Epicardial Border Zone Overexpression of Skeletal Muscle Sodium Channel SkM1 Normalizes Activation, Preserves Conduction, and Suppresses Ventricular Arrhythmia
An In Silico, In Vivo, In Vitro Study

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Background—In depolarized myocardial infarct epicardial border zones, the cardiac sodium channel (SCN5A) is largely inactivated, contributing to low action potential upstroke velocity (Vmax), slow conduction, and reentry. We hypothesized that a fast inward current such as the skeletal muscle sodium channel (SkM1) operating more effectively at depolarized membrane potentials might restore fast conduction in epicardial border zones and be antiarrhythmic.

Methods and Results—Computer simulations were done with a modified Hund-Rudy model. Canine myocardial infarcts were created by coronary ligation. Adenovirus expressing SkM1 and green fluorescent protein or green fluorescent protein alone (sham) was injected into epicardial border zones. After 5 to 7 days, dogs were studied with epicardial mapping, programmed premature stimulation in vivo, and cellular electrophysiology in vitro. Infarct size was determined, and tissues were immunostained for SkM1 and green fluorescent protein. In the computational model, modest SkM1 expression preserved fast conduction at potentials as positive as −60 mV; overexpression of SCN5A did not. In vivo epicardial border zone electrograms were broad and fragmented in shams (31.5±2.3 ms) and narrower in SkM1 (22.6±2.8 ms; P=0.03). Premature stimulation induced ventricular tachyarrhythmia/fibrillation >60 seconds in 6 of 8 shams versus 2 of 12 SkM1 (P=0.02). Microelectrode studies of epicardial border zones from SkM1 showed membrane potentials equal to that of shams and Vmax greater than that of shams as membrane potential depolarized (P<0.01). Infarct sizes were similar (sham, 30±2.8%; SkM1, 30±2.6%; P=0.86). SkM1 expression in injected epicardium was confirmed immunohistochemically.

Conclusions—SkM1 increases Vmax of depolarized myocardium and reduces the incidence of inducible sustained ventricular tachyarrhythmia/fibrillation in canine infarcts. Therapy to normalize activation by increasing Vmax at depolarized potentials may be a promising antiarrhythmic strategy. (Circulation. 2009;119:19-27.)

Key Words: arrhythmia ■ gene therapy ■ ion channels ■ myocardial infarction ■ tachyarrhythmias

Reentry accounts for ≈85% of serious arrhythmias complicating ischemic heart disease. Prevention and treatment are rooted in early 20th century research on reentry. The goals are to create bidirectional conduction block (with drugs that block Na+ channels, surgery, or ablation), to prolong refractoriness so that reentry fails (with drugs that usually prolong repolarization), or both in combination. These therapies have drawbacks ranging from incomplete success to toxicity, including proarrhythmia.

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Less attention has been paid to another therapeutic approach suggested many years ago: reentry should terminate if an activating waveform persists in conducting at normal velocity, even through depolarized tissues. Therefore, we hypothesized that “improving” the efficiency of propagation through depolarized regions by increasing the maximum...
upstroke velocity ($V_{\text{up}}$) of the action potential (AP) might be antiarrhythmic.

Testing our hypotheses required (1) reviewing the literature to identify Na$^+$ channels with activation/inactivation characteristics that suggested they might increase conduction velocity at the low membrane potentials characterizing myocytes in infarct epicardial border zones (EBZs), (2) mathematically modeling ventricular AP to test whether the optimal candidate identified, the skeletal muscle Na$^+$ channel SkM1 might improve conduction,6,7 and (3) studying infarcted canine hearts in which cellular and in situ electrophysiological consequences of the interaction between SkM1 overexpression and arrhythmogenic substrate were observed. We found that SkM1 overexpression increases $V_{\text{max}}$ in depolarized tissue, reduces fragmentation of electrogastrogram activity that reflects disordered conduction in infarction, and appears to be antiarrhythmic.

Methods

Computer Models of Canine Ventricular AP

We implemented the Hund-Rudy (HR) mathematical model7 describing AP in canine ventricular myocytes and modified the model to add a parameter permitting shifting of the midpoint of the product of the Na$^+$ channel inactivation gating variables ($h_1, j_1=0.5$) along the voltage ($V_m$) axis. In addition, because our goal was to simulate the effects of a second Na$^+$ channel exhibiting different inactivation voltage dependence, we added a second Na$^+$ current given by the following:

$$I_{\text{NaI}} = g_{\text{NaI}}(m_h h_j)(V_m - E_Na),$$

where $g_{\text{NaI}}$ is the maximum conductance of the channel, m is the activation gating variable, and $h_j$ and $j_j$ are the rapid and slow, respectively, inactivation gating variables. Equations describing kinetics and voltage dependence of $h_j$ and $j_j$ were the same as those describing inactivation in the first Na$^+$ channel ($h$ and $j$), except that the midpoint of $h$ and $j$ could be set to a value independent of $h_j$. We refer to this modified model as the mHR model. Two versions were implemented: a membrane model of an isolated myocyte and a propagated model simulating 1-dimensional conduction around a 2-cm ring of tissue (see the online-only Data Supplement for details).

Intact Animal and Isolated Tissue Methods

Protocols were performed according to American Physiological Society recommendations and reviewed and approved by the Columbia University Animal Care and Use Committee.

SkM1 Adenovirus Preparation

We inserted the skeletal Na$^+$ channel SkM1 (kindly provided by Dr Gail Mandel), into the pDC516 shuttle vector (Microbix, Toronto, Canada) in 2 sections, prepared an adenovirus from this transgene using the Admax system (Microbix) and HEK293 cells, and, after plaque purification, extracted total DNA from infected cells (Qiagen, Valencia, Calif). The cDNA transgene was amplified by polymerase plaque purification, extracted total DNA from infected cells (Qiagen, Canada) in 2 sections, prepared an adenovirus from this transgene using the Admax system (Microbix) and HEK293 cells, and, after plaque purification, extracted total DNA from infected cells (Qiagen, Valencia, Calif). The cDNA transgene was amplified by polymerase chain reaction and sequenced to confirm the absence of mutations, and the titer was determined using fluorescent focus assay with mouse anti-adenovirus antisera (Advanced ImmunoChemical, Long Beach, Calif) and goat anti-mouse antisera (Santa Cruz Biotechnology, Santa Cruz, Calif).

Canine Infarct Preparation and Virus Injection

Adult male mongrel dogs (22 to 25 kg; Chestnut Ridge Kennels, Chippensburg, Pa) were anesthetized with thiopental (17 mg/kg IV) and mechanically ventilated. Anesthesia was maintained with isoflurane (1.5% to 3.0%). A left thoracotomy was performed by sterile techniques, and sites of coronary ligation was selected on the basis of the distribution of the left anterior descending artery and collateral circulation from the left circumflex.8 A bipolar platinum electrode was used to record epicardial electrograms. Adenoviral constructs expressing SkM1 and/or green fluorescent protein (GFP) (6×10⁹ fluorescent-focus units of each in 0.8 mL solution divided into 4 separate aliquots) were injected within 5 mm of one another in a supranormal flow to the left circumflex via the epicardial sites at 1- to 2-mm depth with a modified Hamilton (30-gauge) syringe. The chest was closed, lidocaine infusion (50 μg·kg⁻¹·min⁻¹) was initiated during surgery, and prophylactic lidocaine continued for 24 to 48 hours postoperatively. At 5 to 7 days of recovery, dogs were anesthetized, the heart was exposed, and ECGs and electrograms were acquired, digitized, and stored on a personal computer (EMKA Technologies, Falls Church, Va).

Induction of Ventricular Tachyarrhythmia

Pacing threshold was determined by incrementally increasing the current until ventricular capture and pacing were performed at 2 times the pacing threshold. Extrastimulus pacing with a programmable stimulator (Bloom Associates, Reading, Pa) was performed sequentially in the high septum, infarct lateral border zone, and viral injection site and within the infarction. Pacing trains began with 10 stimuli at a cycle length of 350 to 400 ms. SS was initiated at 250 ms, and S1-S2 was decreased in 10-ms steps until loss of capture. For S3 pacing, S1-S2 was set at the shortest interval with reliable S2 capture. S3 was initiated at an initial coupling interval of 100 ms and increased in 10-ms steps until S3 capture occurred. If tachyarrhythmia (VT) was ≥60 seconds, the heart was removed and prepared for microelectrode study, histology, and infarct sizing.

Microelectrode Methods

Hearts were removed and immersed in a previously described Tyrode’s solution9 equilibrated with 95% O₂/5% CO₂. Epicardial strips (~10×5×0.5 to 1 mm) were filleted parallel to the left ventricular free wall surface from sites injected with SkM1 and/or GFP, from infarcted noninjected regions, and from normal myocardium. Preparations were pinned to the bottom of a 4-mL tissue bath (epicardial surface up) and superfused (36°C, pH 7.35±0.05) at 12 mL/min. Standard techniques were used to pace the preparations at a cycle length of 500 ms and to make AP and membrane responsiveness recordings at 30 to 50 sites per preparation after 3 hours of equilibration. We have previously demonstrated that 3 hours is essential to ensure steady-state properties of the tissue in situ.9 In some experiments, the effects of tetrodotoxin (100 nmol/L) (Sigma, St Louis, Mo) were observed on $V_{\text{rep}}$ and membrane responsiveness.

Infarct Sizing

After epicardial tissues were removed for microelectrode study and histology, the heart was cooled to 4°C and cut into 1-cm-thick transverse slices from apex to base. Slices were incubated for 20 minutes in 1% triazolium red (pH 7.4 buffer at 37°C), immersed in 10% formalin for 15 minutes, and pressed between 2 glass plates to obtain uniform 1-cm thickness. Apical sides of slices were photographed, and a digital image was obtained. Planimetry (Image J Analysis 1.40g, National Institutes of Health, Bethesda) was used to determine overall infarct size. The volume of infarcted myocardium was calculated by multiplying planimetered areas by slice thickness and expressed as percentage of total left ventricular volume.

Histology and Immunochrometry

Tissue sections were snap-frozen in liquid nitrogen, and 5 μm serial sections were cut with a cryostat (Microm HM505E) and air dried. Sections were washed in PBS, blocked for 20 minutes with 10% goat serum, and incubated overnight at 4°C with anti-SkM1 antibody (1:200, Sigma-Aldrich, St Louis, Mo). Antibody bound to target antigen was detected by incubating sections for 2 hours with goat anti-mouse IgG labeled with Cy3 (red fluorescence), together with detection of GFP with a Nikon E800 fluorescence microscope.

Serial sections also were fixed in formalin and stained with hematoxylin and eosin. For peroxidase staining for GFP, the primary...
antibody was linked to peroxidase with an avidin-biotin-peroxidase kit (Vector Laboratories, Burlingame, Calif) according to the manufacturer’s recommendation. 3,3’ Diaminobenzidine was the peroxidase substrate (brown reaction product) to detect the distribution of the primary antibody bound to the target antigen.

Statistical Analysis
Data are expressed as mean±SEM. Arrhythmia incidence (the ability of ventricular pacing to induce sustained VT) in sham and SKM1-treated animals was analyzed by Fisher’s exact test. Analysis of the difference in electrogram width in control versus SKM1-injected zones was done with Student t test for independent samples. Dose-response curves were analyzed with 2-way ANOVA for nonrepeated measurements. Values of P≤0.05 were considered significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
SKM1 Is Predicted to Be More Effective Than SCN5A in Propagating Through Depolarized Tissue
AP propagation depends on the magnitude of the available Na+ conductance, which in turn depends on the voltage dependence of channel inactivation. Table 1 provides midpoints and slope factors for h_j, curves in canine myocardium for normal ventricular muscle, myocytes in the EBZ, and surviving myocytes in the center of the infarct10 and the SkM1 channel.11 The values in Table 1 are presented in the formalism of the HR model,7 in which total Na+ conductance is described by an equation of the form of Equation 1, in which recovery from inactivation is described by a gating variable j. In the experiments, steady-state inactivation was measured and referred to solely as h_j, but is equivalent to h_j, in the HR representation. Although the slope factors do not differ, the midpoints of the h_j, curves do.

To understand the consequences of the differences in availability, the values of h_j, were tabulated for each condition at −90 to −60 mV (Table 2). Comparing the most extreme examples, we see that if one added an equal amount of cardiac or skeletal muscle Na+ conductance to the cells at the center of an infarct, SkM1 would have ≈17% more availability at −90 mV and ≈9 times the availability at −60 mV. Thus, adding a given number of Na+ channels to each gene should have similar effects in fully polarized myocytes, but SkM1 should be dramatically more effective in depolarized tissue (eg, in the EBZ or center of a myocardial infarct).

Membrane Model Simulations
Simulations were performed using the mHR membrane model in which end-diastolic (resting) potential was altered by changing the extracellular K+ concentration (range, 4 to 12 mmol/L). A, V_max plotted as a function of potential. Solid squares indicate results in EBZ cells alone; open circles, effects of adding 10% more Na+ conductance (simulating effects of additional cardiac SCN5A channels); and solid circles, effects of adding 10% more Na+ conductance where the midpoint of h_j, was set to −68 mV (simulating addition of SkM1 channels). B, Relative percent increase in V_max as a result of the additional 10% Na+ conductance. Open circles indicate addition of SCN5A channels; solid circles, addition of SkM1 channels.

Table 1. Inactivation Properties of Cardiac and Skeletal Muscle Na+ Channels

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Midpoint of Curve, mV</th>
<th>Slope Factor, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal myocyte</td>
<td>−74</td>
<td>−5.3</td>
</tr>
<tr>
<td>Ischemic zone border</td>
<td>−77</td>
<td>−5.8</td>
</tr>
<tr>
<td>Ischemic zone center</td>
<td>−81</td>
<td>−5.6</td>
</tr>
<tr>
<td>Skeletal muscle (SkM1)</td>
<td>−68</td>
<td>−5.4</td>
</tr>
</tbody>
</table>

Table 2. Effects of Voltage on Inactivation in Cardiac and Skeletal Muscle Na+ Channels

<table>
<thead>
<tr>
<th>Preparation</th>
<th>At −90 mV</th>
<th>At −80 mV</th>
<th>At −70 mV</th>
<th>At −60 mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal myocyte</td>
<td>0.95</td>
<td>0.76</td>
<td>0.32</td>
<td>0.07</td>
</tr>
<tr>
<td>Ischemic zone border</td>
<td>0.90</td>
<td>0.63</td>
<td>0.23</td>
<td>0.05</td>
</tr>
<tr>
<td>Ischemic zone center</td>
<td>0.83</td>
<td>0.46</td>
<td>0.12</td>
<td>0.02</td>
</tr>
<tr>
<td>Skeletal muscle (SkM1)</td>
<td>0.98</td>
<td>0.89</td>
<td>0.59</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Figure 1. Simulations computed using the mHR membrane model in which end-diastolic (resting) potential was altered by changing the extracellular K+ concentration (range, 4 to 12 mmol/L). A, V_max plotted as a function of potential. Solid squares indicate results in EBZ cells alone; open circles, effects of adding 10% more Na+ conductance (simulating effects of additional cardiac SCN5A channels); and solid circles, effects of adding 10% more Na+ conductance where the midpoint of h_j, was set to −68 mV (simulating addition of SkM1 channels). B, Relative percent increase in V_max as a result of the additional 10% Na+ conductance. Open circles indicate addition of SCN5A channels; solid circles, addition of SkM1 channels.
required to achieve propagation becomes increasingly greater than the amount of SkM1 channel conductance as the membrane potential depolarizes. This demonstrates the potential advantages of a more depolarized h_{Na} curve (midpoint of -68 mV) in restoring failed conduction.

Figure 2B shows the corresponding conduction velocities. At depolarized potentials, dramatically smaller amounts of additional SkM1 conductance are required to generate the same conduction velocities; an amount only slightly higher than the native conductance (1.1 x g_{Na}) suffices to restore conduction at the highly depolarized potential of -60 mV. It is also notable that successful propagation of an AP to 1 cm results in a minimum conduction velocity of ~27 cm/s; conduction fails altogether at slower velocities.

Given the results of the membrane model and propagation simulations, it seemed reasonable to test the effects of SkM1 gene delivery on V_{max} and electrogram characteristics, reflecting conduction, in the myocardial infarct EBZ. The next section reports such experiments.

**Studies in the Canine Model**

We administered SkM1/GFP-expressing adenovirus subepicardially into EBZ regions of 12 dogs with left anterior descending artery occlusion and GFP-expressing adenovirus into EBZ of 9 shams with left anterior descending artery occlusion. One sham died of intractable VT at 1.5 days after surgery and is not included in the analysis. Animals were studied 5 to 7 days after infarction and adenovirus injection.

**Incidence of Inducible Arrhythmias**

Using programmed electric stimulation (see Methods), sustained VT was induced in 6 of 8 shams and 2 of 12 SkM1-administered dogs (P=0.02). VT was polymorphic except for in 1 sham, in which it was monomorphic. Occasional ventricular premature depolarizations and/or nonsustained VT were inducible in 7 SkM1 dogs and 2 shams.

**Electrogram and Cellular Electrophysiological Studies**

We recorded local EBZ electrograms at 9 to 13 sites per heart. Electrograms were significantly broader and more fragmented in shams than SkM1 (Table 3 and Figure 3). No differences were detected among electrograms recorded from normal myocardium. The preservation of normal local electrograms in EBZs expressing SkM1 suggested a decrease in activation dispersion. No difference was found between sham and SkM1 effective refractory period (Table 3).

In Figure 3A (representative experiment), site 1 is an SkM1-injected EBZ. The electrogram is narrow, similar to noninfarcted regions (sites 3 and 4). In contrast, the noninjected EBZ electrogram (site 2) is broad and fragmented.

In microelectrode studies, membrane potential is equivalent at sites 1 and 2 (Figure 3B), but site 1 tissue (SkM1) shows V_{max} nearly 2 times that of site 2 (no SkM1). Hema-toxylin and eosin staining of both sites is consistent with infarction (Figure 3B), whereas the insets show that site 1 is GFP positive (peroxidase stain) and site 2 is GFP negative. Figure 3C (left) demonstrates that V_{max} is increasingly greater than membrane potential depolarizes in the SkM1 population.
Dependence of the High $V_{\text{max}}$ and Membrane Responsiveness in SkM1-Injected Regions on the SkM1 Channel

We questioned whether the higher $V_{\text{max}}$ and membrane responsiveness in individual cells (Figure 3) and the more organized regional activation (narrow, spiked electrograms) in the SkM1 derive from functional SkM1 channels. SkM1 current should be reduced by 100 nmol/L tetrodotoxin, whereas that of native SCN5A channels should be minimally affected.12 Figure 4 demonstrates differential tetrodotoxin sensitivity; SkM1-treated sites showed higher $V_{\text{max}}$ (especially at depolarized potentials) and a significant response to tetrodotoxin not seen in non–SkM1-treated tissues. Hence, the tetrodotoxin response of the SkM1 site is consistent with an SkM1 channel and that of the non-SkM1 site is consistent with SCN5A. Figure 4E shows that the adenovirus and GFP accompanying SkM1 do not affect membrane responsiveness. In addition, no effect was found of GFP virus alone on $V_{\text{max}}$ in multiple cell impalments (data not shown). Hence, the SkM1 construct rather than the associated virus or GFP induced the effects on $V_{\text{max}}$ reported here.

Infarct Size and Histology

No differences were seen in infarct size between the sham (30±2.8%) and SkM1 (30±2.6%) groups ($P=0.86$). Immunohistochemistry (Figure 5) demonstrates that in an animal receiving only GFP, positive GFP fluorescence is found but no SkM1 fluorescence, whereas an animal receiving SkM1+GFP shows GFP staining and SkM1 fluorescence.

Discussion

We have performed in silico experiments predicting that SkM1 would have a specific effect on Na$^+$ current and identified the antiarrhythmic, electrophysiological, and biochemical outcomes of SkM1 administration in the canine heart. Our results suggest that SkM1 delivered locally to sites of slow/fragmented conduction fostering reentry may have beneficial effects on activation and rhythm.

Speeding Conduction as an Antiarrhythmic Intervention

More than 100 years ago, Mayer13 described circulating excitation in Scyphomedusae, paving the way for our understanding of reentry. Soon thereafter, Mines2,3 and Garrey14 used vertebrate hearts to expand on Mayer’s experiments. Mines characterized reentry in terms of path length, conduction velocity, and refractoriness, applying this knowledge to paroxysmal arrhythmias in human subjects. Mines’ concepts were later tied to the pathological anatomy of the heart by Schmitt and Erlanger.4

This early work suggested that interventions permitting an activating waveform to proceed at a normal velocity and/or to encroach on the refractory tail in a reentrant pathway would result in failure of that waveform to propagate further. Attempts to speed propagation have since included catecholamines to hyperpolarize cells and increase $V_{\text{max}}$,15 connexin overexpression (largely in tissue culture) to improve gap junctional conductance,16 and antiarrhythmic drugs to accelerate repolarization (eg, lidocaine, mexiletine). With antiarrhythmic drugs, improved conduction results from premature impulses arising at more negative membrane potentials than would otherwise be the case.

These approaches, although improving conduction, have limitations. Catecholamines are arrhythmogenic,1,15 Connexin overexpression can increase gap junctional conductance, but the activating waveform is still required as the driving force for the cardiac impulse (If the membrane potential is depolarized and $V_{\text{max}}$ is low, the signal will continue to propagate slowly). As for antiarrhythmic drugs that accelerate repolarization, lidocaine is used during acute phases of some myocardial infarcts, and mexiletine is of limited potential in long-term postinfarction settings.5

Gene Therapy to Overexpress an Na$^+$ Channel to Improve Conduction

A potential advantage of gene therapy over the approaches reviewed above is that we are not limited to interventions

<table>
<thead>
<tr>
<th>Site</th>
<th>EBZ Normal</th>
<th>EBZ Normal</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>In vivo: EG duration, ms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV anterior</td>
<td>32±2.3</td>
<td>23±2.8</td>
<td>0.03</td>
</tr>
<tr>
<td>LV basal</td>
<td>19±1.1</td>
<td>18±0.9</td>
<td>0.25</td>
</tr>
<tr>
<td>RV anterior</td>
<td>18±0.9</td>
<td>20±0.8</td>
<td>0.12</td>
</tr>
<tr>
<td>In vivo: ERP, ms</td>
<td>159±3.4</td>
<td>153±4.8</td>
<td>0.42</td>
</tr>
<tr>
<td>144±5.2</td>
<td>146±5.6</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>In vitro: APs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDP, −mV</td>
<td>78±1</td>
<td>76±1</td>
<td>0.19</td>
</tr>
<tr>
<td>$V_{\text{max}}$, mV/ s</td>
<td>179±14</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>APD30, ms</td>
<td>40±5</td>
<td>39±3</td>
<td>0.85</td>
</tr>
<tr>
<td>APD50, ms</td>
<td>59±7</td>
<td>59±5</td>
<td>1.00</td>
</tr>
<tr>
<td>APD90, ms</td>
<td>103±12</td>
<td>114±6</td>
<td>0.38</td>
</tr>
</tbody>
</table>

**Table 3. In Vivo and In Vitro Electrophysiological Characteristics**

EG indicates electrogram; LV, left ventricular; RV, right ventricular; ERP, effective refractory period; MDP, maximum diastolic potential; and APD, AP duration. EG recordings were done in sinus rhythm (cycle length: sham, 576±23 ms, n=8; SkM1, 535±18 ms, n=12; $P=0.17$). In ERP recordings, pacing was from basal LV anterior wall epicardium for the normal zone and infarct epicardium for the EBZ. S1S1=350 to 400 ms, n=7 sham; n=11 SkM1. For APs, mean values were calculated in 2 stages. First, we calculated individual means from 20 to 40 impalements for each dog. Then, from individual means, we calculated mean±SEM for 2 groups (n=8 and 12). Thus, the "weight" of data from each dog was the same.

The right side shows membrane responsiveness curves for a subset of the same cells. At the more positive potentials, a greater $V_{\text{max}}$ is found for the SkM1 site. This same result was seen for all animals (data not shown).

Although $V_{\text{max}}$ was significantly greater in SkM1 than sham tissues, maximum diastolic potentials for all SkM1-injected and non–SkM1-injected EBZs did not differ (Table 3). Moreover, SkM1 did not alter AP duration (Table 3), and afterdepolarizations were never seen.

The effectiveness of gene therapy for heart disease is well documented, with a number of potential advantages over the currently available approaches. SkM1, which is known to be selectively expressed in skeletal muscle, offers the advantage of being a high-kinetic gain Na$^+$ channel that can increase conduction and decrease repolarization, which is critical for preventing reentry. Moreover, SkM1 is not normally found in the heart, making it an ideal target for gene therapy. In conclusion, our findings support the potential use of SkM1 gene therapy for the treatment of atrial fibrillation.
targeting the channels and transporters expressed by native cardiac myocytes. Instead, channels resident in other tissues or man-made mutants or chimeras with more favorable biophysical properties can be used. Such a unique arsenal of antiarrhythmic tools allows a “rational” approach to antiarrhythmic therapy in which the biophysical properties of an ideal therapeutic agent are defined, synthesized, and delivered. This is not to say that prior attempts made with antiarrhythmic drugs were irrational but rather that the pharmacological tools available did not have the selectivity, specificity, and potential for targeting that gene therapy offers.

Because conduction velocity of the cardiac impulse depends on the magnitude of the Na⁺ current elicited by a stimulus and because this depends on both the number of Na⁺ channels in the membrane and their availability to be opened by depolarization, we selected the Na⁺ channel as a therapeutic intervention. We understood that conduction abnormalities in the 5- to 7-day EBZ are multifactorial in cause and have been attributed to remodeling of I_{Na} and I_{Ca,L} and to altered gap junctional coupling; we also understood that our proposed intervention targets only 1 factor, I_{Na}. However, upregulating I_{Na} might overcome other forms of remodeling, eg, by counteracting the effects of reduced gap junctional coupling to slow conduction.

In seeking noncardiac mammalian Na⁺ channels that might have more favorable properties than the cardiac Na⁺ channel, we focused on 2 situations favoring slow conduction: membrane depolarization and high-frequency stimulation. In both cases, the cell membrane is depolarized but rather that the pharmacological tools available did not have the selectivity, specificity, and potential for targeting that gene therapy offers.

Figure 3. Effect of SkM1 adenoviral infection of epicardial site. The canine heart was infarcted and injected with SkM1 + GFP adenovirus 7 days earlier. A, Left ventricular epicardial surface. Each panel displays a surface ECG (top) and a bipolar local electrogram (bottom). Broken line demarcates infarcted (bottom) from noninfarcted (top) myocardium. The electrogram in noninfected infarcted site 2 is markedly fragmented; infarcted site 1 (SkM1 injected) shows a normal electrogram, as do noninfarcted sites 3 and 4. B, Hematoxylin and eosin staining of tissues from sites 2 (no SkM1) and 1 (with SkM1) shows infarcted myocardium (×100). Inset in site 1 is GFP positive; that in site 2 is GFP negative (×400). Representative AP recorded from site 1 has higher V_{max} and amplitude than that from site 2. C, Left, Multiple impalements from SkM1-injected (red) and noninjected (black) zones show higher V_{max} in the AP from SkM1-injected sites (P<0.05). The same is true for membrane responsiveness curves (C, right; P<0.05).
We validated our hypothesis that Na⁺ channels with more favorable biophysical properties than SCN5A might be a useful antiarrhythmic therapy in silico with a model of canine ventricle and in vivo with viral delivery into the EBZ of a canine infarct. As suspected, in silico addition or substitution of the inactivation voltage dependence of SkM1 increased $V_{\max}$ and preserved fast conduction in depolarized tissue far more effectively than overexpressing SCN5A. Experimentally delivering SkM1 to the EBZ of an infarct increased $V_{\max}$ and decreased the inducibility of arrhythmias, validating our hypothesis in this experimental model.

Although our results are promising, the gene therapy of VT/ventricular fibrillation offers new challenges. For example, whereas infarcts can be thought of as requiring local therapy, variations in anatomy among patients require extensive mapping to determine sites at which to localize therapy.

**Figure 4.** Use of 100 nmol/L tetrodotoxin to discriminate SkM1 channels from SCN5A channels in canine infarct 7 days after SkM1/GFP adenoviral injection. A and B, Multiple impalements in noninjected (A) and SkM1-injected (B) EBZ before and after tetrodotoxin. C and D, Membrane responsiveness curves in the same 2 regions before and after tetrodotoxin. E, Membrane responsiveness from EBZ of region injected with GFP-only adenovirus (no SKM1) vs noninjected. Results are expressed as mean±SE for 30 to 40 impalements in A and B and 5 to 6 cells for each membrane responsiveness curve in B, C, and E. *P<0.05 vs other curve (2-way ANOVA for nonrepeated measures).

**Figure 5.** Immunohistochemical staining for GFP and SkM1 in 2 dogs. Top, GFP adenovirus only. Bottom, SkM1+GFP adenoviruses. Left, DAPI staining for nuclei. Center, GFP immunostaining reveals the presence of GFP in both dogs. Right, SkM1 immunostaining occurs only in the dog receiving SkM1 adenovirus.
Clinical Applicability

We studied VT/ventricular fibrillation in dogs 5 to 7 days after infarction, a model relevant to the initial postinfarction period but not the chronic infarct. The Defibrillator in Acute Myocardial Infarction Trial (DINAMIT)\(^2\) considered primary prevention of ventricular arrhythmias with implantable converter-defibrillators in patients 6 to 40 days after infarction and concluded that prophylactic implantable converter-defibrillator therapy was associated with a reduced rate of arrhythmic death that was offset by increased deaths from nonarrhythmic causes. Even if no antiarrhythmic efficacy had been seen in DINAMIT, the trial is only tangentially applicable to our own study; DINAMIT delivered a shock to terminate an arrhythmia in patients, whereas we injected a viral construct to prevent VT from occurring or to limit its duration in experimental animals. In any event, the proof-of-concept data in our study require more investigation and validation before application to human populations can be considered.

Study Limitations

We questioned whether the SkM1 intervention might be proarrhythmic. In silico, simulated membrane and propagated AP demonstrated neither afterdepolarizations nor prolonged repolarization, suggesting the absence of proarrhythmic potential. We did not simulate high stimulation frequencies or intercalated beats in which the differences in time course of recovery from inactivation would be relevant, so a definitive statement on proarrhythmic potential cannot be made for these conditions. Such simulations would require characterizing the kinetics of inactivation (derived from patch-clamp measurements in myocytes overexpressing SkM1) to confirm that the more rapid recovery from inactivation of the channel is preserved when it is overexpressed in cardiac tissue.

We also considered whether overexpression of SkM1 might interfere with Ca\(^{2+}\) kinetics to be proarrhythmic. Here, we performed simulations with the mHR model, which includes intracellular ion concentrations and Ca\(^{2+}\) cycling. At 1 Hz, overexpression by 100% of an Na\(^+\) channel with SkM1 properties increased \(V_{\text{max}}\) from 285 to 486 V/s. However intracellular Na\(^+\) concentration increased by only 6% (from 13.6 to 14.4 mmol/L), diastolic intracellular Ca\(^{2+}\) concentration increased by only 5% (134 to 141 nmol/L), and peak systolic Ca\(^{2+}\) concentration increased by 21% (679 to 817 nmol/L). Thus, no major change in internal Na\(^+\) or Ca\(^{2+}\) concentrations resulted. This is the expected outcome because when Na\(^+\) conductance is doubled, the AP attains peak amplitude much more quickly. An estimate of Na\(^+\) entry can be obtained from \(Q = CV\), where \(Q\) is charge, \(C\) is capacitance, and \(V\) is voltage across the capacitance. \(V\) (AP amplitude) was increased by 15% in this simulation. Note that other than \(V_{\text{max}}\), AP characteristics in SkM1-administered animals were not significantly altered (Figure 3 and Table 3), and we never saw afterdepolarizations.

Another possibility with regard to proarrhythmia is that SkM1 might restore function and slow conduction in a path in which depolarized fibers were previously silent, and we cannot state with certainty that this might not happen. The same polymorphic pattern seen in shams was seen in the 2 SkM1-treated dogs with VT. We believe it is likely that the site of SkM1 injection was not importantly involved in the reentrant circuit in these 2 animals.

Finally, if proarrhythmia were to emerge, the brief life of the adenovirus would limit the persistence of SkM1 expression. In addition, as noted by Marban et al,\(^2\) in the event of proarrhythmia, local gene therapy site(s) could be ablated.

Conclusions

Using literature review and modeling, we identified an ion channel construct with activation/inactivation properties that suggested the enabling of acceleration of conduction in depolarized tissues overlying myocardial infarcts. Studies in a canine infarct model demonstrated antiarrhythmic activity. Although possible proarrhythmic activity must be explored further, the facts that the greatest increase in \(V_{\text{max}}\) occurs at depolarized potentials and that little or no change is seen in normally polarized fibers suggest that the function of the construct to alter conduction will be limited largely to depolarized regions. This finding validates the concept of Mines\(^2\) that speeding conduction can be used as an antiarrhythmic intervention and represents the first demonstration that this outcome can be effected via manipulation of ion channels as opposed to (or as a complement to) connexins.

Acknowledgments

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Disclosures

None.

References


**CLINICAL PERSPECTIVE**

Reentry accounts for ≈85% of serious tachyarrhythmias complicating ischemic heart disease. Current antiarrhythmic therapies attempt to prevent or treat these arrhythmias by creating bidirectional conduction block, prolonging refractoriness, or both in combination. Implantable defibrillators have had great success in terminating sustained ventricular tachyarrhythmias. However, these therapies possess drawbacks ranging from incomplete arrhythmia suppression to overt toxicity, including proarrhythmia, and the psychological consequence of painful and sometimes inappropriate defibrillation.

A key contributor to many reentrant arrhythmias is slow conduction within part of the reentrant circuit such as a depolarized surviving myocytes within the border zone; would be antiarrhythmic. We focally introduced an adenovirus expressing a skeletal muscle sodium channel. *J Gen Physiol*. 1989;3:33–49. 

The sodium channel β1 subunit in rat skeletal muscle is selectively associated expressed by cardiomyocytes but allow delivery of foreign channels with more favorable biophysical properties.
Epicardial Border Zone Overexpression of Skeletal Muscle Sodium Channel SkM1 Normalizes Activation, Preserves Conduction, and Suppresses Ventricular Arrhythmia: An In Silico, In Vivo, In Vitro Study

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Computer models of canine ventricular action potentials

The mHR model is described by 30 ordinary differential equations (ODEs): one equation describing $V_m$, six equations describing sarcoplasmic and sarcoplasmic-reticulum ion concentrations and 23 equations describing channel gating. Unless otherwise noted, all equations and parameter values are identical to those in the HR model\(^1\) (note that variable initial conditions can be found online at rudylab.wustl.edu).

All computations were done using the MATLAB\(^\circ\) mathematical programming environment (see www.mathworks.com). Code was written that preserved accuracy and avoided computational singularities, cf., in the evaluation of $\frac{z}{(e^z - 1)}$ when $|z| \approx 0$.

Two programs were written: one simulating an isolated myocyte (membrane model), and one simulating a one-dimensional ring of ventricular tissue (propagated model).

Numerical solution of the membrane model

The mHR-model ODEs are stiff, with time constants of $I_{Na}$ being <1 ms, while time constants of changes in sarcoplasmic concentrations being >10 s. Integration of the equations was performed using the MATLAB\(^\circ\) library routine ode15s, a program that excels in solving stiff initial-value problems. The program is an adaptive algorithm that automatically adjusts integration time increments to maintain a relative tolerance of better than $10^{-3}$, or an absolute tolerance of better than $10^{-6}$.
Action potentials were elicited by imposing a brief (typically 0.5-ms) depolarizing current pulse (typically \(-100 \mu A/\mu F\)) at time \(t = 0\). After any change in a model parameter or condition, the model was “paced” (1 Hz) to a steady state, whereby equation values following a pacing cycle were supplied as initial conditions to the next cycle. Pacing to a steady state, as assessed by successive identical voltage traces, typically required between 40 and 100 cycles.

Supplement Figure 1 shows membrane model action potentials comparing the HR and mHR models, with action potentials elicited by a \(-80 \mu A/cm^2\) current pulse of 1 ms duration. The action potentials superimpose. The midpoint of \(h_n, j_x\) in the HR model occurs at \(-70.3\) mV, while in the mHR model, the midpoint was shifted to \(-74.0\) mV, a value more typical for a normal ventricular myocyte (see Table 1A). The two plots superimpose when the maximum Na\(^+\) conductance \(g_{Na}\) was increased by 15% from 8.25 mS/\(\mu F\) (HR) to 9.49 mS/\(\mu F\) (mHR), reflecting the fact that the 3.7-mV left-shift in the \(h_n, j_x\) curve resulted in slightly less inward Na\(^+\) current. In both models, \(V_{max}\) was 193 V/s, a value less than is seen experimentally where typical values are closer to \(-300\) V/s. As such, in subsequent simulations using the mHR model, we increased \(g_{Na}\) by and additional 50% to 14.2 mS/\(\mu F\), which produced a \(V_{max}\) of 289 V/s. Finally, the inset of Figure 1 compares the two superimposed action potentials quantitatively (see legend for description of the symbols).

Numerical solution of the propagated model
To determine conduction velocity and to investigate action-potential shape and duration of a propagating action potential, the 30 ODEs describing the membrane action potential were inserted into the cable equation, which resulted in a partial differential equation describing membrane potential as a function of time ($t$) and one-dimensional space ($x$):

$$\frac{\partial V_m}{\partial t} = \frac{1}{S_vR_aC_m} \frac{\partial^2 V_m}{\partial x^2} - \frac{I_m(x)}{C_m}. \quad (1)$$

$S_v$ is the cell surface-to-volume ratio and was set to 4037 cm²/cm³, the ratio of capacitive membrane area ($A_{cap}$) to cell volume ($V_{cell}$) reported for the HR model. $R_a$ is the axial resistance and was set to 280 Ωcm, a value intermediated between values measured in different myocardial preparations ranging from 180 Ωcm to 470 Ωcm.²-⁴ Note that $R_a$ reflects not only the sarcoplasmic resistivity (~100 Ωcm), but also the resistance through gap junctions. $C_m$ is the cell membrane capacitance, and $I_m$ is the total membrane ionic current generated by the membrane action potential. Although the model computes values for some sarcoplasmic ion concentrations, it was assumed that the axial currents during a propagating action potential result in negligible changes in intracellular solute composition.

The geometry of the interconnected fibers used in our solution of Supplement equation (1) consists of a closed one-dimensional ring of electrically coupled cells with circumference $\ell$, and this results in the (Dirichlet) boundary condition $V(x=0) = V(x=\ell)$. The ring is broken into discrete segments (nodes), each with length significantly less than the resting length constant. We used 101 nodes of length $\Delta x = 0.02$ cm, for a total circumference $\ell = 2.02$ cm. The membrane action potential in each node is described
by the 30 ODEs discussed above, resulting in a total of $101 \times 30 = 3030$ equations that must be integrated.

Integration of Supplement equation (1) was done using Strang splitting\textsuperscript{6}, an algorithm that integrates the diffusion equation using the Crank-Nicolson method\textsuperscript{5}, coupled with a two-point Runge-Kutta algorithm\textsuperscript{5} for integrating the membrane model at each node. The combined algorithm is “second order” both in time and space. We verified the accuracy of the algorithm by comparing results computed with $\Delta t = 0.005$ ms with the same integration with $\Delta t = 0.002$ ms. To improve computational speed, we pre-computed a look-up table for all model equations that were time independent ($V_m$ resolution of 0.001 mV).

Initial conditions for each of the 101 nodes of the ring were set to values determined by pacing the membrane model to steady state. Action potentials were initiated at time $t = 0$ using a brief (typically 0.5 ms) depolarizing current pulse (typically $-200 \, \mu A/\mu F$) applied simultaneously at nodes 1 and 101. This produced two propagating action potentials in the ring: one propagating in the clockwise direction from nodes 1 to 50 (0 to 1 cm), and the other propagating in the counterclockwise direction from nodes 101 to 50 (2.02 to 1 cm).

Supplement Figure 2 shows propagating action potentials (midpoint of $h, j_e$ at $-74.0$ mV and $g_{Na}$ equal to $14.2$ mS/$\mu F$) where $V_m(x)$ is plotted as a function of time at the following distances ($x$): 0, 0.2, 0.4, 0.6, 0.8 and 1.0 cm (left to right, respectively). The action potential at 0 cm (leftmost trace) is distorted by the depolarizing current pulse. The action potential at 1.0 cm (rightmost trace) is distorted due to the “collision” of the two action potentials arriving from the clockwise and counterclockwise directions;
the resulting larger axial current thus elicits a larger inward current. At distances between 0.2 cm and 0.8 cm (and between 1.8 cm and 1.2 cm, not shown), the action potentials appear nearly identical in shape, reflecting uniform conduction at these regions of the tissue ring. This is also seen in the inset to Supplement Figure 2, which shows the “foot” of the propagating action potential at 0.6 cm plotted in an expanded time scale; one observes the expected exponentially rising potential as the axial current charges $C_m$. Conduction velocity was computed from the difference of the times at peak amplitude measured at 0.4 cm and 0.6 cm, and equals 46.0 cm/s.

References


Figure Legends:

Figure 1. Two steady-state action potentials (paced at 1 Hz) comparing the HR and mHR models. The two plots superimpose. Inset: $APD_{90}$ action-potential onset time to 90% repolarization; $APO$, overshoot potential; $APA$, action-potential amplitude; $EDP$, end-diastolic potential; $V_{\text{max}}$, maximum upstroke velocity; $h_{\text{midpt.}}$, potential where $h_{\infty}, j_{\infty}$ equals 0.5; $\bar{g}_{Na}$, maximum Na$^+$ conductance.

Figure 2. Propagating action potentials in mHR model showing $V_m(x)$ at distances 0, 0.2, 0.4, 0.6, 0.8 and 1.0 cm (left to right). The action potentials are elicited by a brief depolarizing current pulse administered at nodes 1 and 101 (0 and 2.02 cm) at time $t = 0$. Inset: action potential at 0.6 cm plotted on an expanded time scale (10–15 ms). Conduction velocity is 46.0 cm/s.
Supplement Figure 1

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Supplement Figure 2