Cae

catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited syndrome characterized by adrenergically mediated ventricular tachycardias, often resulting in syncope and sudden cardiac death. First described more than 20 years ago by Coumel, recent genetic studies have demonstrated that mutations in the ryanodine receptor (RyR2) and calsequestrin (CASQ2) genes underlie some cases of this syndrome, implicating aberrant control of Ca²⁺ homeostasis in the molecular pathogenesis. The specialized cardiac conduction system comprises a heterogeneous network of cells that orchestrate the initiation and propagation of a wave of electric excitation throughout the myocardium. Purkinje cells (PCs) are the most distal component of the cardiac conduction system, and they deliver
the depolarizing impulse to the working myocytes of the ventricular myocardium. More than 40 years ago, Hoffman and colleagues proposed that PCs might “be a significant factor in human arrhythmias.” Since that time, substantial, but somewhat indirect, experimental data have accumulated supporting the concept that PCs play a key mechanistic role triggering a broad range of life-threatening ventricular arrhythmias. Indeed, recent optical mapping data of RyR2R4496C/− mutant mice, which to a remarkable extent phenocopy human CPVT, support the hypothesis that Ca2+-dependent arrhythmic triggers may be arising from the Purkinje fiber network.17

A mechanistic basis for the proarrhythmic behavior of PCs is slowly emerging. Underlying ultrastructural features, such as a decreased T-tubular density,8 the presence of IP3-sensitive Ca2+ channels,9 and susceptibility to Ca2+ overload attributable to greater Na+ load,10 may, in part, account for the propensity of these specialized cells to develop abnormal Ca2+-release events. Moreover, recent data suggest that higher diastolic Ca voltage coupling gain in Purkinje fibers may underlie the generation delayed afterdepolarizations (DADs) and triggered beats.11

In addition to these cell-autonomous proarrhythmic properties, the macroscopic structural features of the specialized ventricular conduction system network may also contribute to the enhanced capacity of PCs to trigger arrhythmias. Whereas electric loading will normally prevent spontaneous Ca2+ release events (SCaEs) in ventricular myocytes (VMs) from evoking propagated action potentials, the same is not true of events arising within PCs. Electrotonic interactions between PCs and the underlying myocardium at Purkinje–ventricular junctions are limited, such that stochastic Ca2+ release events in PCs may trigger APs that successfully propagate into working myocytes.12–14 Moreover, propagation across the Purkinje–ventricular junction is highly sensitive to the extent of coupling, as evidenced by a variety of experimental approaches, including computational studies and genetically engineered mouse model systems.12,15

As a consequence of these multiscale mechanisms, when challenged with stressors that promote electric remodeling or changes in intracellular Ca2+ handling, such as mutations in Ca2+-handling proteins as seen in CPVT, PCs may be preferentially “primed” to develop arrhythmic triggers.16–22

Through a transcriptional screen, our laboratory recently identified a novel cell adhesion molecule named Cntn2, which is specifically expressed in cells of the specialized cardiac conduction system, including the distal Purkinje fiber network.23 Moreover, we demonstrated that genetically engineered mice harboring a Cntn2-EGFP BAC transgene faithfully recapitulated expression of the endogenous protein, thereby providing a tool to specifically identify and distinguish PCs from working SCaEs. Interestingly, in our initial study, we found that even wild-type PCs were more arrhythmogenic than VMs, with more frequent unstimulated Ca2+-release events, DADs, and triggered beats. Using this same approach, we now have examined intracellular Ca2+ dynamics in dissociated adult PCs and VMs from both wild-type and RyR2R4496C/− mutant mice, at baseline and following catecholaminergic stimulation, as might be seen during arrhythmic episodes in CPVT. We also determined the antiarrhythm-
Kinetic Properties in PCs and VMs

Fluorescence photometry with the calcium-sensitive dye x-Rhod-1 was used to study intracellular Ca\(^{2+}\) dynamics (Figure 2). Confirming our previous observation, wild-type PCs displayed significantly slower kinetics of activation and relaxation compared to VMs, with rise times of 117±6.6 (n=8) versus 68±2.3 ms (n=10); P<0.00001; and \(\tau_{\text{decay}}\) of 225±24 (n=8) versus 155±10 ms (n=10); P<0.01. A similar large difference in kinetics was observed comparing the RyR\(^{R4496C/+}\) mutant PCs and mutant myocytes, with rise times of 129±6.5 (n=10) versus 71±5.4 ms (n=10); P<0.000002; and \(\tau_{\text{decay}}\) of 312±23 (n=10) versus 201±15 ms (n=10); P<0.001. Interestingly, the \(\tau_{\text{decay}}\) in the mutant PCs was significantly longer than in wild-type PCs (P<0.01).

SCaEs in RyR\(^{R4496C/+}\) PCs

We next determined the propensity of VMs and PCs to develop SCaEs following field stimulation at a range of...
pacing frequencies. We first examined wild-type VMs and PCs. As expected, wild-type VMs were quite stable; none of 15 cells studies developed SCaEs at 1 Hz, and only 1 cell displayed unpaced SCaEs after pacing at 3 or 5 Hz. Wild-type PCs had a greater tendency to develop unpaced SCaEs compared to the VMs, especially following higher pacing frequencies (3 of 10 cells at 3 and 5 Hz), as shown in Figure 3. We next examined the behavior of cells from RyRR4496C/H11001 mutant mice. Mutant VMs and mutant PCs were more arrhythmogenic than their wild-type counterparts, both with respect to the proportion of cells displaying SCaEs and the number of SCaEs per cell. This was especially the case for the RyR2R4496C mutant PCs, with as many as 62% of cells studied showing arrhythmic activity, as shown in Figure 3 and summarized in Table 1.

### Increased Sensitivity to Catecholaminergic Stimulation

Inasmuch as arrhythmic behavior in patients and animal models of CPVT is provoked during catecholaminergic stimulation, the effects of isoproterenol treatment (30 nmol/L) on intracellular Ca²⁺ dynamics in wild-type and mutant VMs and PCs was assessed. When paced at 1 Hz, wild-type VMs, wild-type PCs, and mutant VMs all showed modest increases in the proportion of cells that developed SCaEs compared to untreated controls, as shown in Figure 4 and Table 1. However, the effect was most profound in mutant PCs; almost all (90%; 9 of 10) developed arrhythmogenic Ca²⁺ activity. Moreover, the quantity of SCaEs per cell was significantly greater for the RyR2R4496C PCs (12.2 ± 1.5/cell) compared to RyR2R4496C VMs (1.1 ± 0.48/cell). Furthermore, even at the slow pacing frequency of 1 Hz, the PCs developed marked abnormalities in intracellular Ca²⁺ handling, including oscillations, alternans, and sustained salvos of presumed triggered beats following the end of the pacing train (Figure 4D through 4G). To confirm that these abnormalities in calcium regulation were indeed sufficient to trigger action potentials, we performed simultaneous Ca²⁺ imaging and recordings of transmembrane potentials in whole-cell current-clamp mode. These studies indeed demonstrated that SCaEs could successfully trigger action potentials in the mutant PCs (Figure 4H). Thus, the RyR2R4496C mutant PCs appeared to be especially sensitive to the proarrhythmic effects of catecholaminergic stimulation.

### Preferential Suppression of SCaEs in Mutant PCs

The antiarrhythmic agent flecainide has shown efficacy in preventing ventricular arrhythmias in mice and humans with Casq2-associated CPVT. We therefore tested the effects of flecainide on the magnitude of SCaEs in VMs and PCs. In agreement with previous studies, the modest arrhythmic activity observed in wild-type VMs was diminished with flecainide, both with respect to the number of cells developing SCaEs and the number of SCaEs per cell. We also

### Table 1. SCaEs in VMs and PCs

<table>
<thead>
<tr>
<th></th>
<th>Cells With SCaEs</th>
<th>SCaEs per Cell</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Without ISO</td>
<td>With ISO</td>
<td>Without ISO</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 Hz</td>
<td>3 Hz</td>
<td>5 Hz</td>
<td>1 Hz</td>
</tr>
<tr>
<td>WT VM</td>
<td>0% (15)</td>
<td>7% (15)</td>
<td>7% (15)</td>
<td>14% (7)</td>
</tr>
<tr>
<td>WT PC</td>
<td>10% (10)</td>
<td>30% (10)</td>
<td>30% (10)</td>
<td>57% (7)</td>
</tr>
<tr>
<td>MUT VM</td>
<td>22% (41)</td>
<td>24% (41)</td>
<td>29% (41)</td>
<td>40% (10)</td>
</tr>
<tr>
<td>MUT PC</td>
<td>52% (29)</td>
<td>62% (29)</td>
<td>62% (29)</td>
<td>90% (10)</td>
</tr>
<tr>
<td></td>
<td>0±0 (15)</td>
<td>0.07±0.07 (15)</td>
<td>0.07±0.07 (15)</td>
<td>0.14±0.14 (7)</td>
</tr>
<tr>
<td></td>
<td>0.10±0.10 (10)</td>
<td>0.40±0.22 (10)</td>
<td>0.40±0.22 (10)</td>
<td>3.00±1.38 (7)</td>
</tr>
<tr>
<td></td>
<td>0.29±0.09 (41)</td>
<td>0.37±0.11 (41)</td>
<td>0.34±0.09 (41)</td>
<td>1.10±0.48 (10)</td>
</tr>
<tr>
<td></td>
<td>1.07±0.32 (29)</td>
<td>1.72±0.36 (29)</td>
<td>1.65±0.36 (29)</td>
<td>12.2±1.48 (10)</td>
</tr>
</tbody>
</table>

Data are shown as percentages of cells with SCaEs and the means±SEM SCaEs/cell at each pacing frequency. The no. of cells studied for each condition is shown in parentheses. ISO indicates isoproterenol; MUT, mutant; WT, wild type.
observed efficacy in wild-type PCs from these same hearts, as shown in Figure 5 and Table 2. As expected, cells from RyR2R4496C/H11001 mutant mice were substantially more arrhythmic at baseline than their wild-type counterparts. Surprisingly, however, the mutant VMs appeared resistant to the antiarrhythmic effects of flecainide, whereas the mutant PCs showed a significant reduction in the number of cells with SCaEs, as well as a reduction in SCaEs per cell, as shown in Figure 5B and 5D. We also tested the effects of tetracaine, which preferentially reduces RyR2 open probability.28 In contrast to flecainide, this agent suppressed SCaEs in mutant PCs and VMs with equal efficacy, as shown in Figure 5E and 5F. These data are summarized in Table 2.

**Discussion**

Recent data suggest that SR Ca\(^{2+}\)-release channels comprised of the CPVT-causing RyR2R4496C mutant protein display increased sensitivity to both cytosolic and luminal Ca\(^{2+}\), predisposing them to the development of arrhythmogenic DADs and triggered beats.29–32 In this study, we show that PCs from mice harboring this same mutation are preferentially and exquisitely sensitive to this underlying molecular defect. Compared to genetically identical VMs, the mutant PCs show more profound abnormalities in intracellular Ca\(^{2+}\) handling, including the development of SCaEs at baseline and, as demonstrated by simultaneous calcium and voltage measurements, the progression to triggered beats in response to relatively modest catecholaminergic stimulation.

This study extends our prior observation indicating that even wild-type PCs are more likely to develop DADs and triggered beats compared to VMs.23 The basis for this heightened proclivity of PCs to develop arrhythmic behavior is not fully known, although the diminished T-tubular density,8 altered action potential morphology,33 altered kinetics of Ca\(^{2+}\) handling (Figure 2), and increased intracellular Na\(^{+}\) load10 may all be contributory factors. In addition, the participation of IP\(_3\)-sensitive Ca\(^{2+}\) channels as part of the excitation–contraction coupling machinery may also influence this arrhythmogenic behavior.9 As such, when confronted with another perturbation, in this case, a defective intracellular Ca\(^{2+}\) release channel, SCaEs may arise. If of sufficient number, magnitude, or location, these events may result in DADs. Moreover, PCs appear especially vulnerable to the development of DADs by virtue of their increased diastolic Ca\(_i\).
voltage coupling gain, behavior which may be due in part to diminished inwardly rectifying potassium currents ($I_{K1}$).\textsuperscript{11} Furthermore, in contrast to well-coupled and electrically loaded working myocytes, in PCs, oscillations in membrane potential are uniquely capable of evoking propagated action potentials that can traverse the Purkinje–ventricular junction into the myocardium proper.\textsuperscript{12}

Our analysis of myocytes from RyR2R\textsuperscript{R4496C} mutant mice strongly support the recent suggestion that the Purkinje network may serve as the arrhythmogenic trigger in CPVT associated with RyR2 mutations. This initial hypothesis was based, in part, on the characteristic bidirectional ventricular tachycardia seen in patients and mice with this syndrome\textsuperscript{24,34} and data demonstrating that ablation of the right ventricular subendocardium (where Purkinje fibers are found) with Lugol’s solution converted the biventricular tachycardia to a monomorphic arrhythmia.\textsuperscript{7} Whether or not PCs serve as arrhythmic triggers in CPVT associated with mutations in the calsequestrin gene remains to be seen,\textsuperscript{35,36} but comparable studies in Casq2 mutant mice should be informative. More broadly, the Purkinje fiber network has been implicated in the initiation of ventricular tachycardia not only in CPVT but also following myocardial infarction, in dilated cardiomyopathies, in idiopathic ventricular fibrillation, in postshock arrhythmias, and other inherited channelopathies including Brugada and long QT syndromes.\textsuperscript{7,37–42} A similar analysis of PC behavior at the cellular level of resolution in these diseases should be equally revealing.

Several experimental and clinically approved agents have recently been shown to inhibit arrhythmogenic Ca\textsuperscript{2+}/H\textsuperscript{11001} waves.\textsuperscript{26,27,43} In particular, the class 1C antiarrhythmic agent flecainide was shown to inhibit RyR2 channel activity by reducing the duration of channel openings, especially when the RyR2 channel was activated by high luminal Ca\textsuperscript{2+} concentration. Moreover, flecainide showed efficacy in preventing ventricular arrhythmias in mice and in humans with Casq2-associated CPVT. Here, we show that flecainide is indeed effective in suppressing SCAEs not only in VMs but also in PCs. Unexpectedly, however, whereas flecainide appears equally efficacious in VMs and PCs from wild-type mice, in RyR2R\textsuperscript{R4496C}/H\textsuperscript{11001} mutant mice, the beneficial effect appears restricted to only the PCs. The suppressive effect we observed in PCs occurs at substantially lower concentrations than the reported IC\textsubscript{50} for inhibition of RyR2 open probability.\textsuperscript{27} Conceivably, flecainide may be acting through additional targets such as voltage-gated sodium channels, secondarily influencing intracellular Ca\textsuperscript{2+} load and the propensity for arrhythmogenic SCAEs. Interestingly, we have recently found that Na\textsubscript{1.8}/Scn10a is preferentially expressed in PCs with mutations in the calsequestrin gene remains to be seen,\textsuperscript{35,36} but comparable studies in Casq2 mutant mice should be informative. More broadly, the Purkinje fiber network has been implicated in the initiation of ventricular tachycardia not only in CPVT but also following myocardial infarction, in dilated cardiomyopathies, in idiopathic ventricular fibrillation, in postshock arrhythmias, and other inherited channelopathies including Brugada and long QT syndromes.\textsuperscript{7,37–42} A similar analysis of PC behavior at the cellular level of resolution in these diseases should be equally revealing.

Figure 5. Effects of Flecainide and Tetracaine on SCAEs. Proportion of cells displaying SCAEs (A, B, and E) and number of SCAEs per cell (C, D, and F) in dissociated VMs and PCs from wild-type (WT) and RyR2R\textsuperscript{R4496C} mutant (MUT) mice in the absence (CON) or presence of flecainide (FLEC; 5 \mu m) or tetracaine (TETR; 50 \mu m). *P<0.05 compared to control cells.

Table 2. Antiarrhythmic Effects of Flecainide and Tetracaine in VMs and PCs

<table>
<thead>
<tr>
<th></th>
<th>Cells With SCAEs</th>
<th>SCAEs per Cell</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>CON</td>
<td>FLEC/TETR</td>
</tr>
<tr>
<td>WT VM</td>
<td>14% (28)</td>
<td>3% (36)</td>
</tr>
<tr>
<td>MUT VM</td>
<td>43% (56)</td>
<td>34% (71)</td>
</tr>
<tr>
<td>MUT PC</td>
<td>76% (25)</td>
<td>27% (37)</td>
</tr>
<tr>
<td></td>
<td>TETR</td>
<td>TETR</td>
</tr>
<tr>
<td>WT VM</td>
<td>54% (24)</td>
<td>16% (25)</td>
</tr>
<tr>
<td>MUT PC</td>
<td>89% (19)</td>
<td>27% (11)</td>
</tr>
</tbody>
</table>

Data are shown as percentages of cells with SCAEs and the means±SEM SCAEs/cell. The number of cells studied for each condition are shown in parentheses. CON indicates control; FLEC, flecainide; MUT, mutant; TETR, tetracaine; WT indicates wild type.
This study was facilitated by our recent discovery of Cntn2 in the specialized conduction system and the recognition that Cntn2-EGFP mice could facilitate the identification and isolation of conduction system cells, including PCs.23 This approach is similar in principal to using Cx40-EGFP knock-in mice,44 although that strategy may be less specific, because Cx40 is expressed not only in the conduction system but also in atrial myocytes and vascular endothelium and smooth muscle cells.45

In summary, our study provides compelling evidence at the cellular level that the Purkinje fiber network is a critical contributor to arrhythmia initiation in CPVT and that defects in intracellular Ca2+ regulation underlie the disease phenotype. These data add to an accumulating body of literature suggesting that the unique structural and functional properties of PCs and the network they comprise play a mechanistic role in a broad range of arrhythmic syndromes. Moreover, our data provide impetus for focusing on PCs as specific antiarrhythmic targets.

Sources of Funding

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Disclosures

None.

References


What Is Known?

- Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmia that is characterized by aberrant regulation of intracellular calcium handling.
- Studies at the organ or organismal level of resolution suggest that the Purkinje fiber network may serve as the arrhythmic trigger in CPVT.

What New Information Does This Article Contribute?

- We determined the kinetic properties of intracellular calcium transients in adult ventricular myocytes and Purkinje cells from both wild-type and RyR2<sup>3337IQ/3337IQ</sup> mutant mice.
- We found that both wild-type and RyR2<sup>3337IQ/3337IQ</sup> mutant Purkinje cells have a great propensity to develop unstimulated spontaneous calcium release events and triggered action potentials compared to working ventricular myocytes of the same genotype.
- We determined that flecainide and tetracaine both potently suppress spontaneous calcium release events in Purkinje cells.

Multiple lines of evidence suggest that the cardiac Purkinje fiber network serves as the arrhythmic trigger in a range of genetic and acquired forms of heart disease. However, to date, the evidence is largely indirect, including electric recordings from the subendocardium in the intact or isolated heart or suppression of arrhythmias following ablation of presumptive cells from the Purkinje fiber network. Here, using a novel genetic reporter gene to specifically identify dissociated adult murine Purkinje cells, we show for the first time that Purkinje cells are indeed more likely than working ventricular myocytes to develop unstimulated spontaneous calcium release events that are capable of triggering action potentials. This preferential behavior is exacerbated in mice harboring a CPVT disease—causing mutation in the RyR2 channel, especially so in the presence of isoproterenol. Moreover, we show that Purkinje cells can respond to antiarrhythmic agents, but their sensitivities may differ from working ventricular myocytes. Our study provides new insight into disease pathogenesis at the cellular level of resolution and also provides impetus for the development of Purkinje cell–specific antiarrhythmic strategies. Such strategies may find utility both in inherited arrhythmias such as CPVT but potentially also in acquired syndromes including postmyocardial infarction ventricular arrhythmias.
Purkinje Cells From RyR2 Mutant Mice Are Highly Arrhythmogenic But Responsive to Targeted Therapy
Guoxin Kang, Steven F. Giovannone, Nian Liu, Fang-Yu Liu, Jie Zhang, Silvia G. Priori and Glenn I. Fishman

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Online Supplemental Methods

**Mutant Mice.** All experiments were performed according to protocols approved by the NYU School of Medicine Institutional Animal Care and Use Committee and conformed to the National Institutes of Health (NIH) guidelines for the care and use of Laboratory Animals. *Cntn2-EGFP* BAC transgenic mice and RyR2<sup>R4496C/+</sup> mutant mice have both been previously described<sup>1,2</sup>. All mice studied were F1 crosses between the two strains and used at 8-12 weeks of age. To minimize experimental variability, data from VMs and PCs from the same preparation were always collected on the same day.

**Immunohistochemistry.** Mouse hearts were fixed in 4% paraformaldehyde (PFA), then equilibrated in 10% sucrose and embedded in OCT, as previously described<sup>1</sup>. Cryosections, 6-10μM thick, were post-fixed for 10 min in 4% PFA (Cx40), permeabilized for 10 min in 0.5% Triton X-100 and blocked for 1 h at 37°C in 5% Normal Donkey Serum/0.1% Triton X-100/0.1% NP-40. Slides were then incubated overnight at 4°C with the primary antibodies directed against ankyrin-G (rabbit polyclonal, Invitrogen); Scn5a (rabbit polyclonal, Chemicon); and/or α-actinin (mouse monoclonal, Sigma), followed by incubation for 1 hr at 37°C with the appropriate secondary antibody and mounted with Vectashield. Confocal images were acquired with a SP5 confocal laser system (Leica), using 20x (water) and 40x (dry) objectives, and Ar (488 nm line; green fluorescence) or Green HeNe (543 nm line; red fluorescence) lasers.

**Ca<sup>2+</sup> imaging.** Adult myocytes were isolated using an established enzymatic digestion protocol<sup>1</sup> yielding 60-80% rod-shaped, Ca<sup>2+</sup>-tolerant myocytes. Cells were exposed to a dye loading solution consisting of a standard Tyrodes containing (in mm): 140 NaCl, 4 KCl, 2 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 10 HEPES, and 5.6 glucose. The Tyrodes solution was supplemented with 2.5 μM x-Rhod-1 acetoxymethyl ester (x-Rhod-1/AM; Invitrogen Inc., Eugene, OR). Cells were exposed to x-Rhod-1/AM for 6 min at 22°C. The loading solution was removed, and cells were washed and equilibrated in fresh Tyrodes solution for 30 min at 22°C to allow deesterification of the dye before recording. Epifluorescence imaging was used to identify the GFP-expressing and non-expressing cells. Fluorescent signals were acquired using a 40X UVF objective (numerical aperture 1.0, Nikon), and single excitation wavelength microfluorimetry was performed using a PMT system (IonOptix Corp., Milton, MA). Cells were field-stimulated as previously described<sup>23</sup>. In brief, cells were paced at 1Hz to achieve steady-state, then paced for 10 beats at either 1 Hz, 3 Hz or 5 Hz, as described in individual figure legends. Spontaneous Ca<sup>2+</sup> release events (SCaEs) were defined as unstimulated increases in intracellular Ca<sup>2+</sup> and were quantified during the 6 second unpaced interval following the 10 beat pacing train.

**Dual Ca<sup>2+</sup> imaging and voltage measurements.** Ventricular myocytes were isolated using an established enzymatic digestion protocol yielding to 60-80% rod-shaped, calcium-tolerant myocytes.<sup>1,3</sup> Within 6 hours from isolation, laminin-coated dishes containing isolated ventricular myocytes were mounted on the stage of an inverted microscope. The myocytes were bathed with the solution containing (mmol/L): 140 NaCl, 4 KCl, 2 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 10 HEPES and 5 glucose, pH adjusted to 7.4 with NaOH at 22°C. Transmembrane potentials were recorded in whole cell current-clamp mode as
previously described. All signals were acquired at 5 kHz (Digidata 1322A, Axon Instruments) and analyzed with pCLAMP version 9.2 software (Axon Instruments). Patch electrodes had a resistance of 1-2 M when filled with patch electrode solutions containing (mmol/L): 120 potassium aspartate, 20 KCl, 1 MgCl2, 4 Na2ATP, 0.1 GTP, 10 HEPES, 10 glucose and 0.05 Rhod-2-potassium, pH adjusted to 7.2 with NaOH. Myocytes were electrically stimulated by intracellular current injection through patch electrodes using depolarizing pulses with duration of 5 ms and amplitude of 0.5-1 nA. DADs were defined as phase 4 positive (depolarizing) deflections of the membrane potential. EADs were defined as positive (depolarizing) oscillations occurring during phase 2 or 3 of action potential. For the induction of triggered activity, isoproterenol (1 μmol/L) was used.

Statistics. Kinetic parameters were compared using Student’s t-test. The proportion of cells displaying SCaEs was compared using Fisher’s exact test. The number of SCaEs per cell was compared by the Mann-Whitney test. P values < 0.05 were considered significant.

References

