Cellular mechanism of premature ventricular contraction–induced cardiomyopathy

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BACKGROUND Frequent premature ventricular contractions (PVCs) are associated with increased risk of sudden cardiac death and can cause secondary cardiomyopathy.

OBJECTIVE We sought to determine the mechanism(s) responsible for prolonged refractory period and left ventricular (LV) dysfunction demonstrated in our canine model of PVC-induced cardiomyopathy.

METHODS Single myocytes were isolated from LV free wall of PVC and control canines and used for patch-clamp recording, intracellular Ca^{2+} measurements, and immunocytochemistry/confocal microscopy. LV tissues adjacent to the area of myocyte isolation were used for the immunoblot quantification of protein expression.

RESULTS In the PVC group, LV ejection fraction decreased from 57.6% ± 1.5% to 30.4% ± 3.1% after ≥4 months of ventricular bigeminy. Compared to control myocytes, PVC myocytes had decreased densities of both outward (transient outward current [I_{to}] and inward rectifier current [I_{K1}]) and inward (L-type Ca current [I_{CaL}]) currents, but no consistent changes in rapid or slow delayed rectifier currents. The reduction in I_{to}, I_{K1}, and I_{CaL} was accompanied by decreased protein levels of their channel subunits. The extent of reduction in I_{to}, I_{K1}, and I_{CaL} varied among PVC myocytes, creating marked heterogeneity in action potential configurations and durations. PVC myocytes showed impaired Ca-induced Ca release from the sarcoplasmic reticulum (SR), without increase in SR Ca leak or decrease in SR Ca store. This was accompanied by a decrease in dyad scaffolding protein, junctophilin-2, and loss of Cav1.2 registry with Ca-releasing channels (ryanodine receptor 2).

CONCLUSION PVCs increase dispersion of action potential configuration/duration, a risk factor for sudden cardiac death, because of the heterogeneous reduction in I_{to}, I_{K1}, and I_{CaL}. The excitation-contraction coupling is impaired because of the decrease in I_{CaL} and Cav1.2 misalignment with respect to ryanodine receptor 2.

KEYWORDS Premature ventricular contraction; Cardiomyopathy; Electrical remodeling; Excitation-contraction coupling

ABBREVIATIONS **AP** = action potential; **APD** = action potential duration; **$[Ca^{2+}]_i$** = intracellular Ca^{2+} concentrations; **CM** = cardiomyopathy; **CON** = control; **E-C** = excitation-contraction; **I_{CaL}** = L-type Ca current; **I_{K1}** = inward rectifier current; **I_{Kr}** = rapid delayed rectifier current; **I_{Ks}** = slow delayed rectifier current; **I_{to}** = transient outward current; **JPH-2** = junctophilin-2; **LV** = left ventricular; **PVC** = premature ventricular contraction; **RyR2** = ryanodine receptor 2; **SCD** = sudden cardiac death; **SDSD** = standard deviation of successive difference; **SR** = sarcoplasmic reticulum; **t-tubules** = transverse tubules; **WGA** = wheat germ agglutinin

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Introduction

Frequent premature ventricular contractions (PVCs) have been associated with an increased risk of sudden cardiac death (SCD) and identified as a possible cause of nonischemic cardiomyopathy (CM). Our canine model of PVC has shown that chronic exposure (12 weeks) of frequent PVCs (ventricular bigeminy; a coupling interval of 240 ms) impaired ventricular contractile function.[1] These observations established frequent PVC as an entity causing CM (PVC-induced CM). Surprisingly, despite left ventricular (LV) contractile dysfunction, there were no detectable structural abnormalities.[1] Therefore, PVC-induced CM appears to be a functional abnormality. Consistent with these findings, LV ejection fraction returned to normal within 2–4 weeks after discontinuation of PVCs.[1]

The mechanism for PVC-induced CM is not clear. Furthermore, it is uncertain whether or how chronic PVCs may induce electrical remodeling that may contribute to the increased risk of SCD observed in some patients with frequent PVCs. The present study was designed to address these issues using 4 complementary approaches. First, patch-clamp recordings of single LV myocytes were used to study how chronic PVCs affected action potentials (APs) and key ionic currents shaping the APs. Second, immunoblots of whole tissue lysates were used to quantify the expression levels of proteins of interest. Third, monitoring of

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intracellular Ca^{2+} ($[Ca^{2+}]_i$) in LV myocytes was used to evaluate whether chronic PVCs affected $[Ca^{2+}]_i$ handling. Fourth, immunocytochemistry in conjunction with confocal microscopy was used to assess the organization of t-tubules and junctional sarcoplasmic reticulum (SR; dyads) and proteins involved in excitation-contraction (E-C) coupling.

Methods

Animal model

Under general anesthesia, 5 mongrel dogs (>10 months old; weight 35–45 lb) underwent implantation of an experimental pacemaker via left thoracotomy. After recovery in 2–3 weeks, ventricular bigeminy (50% PVC burden; a coupling interval of 240 ms) originating from the right ventricular apex was initiated for ≥4 months using a pacemaker with a unique premature pacing algorithm, as described previously.[1] Four additional healthy mongrel canines were used as control. Animal studies conformed to the *Guide for the Care and Use of Laboratory Animals*. Experiments were approved by the Institutional Animal Care and Use Committee of the McGuire VA Medical Center.

Myocyte isolation

The procedures were as described previously.[2] Briefly, LV free wall was cannulated through the left circumflex coronary artery and mounted on a Langendorff apparatus. Tissue was perfused with Ca-free Tyrode solution containing bovine serum albumin (0.5 mg/mL) for ~15 minutes and then with the same solution containing collagenase type II (1 mg/mL, Worthington) and pronase E (type XIV, 0.05 mg/mL, Sigma-Aldrich) for 12–14 minutes till tissue was softened. Myocytes were mechanically disaggregated, filtered through a nylon mesh, transferred to the Kraft-Bruhe solution, and kept at room temperature for patch-clamp recordings and $[Ca^{2+}]_i$ experiments (in ≤8 hours). Myocytes were also fixed on polylysine-coated coverslips for immunocytochemistry/confocal microscopy.

Patch-clamp experiments

APs and whole cell currents were recorded using the patch-clamp method as described previously.[2] Experiments were controlled by pCLAMP 10 via Digidata 1440A, using an Axopatch 200B amplifier. Pipette tip resistance was 2–3 MΩ, and tip potential was zeroed before making gigaohm seal.

A liquid junction potential of 10 mV was corrected during data analysis. Currents were low-pass filtered at 1 kHz (Frequency Devices, Ottawa, IL) and stored for off-line analysis. Data analysis was done using Clampfit (Molecular Devices, Sunnyvale, CA), Excel (Microsoft, Redmond, WA), PeakFit (Jandel Scientific, Corte Madera, CA), and SigmaStat.

Immunoblot experiments

Whole tissue lysates were prepared as described previously.[2] Proteins were fractionated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and blotted to polyvinylidene fluoride (PVDF) membranes, and the levels of proteins of interest were quantified by using enhanced chemiluminescence.

Immunocytochemistry, confocal microscopy, and image analysis

Myocytes were permeabilized and incubated with primary/Alexa-conjugated secondary antibody pairs. Plasma membrane was stained by Alexa-conjugated wheat germ agglutinin (WGA). Nuclei were stained with 4′,6-diamidino-2-phenylindole. Fluorescence images were obtained with a Zeiss 710 confocal microscope and analyzed using National Institutes of Health's ImageJ.

Monitoring $[Ca^{2+}]_i$

Fluo-4–loaded myocytes were placed on the stage of an epifluorescence microscope, superfused with the bath solution, and paced by field stimulation (cycle length 2 seconds). Fluo-4 was excited by 490/10 nm light, and emission was measured at 535/50 nm.

Statistical analysis

Statistical analysis was done using SigmaStat (v. 2). A *t* test was used to compare 2 groups (eg, PVC vs CON), and a difference reaching $P < .05$ was considered significant.

Online supplemental data

Details of experimental setups, data acquisition, and analysis are provided in Online Supplemental Detailed Methods. Numerical data values are listed in Online Supplemental Tables 1–3.

Results

On average, PVC myocytes had larger values of cell capacitance than did CON myocytes (225 ± 6 pF vs 189 ± 5 pF; n = 50 and n = 51; $P < .001$), suggesting larger cell sizes in PVC-induced CM. PVC myocytes also exhibited significantly larger length-to-width ratios than did CON myocytes (Figure 1A), which was similar to cellular enlargement seen in chamber dilation. This indicates that although there was no detectable sign of myocyte loss in PVC-induced CM,[1] the myocytes did respond to the chronic stress of ventricular bigeminy by cellular hypertrophy.

AP configurations and durations

Figure 1B depicts representative AP traces recorded from CON and PVC myocytes. We compared the action potential duration (APD; Figure 1C) and the degrees of "beat-to-beat" variations in APDs between these 2 groups of myocytes (Figures 1D and 1E). The latter is believed to be a better predictor of arrhythmia risk.[3]

CON myocytes had an average APD of 455 ± 20 ms. There was a modest degree of beat-to-beat variations in APD. One example is shown in Figure 1D, which plots APD_{n+1} against APD_n for 50 consecutive APs in a CON myocyte. The beat-to-beat variations in APD were quantified by the standard deviation of successive difference (SDSD) values in