Conditional FKBP12.6 Overexpression in Mouse Cardiac Myocytes Prevents Triggered Ventricular Tachycardia Through Specific Alterations in Excitation-Contraction Coupling

Barnabas Gellen, MD; María Fernández-Velasco, PhD*; François Briec, MD*; Laurent Vinet, MS*; Khai LeQuang, MD; Patricia Rouet-Ben zincin, PhD; Jean-Pierre Bénitah, PhD; Mylène Pezet, PhD; Gael Palais, MS; Noémie Pellegrin, MS; Andy Zhang, MS; Romain Perrier, MS; Brigitte Escoubet, MD; Xavier Marniquet, MS; Sylvain Richard, PhD; Frédéric Jaisser, MD, PhD; Ana María Gómez, PhD; Flavien Charpentier, PhD; Jean-Jacques Mercadier, MD, PhD

Background—Ca\(^{2+}\) release from the sarcoplasmic reticulum via the ryanodine receptor (RyR2) activates cardiac myocyte contraction. An important regulator of RyR2 function is FKBP12.6, which stabilizes RyR2 in the closed state during diastole. β-Adrenergic stimulation has been suggested to dissociate FKBP12.6 from RyR2, leading to diastolic sarcoplasmic reticulum Ca\(^{2+}\) leakage and ventricular tachycardia (VT). We tested the hypothesis that FKBP12.6 overexpression in cardiac myocytes can reduce susceptibility to VT in stress conditions.

Methods and Results—We developed a mouse model with conditional cardiac-specific overexpression of FKBP12.6. Transgenic mouse hearts showed a marked increase in FKBP12.6 binding to RyR2 compared with controls both at baseline and on isoproterenol stimulation (0.2 mg/kg IP). After pretreatment with isoproterenol, burst pacing induced VT in 10 of 23 control mice but in only 1 of 14 transgenic mice (P<0.05). In isolated transgenic myocytes, Ca\(^{2+}\) spark frequency was reduced by 50% (P<0.01), a reduction that persisted under isoproterenol stimulation, whereas the sarcoplasmic reticulum Ca\(^{2+}\) load remained unchanged. In parallel, peak L\(_{Ca}\) density decreased by 15% (P<0.01), and the Ca\(^{2+}\) transient peak amplitude decreased by 30% (P<0.001). A 33.5% prolongation of the caffeine-evoked Ca\(^{2+}\) transient decay was associated with an 18% reduction in the Na\(^+\)–Ca\(^{2+}\) exchanger protein level (P<0.05).

Conclusions—Increased FKBP12.6 binding to RyR2 prevents triggered VT in normal hearts in stress conditions, probably by reducing diastolic sarcoplasmic reticulum Ca\(^{2+}\) leak. This indicates that the FKBP12.6-RyR2 complex is an important candidate target for pharmacological prevention of VT. (Circulation. 2008;117:1778-1786.)

Key Words: arrhythmia ■ calcium ■ catecholamines ■ sarcoplasmic reticulum ■ stress

The cardiac ryanodine receptor (RyR2), a tetrameric Ca\(^{2+}\) channel located in the sarcoplasmic reticulum (SR), plays a key role in excitation-contraction coupling. During an action potential, membrane depolarization promotes transmembrane Ca\(^{2+}\) influx through voltage-gated L-type Ca\(^{2+}\) channels. The resulting local elevation of the free intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) leads to the release of a larger amount of Ca\(^{2+}\) from the SR via RyR2, thereby triggering cell contraction.1 Subsequently, cytoplasmatic Ca\(^{2+}\) is pumped back into the SR via the SR Ca\(^{2+}\)-ATPase (SERCA2a) and extruded from the cell via the Na\(^+\)–Ca\(^{2+}\) exchanger (NCX), leading to cell relaxation. RyR2 is at the center of a macromolecular complex of several proteins such as FKBP12.6 (also known as calstabin 2), calmodulin, CaM kinase II\(\gamma\) (CaMKII), sorcin, and phosphatases PP1 and PP2A.2 FKBP12.6 is a small cytosolic protein with cis-trans peptidyl-prolyl isomerase activity that binds to the RyR2 channel.3 FKBP12.6 binding stabilizes the RyR2 in its closed state during diastole.4 Disruption of this binding by the immunosuppressive agent FK506 increases the open proba-
bility of the RyR2 channel, thereby leading to an aberrant increase in diastolic [Ca\(^{2+}\)]\(_i\);\(^5\)\(^7\) and increasing the risk of ventricular arrhythmias.\(^5\)\(^7\)

**Clinical Perspective p 1786**

It has been proposed that β-adrenergic stimulation during physical or emotional stress, as well as chronic activation of the sympathetic nervous system in heart failure, reduces FKBP12.6 binding to RyR2.\(^8\) However, the role of protein kinase A–dependent RyR2 phosphorylation in FKBP12.6 dissociation is controversial.\(^5\)\(^6\)\(^7\) FKBP12.6-deficient mice (FKBP12.6\(^{-/-}\)) consistently exhibit exercise-induced ventricular arrhythmias.\(^11\) In mice with reduced FKBP12.6 levels (FKBP12.6\(^{+/}\)), pretreatment with the 1,4-benzoiazepine derivative JTV519 prevents death caused by exercise-induced ventricular tachycardia, probably by enhancing FKBP12.6 binding to RyR2.\(^12\)\(^13\) Recently, Huang et al\(^14\) used a constitutive model to show that cardiac FKBP12.6 overexpression protects in part against post–myocardial infarction infarct remodeling. In vitro, FKBP12.6 overexpression has been shown to reduce diastolic SR Ca\(^{2+}\) efflux.\(^5\)\(^15\) To the best of our knowledge, it has not yet been determined whether FKBP12.6 overexpression protects from arrhythmias. We therefore used the Tet-Off system to create a model of conditional FKBP12.6 overexpression. Induction of FKBP12.6 overexpression was started at weaning, and mice were examined 6 to 8 weeks later for cardiac morphology, cardiac function, level of FKBP12.6 binding to RyR2, RyR2 phosphorylation, and susceptibility to triggered arrhythmias; excitation-contraction coupling and SR Ca\(^{2+}\) handling were examined in isolated ventricular myocytes.

**Methods**

**Generation of Conditional FKBP12.6-Overexpressing Mice**

Transgenic (TG) mice with single-copy transgene insertion were generated as described\(^16\) using a 491-bp cDNA fragment containing the full-length coding sequence of the mouse FKBP12.6 gene (BC061121, Genebank). Double-TG (DT) mice were obtained by crossing the tetO-FKBP12.6 mice with the β-MHC-tTA transactivator (β-MHCtTA) transgenic (TG) mouse strain (kindly provided by G.I. Fishman, Mount Sinai School of Medicine, Queens, NY).\(^17\) allowing cardiac-specific and doxycycline-regulated expression of FKBP12.6. Wild-type, α-MHCtTA, tetO-FKBP12.6, and DT mice were identified by Southern blot. All mice used in the present study were maintained on 12-hour light/dark cycles, 21°C to 23°C.

Immunobots of Cardiac Lysates

Immunoblots were prepared from homogenates of ventricular tissue and on isolated cells collected after enzymatic dissociation\(^18\) using anti-FKBP12 (1:1000), anti-RyR2 (C3–33, 1:1000), anti-PLB (1:5000), and anti-calsequestrin (1:2500) antibodies from Affinity Bioreagents (Golden, Colo); a custom-made anti-FKBP12.6 (1:100) antibody from Eurogentec; anti-SERCA2a antibody (N-19, Santa Cruz Biotechnology Inc, Santa Cruz, Calif; 1:200); and anti-NCX antibody (R3F1, Swant, Bellinzona, Switzerland; 1:1000). RyR2-FKBP12.6 was communoprecipitated as described.\(^19\) Immunoblots of RyR2 phosphorylation were performed from heart homogenates using anti–RyR2-PS2808 (1:2000) and anti–RyR2-dePS2808 (1:2000) antibodies from Badrilla (Lees, West Yorkshire, UK) and anti–RyR2-PS2814 (1:5000) antibody generously provided by Dr A.R. Marks (Columbia University, New York, NY).

**ECG Recording and Intracardiac Recording and Pacing**

ECG recordings were performed as described on mice anesthetized by intraperitoneal injection of ketamine (50 mg/kg, Janssen-Cilag, Belgium).\(^20\) After ECG recording, anesthesia was prolonged by an additional intraperitoneal injection of ketamine. The extremity of a 2F quadripolar catheter specially designed by Biosense (Johnson & Johnson) was placed in the right ventricle through the right internal jugular vein. Standard pacing protocols were used to determine the ventricular effective refractive periods and to induce ventricular arrhythmias. The inducibility of ventricular arrhythmias was assessed at baseline and after infusion of isoproterenol 0.2 mg/kg IP by using the programmed electric stimulation (PES) method with 1 to 3 extrastimuli and burst pacing. Burst pacing consisted of trains of 20 to 100 paced beats at a cycle length of 50 ms, with at least a 4-second interval between 2 successive trains for an overall duration of 2 minutes. PES was started 2 to 3 minutes after injection of isoproterenol and lasted 15 to 17 minutes. Ventricular tachycardia (VT) was defined as the occurrence after the last paced beat of at least 4 consecutive QRS complex beats with a morphology different from that seen in normal sinus rhythm.\(^21\) VT of >10 cycles was defined as sustained VT.

**Results**

**Baseline Characterization of TG Mice**

No deaths occurred during transgene induction. Six to 8 weeks after doxycycline withdrawal, control and DT mice did not differ in terms of morphological or functional parameters (the Table). Myocardial sections stained by Masson’s trichrome, Sirius red, and hematoxylin and eosin did not show any histological alteration in DT mice (not shown). Reverse-transcription polymerase chain reaction specific for TG FKBP12.6 mRNA showed strong TG expression in the
ventricular myocardium, whereas TG expression in other tissues was nearly (brain, lung) or totally (kidney, liver, skeletal muscle) undetectable at 30 polymerase chain reaction cycles using 100 ng total cDNA per organ (Figure 1A).

Western blot analysis using the anti-FKBP12 antibody yielded a strong FKBP12.6 signal in ventricular homogenates from DT mice, whereas the endogenous FKBP12.6 was not detected in control mice (Figure 1B). Our anti-FKBP12.6-specific antibody detected the endogenous FKBP12.6 in control mice (Figure 1B). Our anti-FKBP12 antibody shows marked FKBP12.6 overexpression in DT mice. The specific antibody detected the endogenous FKBP12.6 in control mice (Figure 1B). Our anti-FKBP12 antibody shows marked FKBP12.6 overexpression in DT mice.

Table. Mouse Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control Mice (n=13)</th>
<th>DT Mice (n=11)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>29.3±0.7</td>
<td>30.0±0.9</td>
<td>NS</td>
</tr>
<tr>
<td>HW, mg</td>
<td>141.5±6.0</td>
<td>147.1±5.6</td>
<td>NS</td>
</tr>
<tr>
<td>HW/BW, mg/g</td>
<td>4.8±0.2</td>
<td>4.9±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Echocardiography, n</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>523±9</td>
<td>545±10</td>
<td>NS</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>4.21±0.08</td>
<td>4.18±0.09</td>
<td>NS</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>2.26±0.10</td>
<td>2.22±0.08</td>
<td>NS</td>
</tr>
<tr>
<td>FS, %</td>
<td>46±1.9</td>
<td>47±1.3</td>
<td>NS</td>
</tr>
<tr>
<td>SWT, mm</td>
<td>0.93±0.11</td>
<td>0.94±0.13</td>
<td>NS</td>
</tr>
<tr>
<td>PWT, mm</td>
<td>0.81±0.11</td>
<td>0.79±0.09</td>
<td>NS</td>
</tr>
<tr>
<td>dP/dt, mm Hg/s</td>
<td>10 323±277</td>
<td>10 961±313</td>
<td>NS</td>
</tr>
<tr>
<td>−dP/dt, mm Hg/s</td>
<td>−8720±204</td>
<td>−8713±307</td>
<td>NS</td>
</tr>
</tbody>
</table>

BW indicates body weight; HW, heart weight; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; FS, fractional shortening; SWT, septal wall thickness; PWT, posterior wall thickness; PV, pressure-volume; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEDP, left ventricular end-diastolic pressure; and dP/dt and −dP/dt, maximal and minimal first derivatives of LV pressure.

ECG, Intracardiac Recording, and Pacing

Surface ECG parameters were examined in 14 DT and 28 control mice before catheter introduction and ventricular pacing (Figure 2A). No significant differences were observed in RR (DT, 142±6 ms; controls, 136±5 ms), PQ (DT, 37±1 ms; controls, 36±1 ms), QRS (DT, 14±0 ms; controls, 14±0 ms), or QTc (DT, 56±2 ms; controls, 54±1 ms) intervals.

Intracardiac ECG recordings in DT and control mice also were similar (Figure 2B). In baseline conditions, FKBP12.6 overexpression had no effect on the ventricular effective refractory period measured at a basic cycle length of 100 ms (DT, 36±2 ms; controls, 39±2 ms; P=NS) or under sinus rhythm (DT, 32±2 ms; controls 35±2 ms; P=NS). Paced extrasystoles induced VT in 5 of 28 controls (18%) and in 5 of 14 DTs (36%; P=NS; data not shown). After intraperitoneal injection of isoproterenol, the spontaneous heart rate was too fast to pace at a cycle length of 100 ms. Ventricular effective refractory period under sinus rhythm was reduced to 27±1 ms in DT (P<0.01 versus baseline) and 28±1 ms in controls (P<0.001 versus baseline; P=NS versus DT). ECG parameters also were similar in the 2 groups (data not shown).

VT was induced by PES in 14 of 28 controls (50%) and 7 of 14 DTs (50%; data not shown).

In baseline conditions, burst pacing at a cycle length of 50 ms induced VT in 5 of 28 (18%) control mice (nonsustained VT in 2, sustained VT in 3) but in none of the DT mice (P=NS). After pretreatment with isoproterenol, the same burst pacing protocol induced VT in 12 of 27 controls (44%) but in only 1 of 14 DT (7%; P=0.03; Figure 2C and 2D). Of the 12 control mice with VT, 9 had sustained VT, sometimes lasting >5 seconds. The DT mouse with VT had only a short salvo of 6 cycles. Thus, after pretreatment with isoproterenol, FKBP12.6 overexpression prevented VT induced by burst pacing.

Ca2+ Imaging and Cellular Electrophysiology

In baseline conditions, Ca2+ spark frequency was decreased by 50% in DT myocytes compared with controls (P<0.01; Figure 3A). This was associated with a slight but significant
increase in the averaged peak amplitude ($F/F_0$; $P<0.01$), full width at half-maximum amplitude ($P<0.05$), and full duration at half-maximum peak ($P<0.05$), indicating increased spark size (Figure 3B). On isoproterenol application, $Ca^{2+}$ spark frequency increased by 41.2±16.8% ($P<0.05$) in control cells and by 26.0±16.0% in DT myocytes ($P<0.05$, $P=NS$ versus controls; Figure 3C). In DT myocytes, SR $Ca^{2+}$ load was unchanged compared with controls (Figure 3D). However, the $Ca^{2+}$ signal decay was prolonged, reflecting decreased NCX activity in DT myocytes ($P<0.05$).

In baseline conditions, at a stimulation frequency of 1 Hz, $Ca^{2+}$ transient peak amplitude was decreased by 30% in DT cells compared with controls ($P<0.001$; Figure 4A). Isoproterenol increased [$Ca^{2+}$] transient, by 17.8±5.5% in control cells ($P<0.001$) and by 32.5±3.6% in DT myocytes ($P<0.001$, $P<0.001$ versus controls). In baseline conditions, $Ca^{2+}$ transient decay was prolonged in DT myocytes ($P<0.001$; Figure 4B), with no change in cell fractional shortening (data not shown).

Whole-cell patch-clamp recordings showed a reduction in $I_{Ca-L}$ in myocytes isolated from DT mice compared with controls (Figure 4C), with no difference in cell capacitance (211.5±11.6 pF [n=32] versus 217.6±15.3 pF [n=29]; $P=NS$). Sequential comparisons showed a significant decrease in $I_{Ca-L}$ density in DT myocytes in the ~20- to 10-mV range. Peak $I_{Ca-L}$ density was 15% smaller in DT cells compared with controls (at −10 mV, −9.8±0.4 pA/pF [n=29] versus −11.8±0.5 pA/pF [n=32], respectively; $P<0.01$). These changes were not associated with changes in the voltage- or time-dependent properties of $I_{Ca-L}$ (data not shown). No change in the level of $\alpha_{1C}$-subunit mRNA was detected by quantitative reverse-transcription polymerase chain reaction (data not shown). In contrast, NCX protein level was decreased by 18% in DT mice compared with controls ($P<0.05$), in line with prolonged $Ca^{2+}$ signal decay during caffeine exposure (Figure 3D).

**RyR2-FKB12.6 Coimmunoprecipitation and RyR2 Phosphorylation**

Coimmunoprecipitation experiments showed that the increased expression of FKB12.6 in DT hearts resulted in markedly increased FKB12.6 binding to RyR2 in baseline conditions that persisted on isoproterenol stimulation in both control and DT mice (Figure 5A). Immunoblot analysis of phosphorylated and dephosphorylated forms of RyR2 at S2808 showed no difference between DT and controls in basal conditions and on isoproterenol stimulation (Figure 5B and 5C). In the latter condition, most S2808 sites appeared to be phosphorylated. Unexpectedly, in basal conditions, S2814 phosphorylation was found to be increased >2-fold in DT compared with control mice ($P<0.01$; Figure 5B and 5D). Isoproterenol stimulation increased S2814 phosphorylation almost 5-fold in control ($P<0.01$) compared with only 2-fold in DT ($P=NS$) mice to reach similar levels.
Discussion

In our conditional mouse model, cardiac FKBP12.6 overexpression increased FKBP12.6 binding to RyR2 and prevented ventricular tachycardia induced by burst pacing after pretreatment with isoproterenol. Data obtained with isolated ventricular myocytes suggest that this protection is, at least in part, due to decreased diastolic SR Ca\(^{2+}\) release.

FKBP12.6 Overexpression Prevents Catecholamine-Promoted Ventricular Arrhythmias

PES is widely used to test the propensity of patients and experimental animals to develop ventricular arrhythmias. The electric vulnerability of mouse hearts is low and strongly dependent on strain, sex, and age. The C57Bl/6 genetic background is one of the least susceptible to develop VT after

![Figure 3](image3.png)

**Figure 3.** FKBP12.6 overexpression decreases Ca\(^{2+}\) spark frequency and increases Ca\(^{2+}\) spark size without altering SR Ca\(^{2+}\) load. A, Left, Line-scan image of a freshly isolated ventricular myocyte from a control mouse (top) and a DT mouse (bottom). Right, Bar graph showing mean Ca\(^{2+}\) spark frequency measured in 37 control and 19 DT myocytes (**P<0.01**). B, Bar graphs showing increased spark amplitude (F/F\(_0\)), increased spark width at half its maximal amplitude (FWHM), and increased spark duration at half its maximal amplitude (FDHM) in DT myocytes vs controls (**P<0.05; ***P<0.01; n=112 control, n=232 DT Ca\(^{2+}\) sparks). C, Isoproterenol (ISO) effect on increasing Ca\(^{2+}\) spark frequency on 4 control and 8 DT myocytes. D, Left, Examples of line-scan images of a control (top) and DT (bottom) myocyte after caffeine application. Middle, The corresponding bar graph shows no significant difference in SR Ca\(^{2+}\) content between DT and control myocytes (**P<0.05). Right, The Ca\(^{2+}\) signal decay time during caffeine application is prolonged in DT myocytes (**P<0.05).

![Figure 4](image4.png)

**Figure 4.** SR Ca\(^{2+}\) transient and L-type Ca\(^{2+}\) channel current. A, Line-scan image of a control and a DT myocyte during field stimulation at 1 Hz. Bar graph shows a marked decrease in the SR Ca\(^{2+}\) transient peak amplitude in DT cells in baseline conditions and under isoproterenol (Iso) stimulation (**P<0.001; n=23 control, n=40 DT cells). B, Bar graph shows the prolonged decay time of the Ca\(^{2+}\) transient in DT cells in baseline conditions (**P<0.001). C, I\(_{\text{Ca,L}}\) density was reduced in DT cardiomyocytes at voltages between −20 and 10 mV (**P<0.05; ***P<0.01; n=33 control, n=33 DT cells). D, Immunoblot and bar graph show decreased NCX expression (full-length protein) in DT mice (**P<0.05).
PES or burst pacing. Indeed, the incidence of VT after PES or burst pacing in baseline conditions was very low in our control animals, making it impossible to detect a potential antiarrhythmic effect of FKBP12.6 overexpression.

Interestingly, in stress conditions, FKBP12.6 overexpression significantly reduced the incidence of arrhythmias induced by burst pacing but not by extrastimuli. PES-induced VTs are known to reflect reentrant mechanisms secondary to altered conduction and/or refractory periods. Because neither ventricular conduction nor refractory periods were affected by FKBP12.6 overexpression, the absence of protection against reentries in DT mice is not surprising.

The mechanism underlying arrhythmias triggered by burst pacing during β-adrenergic stimulation is most likely linked to delayed afterdepolarizations, as shown in mouse isolated ventricular myocytes in which delayed afterdepolarizations have been triggered in experimental conditions close to those used here. Indeed, the occurrence of delayed afterdepolarizations is favored by abrupt increases in heart rate and by catecholamines, conditions that lead to intracellular Ca²⁺ overload and store overload–induced spontaneous Ca²⁺ release via RyR2. This activates the NCX, thus generating the transient inward current (Iᵣ) at the origin of delayed afterpolarizations. Alternatively, spontaneous SR Ca²⁺ release may result from changes in RyR2 intrinsic activity as seen, for instance, with RyR2 mutations responsible for catecholaminergic polymorphic ventricular tachycardia or with RyR2 phosphorylation. Our results suggest that the protective effect of FKBP12.6 overexpression against ventricular tachycardia induced by burst pacing after pretreatment with isoproterenol is due to decreased diastolic SR Ca²⁺ release (see below), although a decrease in Iᵣ likely also plays a role.

FKBP12.6 Overexpression Reduces RyR2-Mediated Diastolic Ca²⁺ Leakage
FKBP12.6 overexpression in vitro or enhanced FKBP12.6 binding to RyR2 by JTV51913 reduces SR Ca²⁺ release events in resting cardiac myocytes, which has been proposed to occur by stabilizing RyR2 in its closed state during diastole. The marked reduction in Ca²⁺ spark frequency, which we report here in myocytes isolated from DT mice, is consistent with this hypothesis. Our results indeed show that FKBP12.6 overexpression is associated with a decreased occurrence of spontaneous openings of RYR2. Importantly, isoproterenol increased Ca²⁺ spark frequency in a similar proportion in control and DT myocytes, thus maintaining a reduced Ca²⁺
leak in DT myocytes compared with controls, consistent with the decreased incidence of ventricular arrhythmias after burst pacing in DT mice pretreated with isoproterenol.

The mechanisms by which FKBP12.6 overexpression decreased spark frequency in basal and in stress conditions in DT mice are probably complex and multiple. Our results suggest that this is due, at least in part, to the major increase in FKBP12.6 binding to RyR2. The marked apparent difference in FKBP12.6 binding to RyR2 in our communoprecipitation experiments raises questions about the precise baseline stoichiometry of FKBP12.6 to RyR2. If 1 RyR2 tetramer cannot bind >4 FKBP12.6 molecules, our results suggest that the baseline binding ratio in mice is much lower that the value of 3.6 reported for the dog heart.28,29 Alternatively, it cannot be excluded that the maximal binding capacity exceeds 4 FKBP12.6 molecules per RyR2. Most importantly, such an increased binding persisted with isoproterenol exposure. If this holds true in isolated myocytes and in vivo, it may explain both the decreased diastolic SR Ca$^{2+}$ leakage and the decreased incidence of ventricular tachycardia induced by burst pacing in stress conditions.

In contrast to our study, in cultured rabbit15,27 and rat9 cardiac myocytes with adenoviral FKBP12.6 overexpression, decreased Ca$^{2+}$ spark frequency was associated with both a decrease in Ca$^{2+}$ spark size and an increase in SR Ca$^{2+}$ load. Differences in animal species, experimental conditions, and adaptive mechanisms linked to interdependence between SR Ca$^{2+}$ content and transsarcolemmal Ca$^{2+}$ fluxes may explain this discrepancy. In cultured ventricular myocytes with adenoviral FKBP12.6 overexpression, Ca$^{2+}$ spark characteristics are significantly different from those observed in freshly dissociated myocytes,15 possibly because of alterations in cell architecture (eg, loss of t tubules) and in the phosphorylation status and expression level of Ca$^{2+}$-handling proteins. Moreover, differences also may be due to the duration of FKBP12.6 overexpression (short term in the transfection experiments versus long term in DT mice).

**RyR2 Phosphorylation in Basal Condition and During Stress**

There is a consensus that RyR2 can be protein kinase A and CaMK phosphorylated11,30–32 but not on whether RyR2 phosphorylation dissociates FKBP12.6 from its binding sites.8–10 β-Adrenergic stimulation increased S2808 phosphorylation to a similar extent in DT and control mice (Figure 5C). Similarly, RyR2 phosphorylation at S2814 increased to comparable levels in control and DT mice, consistent with the dual effect of β-adrenergic stimulation on protein kinase A (S2808) and CaMKII (S2808 and S2814) phosphorylation sites.33,34 Most importantly, our communoprecipitation experiments indicated that these phosphorylations, submaximal in the case of S2808, were not accompanied by a significant dissociation of FKBP12.6 from RyR2, in agreement with previous reports.10,19,35 Altogether, our results suggest that decreased SR Ca$^{2+}$ leakage on isoproterenol stimulation in DT mice resulted from increased FKBP12.6 binding to RyR2 despite increased RyR2 phosphorylation.

Intriguingly, in contrast to similar S2814 phosphorylation levels in DT and control mice in stress conditions, the baseline phosphorylation level at S2814 in the former was more than twice that in the latter. In view of several reports indicating an increase in Ca$^{2+}$ spark frequency and SR Ca$^{2+}$ leakage in the presence of increased CaMKII activity and/or CaMKII-mediated S2814 RyR2 phosphorylation,19,26,36 it is possible that the increased baseline S2814 phosphorylation represents a long-term adaptive mechanism aimed at maintaining normal SR Ca$^{2+}$ load by preventing excessive decrease in SR Ca$^{2+}$ efflux. However, this hypothesis should be taken cautiously because the effects of S2814 phosphorylation on RyR2 function remain controversial.37,38

**FKBP12.6 Overexpression Effects on Transsarcolemmal Ca$^{2+}$ Movements**

The reasons for the observed decrease in I_{Ca,L} density are unclear and require further study. The decrease could be due to FKBP12.6-induced alterations in the L-type Ca$^{2+}$ channel–RyR2 interaction.2 The decrease in [Ca$^{2+}$], transient amplitude may simply result from the reduced trigger (I_{Ca,L}). Alternatively, it could be due to FKBP12.6 overexpression favoring the closed state of RyR2. Finally, it is possible that the prolonged duration of the [Ca$^{2+}$], transient amplitude, resulting in unaltered fractional shortening of isolated myocytes. Cardiac myocytes are known to adapt to alterations in RyR2 function by rapidly returning to steady-state SR Ca$^{2+}$ load.19,40 Such homeostasis is achieved by adaptation of transsarcolemmal Ca$^{2+}$ movements. In the present study, slower Ca$^{2+}$ extrusion via decreased NCX activity might have counterbalanced decreased Ca$^{2+}$ entry via I_{Ca,L}. In addition, the reduced Ca$^{2+}$ leak that tends to increase the SR Ca$^{2+}$ load might have been counterbalanced by the decreased I_{Ca,L}, which tends to decrease the SR Ca$^{2+}$ load.

**Conclusions**

Our study shows that cardiac FKBP12.6 overexpression prevents triggered ventricular arrhythmias in stress conditions without altering baseline ECG parameters or myocardial performance. The antiarrhythmic effect is probably linked to a reduced diastolic SR Ca$^{2+}$ leak, itself a result of increased FKBP12.6 binding to RyR2, even when the latter is heavily phosphorylated. Our results also underline the importance of in vivo studies for investigating the impact of manipulating SR Ca$^{2+}$ handling and support the hypothesis that FKBP12.6 binding to RyR2 is an important potential target for the development of new drugs aimed at preventing ventricular arrhythmias.

**Acknowledgment**

This work is dedicated to the memory of Denis Escande, MD, PhD (1953 to 2006), a fighter against sudden cardiac death.

**Sources of Funding**

This work was supported in part by a grant from Programme National de Recherche Cardiovasculaire of Inserm and by a grant from Agence Nationale de la Recherche “Cardiologie, obésité, diabète” (ANR-05-PCOD-037-02). Dr Gellen was supported by grants from the Fondation Lefoulon-Delalande, the European Union (Marie Curie Fellowship), Groupe de Réflexion sur la Recherche Cardiovasculaire de la Société Française de Cardiologie, and...
Disclosures

None.

References


9. Xiao B, Sutherland C, Walsh MP, Chen SR. Protein kinase A phosphorylation at serine-2808 of the cardiac Ca\(^{2+}\)-release channel (ryanodine receptor) does not dissociate 12.6-kDa FK506-binding protein receptor (FKBP12.6). *Circ Res*. 2004;94:487–495.


Ventricular arrhythmias are a frequent fatal outcome during chronic heart failure. As is the case in catecholaminergic ventricular tachycardia, they seem to result from a leak out of the sarcoplasmic reticulum (SR) during diastole, itself favored by stress. The ryanodine receptor (RyR2) is the SR channel through which calcium normally comes out of the SR during systole to trigger contraction and leaks out of the SR during diastole. It has been suggested that RyR2 leakage may be favored by the unbinding from RyR2 of the small regulatory protein FKBP12.6, also known as calstabin 2. In the present study, we show in a mouse model that increasing the expression level of FKBP12.6 in cardiac myocytes results in increased FKBP12.6 binding to RyR2, even when the latter is hyperphosphorylated, a feature associated with a decreased rate of ventricular tachycardia triggered by burst pacing in stress conditions, and a reduced SR calcium leak in isolated myocytes. Our results firmly support the hypothesis that the FKBP12.6-RyR2 complex is an important candidate target for pharmacological prevention of ventricular tachycardia. Other studies are now needed to determine precisely how FKBP12.6 binding to a hyperphosphorylated RyR2 exerts this beneficial effect and to identify new molecules that may favor this binding.
Conditional FKBP12.6 Overexpression in Mouse Cardiac Myocytes Prevents Triggered Ventricular Tachycardia Through Specific Alterations in Excitation-Contraction Coupling

Barnabas Gellen, María Fernández-Velasco, François Briec, Laurent Vinet, Khai LeQuang, Patricia Rouet-Benzineb, Jean-Pierre Bénitah, Mylène Pezet, Gael Palais, Noémie Pellegrin, Andy Zhang, Romain Perrier, Brigitte Escoubet, Xavier Marniquet, Sylvain Richard, Frédéric Jaisser, Ana María Gómez, Flavien Charpentier and Jean-Jacques Mercadier

Circulation. 2008;117:1778-1786; originally published online March 31, 2008; doi: 10.1161/CIRCULATIONAHA.107.731893

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/117/14/1778

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/