CARDIAC DYSFUNCTION AND REMODELING IN CHRONIC HEART FAILURE

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I am an Assistant Professor for the Department of Physiology and Cell Biology at Ohio State University. I received my Ph.D. in Biophysics in 1995 from the Department of General Neurophysiology (Mentors Prof. Ihor Mahura and Prof. Platon Kostyuk), Bogomoletz Institute of Physiology, NAS Ukraine. I took post doctoral training in the developmental and cellular cardiology at the University of Cologne (Germany), Texas Tech University and Ohio State University in the United States.

My research is mostly in the realm of cardiac remodeling which underlie the development of cardiac pathology and perpetuation of cardiac arrhythmias. Current areas of investigation include molecular modulation of cardiac contractility in early cardiogeneses (using stem cells plasticity), mechanism of intracellular calcium release termination by luminal proteins, genetic diseases linked to the mutations of calsequestrin, cardiac myopathy in diabetes, pathology of cardiac excitation-contraction coupling in ischemia and heart failure. I provide research from single-molecular level to the whole heart, using biophysical methods (electrophysiological technique, fluorescent optical measurements) in combination with biochemistry and molecular biology.

We have found that genetic disease CPVT, linked to the mutations of luminal cardiac regulatory protein calsequestrin alters termination of calcium release from sarcoplasmic reticulum lumen. Using adenovirus-mediated gene transfer to the primary cell culture of rat ventricular myocytes, we proposed two possible mechanisms of the CPVT development.

Stem cell transplantation is a promising technique to improve cardiac function. However, proarrhythmia is a major concern surrounding the use of ventricular regeneration. My goal is to determine the cellular and molecular mechanisms underlying modulation of spontaneous contractility in early cardiac myocytes and to find ways to synchronize early cardiac contractility. Our preliminary studies of in vitro model of cardiac differentiation also indicate that establishment of calcium induced calcium release in early cardiogenesis is critical for synchronization of early cardiac contractility.

The cardiac activation-relaxation cycle

Myocardial excitation-contraction (EC) coupling begins with membrane depolarization, a process that activates voltage-dependent calcium (Ca) channels (dihydropyridine receptors, DHPRs) in the plasma membrane and allows a relatively small amount of Ca to enter the cell (Bers, 2002). This Ca serves as a trigger to activate the Ca release channels (ryanodine receptors, RyRs) in the sarcoplasmic reticulum (SR), a mechanism known as Ca-induced Ca release (CICR) (Bers, 2002; Fabiato, 1985). It was also shown that Ca entry via DHPR and reverse mode of sodium-calcium exchanger (NCX) could synergistically activate Ca release from RyRs (Viatchenko-Karpinski et al, 2001, 2005). The combination of Ca influx and SR Ca release increases the cytosolic free [Ca] ([Ca]c), allowing Ca to bind to troponin C, which then initiates formation of actin-myosin cross-bridges causing contraction. For myofilaments to relax, [Ca]c must be returned to its basal level. This is predominantly achieved by the SR Ca pump (SERCA), which transports cytosolic Ca to the SR luminal compartment. Accumulated SR Ca is bound to a low-affinity high-capacity protein calsequestrin (CSQ). While most of the Ca constituting the cytosolic Ca transient is taken up by the SR, some Ca is extruded from the cell by the NCX to make up for one which entered via the Ca channels in the plasmalemma. RyR open probability changes as a direct function of [Ca] at the luminal side of the channel (Gyorke and Gyorke, 1998). The responsiveness of RyRs to luminal Ca seems to be mediated by the auxiliary proteins triadin, junctin, and CSQ, which are coupled to RyRs at the luminal surface of the SR (Gyorke et al, 2004). During the release process, the reduction in SR luminal free [Ca] ([Ca]SR) leads to deactivation or closure of RyRs, contributing to Ca-induced Ca release termination (Terentyev et al, 2002). At the same time, stimulatory effects of high luminal Ca on RyR channel open probability are responsible for the Ca leak pathway activation, which plays a role in setting the SR Ca content during the diastolic phase via leaking excess Ca from the SR.

Heart failure (HF) occurs when there is a reduction in cardiac output that is inadequate to meet the metabolic demands of the body. The most common defect in HF is impaired contractility of the ventricles. Evidence suggests that the amount of Ca released from the SR into the cytosol is reduced, accounting for, or contributing to, the reduced contractile force generated by the failing heart. Reduced SR Ca release has been shown to be associated with a decrease in the SR Ca content (Bers, 2002). Several explanations have been put forth for the diminished SR Ca stores in HF. In a number of reports, the reduction of SR Ca content has been attributed to depressed SR Ca-ATPase function and/or enhanced NCX activity during HF (Shannon et al, 2002; Houser et al, 2000). These changes are expected to facilitate Ca removal from the cell at the expense of its uptake into the SR and result in underfilled SR Ca stores in HF. Another potential cause of reduced SR Ca

content is enhanced diastolic leak of Ca via the RyRs (Marx et al, 2000; Shannon et al, 2003; Ai et al, 2005; Curran et al, 2007).

Hear we present an investigation of subcellular and molecular features of HF using the canine model of chronic tachypacing-induced HF (Kubalova et al, 2005; Nishijima et al, 2007). To investigate the causes of aberrant SR Ca function in chronic HF, we performed measurements of myocyte cytosolic and SR luminal Ca, and conducted recordings from single-RyR channels. This work shows that the diminished SR Ca release, which is characteristic of failing. One space must be present before and after the Title myocardium can be explained by increased sensitivity of RyRs to luminal Ca, leading to enhanced spark-mediated SR Ca leakage and reduced intra-SR [Ca]. We also show that cardiac resynchronizaiton therapy (CRT) reversed altered Ca signaling in HF.

Methods

Canine Heart Failure and CRT. Chronic, stable left ventricular dysfunction was induced by right ventricular apical tachypacing in the canine model for 24 months. Detailed material and methods can be easily found in the literature. We used three groups of adult mongrel dogs (ages 1.5-3 years; weights 20-33 kg): normal controls (n = 4), HF for 24 months (n = 6) or HF for 15 months with subsequent CRT for 9 months (n = 3). CRT utilizes atrial-synchronized, bi-ventricular pacing to optimize cardiac synchrony and function. Recent studies have found that CRT is beneficial in patients with chronic HF. Specifically, CRT reduces left ventricular (LV) size and hospitalization, while improving symptoms, exercise capacity, systolic function, and survival (16, 17). Although CRT exerts a beneficial effect in HF heart failure, the underlying mechanisms contributing to improved ventricular function remain poorly understood. In HF patients, prolongation of the QRS interval or signs of mechanical dyssynchrony by echocardiography are most frequently used as a criterion for CRT in clinical trials and symptomatic HF patients.

CRT was started (by pacing the right atrium, right ventricular endocardium, and left ventricular epicardium. Atrioventricular (AV) delays were 110 ms, 90 ms, and 100 ms, optimized by maximal aortic stroke volume (velocity-time integral). V-V delays between stimulations of the right ventricular endocardium and left ventricular epicardium were 12 ms, 4 ms, and 4 ms, respectively, optimized by tissue velocity imaging to minimize mid-ventricular LV dyssynchrony (Fig. 1).

Myocytes isolation. Myocytes were isolated by using standard techniques (14, 15).

Electrophysiological and confocal fluorescence experiments. Electrophysiological and calcium measurements were made using previously described methods (14, 15) and an Olympus Fluoview 1000 laser scanning confocal microscope with an Axopatch 200B patch-clamp amplifier.

Results and Discussion

In vivo results. While the untreated HF dogs had evidence of continued increases in LV mass and LV volumes, CRT were either attenuated or reversed adverse structural remodeling. CRT resulted in a significant improvement in LV end diastolic volume, LV end systolic CRT also *has not* statistically significant tendency to LV fractional shortening.

ICa, intracellular Ca transients, and SR Ca content in intact myocytes. Cell capacitance in HF myocytes was dramatically increased (126 ± 21 pF, n = 8, in control, and 214 ± 39 pF, n = 9 in HF myocytes), consistent with ventricular hypertrophy.

Depolarization-induced ICa and spatially resolved intracellular Ca transients were measured in cardiomyocytes dialyzed with fluo-3. Fig. 2 shows representative traces of ICa and confocal line-scan images along with the spatial average of Ca transients recorded during the depolarizing step from -50 to 0 mV in myocytes from control and failing hearts. The amplitude of Ca transients was reduced and their duration prolonged in HF myocytes, whereas the amplitude of current density (ICa) did not change and the time constant of ICa inactivation was slowed in HF myocytes, apparently because of diminished Ca-dependent inactivation.

Statistically, the observed decreases in Ca transient amplitude in HF myocytes with respect to control were significant within the whole range of membrane potentials tested (from –40 to 60 mV). At the same time, ICa density was preserved, suggesting that the reduced SR Ca release is not simply due to the reduced Ca trigger in myocytes from failing heart. The SR Ca content was inferred from recordings of Ca transients and NCX exchange currents recorded on application of caffeine and reduced to ≈65% of control in HF myocytes. After nine months of CRT, there was observed restoration of the calcium transient amplitude. However, the CRT-induced changes in the intracellular calcium transient were not fully normalized and remained different from control values.

Ca sparks and SR Ca content. In control myocytes, spontaneous Ca sparks occurred with an average frequency of ≈3.0 ± 0.3 s^{-1}. In myocytes from failing hearts, spark frequency increased to 6.6 ± 1.2 s^{-1}, whereas the event amplitude significantly decreased. CRT markedly decreased Ca spark frequency (3.7 ± 0.5 s^{-1}).

Strikingly, the SR Ca signals revealed a dramatic reduction in the basal [Ca] SR in HF myocytes compared with control ones. Additionally, the amplitudes of both global and focal Ca depletion signals (i.e., during Ca waves and sparks, respectively) were diminished in HF cells compared with control. These results show that SR luminal [Ca] is reduced in HF myocytes. They also demonstrate that generation of spontaneous Ca waves and sparks in HF myocytes occurs at abnormally low intra-SR Ca levels, thereby accounting for, or contributing to the reduced sequestration of Ca in the SR in HF myocytes.

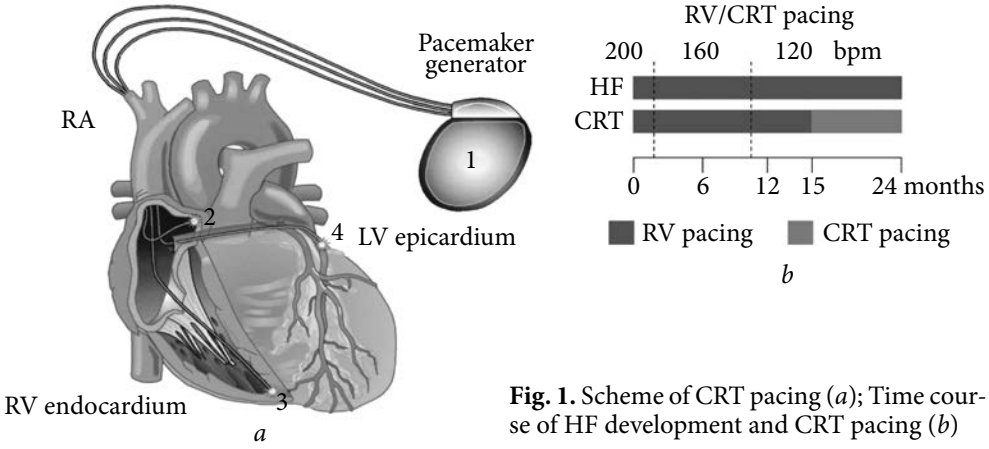

Fig. 1. Scheme of CRT pacing (*a*); Time course of HF development and CRT pacing (*b*)

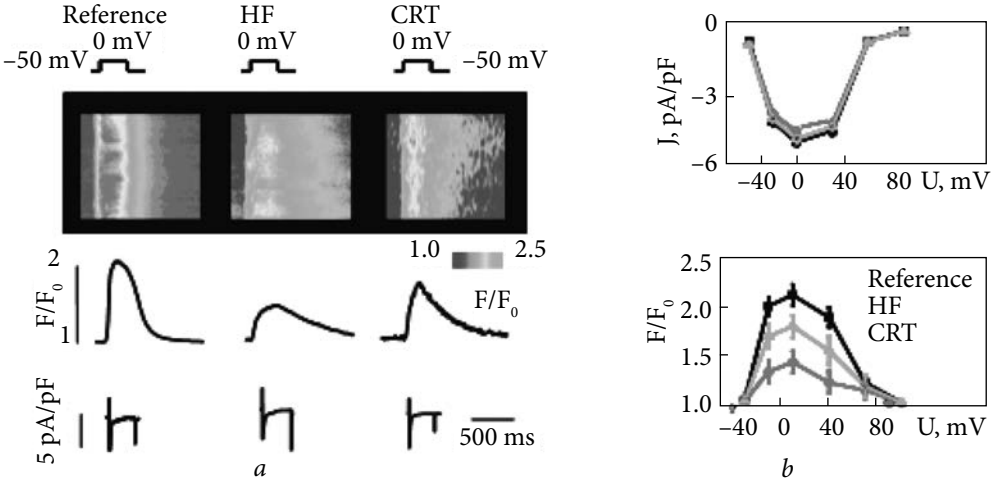

Fig. 2. CRT improves parameters of intracellular Ca signaling: *a* — representative confocal line-scan images of Ca transients (top) along with their spatial averages (middle) and recordings of ICa (bottom), induced by a depolarizing step of from –50 to 0 mV, in control (left), HF (middle) myocytes, and CRT-treated HF myocytes (right); *b* — averages of Ca transient amplitudes and peak ICa densities as functions of membrane potential for control and HF and CRT myocytes

Single-RyR channel activity. To directly compare the sensitivities of RyR channels from normal and failing hearts to luminal Ca, we performed single-RyR channel recordings using the planar lipid bilayer technique. In RyRs isolated from failing hearts, the luminal Ca sensitivity was profoundly shifted toward lower concentrations compared with controls so that the channel was substantially activated at trans [Ca] as low as 20 μmol/l (EC50 ~5 μmol/l) In HF myocytes, reduction in luminal Ca below 1 μmol/l was required to abolish luminal Ca activation. These results indicate that RyR sensitivity to luminal Ca is greatly enhanced in HF.

Conclusions

Using the canine model of chronic HF, we showed that the diminished SR Ca release characteristic of failing myocardium is at least partially due to increased sensitivity of RyRs to luminal Ca, leading to enhanced spark-mediated SR Ca leak and reduced intra-SR [Ca]. The biochemical causes for the abnormal RyR luminal Ca-dependent gating behavior are not known. They could involve disrupted protein–protein interactions within the RyR complex, altered covalent modification of RyRs such as phosphorylation, or acquired defects caused by reactive intracellular metabolites. Sensitization of RyRs to luminal Ca in HF can reflect an adaptive transformation, allowing the SR to operate at reduced SR Ca loads (and reduced energy costs) in the ATP-starved failing myocardium. Alternatively, the RyR sensitization may play a pathophysiologic or maladaptive role in HF. Also, our studies show that CRT improves cellular electrophysiology and intracellular calcium regulation. These findings suggest that *in vivo* function and myocyte calcium regulation are linked, not only during the development of heart failure, but also during reverse remodeling. In the future it may be possible to further elucidate specific signaling mechanisms linked to myocyte contractile performance and to change in functional parameters.

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