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Original article

Reduced sarcoplasmic reticulum Ca^{2+} load mediates impaired contractile reserve in right ventricular pressure overload

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Abstract

Myocardial contractile reserve is significantly attenuated in patients with advanced heart failure. The aim of this study was to identify mechanisms of impaired contractile reserve in a large animal model that closely mimics human myocardial failure. Progressive right ventricular hypertrophy and failure were induced by banding the pulmonary artery in kittens. Isometric contractile force was measured in right ventricular trabeculae ($n=115$) from age-matched Control and Banded feline hearts. Rapid cooling contractures (RCC) were used to determine sarcoplasmic reticulum (SR) Ca^{2+} load while assessing the ability of changes in rate, adrenergic stimulation and bath Ca^{2+} to augment contractility. The positive force–frequency relationship and robust pre- and post-receptor adrenergic responses observed in Control trabeculae were closely paralleled by increases in RCC amplitude and the RCC2/RCC1 ratio. Conversely, the severely blunted force–frequency and adrenergic responses in Banded trabeculae were paralleled by an unchanged RCC amplitude and RCC2/RCC1 ratio. Likewise, supraphysiologic levels of bath Ca^{2+} were associated with severely reduced contractility and RCC amplitude in Banded trabeculae compared to Controls. There were no differences in myofilament Ca^{2+} sensitivity or length-dependent increases in contractility between Control and Banded trabeculae. There was a significant decrease in SR Ca^{2+}-ATPase pump abundance and phosphorylation of phospholamban and ryanodine receptor in Banded trabeculae compared with Controls. A reduced ability to increase SR Ca^{2+} load is the primary mechanism of reduced contractile reserve in failing feline myocardium. The similarity of impaired contractile reserve phenomenology in this feline model and transplanted hearts suggests mechanistic relevance to human myocardial failure.

Keywords: Contractile reserve; Sarcoplasmic reticulum; Calcium; Force–frequency; Beta-adrenergic signaling; Frank-Starling; Rapid cooling contracture; Myofilament calcium sensitivity

1. Introduction

In human heart failure, myocardial contractility is depressed and the ability of the heart to increase its contractility in response to physiologic stress, such as exercise, is diminished. This loss of contractile reserve is evident in vivo by the aberrant response to increases in heart rate, adrenergic drive and/or preload. It is well accepted that frequency- and adrenergic-dependent contractile responses are abnormal in human heart failure, as evidenced by a blunted or negative force–frequency relationship and reduced adrenergic responsiveness [1–4]. While most investigators suggest that the Frank-Starling mechanism is intact in failing hearts [5,6], it has yet to be determined whether it is altered. Nonetheless, the ability of the failing heart to modulate its contractile performance in response to physiologic stress is impaired.

Despite the practical advantages of utilizing human tissue obtained at the time of heart transplantation, it must be recognized that such studies engender several unavoidable shortcomings that may confound the insights into the basis for contractile dysfunction in human heart failure. Differences in age, sex, heart failure etiology and therapeutic interventions

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collectively contribute to heterogeneity between hearts. Moreover, because failing human hearts are procured exclusively from the very advanced cases of heart failure, they provide no opportunity for mechanistic studies before the development of end-stage disease. Finally, because most normal or nearly normal human donor hearts are used for transplantation, non-failing control hearts usually have some type of pathologic abnormality that could affect the interpretation of comparisons between failing hearts.

Despite the potential utility of animal models in this context, not all models are ideally suited for deriving insights relevant to the biology of contractile reserve in failing human hearts. Rodent hearts, used in many previous studies, exhibit substantial differences in Ca^{2+} handling dynamics compared to larger mammals [7]. For example, normal rats exhibit a negative force–frequency response [8,9] as compared to the positive force–frequency response commonly associated with non-failing humans. In addition, the action potential waveshape, which influences excitation–contraction coupling, is likewise quite different in rodents than in larger mammals [10]. These considerations make it preferable to utilize a large animal model with disease-free phenomenology that better parallels that observed in nondiseased human hearts. Specifically, larger animals increase their contractility in response to physiologic stressors (heart rate, adrenergic drive and/or preload), in a similar manner compared to humans.

Independent of the inciting event (hypertension, myocardial infarction, valvular lesions, etc.), myocardial failure often develops as a result of a chronic hemodynamic overload. In response to this overload, the myocardium remodels at the tissue level [11,12] and undergoes numerous alterations at the cellular and molecular level [13–15], which eventually lead to the deterioration of normal myocardial structure and function. These considerations support the use of hemodynamic overload as a clinically relevant trigger for myocardial failure. Accordingly, the objective of these studies was to investigate contractile reserve in a uniform large animal model of right ventricular progressive pressure overload producing myocardial failure. Based on recent studies in isolated myocytes, we hypothesized that defects in frequency- and adrenergic-dependent contractile reserve are closely linked to defects in the ability to load the sarcoplasmic reticulum with calcium. By demonstrating a phenomenology that closely mimics defects observed in failing human hearts, our studies provide unique and relevant insights into the cellular and molecular mechanisms responsible for impaired contractile reserve in failing human hearts.

2. Materials and methods

2.1. Pulmonary artery banding

Pre-emptive analgesia (buprenorphine, 0.005–0.01 mg/kg S. Q. BID) was used. Young cats (8–10 weeks old) were anesthetized with an intramuscular injection of Ketamine (50 mg/kg) and Acepromazine (0.5 mg/kg), intubated and ventilated with room air. Surgical anesthesia was maintained with 1% isoflurane during the operative procedure. Using aseptic technique, a right thoracotomy and pericardiotomy were performed and the pulmonary artery was dissected free from the aorta. A 3.0 mm (internal diameter) clip was then placed around the pulmonary artery. The lungs were fully expanded and the chest was closed. Initially, both age-matched sham-operated and age-matched non-operated animals were used as controls. However, after echocardiographic imaging, force–frequency experiments and isoproterenol challenges revealed no functional difference between sham-operated animals and non-operated controls, we used only age-matched non-operated animals as controls for the remaining experiments. All experiments were conducted in accordance with the ILAR Guide for the Care and Use of Laboratory Animals and all protocols were approved by the appropriate institutional animal care and use committees.

2.2. Echocardiographic assessment

A Hewlett-Packard SONOS 5500 Echocardiography machine and 12S probe were utilized to non-invasively measure in vivo cardiovascular parameters, including ventricular chamber

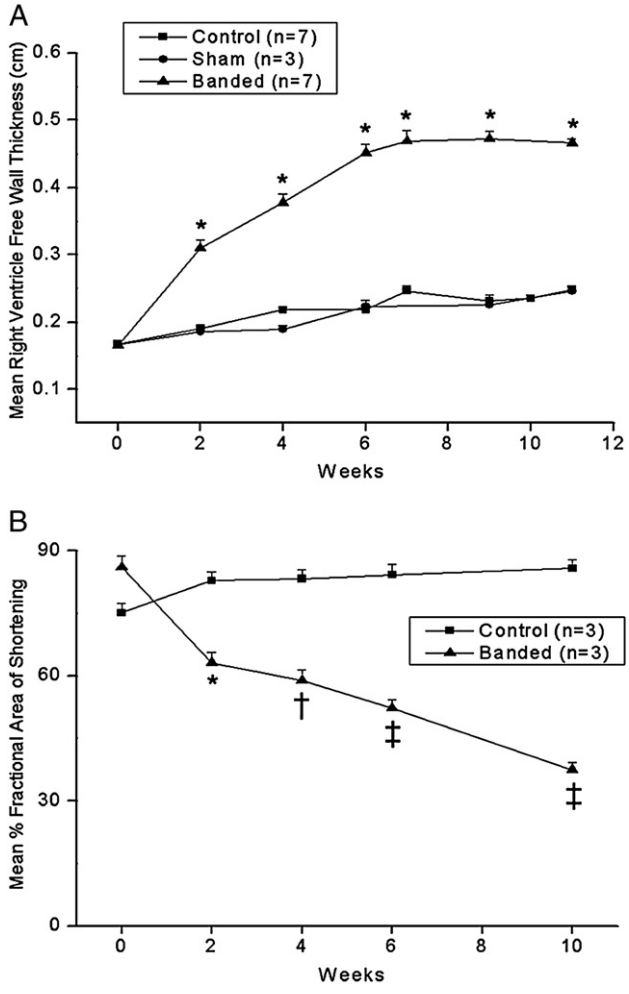

Fig. 1. (A) Mean right ventricular free wall thickness as a function of time in control, sham and banded cats. *$P<0.05$ compared to control and sham group. (B) Mean right ventricular percent fractional area of shortening as a function of time in control and banded cats. *$P<0.05$; †$P<0.01$; ‡$P<0.001$ compared to control group.