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-Benazine, PhD; Jean-Pierre Bénitah, PhD; Mylène Pezet, PhD; Gael Palais, MS; Noémie Pellegrin, MS; Andy Zhang, MS; Romain Perrier, MS; Brigitte Escoubet, MD; PhD; 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\(^{\text{Ca}}\) 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
bility of the RyR2 channel, thereby leading to an aberrant increase in diastolic \( [\text{Ca}^{2+}]_i \), and increasing the risk of ventricular arrhythmias.\(^6\)\(^,\)\(^7\) 

Clinical Perspective p 1786

It has been proposed that \( \beta \)-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.\(^5\)\(^–\)\(^10\) FKBP12.6-deficient mice \((\text{FKBP12.6}^{-/-})\) consistently exhibit exercise-induced ventricular arrhythmias.\(^11\) In mice with reduced FKBP12.6 levels \((\text{FKBP12.6}^{+/-})\), pretreatment with the 1,4-benzothiazepine 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 remodeling. In vitro, FKBP12.6 overexpression has been shown to reduce diastolic SR \( \text{Ca}^{2+} \) 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 \( \text{Ca}^{2+} \) 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 transactivator \((\text{tTA})\) 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, \( \text{\alpha-MHCtTA} \), tetO-FKBP12.6, and DT mice were identified by Southern blot. All mice used in the present study were maintained on a 21°C to 24°C. 

Immunoblots 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 (25 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 refractory 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.

Ca\(^{2+}\) Imaging and Cellular Electrophysiology

Single ventricular myocytes were prepared by enzymatic dissociation as described.\(^18\) Myocytes were loaded with the cell-permeant \( \text{Ca}^{2+} \) fluorescent dye fluo-3 AM. Confocal imaging \((\text{Meta Zeiss LSM 510})\) were acquired in the line-scan mode at 1.5 ms per line. L-type \( \text{Ca}^{2+} \) current \((I_{\text{CaL}})\) was measured with the whole-cell patch-clamp technique \((\text{Axopatch-1D amplifier, Axon Instruments})\) with 1.0- to 1.8-M\(\Omega\)-resistance micropipettes. Calcium imaging and electrophysiology experiments were performed at room temperature \((21^\circ\text{C} \text{ to } 24^\circ\text{C})\).

Data Analysis

A detailed Methods section can be found in the online Data Supplement.

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 and showed a marked FKBP12.6 overexpression in DT mice (Figure 1C). These data demonstrated strong and cardiac-specific FKBP12.6 overexpression in DT mice. The expression levels of FKBP12, RyR2, SERCA2a, phospholamban, and calsequestrin were not significantly altered (Figure 1B and 1C). Thus, FKBP12.6 overexpression for 6 to 8 weeks did not alter cardiac morphofunctional parameters or the expression level of the main Ca\(^{2+}\)-handling SR proteins.

**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.

**Ca\(^{2+}\) Imaging and Cellular Electrophysiology**

In baseline conditions, Ca\(^{2+}\) 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

---

**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>PV catheterization, n</td>
<td>13</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>507±10</td>
<td>487±13</td>
<td>NS</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>107±3</td>
<td>113±2</td>
<td>NS</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>70±2</td>
<td>76±2</td>
<td>NS</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>6±1</td>
<td>6±1</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>

**Figure 1. DT mice overexpress FKBP12.6 in the myocardium.** A, Reverse-transcription (RT) polymerase chain reaction specific for TG FKBP12.6 shows marked expression of the transgene in the myocardium, whereas transgene expression in other tissues is very weak or absent. B, Immunoblot of proteins from ventricular homogenates using anti-FKBP12/12.6 antibody shows marked FKBP12.6 expression in DT cells. C, Immunoblot of proteins from ventricular homogenates using anti-FKBP12.6-specific antibody shows marked FKBP12.6 overexpression in DT mice. D, No change in the expression level of the SR Ca\(^{2+}\) regulatory proteins RyR2, SERCA2a, phospholamban (PLB), or calsequestrin was found by Western blot in FKBP12.6-overexpressing mice. RP indicates FKBP12 and FKBP12.6 mouse recombinant proteins. WT indicates wild type.
increase in the averaged peak amplitude (F/F0; 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, Ca²⁺ 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 Ca²⁺ load was unchanged compared with controls (Figure 3D). However, the Ca²⁺ signal decay was prolonged, reflecting decreased NCX activity in DT myocytes (P<0.05).

In baseline conditions, at a stimulation frequency of 1 Hz, Ca²⁺ transient peak amplitude was decreased by 30% in DT cells compared with controls (P<0.001; Figure 4A). Isoproterenol increased [Ca²⁺] transient frequency increased by 14.2±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 Ca²⁺ load was unchanged compared with controls (Figure 3D). However, the Ca²⁺ signal decay was prolonged, reflecting decreased NCX activity in DT myocytes (P<0.05).

Whole-cell patch-clamp recordings showed a reduction in ICa,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 ICa,L density in DT myocytes in the −20- to 10-mV range. Peak ICa,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 ICa,L (data not shown). No change in the level of α1C-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 Ca²⁺ signal decay during caffeine exposure (Figure 3D).

**RyR2-FKBP12.6 Coimmunoprecipitation and RyR2 Phosphorylation**

Coimmunoprecipitation experiments showed that the increased expression of FKBP12.6 in DT hearts resulted in markedly increased FKBP12.6 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...
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 Ca$^{2+}$ overload– and store overload–induced spontaneous Ca$^{2+}$ release via RyR2. This activates the NCX, thus generating the transient inward current (I\textsubscript{t,i}) at the origin of delayed afterdepolarizations. Alternatively, spontaneous SR Ca$^{2+}$ 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 Ca$^{2+}$ release (see below), although a decrease in I\textsubscript{t,i} likely also plays a role.

**FKBP12.6 Overexpression Reduces RyR2-Mediated Diastolic Ca$^{2+}$ Leakage**

FKBP12.6 overexpression in vitro or enhanced FKBP12.6 binding to RyR2 by JTV51913 reduces SR Ca$^{2+}$ 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 Ca$^{2+}$ 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 Ca$^{2+}$ spark frequency in a similar proportion in control and DT myocytes, thus maintaining a reduced Ca$^{2+}$

---

**Figure 5.** RyR2-FKBP12.6 coimmunoprecipitation and RyR2 phosphorylation. A, Typical immunoblot (among 3) of FKBP12.6 and RyR2 coimmunoprecipitated with the anti-RyR2 antibody from control (Ctr; wild type [WT]) and tTA) and DT heart homogenates of mice injected with isoproterenol (ISO) or vehicle using the anti-RYR2 antibody (top) and anti-FKBP12.6 antibody (bottom) B, Immunoblots of proteins from ventricular homogenates of DT and control mice injected with isoproterenol or vehicle using anti-RyR-PS2808, anti-RyR-dePS2808, and anti–RyR-PS2814–specific antibodies. C, Bar graph showing the ratio of phosphorylated to dephosphorylated forms of RyR2 at S2808 shown as a percentage of baseline value in controls. D, Bar graph showing the ratio of phosphorylated form of RyR2 at S2814 to total RyR2 as a percentage of baseline value in controls. **P < 0.01, *P < 0.05 vs values at baseline; n = 6 for control, n = 5 for DT.
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 than 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 rat\(^{6}\) 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).

**RyR2Phosphorylationin Basal Condition and During Stress**

There is a consensus that RyR2 can be protein kinase A and CaMK phosphorylated\(^ {11,30-32} \) but not on whether RyR2 phosphorylation dissociates FKBP12.6 from its binding sites.\(^ {8-10} \) \( \beta \)-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 \( \beta \)-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 \( I_{\text{Ca,L}} \) 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 (\( I_{\text{Ca,L}} \)). 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.\(^ {39,40} \) 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 \( I_{\text{Ca,L}} \). In addition, the reduced Ca\(^{2+}\) leak that tends to increase the SR Ca\(^{2+}\) load might have been counterbalanced by the decreased \( I_{\text{Ca,L}} \), 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...
Académie Nationale de Médecine. Dr Fernández-Velasco is a fellow of the Spanish Ministry of Education and Science. L. Vinet was supported by the Ministère de l’Enseignement Supérieur et de la Recherche and Institut de Recherches Servier (Suresnes, France). Drs Gómez and Richard are scientists at the Centre National de la Recherche Scientifique. Dr Gellen, L. Vinet, Dr Rouet-Benzineb, N. Pellegrin, and Dr Mercadier are supported by Inserm, Université Paris Diderot, Association Française du Cœur, Fondation de France, and EU FP6 grant LSHM-CT-2005-018833, EUGeneHeart.

Disclosures

None.

References

15. Xiao B, Sutherland C, Walsh MP, Chen SR. Protein kinase A phosphorylation mutants of recombinant skeletal muscle (Ser-2843) and cardiac muscle (Ser-2809) ryanodine receptors: modulation by Mg2+/H11001. *J Physiol*. 2001;546:919–934.


**CLINICAL PERSPECTIVE**

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/