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Purkinje Cell Calcium Dysregulation Is the Cellular Mechanism that Underlies Catecholaminergic Polymorphic Ventricular Tachycardia

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Abstract

Background—Inherited arrhythmias can be caused by mutations in the cardiac ryanodine receptor (RyR2). The cellular source of these arrhythmias is unknown. Isolated RyR2^{R4496C} mouse ventricular myocytes display arrhythmogenic activity related to spontaneous Ca²⁺ release during diastole. On the other hand, recent whole heart epicardial and endocardial optical mapping data demonstrate that ventricular arrhythmias in the RyR2^{R4496C} mouse model of CPVT originate in the His-Purkinje system, suggesting that Purkinje cells, and not ventricular myocytes, may be the cellular source of arrhythmogenic activity. The relative effect of the RyR2^{R4496C} mutation on calcium homeostasis in ventricular myocytes versus Purkinje cells is unknown.

Objective—To determine which cardiac cell type is more severely affected, in terms of calcium handling, by expression of the RyR2^{R4496C} mutant channel: the ventricular myocytes or the Purkinje cells.

Methods and Results—To discriminate Purkinje cells from ventricular myocytes we crossed the RyR2^{R4496C} mouse model of CPVT with the Cx40^{EGFP/+} transgenic mouse. This genetic cross yields Purkinje cells that express eGFP, and therefore fluoresce green when excited by the appropriate wavelength; ventricular myocytes, which do not express Cx40, are not green. Intracellular calcium was measured in each cell type using calcium sensitive probes. Purkinje cells of the RyR2^{R4496C} mouse model of CPVT exhibit a ~2X greater rate (P<0.05) and ~2-3X greater amplitude (P<0.000001) of spontaneous calcium release events than ventricular myocytes isolated from the same heart.

Conclusion—These results demonstrate that focally activated arrhythmias originate in the specialized electrical conducting cells of the His-Purkinje system in the RyR2^{R4496C} mouse model of CPVT.

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Keywords

Inherited arrhythmias; Ryanodine Receptor; Calcium; Cardiac Purkinje cells; Molecular mechanisms of arrhythmia; Ventricular cardiac myocytes

Introduction

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a rare but lethal inherited arrhythmogenic disease that leads to stress-induced sudden cardiac death¹. Autosomal dominant forms of CPVT have been linked to ryanodine receptor gene (RyR2) mutations²⁻⁴. In cardiac muscle cells RyR2 governs the release of calcium from internal stores of the sarcoplasmic reticulum (SR) following membrane depolarization. The calcium released from the SR via RyR2 initiates myocardial contraction. Consequently, inappropriate calcium release from the SR may result in delayed afterdepolarizations (DADs) and cause triggered activity leading to ectopic discharges^{5,6}. Currently more than 70 RyR2 mutations have been identified and RyR2 mutations may account for half of diagnosed cases of CPVT^{2, 3, 7}.

The first cardiac ryanodine receptor mutation identified in a family afflicted with CPVT was RyR2^{R4497C}. When expressed transiently in human embryonic kidney (HEK) cells, the RyR2^{R4496C} (the mouse correlate to the R4497C mutation in the human RyR2 sequence) mutation causes increased basal activity and increased sensitivity to luminal calcium^{8, 9}. These reports demonstrated that the charge and polarity at amino acid residue 4496 is essential for RyR2 channel gating. More recently a knock-in mouse model carrying the RyR2^{R4496C} mutation was developed, which provided important information about the molecular mechanisms that underlie the CPVT phenotype¹⁰. DADs are observed in RyR2^{R4496C} cardiac myocytes when exposed to known stressors such as caffeine and adrenaline¹¹. Fernandez-Velasco et al have recently shown that even untreated RyR2^{R4496C} myocytes have increased spontaneous Ca²⁺ release (SCR) in diastole following electrical pacing when compared with wild-type (WT) myocytes¹².

Recent evidence from the RyR2^{R4496C} knock-in mouse model strongly suggests that the His-Purkinje system is a major source of focal arrhythmias in CPVT¹³. A combination of high resolution epi- and endocardial optical mapping experiments demonstrated that focal activation of the His-Purkinje system causes bidirectional ventricular tachycardia¹³ in this genetic mouse model of CPVT. At the cellular level, single Purkinje cells from RyR2^{R4496C} hearts generated DADs at lower frequencies than those isolated from WT hearts. Similar results were obtained when cardiac myocytes were compared between WT and RyR2 mutant genotypes¹¹. The relative effects of this RyR2 mutation on cardiac myocyte calcium homeostasis versus Purkinje cell calcium homeostasis, however, are unknown. Because both Purkinje cells and myocytes develop DADs, it is unclear whether focally activated arrhythmias in the RyR2^{R4496C} mouse model originate in the 3D myocardium or in the branching network of the His-Purkinje system.

Here we have compared the incidence of spontaneous calcium release (SCR) in WT and RyR2^{R4496C} Purkinje cells and ventricular myocytes. To clearly discriminate between Purkinje cells and ventricular myocytes, we crossed the RyR2^{+/R4496C} CPVT mouse with the Cx40^{eGFP/+} transgenic mouse¹⁴. This genetic cross yields Purkinje cells that express enhanced Green Fluorescent Protein (eGFP) while ventricular myocytes from these animals do not.

Methods

Mouse models

The RyR^{R4496C} mouse was obtained from the laboratory of one of the authors (SP). Details of the production and characterization of the knock-in RyR^{R4496C} mice have been previously described^{10,13}. Cx40^{eGFP/+} mice were generously provided by the laboratory of Dr. Daniel Gros at Université de la Méditerranée, Marseille, France. Generation and characterization of this mouse has been described in detail previously¹⁴. To easily identify the Purkinje cells in the CPVT, heterozygous (RyR^{+ / R4496C}) mouse, we cross-bred this strain of mouse with the Cx40^{eGFP/+} mice to generate the RyR^{+ / R4496C}-Cx40^{eGFP/+} mouse.

Isolation of murine Purkinje cells and ventricular myocytes

The protocol for isolating adult ventricular myocytes and Purkinje cells is a modification of that previously described^{13,15}. All protocols were performed with the approval of the University Committee on Use and Care of Animals at the University of Michigan. Briefly, RyR2 mutant (RyR2^{+ / R4496C} or RyR2^{+ / R4496C}-Cx40^{eGFP/+}) and the respective littermate wild-type (RyR2^{+/+} or RyR2^{+/+}-Cx40^{eGFP/+}) mice (2-4 months old) were injected with heparin (1000 IU/ml, intraperitoneal (IP)) 20 min before heart excision. Animals were then anesthetized with Avertin (tribromoethanol, 125-250 mg/Kg, IP). The heart was quickly removed from the chest and retrograde perfusion was performed via the aorta with constant flow at 37°C for 4 min with a Ca²⁺-free buffer containing (in mmol/L): 113 NaCl, 4.7 KCl, 1.2 MgSO₄, 0.6 Na₂HPO₄, 0.6 KH₂PO₄, 10 KHCO₃, 12 NaHCO₃, 10 HEPES, 10 2,3-butanedione monoxime (BDM, Sigma), 30 taurine, and 5.5 glucose. All solutions were filtered (0.2-μm filter) and equilibrated with 100% O₂ for at least 20 min before use. Enzymatic digestion was initiated by adding collagenase type II (Worthington) (0.25 mg/mL), trypsin (0.14 mg/ml), and CaCl₂ (12.5 μmol/L) to the perfusion solution. After digestion, the ventricles were cut open and Purkinje fibers/webs were extracted from the tissue using fine-tipped forceps and a dissecting microscope. The fibers were teased in solution containing perfusion buffer plus 10% fetal bovine serum and 12.5 μmol/L CaCl₂ to free the Purkinje cells. Remaining tissue from the free ventricular walls was cut out and teased into small pieces to obtain a pure population of rod-shaped myocytes. The extracellular Ca²⁺ concentration was increased gradually to 1 mmol/L. Cells were used for intracellular calcium measurements within 4 hr after isolation.

Intracellular calcium measurements

Cardiac cell suspensions were incubated with cell permeant calcium indicators (rhod-2AM or fura-2AM, 2 μmol/L Molecular Probes) to monitor intracellular calcium concentrations. Briefly, cells were loaded with the appropriate dye for 10 min at room temperature and then washed to remove excess dye. Rhod-2 excitation and emission spectra do not overlap with eGFP fluorescence and was therefore used in experiments utilizing the GFP-positive Purkinje cells. Rhod-2 fluorescence is expressed as $\Delta F/F_0 = (F - F_0)/F_0$, where F is the fluorescence at any given time and F₀ is the baseline fluorescence value.

GFP is a large protein that can potentially affect the intracellular milieu, so experiments were also performed in Purkinje cells isolated from the RyR^{R4496C} mutant mouse since these cells do not express eGFP. In these experiments Fura-2 was used to quantify intracellular calcium concentrations in cells from the RyR^{R4496C} mutant mouse. Purkinje cells were identified based on cell size and morphology as previously described^{13,15}. Importantly, eGFP-positive Purkinje cells were found to be significantly thinner than ventricular myocytes from the same heart, thus providing a way to discriminate mouse Purkinje cells from ventricular myocytes based on morphometry.¹⁵

Spontaneous calcium release (SCR) from internal cellular stores was measured after cessation of electrical pacing. Briefly, cells were paced by field stimulation (40V, 37°C) up to 5-6Hz to load the sarcoplasmic reticulum with calcium and then stimulation was turned off. Intracellular calcium concentrations were continuously recorded during and following electrical pacing using a photomultiplier tube (1 kHz) and commercially available software (IonOptix, Inc. Milton, MA). The same pacing protocol was utilized for cells treated with isoproterenol (10 nmol/L) to determine any cell specific effect of β -adrenergic stimulation.

Fura-2 calibration

The Fura-2 calcium imaging calibration kit (Invitrogen, Molecular Probes) was used to transform Fura ratio units into calcium concentrations (nmol/L). The interrelationship of the free calcium concentration and the fluorescence emission intensity ratio is described by the equation:

$$[Ca^{2+}]_{free} = K_d \times [R - R_{min}/R_{max} - R] \times [F^{380}_{max}/F^{380}_{min}],$$

where R is the ratio of 510 nm emission intensity with alternate (250Hz) excitation between 340 nm and 380 nm ultraviolet light. R_{min} is the ratio at zero free Ca^{2+} ; R_{max} is the ratio at saturating Ca^{2+} (39 μ mol/L); F^{380}_{max} is the fluorescence intensity, with 380 nm excitation, for zero free Ca^{2+} ; and F^{380}_{min} is the fluorescence intensity at saturating free Ca^{2+} . A value of 225 nmol/L was assumed for the dissociation constant for the fura2-calcium binding (K_d).

Results

Purkinje cell isolation and identification

eGFP expression under the control of the Connexin40 (Cx40) promoter was used to clearly discriminate Purkinje cells from ventricular myocytes as previously reported¹⁴. The cross of the RyR2^{+/R4496C} CPVT knock-in mouse with the Cx40^{eGFP/+} transgenic mouse results in Purkinje cell specific eGFP expression. Figure 1 demonstrates typical cardiac cell isolation performed in our laboratory and shows Purkinje cell specific eGFP expression. Dimension analysis reveals that Purkinje cells have more narrow widths compared to the ventricular myocytes (10.1 $\pm$ 0.5 μ m vs. 26.6 $\pm$ 1.3 μ m, n=43, P<0.000001). This difference in the width allows for identification of Purkinje cells without the use of GFP.

Spontaneous calcium release in Purkinje cells and ventricular myocytes

Control experiments were performed in WT littermate mice that were negative for the RyR2 mutation, but positive for eGFP expression under the Cx40 promoter (RyR2^{+/+}-Cx40^{eGFP/+}). Myocytes and Purkinje cells from the wild-type littermate group were compared to cells from mice that were positive for the RyR2 mutation and eGFP expression (RyR2^{+/R4496C}-Cx40^{eGFP/+}).

eGFP positive Purkinje cells expressing the RyR2 mutation were identified on a fluorescent microscope (Nikon Ti-S, 40x CFI Super Fluor oil objective, NA 1.3) before intracellular calcium measurements. Ventricular myocytes were negative for eGFP expression (Figure 1). Figure 2A shows representative intracellular calcium recordings in a ventricular myocyte and Figure 2B shows representative intracellular calcium recordings in a Purkinje cell from the same heart. Average data in Figure 2C demonstrate that Purkinje cells exhibited a higher rate of SCR (0.11 $\pm$ 0.03 s⁻¹, n=7 Purkinje cells from 3 different mice) than ventricular myocytes (0.05 $\pm$ 0.01 s⁻¹, n=7 myocytes from 3 different mice). The amplitude of SCR was greater in RyR2 mutant Purkinje cells ($\Delta F/F_0$ =0.19 $\pm$ 0.02, n=60 SCR events from 7 cells from 3 mice) than in the ventricular myocytes ($\Delta F/F_0$ =0.06 $\pm$ 0.008, n=34 SCR events from 7

cells from 3 mice). Compared to non-transgenic littermates, RyR2 mutant Purkinje cells had a higher rate of SCR. Consistent with a previous study, RyR2 mutant myocytes also had a higher rate of SCR following the pacing protocol¹². A comparison of RyR^{+/+}-Cx40^{eGFP/+} cardiac cells with RyR^{+/R4496C}-Cx40^{eGFP/+} cardiac cells is presented in Figure 3. In both cases, RyR2 mutant cells demonstrate a higher number (panel A) and frequency of SCR (panel B), and in both, the number and frequency of SCR are greater in Purkinje cells than in ventricular myocytes. Altogether, these data demonstrate that the cardiac Purkinje cells are affected to a greater degree than the myocytes of the RyR2^{+/R4496C} CPVT mouse.

To better quantify the intracellular calcium concentrations in Purkinje cells and myocytes, we next used the ratiometric calcium indicator Fura-2 AM. In these experiments GFP was not used to distinguish Purkinje cells from myocytes. On account of the morphological differences between myocytes and Purkinje cells (Figure 1), we could distinguish one cell type from another based on cell width and shape as previously done¹³. As shown in Figure 4, results obtained using Fura-2 are similar to the results obtained using rhod-2 (Figure 2). Resting calcium concentrations after electrical pacing were the same between cell types (Figure 4C, 245.1±1.91nmol/L, n=8 cells, N=3 mice vs. 243.9±4.6nmol/L, n=6 myocytes, N=3 mice). The peak calcium concentration observed for SCR events was greater in Purkinje cells (Figure 4D, 330.5±4.5nmol/L, n=6 cells, N=3 mice vs. 285.7±6.7nmol/L, n=6 myocyte, N=3 mice) and thus the amplitude of SCR events was ~2X greater in Purkinje cells (Figure 4E). The latency from the time cessation of electrical stimulation to the first SCR was shorter in Purkinje cells (Figure 5). The latency period for Purkinje cells was 4.17±0.93 s and the latency period for ventricular myocytes was 8.5±1.7 s (n=7 Purkinje cells and n=5 myocytes from 3 mice).

Spontaneous calcium release in Purkinje cells and ventricular myocytes after β -adrenergic stimulation

Ventricular arrhythmias are induced by catecholamines in CPVT patients and in the RyR2^{R4496C} mouse model of CPVT *in vivo*^{1,10}. Therefore, we next tested the effect of isoproterenol (10 nmol/L) on Purkinje cell and ventricular myocyte calcium homeostasis following the same electrical pacing protocol shown in Figure 2. The effect of isoproterenol on SCR in each cell type is shown in Figure 6. Panel A shows a representative recording of intracellular calcium in a mutant Purkinje cell following electrical pacing (6Hz). Figure 6B shows a representative intracellular recording in a mutant ventricular myocyte following the same pacing regimen. As predicted, SCR in the Purkinje cells are more frequent than in myocytes after β -adrenergic stimulation (Figure 6C).

Discussion

Our data demonstrate that the RyR2^{R4496C} mutation has more profound effects on Purkinje cell calcium activation than on ventricular myocytes. This is manifested as greater frequency, amplitude and duration of SCR in Purkinje cells than in the ventricular myocytes. These results strongly support the contention that cardiac Purkinje cells represent a cellular basis for arrhythmia initiation in CPVT. We utilized a unique genetic approach to discriminate Purkinje cells from ventricular myocytes based on Connexin40-driven eGFP expression¹⁴. These new cellular data strongly support previous whole heart optical mapping studies reporting that the His-Purkinje system is the likely source of focally activated arrhythmias including bidirectional ventricular tachycardia and polymorphic ventricular tachycardia that can degenerate into ventricular fibrillation¹³. Single Purkinje cells and ventricular myocytes isolated from this mouse model are reported to generate delayed afterdepolarizations (DADs)^{12, 13}. Taken together, the data presented by Cerrone et al¹³ and our results demonstrate that DADs caused by calcium overload are of more common

occurrence in Purkinje cells than in ventricular myocytes, both at baseline and after β -adrenergic stimulation.

Purkinje cells are very different from ventricular myocytes morphologically and electrophysiologically (reviewed recently¹⁶). Importantly, the intracellular calcium homeostasis mechanisms are very distinct between Purkinje cells and ventricular myocytes^{6,17}, and Purkinje cells have a higher sodium content than ventricular myocytes¹⁸. In addition, unlike ventricular myocytes, Purkinje cells are devoid of t-tubules and have two types of sarcoplasmic reticulum: junctional and corbular¹⁹. This morphological difference dictates a unique calcium activation process in Purkinje cells compared to the ventricular myocytes²⁰. Upon electrical stimulation in canine Purkinje cells there is an initial uniform calcium rise under the cell membrane that propagates to the central core region via the well known calcium induced calcium release mechanism²¹. Thus the calcium release units, which are comprised of RyR2, in the core of the cell do not respond to membrane voltage, but to calcium diffusion as a wave in forward mode. In the case of the R4496C mutation of RyR2 it is conceivable that this calcium wave propagation in Purkinje cells operates in reverse mode. In other words, it is possible that leaky mutant RyR2 channels can initiate a calcium wave that propagates from the core of a Purkinje cell back to the membrane and can cause depolarization. This reverse propagation of calcium as a wave may then travel through the single Purkinje cell as well as between cells and along a fiber²⁰. This potential mechanism is referred to as reverse mode excitation-contraction coupling and warrants further study to shed light on mechanisms of arrhythmia initiation and maintenance not only in genetic cases of CPVT, but also in more common forms of heart disease such as ischemic heart failure.

Clinical implications

CPVT is an inherited arrhythmia syndrome with a high mortality rate in young individuals¹⁻³. Current therapies such as β -adrenergic blockers and implantable cardioverter defibrillators can reduce the arrhythmia propensity and mortality rate in CPVT patients, but present some limitations². For example, inappropriate shocks from ICDs can be painful for patients and trigger further adrenergic stress and death in CPVT patients. Thus, there is a great need to develop novel cellular and molecular based therapies. The results presented here suggest that Purkinje cell specific therapies to correct the calcium dysregulation in CPVT hearts may offer a tantalizing new strategy for anti-arrhythmic therapies.

Non-Standard Abbreviations and Acronyms

CPVT	Catecholaminergic Polymorphic Ventricular Tachycardia
Cx40	Connexin 40
RyR2	Ryanodine Receptor type 2
SCR	Spontaneous Calcium Release
EGFP	Enhanced Green Fluorescent Protein

Reference List

1. Leenhardt A, Lucet V, Denjoy I, Grau F, Ngoc DD, Coumel P. Catecholaminergic Polymorphic Ventricular Tachycardia in Children : A 7-Year Follow-up of 21 Patients. *Circulation* March 1;1995 91(5):1512–9. [PubMed: 7867192]
2. Priori SG, Napolitano C, Memmi M, et al. Clinical and Molecular Characterization of Patients With Catecholaminergic Polymorphic Ventricular Tachycardia. *Circulation* July 2;2002 106(1):69–74. [PubMed: 12093772]

3. Laitinen PJ, Brown KM, Piippo K, et al. Mutations of the Cardiac Ryanodine Receptor (RyR2) Gene in Familial Polymorphic Ventricular Tachycardia. *Circulation* January 30;2001 103(4):485–90. [PubMed: 11157710]
4. Baucé B, Rampazzo A, Basso C, et al. Screening for ryanodine receptor type 2 mutations in families with effort-induced polymorphic ventricular arrhythmias and sudden death: Early diagnosis of asymptomatic carriers. *J Am Coll Cardiol* July 17;2002 40(2):341–9. [PubMed: 12106942]
5. Bers DM. Altered Cardiac Myocyte Ca Regulation In Heart Failure. *Physiology* December 1;2006 21(6):380–7. [PubMed: 17119150]
6. ter Keurs HEDJ, Boyden PA. Calcium and Arrhythmogenesis. *Physiol Rev* April 1;2007 87(2):457–506. [PubMed: 17429038]
7. Priori SG, Napolitano C, Tiso N, et al. Mutations in the Cardiac Ryanodine Receptor Gene (hRyR2) Underlie Catecholaminergic Polymorphic Ventricular Tachycardia. *Circulation* January 16;2001 103(2):196–200. [PubMed: 11208676]
8. Jiang D, Xiao B, Zhang L, Chen SRW. Enhanced Basal Activity of a Cardiac Ca²⁺ Release Channel (Ryanodine Receptor) Mutant Associated With Ventricular Tachycardia and Sudden Death. *Circ Res* August 9;2002 91(3):218–25. [PubMed: 12169647]
9. Jiang D, Xiao B, Yang D, et al. RyR2 mutations linked to ventricular tachycardia and sudden death reduce the threshold for store-overload-induced Ca²⁺ release (SOICR). *Proceedings of the National Academy of Sciences of the United States of America* August 31;2004 101(35):13062–7. [PubMed: 15322274]
10. Cerrone M, Colombi B, Santoro M, et al. Bidirectional Ventricular Tachycardia and Fibrillation Elicited in a Knock-In Mouse Model Carrier of a Mutation in the Cardiac Ryanodine Receptor. *Circ Res* May 27;2005 96(10):e77–e82. [PubMed: 15890976]
11. Liu N, Colombi B, Memmi M, et al. Arrhythmogenesis in Catecholaminergic Polymorphic Ventricular Tachycardia: Insights From a RyR2 R4496C Knock-In Mouse Model. *Circ Res* August 4;2006 99(3):292–8. [PubMed: 16825580]
12. Fernandez-Velasco M, Rueda A, Rizzi N, et al. Increased Ca²⁺ Sensitivity of the Ryanodine Receptor Mutant RyR2R4496C Underlies Catecholaminergic Polymorphic Ventricular Tachycardia. *Circ Res* January 30;2009 104(2):201–9. [PubMed: 19096022]
13. Cerrone M, Noujaim SF, Tolkacheva EG, et al. Arrhythmogenic Mechanisms in a Mouse Model of Catecholaminergic Polymorphic Ventricular Tachycardia. *Circ Res* November 9;2007 101(10):1039–48. [PubMed: 17872467]
14. Miquerol L, Meysen S, Mangoni M, et al. Architectural and functional asymmetry of the His-Purkinje system of the murine heart. *Cardiovasc Res* July 1;2004 63(1):77–86. [PubMed: 15194464]
15. Anumonwo JMB, Tallini YN, Vetter FJ, Jalife J. Action Potential Characteristics and Arrhythmogenic Properties of the Cardiac Conduction System of the Murine Heart. *Circ. Res* Aug; 2001 89:329–335. [PubMed: 11509449]
16. Boyden PA, Hirose M, Dun W. Cardiac Purkinje Cells. *Heart Rhythm* 2010;7(1):127–135. [PubMed: 19939742]
17. Boyden PA, Pu J, Pinto J, Keurs HEDJ. Ca²⁺ Transients and Ca²⁺ Waves in Purkinje Cells : Role in Action Potential Initiation. *Circ Res* March 3;2000 86(4):448–55. [PubMed: 10700450]
18. Cohen CJ, HA Fozzard HA, Sheu SS. Increase in intracellular sodium ion activity during stimulation in mammalian cardiac muscle. *Circ. Res* 1982;50:651–662. [PubMed: 7074728]
19. Sommer J, Johnson E. A comparative study of Purkinje fibers and ventricular fibers. *J Cell Biol* 1968;36:497–526. [PubMed: 5645545]
20. Stuyvers BD, Dun W, Matkovich S, Sorrentino V, Boyden PA, ter Keurs HEDJ. Ca²⁺ Sparks and Waves in Canine Purkinje Cells: A Triple Layered System of Ca²⁺ Activation. *Circ Res* July 8;2005 97(1):35–43. [PubMed: 15947247]
21. Boyden PA, Barbaiya C, Lee T, ter Keurs HEDJ. Nonuniform Ca²⁺ transients in arrhythmogenic Purkinje cells that survive in the infarcted canine heart. *Cardiovasc Res* March 1;2003 57(3):681–93. [PubMed: 12618230]

Figure 1.

Purkinje cell specific eGFP expression. A, Green fluorescent channel shows eGFP expression exclusively in the Purkinje cells of the cardiac ventricles of the $RyR^{+/R4496C}$ -Cx40^{eGFP/+} transgenic mouse. B, The corresponding bright field image for panel A; M=myocyte and P=Purkinje cell. C, Summary of cell widths for eGFP expressing Purkinje cells and non-eGFP expressing ventricular myocytes. Purkinje cells have more narrow width than the myocytes. * $P < 0.00001$

Figure 2. Purkinje cells of the RyR^{+/R4496C}-Cx40^{eGFP/+} mouse have greater spontaneous calcium release (SCR) frequency and amplitude. A, Typical intracellular calcium recording in a single ventricular myocyte loaded with rhod-2 AM. B, Typical intracellular calcium recording in a GFP positive Purkinje cell. C, Average data shows that Purkinje cells have a higher frequency of SCR than ventricular myocytes, *denotes statistical difference P<0.05. D, Average data shows that Purkinje cells have a greater amplitude of SCR than ventricular myocytes, * denotes statistical difference P<0.000001.

Figure 3.

Comparison between RyR^{+/R4496C}-Cx40^{eGFP/+} and RyR^{+/-}-Cx40^{eGFP} cells. In both cases RyR2 mutant cells demonstrate a higher number (A) and frequency of SCR (B). RyR2^{+/+} mice are littermate controls to the RyR2^{+/R4496C} animals. Black bars, RyR^{+/R4496C}-Cx40^{eGFP/+} Purkinje (n = 7 cells); grey bars, RyR^{+/-}-Cx40^{eGFP/+} (n = 5 cells); White bars, RyR^{+/R4496C}-Cx40^{eGFP/+} ventricular myocytes (n = 7 cells); stripped bars, RyR^{+/-}-Cx40^{eGFP/+} ventricular myocytes (n = 11 cells).

Figure 4. Quantification of SCR in RyR2⁺/RyR2^{R4496C} knock-in mouse myocytes and Purkinje cells. A, SCR events in a Purkinje cell following rapid pacing at 6Hz. B, SCR events in a representative ventricular myocyte after rapid pacing at 6Hz. C, Diastolic calcium concentration was not different between groups. D, The average peak calcium concentration of SCR events was greater in Purkinje cells than in ventricular myocytes, *denotes significant difference from ventricular myocytes $P < 0.000001$. E, The average amplitude of SCR was greater in Purkinje cells (85.4 ± 5.0 nmol/L, $n = 58$ events in 6 Purkinje cells, $N = 3$ mice) than in the ventricular myocytes (41.8 ± 5.0 nmol/L, $n = 40$ events in 6 myocytes, $N = 3$ mice), * denotes significant difference $P < 0.000001$.

Figure 5.

Quantification of SCR latency in RyR2⁺/RyR2^{R4496C} knock-in mouse myocytes and Purkinje cells. The latency to the first SCR event is shorter in Purkinje cells. A, Representative tracing of the latency measurement after 5Hz pacing in a ventricular myocyte. B, Representative intracellular calcium measurement using Fura-2 AM in a Purkinje cell. C, The average data shows that the latency to the first SCR event is significantly shorter in Purkinje cells than in ventricular myocytes. *P<0.05.

Figure 6.

Comparison of spontaneous calcium release in Purkinje cells and myocytes of the RyR2 mutant mouse following isoproterenol treatment. Panel A shows a representative 8s recording of intracellular Purkinje calcium (using fluo-4) following electrical pacing at 5Hz. Similarly, panel B shows a typical 8s recording of intracellular ventricular myocyte calcium following 5 Hz pacing. The Purkinje cells have a higher number of spontaneous calcium release events (SCR) after β -adrenergic stimulation (Panel C).