV, in steps of +10 mV, holding potential of –80 mV) at 22 °C. **(D.)** Peak Current-Voltage (I-V) relationship for I_{ss} at 22 °C, along with experimental data (o).[10] (**E.**) Simulated time-dependent I_{ss} current at 37 °C, and **(F.)** Peak I-V relationship for I_{ss} at 37 °C.

Figure 5. Comparison of simulated I-V relationship of I_{K1} in wild-type (black) and Kir2.1 TG (red) mouse models.

Figure 6. Simulated and experimental[16] mouse ventricular action potentials under control conditions at 1 Hz, at 37 °C and 35 °C respectively.

Figure 7. Mouse ventricular action potential under control conditions (1 Hz, 37 °C) and the underlying ionic currents (I_{K1}, I_{NaCa}, I_{NaK}, and I_B).

Figure 8. Rate dependence of simulated mouse ventricular action potentials at 1 Hz (black), and 10 Hz (red). Corresponding digitized experimental action potentials[20] at 1 and 10 Hz are shown in the inset.

Figure 9. Effect of blocking the maximum conductance value of I_{Kslow} by 80%. The simulated mouse ventricular action potential at I_{Kslow}*2 (red), and the control action potential (black) are shown. Corresponding digitized experimental action potential recordings in control and in the presence of 50 μM 4-AP at 35 °C[9] are shown in the inset.

Figure 10. Effect of blocking the maximum conductance value of I_{to} by 100% on the simulated mouse ventricular action potential is shown in the top panel. In the middle panel, experimental[21] action potential recordings at 22 °C, and depolarization-activated K^+ current recordings along with the corresponding I-V relationships for wild-type and TG (Kv4.2N) mice are shown (Note that the action potentials and currents were recorded from the same myocyte for the wild-type and TG mice respectively). The bottom panel shows simulated action potential when I_{to} was blocked completely, and in addition, both I_{Kslow} and I_{ss} were blocked by 25% respectively.

Figure 11. Simulated mouse ventricular action potentials in control and TG cases (I_{K1} conductance in control case multiplied by 12, and I_{ss} conductance in control case multiplied by 1.36). Corresponding digitized experimental[1] monophasic action potentials in control and Kir2.1 over-expressor mouse heart are also shown in the inset.

Figure 12. Simulated mouse ventricular action potentials in control case and when I_{ss} was blocked completely.

Figure 13. Simulated mouse ventricular action potentials in control case, and when I_{to} and I_{Kslow} were blocked by 100% each, and I_{ss} conductance was reduced by 40% simultaneously are shown. Digitized experimental[22] action potentials at 22 °C in control case and in presence of 5 mM 4-AP are also shown in the inset.

Figure 14. Reentry in a 1x1 cm^2 sheet with WT action potential properties. Frames are 20 msec snapshots of the rotor. After about 2 rotations, the wavefront hits the upper border of the sheet and the rotor self-terminates. Please see Online Supplement for comparison of "WT1b1sim" with "WT2b2sim" movies.

Figure 15. Effect of I_{K1} upregulation on core size and spatial extension of its influence**.** Conduction velocity measurements were carried out in simulated WT and TG 2x2 cm^2 sheets sustaining stable rotating activity. Left panel, CV distribution in a 0.5 x 0.5 cm^2 area during reentry in the WT sheet. Right panel, CV distribution in a 0.5 x 0.5 cm^2 area during reentry in the TG sheet. Color code indicates velocity. Horizontal calibration 0.5 cm

Figure 16. Core diameter measurements from time-space plots (TSP). Simulation. A, TSP of 2 rotations during WT reentry. 1 rotation is indicated by the light blue line. The diameter of the core is the projected distance (green line) on the 2 cm scale of the space between the 2 arms of the TSP. B, TSP of 2 rotations during TG reentry. Experimental. C, TSP of 2 rotations during WT reentry. D, TSP of 2 rotations during TG reentry. The horizontal black arrow indicates the direction of time.

Figure 17. Measurement of the space constant λ. A, The voltage normalized to the voltage at the site of subthreshold current injection is depicted across 10 cells (vertical direction) and a distance of 3 mm (horizontal direction). The color scale indicates voltages from 0 to 100%. The scale bar represents 1 mm. B, Fitting of the voltage normalized to the voltage at the site of injection to a simple exponential decay function of the form $V=V_0e^{-x/\lambda}$. For WT, the fit was: $99e^{-x/0.66}$, R=0.998. For TG, it was: $103e^{-x/0.43}$, R=0.998

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