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Research Article

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

V. A. Maltsev, H. N. Sabbah*, M. Tanimura, M. Lesch, S. Goldstein and A. I. Undrovinas

Henry Ford Hospital, Cardiovascular Research, Education & Research Bldg., Room 4016, 2799 West Grand Boulevard, Detroit (MI 48202-2689, USA), Fax +1 313 876 3001, e-mail: HSABBAH1@hfhs.org

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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 in single 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.

* Corresponding author.

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 as well as heart failure secondary to mitral valve disease [2–5]. The abnormal relaxation in HF was mainly linked to a disturbed Ca^{2+} handling as a result of altered proteins of the sarcoplasmic reticulum (SR) responsible for Ca^{2+} removal from the cytosol [6–13]. Abnormal prolongation of Ca^{2+} 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 Ca^{2+} influx through voltage-dependent Ca^{2+} 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 Ca^{2+} 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 $\leq 40\%$. 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 $29 \pm 2\%$. 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 $\times$ 50 mm $\times$ 10 mm), perfused by this vessel, was dissected as previously described [31]. The wedge was perfused with 1.8 Ca^{2+} 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 Ca^{2+}-free MEM and, subsequently, with Ca^{2+}-free MEM containing 1 mg/ml type II collagenase (Worthington, 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 Ca^{2+} 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 Ca^{2+} transients. Intracellular Ca^{2+} transients were measured in response to field stimula-

tion by means of a fluorescence microscope (Nikon, Diaphot 200) connected to a photomultiplier (PMT-200A, IonOptix, MA, USA) in conjunction with a cell framing adapter and a rectangular aperture (CFA200, IonOptix). A standard optical filter block (B-2A, Nikon) was used to collect the fluorescence signal from a single cardiomyocyte. Cells were loaded with the Ca^{2+} indicator fluo-3/AM prior the measurement. The loading procedure included incubation of the cardiomyocyte suspension with 10 μM of fluo-3/AM for 20 min in 0.3 mM Ca^{2+} containing MEM (pH 7.4) at room temperature. Thereafter, the cardiomyocytes were washed twice with the incubation solution and were then maintained for 20 min in the solution before measurements were made. To minimize indicator bleaching, the intensity of the mercury arc lamp was attenuated by eightfold (filters ND2 and ND4). The measurements of fluo 3 fluorescence were performed in a small (vol. 0.3 ml) perfusion chamber and were reported in arbitrary units (mV of photomultiplier output signal). The chamber was kept at a physiological temperature of 37 °C by a temperature controller Delta T-40N (Warner Instrument, CT, USA).

Recordings of action potential. Action potentials were recorded in current-clamp mode in an amphotericin-B perforated patch clamp configuration to maintain an intact intracellular milieu. Perforation of the membrane patch was achieved by the use of amphotericin-B in the patch pipette solution as previously described [32]. Recordings were performed using an Axopatch 200A patch-clamp amplifier (Axon Instruments, Foster City, CA, USA). The patch pipettes were pulled from borosilicate glass capillaries, (K150F, WPI, Sarasota, FL, USA). After heat polishing, the pipette tip resistance was 2–3 MΩ. The pipette solution contained (in mM): KCl 143, NaCl 10, EGTA 5, MgATP 2, HEPES 10 and amphotericin-B 0.32 (pH 7.4 with KOH). The course of perforation was monitored by measuring the increase in the capacity current evoked with a short (2 ms) voltage ramp pulse. The membrane patch was considered intact (unbroken) when the evoked action potentials were followed by cardiomyocyte contractions. All action potentials were recorded at 37 °C in the Tyrode solution consisting of (in mM): KCl 5.4, NaCl 140, $MgCl_2$ 2, $CaCl_2$ 1.8, Hepes 10 (pH 7.4 with NaOH). The pClamp 6.0 software (Axon Instruments) in conjunction with a 486 computer was used for operation of the current-clamp stimulation protocol. In all instances, cardiomyocytes were stimulated using current pulses of 0.2 ms duration.

Cardiomyocyte contraction measurements. Cardiomyocyte contraction and relaxation were recorded separately or simultaneously with action potentials or Ca^{2+} transients using an edge-detection algorithm. Cardiomyocyte contraction was evoked by electrical field stimulation or by applying a current pulse in perforated patch-clamp configuration. If not specifically stated, the stimulation frequency rate was always 0.2 Hz. The apparatus for measurement of cardiomyocyte contraction/relaxation included a microscope-mounted video camera (Pulnix TM 640) connected to a video motion edge detector (VED 103, Crescent Electronics, Sandy, UT, USA). The sampling period of the detector was 16.7 ms. Before any measurements were made, the edge-detector raster line lockon dot on a video monitor was placed on one of the myocyte ends. The signals from the edge movement detector were recorded either online or stored on video VHS tape for subsequent offline analysis. In all instances, 5 to 10 consecutive contractions of each cardiomyocyte were averaged to improve signal-to-noise ratio. In the case of simultaneous measurements of cardiomyocyte contractions and Ca^{2+} transient signals, the edge detector was connected to the fluorescence microscope through a 565-nm dichroic mirror and a 665-nm red filter. Cardiomyocyte contractions and Ca^{2+} transients were recorded at 37 °C in Tyrode solution with 1.2 mM $CaCl_2$.

Data acquisition and analysis. The signals from the edge detector, patch-clamp amplifier or photomultiplier were filtered at 5 kHz (−3 dB) using a four-pole low-pass Bessel filter and then digitized at a sampling rate of 10 kHz (Digidata 1200, Axon Instruments) and stored on a hard disk of a 486 computer for offline analysis by pClamp 6.0 software (Axon Instruments). Action potential duration was measured at 90% of repolarization (AP_{90}). The duration of intracellular Ca^{2+} transient was measured at 90% of the maximum fluorescent signal (T_{90}). The contraction/relaxation cycle duration was measured at 90% of the relaxation downstroke (CR_{90}). In HF cardiomyocytes, Ca^{2+} transients, action potential and cardiomyocyte contraction and relaxation were measured before and after treatment with saxitoxin (STX, 100 nM) and lidocaine (30 μM). The Na^+ channels blockers STX and lidocaine [33, 34] were used to shorten the duration of the action potential. We assumed that STX and lidocaine produced minimum disturbances to sarcolemmal L-type Ca^{2+} current, the main trigger for Ca^{2+} release from the SR.

Comparisons of AP_{90}, CR_{90} and T_{90} between HF cardiomyocytes and HF cardiomyocytes treated with STX and normal cardiomyocytes was performed using an unpaired *t* test for two means. For this test, a probability of 0.05 or less was considered significant. Comparisons between HF cardiomyocytes treated with or without STX were performed using Student's paired *t* test with significance set at $P < 0.05$. All data are reported as the mean ± SEM.

Chemicals. STX was purchased from Calbiochem, La Jolla, CA, USA. All other chemicals used in the study were purchased from Sigma, St. Louis, MO, USA.

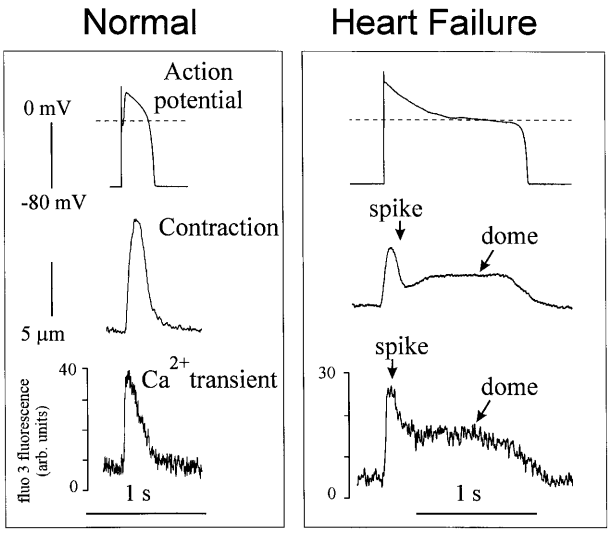

Figure 1. Representative examples of simultaneous recordings of the action potential (top), contraction and relaxation cycle (middle) and Ca^{2+} transients (bottom) obtained in cardiomyocytes from a normal dog (left panel) and a dog with heart failure (right panel) at the stimulation rate of 0.2 Hz. Heart failure cardiomyocytes were characterized by a prolonged action potential and spike-dome shape for both contraction and Ca^{2+} transients. Experiments were performed at 37 °C and 1.2 mM of $[Ca^{2+}]o$.

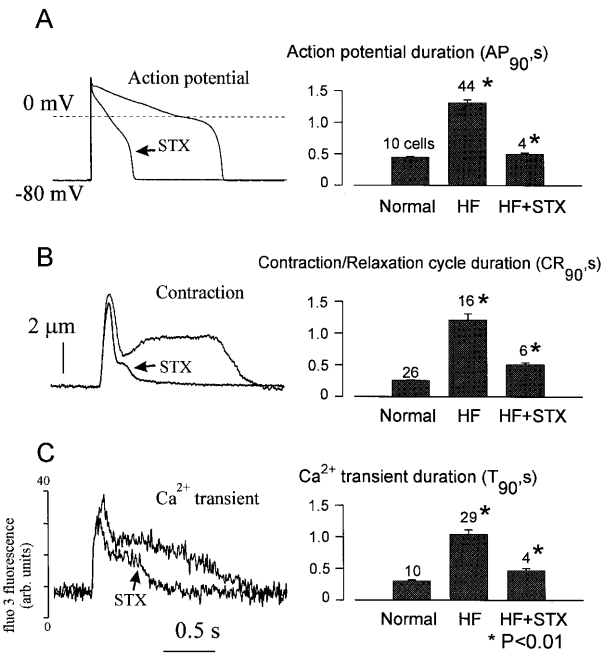

Figure 2. Saxitoxin (STX, 100 nM), a specific blocker of cardiac Na^+ channels, reduced action potential duration (*A*) and suppressed the dome phase of both the contraction/relaxation cycle (*B*) and intracellular Ca^{2+} transients (*C*) in ventricular myocytes of dogs with HF. Data in bar graphs are mean ± SEM, and numbers above the bars indicate the number of cardiomyocytes tested. $*P < 0.01$ normal vs. HF and HF vs. HF + STX. Experiments were performed at 37 °C and 1.2 mM of $[Ca^{2+}]o$. Stimulation rate was 0.2 Hz.

Results

Cardiomyocytes isolated from failed dog hearts exhibited abnormal repolarization, contraction and relaxation patterns and intracellular Ca^{2+} transients. Typical examples of action potentials, cardiomyocyte contraction and relaxation, and Ca^{2+} transients recorded in cardiomyocytes from a normal dog and a dog with HF are shown in figure 1. AP_{90} was significantly prolonged in HF cardiomyocytes compared with normals (1.313 ± 0.044 s vs. 0.450 ± 0.007 s, $P < 0.01$) (fig. 2A). Measurements obtained in 40 normal cardiomyocytes showed a monophasic contraction/relaxation pattern and Ca^{2+} transients. In contrast, an abnormal contraction/relaxation pattern was observed in the majority (58%) of HF cardiomyocytes ($n = 211$ cardiomyocytes tested). The pattern in HF cardiomyocytes was characterized by a short attenuated twitch followed by a tonic contracture (spike-dome configuration) (fig. 1, right panel). The duration of the dome phase in HF cardiomyocytes varied from 1 s to 8 s. CR_{90} was significantly longer in HF cardiomyocytes compared with normals (1.210 ± 0.097 s vs. 0.254 ± 0.075 s, $P < 0.01$) (fig. 2B). The spike-dome configuration was also observed for Ca^{2+} transients in HF cardiomyocytes. T_{90} was also significantly longer in HF cardiomyocytes compared with normals (1.042 ± 0.070 s vs. 0.303 ± 0.015 s, $P < 0.01$) (fig. 2C). In HF cardiomyocytes ($n = 17$) in which simultaneous recordings of action potential, Ca^{2+} transient and contraction/relaxation were obtained, the dome phase was always of the same duration for contraction/relaxation and Ca^{2+} transient and both coincided with the action potential plateau duration.

To reduce AP_{90}, HF cardiomyocytes were treated with two specific cardiac channel Na^+ blockers, namely STX and lidocaine. Administration of STX dramatically reduced AP_{90}, as well as of the dome phase of both the contraction/relaxation cycle and the Ca^{2+} transient (fig. 2). STX had little or no effect on the spike phase of the contraction/relaxation cycle or the spike phase of the Ca^{2+} transients (fig. 2B,C). Treatment of HF cardiomyocytes with STX nearly normalized AP_{90}, CR_{90} and T_{90} (fig. 2). In HF cardiomyocytes ($n = 8$), lidocaine (30 μM) treatment significantly shortened AP_{90} (1.571 ± 0.081 s vs. 0.359 ± 0.005 s, $P < 0.001$, $n = 4$). A typical example of action potential shortening in response to lidocaine administration is illustrated in figure 3A. In HF cardiomyocytes ($n = 4$), lidocaine also partially or completely abolished the abnormal dome phase of the contraction/relaxation cycle (fig. 3B). CR_{90} was signifi-

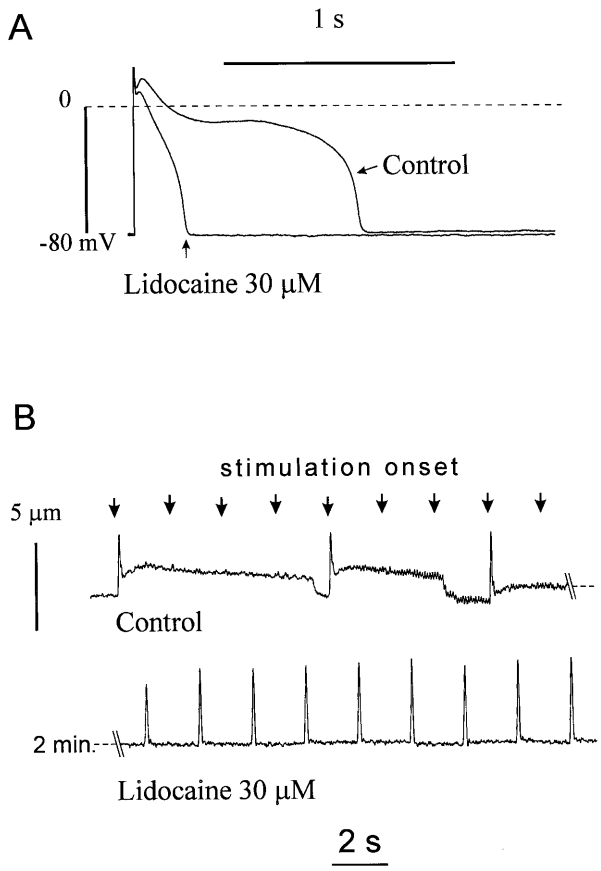

Figure 3. Effect of lidocaine on action potential (*A*) and contraction/relaxation (*B*) of a cardiomyocyte from a dog with heart failure. *A* shows superimposed tracings of action potential before (control) and after administration of lidocaine. *B* shows contraction and relaxation tracings from a failed cardiomyocyte before (upper traces) and after (lower traces) administration of lidocaine. Arrows indicate onset of the stimulus in a stimulation train (0.5 Hz). Note that before lidocaine application, the cardiomyocyte contraction was alternating (4:1). The cardiomyocyte did not contract when the stimulus onset coincided with the dome phase. Two minutes after lidocaine, the cardiomyocyte responded to each stimulus. Experiments were performed at 37 °C and 1.2 mM of $[Ca^{2+}]o$.

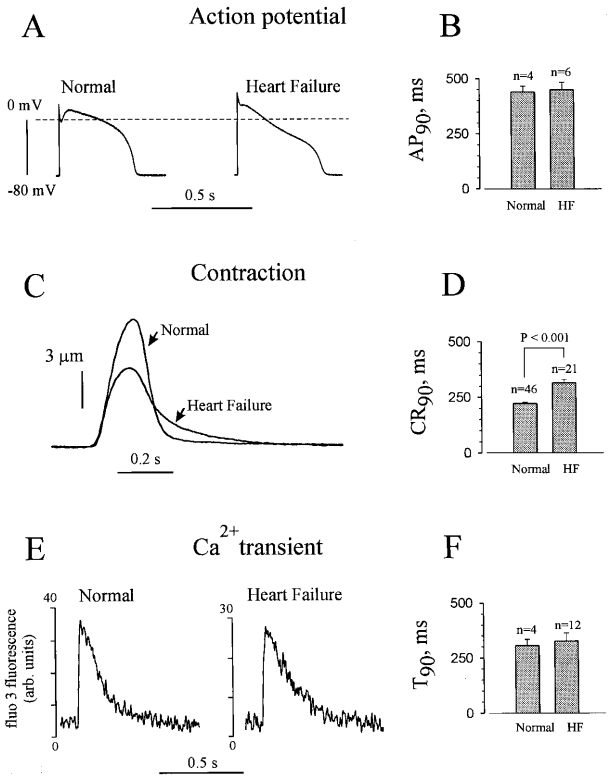

Figure 4. Effect of increased stimulation rate (1 Hz) on action potential, contraction/relaxation and intracellular Ca^{2+} transients in ventricular myocytes of normal and failing dogs. *A* shows a typical example of action potential recorded in a normal and failed cardiomyocyte and after administration of lidocaine. *C* shows superimposed contraction and relaxation tracings from normal and failed cardiomyocytes. Note delayed relaxation in the failed cardiomyocyte. *E* shows intracellular Ca^{2+} transients recorded in normal and failed cardiomyocytes. Transients are presented in arbitrary fluo 3 fluorescence units. Note the absence of dome phase in HF cardiomyocytes at this stimulation rate. Statistical data for duration of action potential (*B*), contraction-relaxation cycle (*D*) and intracellular Ca^{2+} transients (*F*) are presented as bar graphs *B*, *D* and *F*, respectively. Data in bar graphs are mean ± SEM, and the numbers above the bars indicate the number of cardiomyocytes tested. Action potential duration and intracellular Ca^{2+} transient duration was not statistically different between normal and HF cardiomyocytes. However, contraction-relaxation cycle length was statistically different ($*P < 0.01$) in normal vs. HF cardiomyocytes. Experiments were performed at 37 °C and 1.2 mM of $[Ca^{2+}]o$.

cantly reduced by the drug (2.409 ± 0.094 s vs. 0.311 ± 0.025 s, $P < 0.001$) and was not distinguishable from the normal contraction cycle duration.

In the next series of experiments we tested the frequency dependence of the spike-dome pattern in failed cardiomyocytes. The spike-dome contraction and Ca^{2+} transient were more prominent and were found in a higher percentage of HF cardiomyocytes at lower stimulation rates (58% at 0.2 Hz, $n = 211$, 21% at 0.5 Hz, $n = 185$). At a physiological rate of 1 Hz, only 11% ($n = 44$) of HF cardiomyocytes displayed the spike-dome configuration. In fact, increasing the stimulation rate to 1 Hz caused action potential shortening and concomitant decrease in both CR_{90} and T_{90} (fig. 4). However, at this stimulation rate, the cardiomyocyte contraction-relaxation cycle remained significantly longer in HF cardiomyocytes in comparison with normal cardiomyocytes (fig. 4C,D).

Discussion

In the present study, a single ventricular cardiomyocyte preparation was used to examine abnormalities of myo-

cardial E-C coupling in dogs with chronic heart failure. A unique feature of this approach was the ability to obtain simultaneous measurements of the action potential, myocyte contraction and relaxation patterns and intracellular Ca^{2+} transients. The results showed distinct abnormalities of contraction/relaxation patterns in HF cardiomyocytes that were associated with temporally similar patterns of intracellular Ca^{2+} transients. More important, the results of the study showed that prolongation of the action potential in the setting of HF may itself be responsible for the observed abnormalities in cardiomyocyte function and Ca^{2+} homoeostasis.

The prolongation of the action potential at low stimulation rates reported in this study in cardiomyocytes of dogs with chronic HF is consistent with reports by others in different animal models of heart failure as well as in cardiomyocytes and ventricular myocardial fibers isolated from failing human hearts [19–24, 35–37]. At present, the ionic mechanisms underlying action potential prolongation in HF remains to be elucidated. In the pacing-induced HF dog model [23] as well as in human HF hearts [16–18] the major factor leading to action potential prolongation is thought to be downregulation of K^+ channels. Decrease in inward rectifier (I_{K1}) [16, 37], transient outward current (I_{to}) [16] and delayed rectifier current (I_K) [38] can result in a net reduction of outward currents, thereby delaying repolarization. Indeed, alterations in the action potential shown in the present study (figs 1, 2, 4) are in line with the reported downregulation of I_{to}. In addition to downregulation of I_{to}, previous data from our laboratory showed the presence of a sustained tetrodotoxin-sensitive Na^+ inward current that can also contribute to prolongation of the action potential [39]. AP_{90} is dependent on cycle length, and most studies documented action potential prolongation in experimental or clinical HF at long unphysiological cycle lengths [14, 23]. At cycle lengths close to normal or slightly shorter than normal, the action potential was not different or shorter in failing compared with normal hearts [21, 22, 40]. The action potential shortening (compare figs 1 and 4A) in response to the higher stimulation rates can in part be due to incomplete recovery of slowly inactivating Na^+ channels (our unpublished data).

Two patterns of abnormal intracellular Ca^{2+} transients have been described in failed human hearts. Beuckelmann et al. [14, 41] reported a gradual (almost sustained) Ca^{2+} increase immediately after the onset stimulation in single failed cardiomyocytes. A second, spike-dome pattern of Ca^{2+} transients was reported by Morgan et al. [42] in aquorin-loaded trabeculi isolated from hearts of patients with end-stage HF. Both SR- and sarcolemma-related mechanisms were used to explain these patterns. A spike-dome pattern of contraction/relaxation was previously described by Davies et al. [1] in single ventricular cardiomyocytes isolated from patients with HF. However, no mechanisms were suggested for this observation. The observation of a spike-dome pattern for cardiomyocyte contractions and $[Ca^{2+}]i$ in our canine HF model indicates similarities of E-C coupling defects with human HF.

The results of the present study show, for the first time, that the prolonged dome phase observed in both the cardiomyocyte contraction/relaxation cycle and in Ca^{2+} transients in HF share the same sarcolemma-related mechanism, namely that of prolonged action potential. Two separate experiments were conducted to establish this mechanism. First, in simultaneous recordings, we showed that the prolonged duration of the dome phase of Ca^{2+} transients and cardiomyocyte contraction/relaxation always coincided with the plateau of the action potential. Second, decrease in the duration of the action potential plateau, induced either by Na^+-channel blockers STX and lidocaine or by higher stimulation rates, resulted in a concomitant decrease in the duration of both cardiomyocyte contraction/relaxation and Ca^{2+} transients and in the suppression of the dome phase. The action potential could control the time course of the Ca^{2+} transients and subsequent contraction through its effects on Na^+-Ca^{2+} exchange. The Na^+-Ca^{2+} exchanger is the dominant myocardial Ca^{2+} efflux mechanism, and can contribute significantly to relaxation. However, this pathway can also promote Ca^{2+} influx. Ca^{2+} influx would continue during the plateau phase of the action potential until membrane potential repolarizes to -40 mV, the calculated reversal potential for Na^+-Ca^{2+} exchange [43]. At that point Ca^{2+} efflux would begin, resulting in relaxation. Thus, the longer action potential would result in a larger amount of Ca^{2+} entering the cardiomyocyte. The contribution of the $Na+$-Ca^{2+} exchanger to Ca^{2+} handling could be more efficient in HF if one takes into account that messenger RNA (mRNA) as well as protein levels are significantly increased in the failing human heart [9, 44]. Another potential source of Ca^{2+} entry during action potential plateau, the L-type Ca^{2+} "window" current [45], was shown to have a limited contribution to overall Ca^{2+} entry into the cell in human HF [43]. The data on L-type Ca^{2+}-channel expression in HF are still controversial. Takahashi et al. [7] reported a significant decrease in mRNA levels encoding the dihydropiridine (DHP) receptor as well as a decrease in DHP-binding sites in failing human hearts. This is in line with our findings in dogs with HF [28]. However, this is in contrast to findings by Rasmussen et al. [46] which indicate that DHP receptors are not significantly altered in human HF. Functional studies are also controversial. Beuckelmann et al. [47] did not find any changes in L-type Ca^{2+} current in failing cardiomyocytes, which is not in line with more recent

data indicating that HF decreases the density of the current [48, 49].

The dome phase of Ca^{2+} transients in HF cardio myocytes observed in the present study at low stimulation rate (0.2 Hz) is likely a result of poor intracellular Ca^{2+} handling, mainly by SR. In contrast, in normal ventricular cardiomyocytes, prolongation of action potential produced by depolarizing current pulse [25] or by Na^{+} agonists DPI and veratridine [43] does not result in a dome phase of either the contraction/relaxation cycle or intracellular Ca^{2+} transients. There is accumulating evidence that alteration of SR proteins involved in calcium removal from the cytosol plays a key role in impaired Ca^{2+} handling in the pathophysiology of HF. Indeed, decreased $[Ca^{2+}]$i reuptake into the SR was reported in humans [41] and dogs with HF [50]. SR proteins were shown to be significantly altered in the failing human heart. A reduction of mRNA levels encoding SR-Ca^{2+}-ATPase (adenosine triphosphatase), has been consistently shown in HF [6–13]. However, at the level of the protein, findings have been controversial, showing unchanged or slightly decreased SR-Ca^{2+}-ATPase protein levels in HF [9–13, 51]. The altered function of a Ca^{2+}-sensitive SR Ca^{2+} release channel (ryanodine receptor, RyR) could also be involved in impaired Ca^{2+} handling in HF. Several groups have studied mRNA expression of the RyR in human HF and found either slightly reduced or unchanged mRNA levels [8, 52, 53]. At the same time, RyR protein levels were found to be unchanged in HF [12, 54]. Neither finding excludes altered function of SR Ca^{2+} release channels in HF and its possible impact on impaired E-C coupling. Nimer et al. [55] found uncoupled gating of SR calcium release channels from RyR receptors, which may result in uncontrolled increase in Ca^{2+} efflux from the SR and thus contribute to abnormal Ca^{2+} handling in HF. Such Ca^{2+} leaking from the SR requires more energy for Ca^{2+} reuptake by SR ATPase and may disturb the energy supply-demand balance of the energy-deficient failing heart [56–59] and lead to Ca^{2+} overload. Energy deficiency in HF might be the key process underlying impaired myofibrillar cross-bridge cycling, resulting in delayed relaxation as seen in the present study (fig. 4). This is consistent with recent findings from our laboratory which showed that intracellular infusion of ATP increases the velocity of relaxation in HF dog cardiomyocytes [60].

The sarcolemma-related Ca^{2+}-handling defect shown in the present study might be a common mechanism for any type of HF characterized by prolonged action potential. A sustained Ca^{2+} influx during an action potential plateau can adversely impact myocardial contractility, since it directly interferes with diastolic relaxation. In addition to provoking relaxation abnormalities, Ca^{2+} overload can also activate enzymes that affect function of contractile proteins and produce cytoskeleton damage [61]. Finally, cycling of actin-myosin cross-bridges triggered by the sarcolemmal Ca^{2+} influx during diastolic relaxation may further exacerbate the already depleted high-energy metabolite pool in HF cardiomyocytes [56, 60].

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