Research Article

Relationship between action potential, contraction-relaxation pattern, and intracellular Ca$^{2+}$ transient in cardiomyocytes of dogs with chronic heart failure

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Abstract. Abnormalities of contractile function have been identified in cardiomyocytes isolated from failed human hearts and from hearts of animals with experimentally induced heart failure (HF). The mechanism(s) responsible for these functional abnormalities are not fully understood. In the present study, we examined the relationship between action potential duration, pattern of contraction and relaxation, and associated intracellular Ca$^{2+}$ transients seen in cardiomyocytes isolated from the left ventricle (LV) of dogs ($n=7$) with HF produced by multiple sequential intracoronary microembolizations. Comparisons were made with LV cardiomyocytes isolated from normal dogs. Action potentials were measured in isolated LV cardiomyocytes by perforated patch clamp, Ca$^{2+}$ transients by fluo 3 probe fluorescence, and cardiomyocyte contraction and relaxation by edge movement detector. HF cardiomyocytes exhibited an abnormal pattern of contraction and relaxation characterized by an attenuated initial twitch (spike) followed by a sustained contracture ('dome') of 1 to 8 s in duration and subsequent delayed relaxation. This pattern was more prominent at low stimulation rates (58% at 0.2 Hz, $n=211$, 21% at 0.5 Hz, $n=185$). Measurements of Ca$^{2+}$ transients in HF cardiomyocytes at 0.2 Hz manifested a similar spike and dome configuration. The dome phase of both the contraction/relaxation pattern and Ca$^{2+}$ transients seen in HF cardiomyocytes coincided with a sustained plateau of the action potential. Shortening of the action potential duration by administration of saxitoxin (100 nM) or lidocaine (30 μM) reduced the duration of the dome phase of both the contraction/relaxation profile as well as that of the Ca$^{2+}$ transient profile. An increase of stimulation rate up to 1 Hz caused shortening of the action potential and disappearance of the spike-dome profile in the majority of HF cardiomyocytes. In HF cardiomyocytes, the action potential and Ca$^{2+}$ transient duration were not significantly different from those measured in normal cells. However, the contraction-relaxation cycle was significantly longer in HF cells (314 ± 67 ms, $n=21$, vs. 221 ± 38 ms, $n=46$, mean ± SD), indicating impaired excitation-contraction uncoupling in HF cardiomyocytes. The results show that, in cardiomyocytes isolated from dogs with HF, contractile abnormalities and abnormalities of intracellular Ca$^{2+}$ transients at low stimulation rates are characterized by a spike-dome configuration. This abnormal pattern appears to result from prolongation of the action potential.

Key words. Heart failure; single cardiomyocytes; action potential; calcium transient; contraction; relaxation; saxitoxin; lidocaine; perforated patch clamp.

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Introduction

Heart failure (HF) is associated with profound abnormalities of both contractile function and cardiac rhythm. It is generally believed that the contractile dysfunction is related not only to ongoing loss of functional cardiac units, but also to intrinsic abnormalities of cardiomyocyte contraction and relaxation. Studies performed using cardiomyocytes isolated from failed human hearts and animals with experimentally induced heart failure showed profound abnormalities in relaxation [1, 2]. Impaired relaxation was reported in most HF etiologies, including ischaemic cardiomyopathy, idiopathic dilated cardiomyopathy, and the secondary to mitral valve disease [2–5]. The abnormal relaxation in HF was mainly linked to a disturbed Ca2+ handling as a result of altered proteins of the sarcoplasmic reticulum (SR) responsible for Ca2+ removal from the cytosol [6–13]. Abnormal prolongation of Ca2+ transients has been observed in both isolated cardiomyocytes [14] and ventricular muscle strips isolated from end-stage failed human hearts [15].

Cardiomyocytes as well as multicellular preparations isolated from failing animal and human hearts consistently reveal abnormalities of repolarization that are independent of the etiology of heart failure [15–24]. A possible working hypothesis is that abnormal relaxation in HF results from prolongation of the duration of the action potential, a characteristic feature of the heart failure state. Because duration of the action potential can have profound effects on cardiac excitation-contraction [E-C] coupling in terms of regulating Ca2+ influx through voltage-dependent Ca2+ channels [25], it is possible that the abnormality of action potential duration in HF can lead to distortion of the contraction/relaxation pattern. In the present study, we tested the hypothesis that in HF, prolongation of the action potential is associated with abnormalities of E-C coupling. To test this hypothesis, we examined the relationship between action potential duration, the pattern of contraction and relaxation, and associated intracellular Ca2+ transients in single cardiomyocytes isolated from the left ventricle of dogs with HF produced by multiple sequential intracoronary microembolizations. Some of these findings have been reported previously [26].

Materials and methods

Heart failure model. The canine model of chronic HF used in this study was previously described in detail [27]. The model manifests most of the outcomes of HF seen in humans, including marked and sustained depression of ventricular performance (contraction and relaxation), ventricular hypertrophy and dilation, reduced cardiac output, increased systemic vascular resistance, enhanced sympathoadrenergic drive and down-regulation of cardiac β-adrenoceptor density [27, 28]. As in humans with HF, the model also manifests chronic ventricular arrhythmias and progressive left ventricle (LV) dysfunction long after cessation of coronary microembolizations [29, 30].

In the present study, seven healthy mongrel dogs, weighing between 24 and 31 kg, underwent multiple sequential coronary microembolization to produce HF. Embolizations were performed 1–3 weeks apart and were discontinued when LV ejection fraction, determined angiographically, was ≤ 40%. Dogs underwent an average of four to eight embolization procedures to achieve this target ejection fraction. In all instances, coronary microembolizations were performed during cardiac catheterization under general anaesthesia and sterile conditions. The anaesthesia regimen consisted of intravenous administration of oxymorphone (0.22 mg/kg), diazepam (0.17 mg/kg) and sodium pentobarbital (150–250 mg to effect). This anaesthesia regimen was previously shown to be effective in preventing the tachycardia, hypertension and myocardial depression seen with pentobarbital alone and does not alter LV function when compared to the conscious state [30]. At the time of harvesting the heart for cardiomyocyte isolation, 3 to 4 months after the final microembolization, LV ejection fraction was 29 ± 2%. The study protocol was approved by the institution Care of Experimental Animals Committee and conformed to the “Position of the American Heart Association on Research Animal Use” and the guiding principles of the American Physiological Society.

Cardiomyocyte isolation. Under general anaesthesia, the chest of the dog was opened, the heart was rapidly removed and the left circumflex coronary artery was cannulated. A transmural tissue wedge (30 mm × 50 mm × 10 mm), perfused by this vessel, was dissected as previously described [31]. The wedge was perfused with 1.8 Ca2+ containing Eagle’s minimum essential medium (MEM) at 37 °C and 12 ml/min for 5 to 6 min until contractions were restored. Immediately thereafter, the wedge was perfused for 7 min with nominally Ca2+-free MEM and, subsequently, with Ca2+-free MEM containing 1 mg/ml type II collagenase (Warthington, NJ, USA) for 20 to 25 min. Upon completion of the collagenase perfusion, the mid-myocardial tissue was minced, and cardiomyocytes were suspended in MEM with 0.3 mM Ca2+ and stored at room temperature for 5 to 7 h. The yield of viable, quiescent, rod-shaped cardiomyocytes varied from 40 to 60%. In addition to cardiomyocytes isolated from dogs with HF, cardiomyocytes were also isolated from LV myocardium of five normal dogs using the same protocol.

Measurements of Ca2+ transients. Intracellular Ca2+ transients were measured in response to field stimula-