Short Communication: Flecainide Exerts an Antiarrhythmic Effect in a Mouse Model of Catecholaminergic Polymorphic Ventricular Tachycardia by Increasing the Threshold for Triggered Activity

Nian Liu, Marco Denegri, Yanfei Ruan, José Everardo Avelino-Cruz, Andrea Perissi, Sara Negri, Carlo Napolitano, William A. Coetzee, Penelope A. Boyden, Silvia G. Priori

**Rationale:** Flecainide prevents arrhythmias in catecholaminergic polymorphic ventricular tachycardia, but the antiarrhythmic mechanism remains unresolved. It is possible for flecainide to directly affect the cardiac ryanodine receptor (RyR2); however, an extracellular site of action is suggested because of the hydrophilic nature of flecainide.

**Objective:** To investigate the mechanism for the antiarrhythmic action of flecainide in a RyR2R4496C+/− knock-in mouse model of catecholaminergic polymorphic ventricular tachycardia.

**Methods and Results:** Flecainide prevented catecholamine-induced sustained ventricular tachycardia in RyR2R4496C+/− mice. Cellular studies were performed with isolated RyR2R4496C+/− myocytes. Isoproterenol caused the appearance of spontaneous Ca2+ transients, which were unaffected by flecainide (6 μmol/L). Flecainide did not affect Ca2+ transient amplitude, decay, or sarcoplasmic reticulum Ca2+ content. Moreover, it did not affect the frequency of spontaneous Ca2+ sparks in permeabilized myocytes. In contrast, flecainide effectively prevented triggered activity induced by isoproterenol. The threshold for action potential induction was increased significantly (P<0.01), which suggests a primary extracellular antiarrhythmic effect mediated by Na+ channel blockade.

**Conclusions:** Flecainide prevents catecholaminergic polymorphic ventricular tachycardia in RyR2R4496C+/− mice; however, at variance with previous reports, we observed minimal effects on intracellular Ca2+ homeostasis. Our data suggest that the antiarrhythmic activity of the drug is caused by reduction of Na+ channel availability and by an increase in the threshold for triggered activity. (Circ Res. 2011;109:291-295.)

Key Words: ryanodine receptor ■ sodium channel ■ ventricular tachycardia ■ genetics

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited disease characterized by adrenergically mediated polymorphic ventricular tachycardia (VT) that leads to syncope and sudden cardiac death.1 Although β-adrenergic blockers are the mainstay therapy in CPVT patients, approximately 30% of patients receiving this treatment still experience cardiac arrhythmias and eventually require an implantable cardioverter-defibrillator.1 Abnormal diastolic calcium leak from the mutant cardiac ryanodine receptor (RyR2) is responsible for the induction of triggered activity (TA), which is the pivotal arrhythmogenic mechanism in CPVT.1 Potential antiarrhythmic strategies therefore include either targeting the abnormal calcium leak or targeting the ionic mechanisms responsible for the generation of TA. It has been proposed recently that flecainide prevents arrhythmias in calsequestrin knockout mice and in CPVT patients by blocking RyR2 and by decreasing its opening probability.2,3 In the present study, we systematically investigated the antiarrhythmic mechanism of flecainide in our RyR2R4496C+/− knock-in mouse model of CPVT4 and found that inhibition of TA by reducing the availability of sodium channels accounts for the unexpected antiarrhythmic efficacy of the drug in CPVT.

**Methods**

An expanded Methods section is available in the Online Data Supplement at http://circres.ahajournals.org. ECG radiotelemetry monitors were implanted, and drug stress with coinjection of epinephrine and caffeine was performed as described previously in RyR2R4496C+/− (patent number US11/429167) mice.4 Ca2+ imaging and a patch clamp study were performed in isolated myocytes.
ventricular RyR2R4496C+/− myocytes. For flecainide diffusion across cell membrane, intact myocytes were incubated with flecainide for 30 minutes.

**Results**

**Flecainide Prevents Sustained VT in RyR2R4496C+/− Mice**

Sustained VT occurred in 70% of RyR2R4496C+/− mice when challenged with epinephrine (2 mg/kg IP) and caffeine (120 mg/kg IP). In contrast, only 8% (1/12) of the animals had these arrhythmias when pretreated with flecainide (15 mg/kg; P<0.001; Figure 1A). Thus, flecainide exerts a powerful antiarrhythmic activity in RyR2R4496C+/− mice.

**Effects of Flecainide on Intracellular Ca2+**

The antiarrhythmic action of flecainide may be due to a direct action of the drug on intracellular Ca2+ homeostasis. We therefore evaluated this hypothesis in isolated ventricular RyR2R4496C+/− myocytes. Isoproterenol (1 μmol/L) increased the amplitude of Ca2+ transients, accelerated the Ca2+ transient decay, and increased the sarcoplasmic reticulum (SR) Ca2+ load. Despite the blockade of JNa, flecainide (6 μmol/L, incubation for 30 minutes) had no effect on any of these parameters (Figures 1B and 1C), in agreement with Hilliard et al.3 In contrast, tetracaine (50 μmol/L) significantly increased the Ca2+ transient amplitude and SR Ca2+ load, which suggests that tetracaine but not flecainide is able to block RyR2 in intact myocytes.

We next investigated the effects of flecainide on SR function in permeabilized RyR2R4496C+/− myocytes. Tetracaine (50 μmol/L), but not flecainide (25 μmol/L), decreased the spontaneous Ca2+ wave frequency compared with untreated cells (Figure 2A). Flecainide had no effect on the frequency of the sparks, whereas tetracaine decreased the frequency of the sparks by ≅20%, which is consistent with the fact that tetracaine is a RyR2 blocker (Figure 2; Online Table I).5

**Flecainide Does Not Prevent Spontaneous SR Ca2+ Release**

Spontaneous Ca2+ transients (SCaTs) originating from abnormal SR Ca2+ release are a potential arrhythmogenic trigger. We next investigated the occurrence of SCaTs in intact RyR2R4496C+/− myocytes. Under basal conditions, only ≅4% of the myocytes (n=27 cells) developed SCaTs when paced at 1 Hz (Figure 3A). The incidence of SCaTs was increased to 87% (n=30 cells) by isoproterenol (1 μmol/L). Flecainide (6 μmol/L, incubation for 30 minutes) had no effect on the incidence of SCaTs, which occurred in ≅85% of myocytes (n=14 cells; Figure 3B). In contrast, tetracaine (50 μmol/L) reduced the incidence of SCaTs to ≅15% (P<0.001; n=23 cells).

SCaTs often led to the occurrence of triggered events (arrow in Figure 3A). In the presence of flecainide, 7% of myocytes developed triggered events in the presence of isoproterenol at 1 Hz pacing compared with 60% in the absence of flecainide (P<0.001; Figure 3C). These data demonstrate that the SCaTs elicited by isoproterenol were largely unaffected by flecainide, but the drug inhibited the development of triggered events.

**Non-standard Abbreviations and Acronyms**

- AP: action potential
- CPVT: catecholaminergic polymorphic ventricular tachycardia
- DAD: delayed afterdepolarization
- RyR2: ryanodine receptor type 2
- SCaT: spontaneous Ca2+ transient
- SR: sarcoplasmic reticulum
- TA: triggered activity

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**Figure 1.** A, ECG recordings in RyR2R4496C+/− mice. Left trace, bidirectional VT induced in a saline-treated mouse after epinephrine and caffeine administration; right trace, sinus rhythm in a flecainide-treated mouse after epinephrine and caffeine administration. B, Representative caffeine-induced Ca2+ transient in a RyR2R4496C+/− myocyte. C, Averaged Ca2+ transient amplitude, caffeine-induced Ca2+ transient amplitude, and Ca2+ transient decay (n=7 to 15 cells for each group). Iso indicates isoproterenol.
Flecainide Abolishes Isoproterenol-Induced TA

We performed patch-clamp studies to investigate whether flecainide inhibits delayed afterdepolarization (DAD)-mediated TA. As reported previously, DADs and TA developed readily in RyR2<sup>R4496C</sup>/<sup>H11001</sup>/<sup>H11002</sup> myocytes on exposure to isoproterenol (1 μmol/L), whereas exposure to flecainide (6 μmol/L) failed to abolish DADs but fully suppressed TA (n=6 cells; Figure 4A).

In some cells, we performed simultaneous action potential (AP) recording and Ca<sup>2+</sup> imaging to confirm that flecainide abolishes DAD-mediated TA with no effect on the underlying SCaTs (Figure 4B). Collectively, these data argue that at this concentration, flecainide preferentially alters AP properties rather than SR Ca<sup>2+</sup> release in RyR2<sup>R4496C</sup>/<sup>H11001</sup>/<sup>H11002</sup> myocytes. Flecainide directly affected AP properties by reducing the AP amplitude in a frequency-dependent manner (Figure 4C; Online Figure I). These data are consistent with the well-described use dependent block of the drug on the Na<sup>+</sup> channel. We directly examined whether flecainide affects AP initiation by incrementally in-
creasing the stimulus current to determine the threshold for AP induction. Flecainide (6 μmol/L) significantly augmented the minimal current required to elicit an AP (from 657 ± 1100 pA to 1171 ± 269 pA, P < 0.001; n = 7 cells; Figure 4D).

Does Flecainide Have an Intracellular Effect on Excitability?
To determine whether flecainide exerts any effect on myocyte physiology once introduced into the intracellular compartment, we dialyzed flecainide (25 μmol/L) into RyR2R4496C/−/− ventricular myocytes through the patch pipette (for >5 minutes). Intracellular flecainide had no effect on AP properties (Online Figure I and Table II), which suggests an extracellular mechanism of action. Moreover, under these conditions, isoproterenol (1 μmol/L) was still able to induce TA (Online Figure II); thus, only extracellular flecainide was able to abolish TA, which suggests the importance of its Na+ channel-blocking properties.

Discussion
It has been agreed that RyR2 mutations cause instability of the RyR2 complex in response to adrenergic stimulation, which in turn facilitates abnormal diastolic Ca2+ release and development of DADs and TA. There is a pressing clinical need to identify new and effective antiarrhythmic approaches. We observed that flecainide has pronounced antiarrhythmic effects in vivo in our RyR2R4496C/−/− mice, which supports the view that flecainide may be beneficial for the management of CPVT patients. The mechanisms of antiarrhythmic action of flecainide in patients with CPVT are not fully elucidated and may involve inhibition of the SR leak or an inhibitory effect of the electrophysiological consequences of abnormal diastolic calcium release.

Recently, Watanabe et al2 and Hilliard et al3 demonstrated that flecainide directly affects SR Ca2+ leak in myocytes derived from mice with a recessive form of CPVT. These authors showed that flecainide is able to inhibit SCaTs and Ca2+ sparks in intact myocytes derived from a calsequestrin knockout model of CPVT by blocking the release channel in its open state.4 However, we rationalized that given its high pKa (9.3), flecainide is charged primarily at physiological pH: thus, with only a mere 1% of neutral flecainide available for diffusion across the membrane, we considered it unlikely that an in vivo effect on RyR2 could account for the antiarrhythmic efficacy of the drug.6 We tested this hypothesis and found that when we applied flecainide to intact myocytes derived from our RyR2R4496C/−/− knock-in mice,4 we failed to demonstrate a reduction of isoproterenol-induced SCaTs. Similarly, in permeabilized myo-
cytes, we were unable to observe an effect on SR Ca\(^{2+}\) release events even at concentrations as high as 25 \(\mu\)mol/L. When we administered flecainide intracellularly through a patch pipette, we were still unable to observe any effect of flecainide on DADs and TA. However, although extracellular application of flecainide (6 \(\mu\)mol/L) failed to affect SCaTs or DADs, it rapidly inhibited TA, which demonstrates that it is the extracellular presence of flecainide that mediates its antiarrhythmic activity in our CPVT mouse model. It is theoretically possible that the discrepancy between the present findings and those of Knollmann et al are attributable to different experimental conditions or to the use of a different CPVT mouse model.

Our novel interpretation of the antiarrhythmic effect of flecainide is that it reduces the availability of sodium channels, thus preventing the development of triggered APs. It has been proposed that sodium channel blockers can prevent TA in cardiac myocytes by alleviation of elevated cytosol Na\(^+\) in some settings, such as ischemia and digitalis administration.\(^7\) Obviously, the efficacy of flecainide in preventing TA in the present study was independent of the cytosol Na\(^+\) level and therefore represents a novel antiarrhythmic mechanism of the compound. We found that the minimal current required to elicit an AP in RyR2\(^{R4496C+/-}\) myocytes was increased significantly by flecainide, which suggests that an increased DAD amplitude leading to a more depolarized cell membrane would be necessary to elicit an AP in the presence of flecainide. Because the Na\(^+\) channel–blocking effect of flecainide is strongly frequency dependent, it is reasonable to propose that flecainide will have a dramatically enhanced effect to prevent TA at elevated heart rates. It makes the drug particularly effective during fast heart rates, ie, in the conditions in which CPVT patients develop cardiac arrhythmias. Whether the antiarrhythmic effect in CPVT observed with flecainide is shared by all class I antiarrhythmic agents deserves clinical evaluation. Some distinguishing properties of flecainide such as its high bioavailability and long half-life may give the drug a composite profile that makes it the class I antiarrhythmic agent of choice for CPVT patients.

Clinical Implications
The evidence that 30% of CPVT patients taking \(\beta\)-blocker therapy who also have an implantable cardioverter-defibrillator receive appropriate shocks to terminate life-threatening arrhythmias calls for the identification of drugs that act on a different target to potentiate the antiadrenergic effect of \(\beta\)-blockers and increase the “safety margin” to prevent VT. The sodium channel–blocking action of flecainide appears to be a good option to achieve control of arrhythmic episodes and reduce the need for an implantable cardioverter-defibrillator in CPVT patients.

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Disclosures
None.

References
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Flecainide exerts an anti-arrhythmic effect in a CPVT mouse model by increasing threshold for triggered activity

In Vivo Drug Testing

ECG radiotelemetry monitors were implanted as previously described. Flecainide (15mg/Kg) was injected intraperitoneally 30 minutes before drug test. This was followed by a drug-testing protocol with co-injection of epinephrine and caffeine as previously described. All experiments were performed in accordance with the regulations of our Institutional Animal Care and Use Committees.

Isolation of Adult Mice Ventricular Myocytes

Ventricular myocytes were isolated using an established enzymatic digestion protocol. 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$_2$, 1 MgCl$_2$, 10 HEPES and 5 glucose, pH adjusted to 7.4 with NaOH.

Ca$^{2+}$ imaging in intact cardiac myocytes

Isolated ventricular myocytes were incubated with 2.5μM Fluo-4 AM (Invitrogen Inc., Eugene, OR) for 10 minutes at room temperature and washed twice with Tyrode’s
solution, then equilibrated in fresh Tyrodes solution containing 250 μM probenecid for 20 min to allow deesterification of the dye before recording. 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 to achieve steady-state at least 20 beats. Spontaneous Ca\textsuperscript{2+} transients (SCaTs) were defined as unstimulated increases in intracellular Ca\textsuperscript{2+} and were quantified during the 5 second unpaced interval following the 20 beat pacing train. The myocytes were incubated with flecainide (6 μmol/L) for 30 mins or with tetracaine (50 μmol/L) for 3 mins before catecholamine stress. Since too much SCaTs occurred in presence of Iso (1 μmol/L), the measurements of SR Ca\textsuperscript{2+} decay, SR Ca\textsuperscript{2+} load and Ca\textsuperscript{2+} sparks were performed at 0.2 Hz pacing.

**Ca\textsuperscript{2+} imaging in permeabilized cardiac myocytes**

Cardiac myocytes were permeabilized by incubating with 0.01% saponin for 1.5 min and placed in a solution containing (in mmol/L): 120 potassium aspartate, 3 MgATP, 10 phosphocreatine, 5 U/ml creatine phosphokinas, 10 reduced glutathione, 0.5 or 0.05 EGTA, 1 free [Mg\textsuperscript{2+}], 4% dextran (relative molecular mass: 40 000), 0.01 K\textsubscript{4}Fluo-4 and 10 Heps. 0.5 mmol/L EGTA was used for Ca2+ sparks study and 0.05 mmol/L EGTA was used for spontaneous Ca2+ waves study. Free [Ca\textsuperscript{2+}]\textsubscript{i} (100 nmol/L) was calculated by WINMAXC32 2.51. Ca\textsuperscript{2+} images were acquired using a 60x oil immersion objective (N.A.=1.4) The images were obtained by positioning the line scan along the longitudinal axis of the myocytes at the rate of 400 Hz (pixel size 0.097 μm) with a Leica SP5 confocal microscope. The dye was excited at 488 nm and emission was collected at >510 nm. Image analyses were performed with spark master in Imaging J software.
Electrophysiological Recordings in Isolated Ventricular Myocytes

Transmembrane potentials were recorded in whole-cell mode using a multiClamp 700B amplifier (Axon Instruments). For action potential recording the pipette solutions contained (in mM): 120 potassium aspartate, 20 KCl, 1 MgCl2, 4 Na2ATP, 0.1 GTP, 10 HEPES, 5 glucose, pH 7.2, with NaOH. Electrode resistance was 1 to 2 MΩ. All signals were low-pass filtered at 5 kHz (Digidata 1322A, Axon Instruments) and analyzed using pCLAMP version 9.2 software (Axon Instruments). Only quiescent, Ca$^{2+}$-tolerant, rod-shaped cells with clear cross striations were used for electrophysiological recordings. Myocytes were electrically stimulated by intracellular current injection through patch electrodes using depolarizing pulses with duration of 5 ms (30% above threshold). In a small set of experiment, simultaneous current patch clamp and Ca$^{2+}$ imaging study were performed in fluo-4 AM loaded myocytes. Electrodes had resistance of 10-20 MΩ with fluo-4 salt (0.05 mM) in the pipette solution.

Data Analysis

Data were expressed as mean±SEM. Normal distribution of variables was assessed with Kolmogorov-Smirnov test. Differences of means for normally distributed variables were assessed with Student t-test (significance between 2 groups) or one-way ANOVA (multiple comparisons). Bonferroni post-hoc analysis between groups was used for pairwise comparison in ANOVA. Non normally distributed variables were analyzed for statistical significance using Kruskal Wallis test with Dunn test for post-hoc pairwise comparisons. Chi-square and Fisher’s exact test were used as appropriate in
contingency tables (with Bonferroni correction to set significance level for > 2 variable crosstabs). Differences with $p \leq 0.05$ were considered statistically significant.

References


Table I. Ca\textsuperscript{2+} spark parameters in permeabilized RyR2\textsuperscript{R4496C+/−} myocytes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=18)</th>
<th>Flecainide (25 μmol/L, n=21)</th>
<th>Tetracaine (50 μmol/L, n=19)</th>
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</thead>
<tbody>
<tr>
<td>Spark frequency (/100μM/s)</td>
<td>52.6±1.6</td>
<td>55.2±1.1</td>
<td>41.7±0.9*</td>
</tr>
<tr>
<td>Amplitude (ΔF/F₀)</td>
<td>0.50±0.02</td>
<td>0.57±0.02</td>
<td>0.75±0.05*</td>
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<tr>
<td>FWHM (μm)</td>
<td>1.94±0.03</td>
<td>1.88±0.02</td>
<td>2.09±0.02#</td>
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<tr>
<td>FDHM (ms)</td>
<td>19.0±0.2</td>
<td>18.7±0.2</td>
<td>21.5±0.3#</td>
</tr>
<tr>
<td>Tau (ms)</td>
<td>16.2±0.2</td>
<td>16.0±0.3</td>
<td>17.9±0.6*</td>
</tr>
</tbody>
</table>

Ca\textsuperscript{2+} sparks were obtained from permeabilized RyR2\textsuperscript{R4496C+/−} myocytes in Ca\textsuperscript{2+}-buffer solutions (0.5 mmol/L EGTA) with a free [Ca\textsuperscript{2+}] of 100 nmol/L. FWHM: spark full width at half maximum amplitude, FDHM: spark duration at half maximum amplitude, Tau: exponential time constant of the spark decay. n: number of cells analyzed per group.

Flecainide had no effect on the frequency of the spark, whereas tetracaine decreased frequency of the sparks.

* p<0.05 vs Control,

# p<0.01 vs Control
Table II. Intrapipette administration of flecainide did not affect the properties of action potential (n=6-8 cells per group)

<table>
<thead>
<tr>
<th></th>
<th>Resting potential (mV)</th>
<th>Amplitude of action potential (mV)</th>
<th>Action potential duration (ms)</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>-71.4±1.2</td>
<td>125±1.4</td>
<td>68.7±8.6</td>
</tr>
<tr>
<td>Flecainide</td>
<td>-71.6±1.1</td>
<td>126±1.7</td>
<td>74.4±10.7</td>
</tr>
</tbody>
</table>

We dialyzed flecainide (25 μmol/L) into RyR2
R4496C+/− ventricular myocytes through the patch pipette (for >5 min). Action potentials were recorded at 1 Hz pacing. There is no significant difference of resting potential, amplitude of action potential and action potential duration between the control cells and the cells dialyzed with flecainide (25 μmol/L).
Table III. Ca\(^{2+}\) spark parameters in intact RyR2\(^{R4496C+_/-}\) myocytes in presence of Iso (1 μmol/L) at 0.2 Hz pacing.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=34)</th>
<th>Flecainide (6 μmol/L, n=31)</th>
<th>Tetracaine (50 μmol/L, n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spark frequency (/100μM/s)</td>
<td>6.14±0.44</td>
<td>6.18±0.41</td>
<td>2.6±0.47*</td>
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<tr>
<td>Amplitude (ΔF/F(_0))</td>
<td>0.71±0.05</td>
<td>0.76±0.07</td>
<td>0.79±0.2</td>
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<tr>
<td>FWHM (μm)</td>
<td>2.00±0.09</td>
<td>1.89±0.06</td>
<td>2.11±0.33</td>
</tr>
<tr>
<td>FDHM (ms)</td>
<td>36.3±1.8</td>
<td>42.8±2.5</td>
<td>34.2±2.9</td>
</tr>
<tr>
<td>Tau (ms)</td>
<td>53.1±3.5</td>
<td>53.6±3.1</td>
<td>52.6±7.4</td>
</tr>
</tbody>
</table>

FWHM: spark full width at half maximum amplitude, FDHM: spark duration at half maximum amplitude, Tau: exponential time constant of the spark decay, n: number of cells analyzed per group. Flecainide had no effect on the frequency of the spark, whereas tetracaine decreased frequency of the sparks.

* p<0.05 vs Control.
A. Representative action potential recordings at 5 Hz pacing in RyR2<sup>R4496C<sup>+/−</sup> myocytes. Extracellular administration of flecainide (6 μmol/L) significantly reduced the amplitude of action potential, while intrapipette administration of flecainide (25 μmol/L, dialysis for >5 min) did not influence the amplitude of action potential. B. Relative amplitude of action potential at 5 Hz pacing. Extracellular administration of flecainide caused significantly used dependent effect on amplitude of action potential compared to intrapipette administration of flecainide (n=6-8 cells for each group).
Representative DADs and triggered activities presented in RyR2\textsuperscript{R4496C+/−} myocytes treated by intrapipette administration of flecainide (25 μmol/L) in presence of Iso (1 μmol/L). Flecainide (25 μmol/L) was dialyzed into RyR2\textsuperscript{R4496C+/−} ventricular myocytes through the patch pipette (for >5 min), then Iso (1 μmol/L) was perfused. Cell 1 showed typical DADs. Cell 2 showed DADs induced triggered activities at 1 and 5 Hz pacing. Black arrow = field stimulation. Asterisks = triggered activity.
Figure III

The effect of flecainide (6 μmol/L) on the Vmax of action potential upstroke.

RyR2\textsuperscript{R4496C+/-} myocytes were paced at 1 Hz, flecainide (6 μmol/L) was administrated in extracellular solution. Flecainide significantly decreased the Vmax of action potential upstroke (n= 6 cells).
Representative line scan in intact RyR2\textsuperscript{R4496C+/-} myocytes. Ca\textsuperscript{2+} sparks were recorded at 0.2 Hz pacing in presence of Iso (1 μmol/L). Tetracaine (50 μmol/L), but not flecainide (6 μmol/L) decreased Ca\textsuperscript{2+} sparks frequency as compared to untreated cells (see Online table III).