Tachycardia-induced failure alters contractile properties of canine ventricular myocytes

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Abstract

Objective: Rapid cardiac pacing has been used as a model for experimentally-induced cardiomyopathy. However, its relevance to human heart failure is not clear at present because little is known about changes in size and function of ventricular myocytes. We have therefore studied the responses to graded increases in frequency and calcium in canine ventricular myocytes from failing hearts. The aim of our study was to evaluate the resemblance between canine pacing-induced and human end-stage heart failure. Methods: Myocytes were isolated from the left ventricular wall of dogs that were in heart failure after 6 weeks of pacing at 250 beats/min. Cell shortening was measured by edge detection. Results: Clinical signs of failure included dyspnea, ascites, and heart dilatation; the hemodynamic parameters were: LVdP/dt max 1613 ± 149 vs. 4713 ± 304 mmHg/s in 6 control dogs; LVEDP 17.2 ± 4.4 vs. 5.6 ± 1.1 mmHg; LV volume 60.5 ± 6.2 vs. 30-35 ml. Myocytes from failing hearts were longer and thinner than those from controls (form factor: 0.40 ± 0.01 vs. 0.47 ± 0.01, P < 0.001, > 30 cells/heart). With 6 mM Ca²⁺ and at 0.5 Hz, contraction amplitude was significantly attenuated in myocytes from failing hearts: 6.6 ± 0.9% cell shortening vs. 10.0 ± 0.8% in controls (P < 0.05). This deficit was exacerbated at higher stimulation rates. Time-to-peak contraction and time-to-50% relaxation were not altered. There was no difference in sensitivity to thapsigargin. Conclusion: As with cells from human failing hearts, contraction amplitude showed rate-dependent depression in this animal model, whereas features like slowing of contraction and relaxation and reduced sensitivity to thapsigargin, were not reproduced.

Keywords: Frequency responses; Postextrasystolic potentiation; Heart failure; Contractile function; Tachycardia; Thapsigargin; Dog, ventricular myocytes

1. Introduction

Chronic rapid ventricular pacing in the dog produces hemodynamic, echocardiographic and hormonal changes similar to those observed in human congestive heart failure [1,2]. The similarities between the two forms of heart failure include ventricular dilatation [1], reduced number of β-adrenergic receptors, particularly of β₁ subtype [3–5], and systolic and diastolic dysfunction [6].

Both in animal models and in humans, failure-induced changes in contractile properties of heart muscle consist of absence or presence of depression of contraction amplitude [7,8], a flattened force–frequency response [9–11], and impaired function of the Ca²⁺ pump of the sarcoplasmic reticulum [12–14] which is associated with abnormal Ca²⁺ handling at the cellular level [7,15]. These changes are in part due to alterations of myocardial gene expression [12,16].

The contraction amplitude of individual myocytes from human failing hearts is depressed as compared to control cells and the time courses of contraction and relaxation are markedly slowed [17,18]. Block of the SR Ca²⁺ pump with the selective inhibitor thapsigargin [19] causes little additional functional impairment in myocytes from failing hearts.

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hearts, and post-rest (3 min) decay of contraction amplitude which was observed in myocytes from non-failing human hearts was markedly attenuated in myocytes from failing hearts [20]. In the present paper we have investigated the effects of stimulation frequency and of thapsigargin on various contraction parameters of canine myocytes isolated from healthy animals and those in tachycardia-induced failure. The aim was to evaluate the resemblance between this model of experimentally-induced cardiomyopathy and human end-stage heart failure.

2. Methods

2.1. Induction of heart failure

Twelve age- and weight-matched mongrel dogs (weight range 18–25 kg) were used in this study. All studies complied with the United Kingdom Home Office regulations governing the care and use of laboratory animals. Rapid ventricular pacing at a rate of 250 pulses/min was used to induce heart failure in 6 dogs; another 6 animals were sham-operated and served as controls. Instrumentation of the animals was carried out as described previously [21]. In brief, the dogs were anesthetized with 5 mg/kg of 2.5% sodium thiopentone intravenously and maintained in anesthesia with halothane delivered in a mixture of N₂O and O₂ (30:70, vol/vol) via an endotracheal tube. A Medtronic 6957 unipolar pacing electrode was positioned into the right ventricle under fluoroscopic control and fixed into position in the right ventricular apex. A programmable pacemaker generator (Medtronic Spectrax) was connected and buried in a subcutaneous pocket between the scapulae. The generator had been modified to be permanently programmed to 250 pulses/min in the activated and to 30 pulses/min in the inhibited mode. The pacing procedure was started when the dogs had recovered from the surgical procedure for at least 7 days; and was continued for 6 weeks. The pacemaker was turned off 2 days before the dogs were used in a hemodynamic study under general anesthesia with chloralose (dose: 100 mg/kg body weight in control dogs, half this dose in dogs with heart failure). Each animal received 1.5 mg atropine.

2.2. Cell isolation

At the end of an experiment, the animal was given 5000 units of heparin (Monoheparin®). The heart was exposed by thoracotomy and after induction of cardiac fibrillation, a portion of the left ventricular free wall (5–10 g) was excised and transported to the cell laboratory in ice-cold cardioplegic St. Thomas' solution [22]. A surface artery or vein was cannulated for perfusion of the tissue with Krebs-Henseleit solution, the tissue was perfused in a heart failure solution, the tissue was perfused in a low-calcium buffer for 5 min, followed by 2 min of perfusion with recirculated buffer containing Sigma type XXIV protease (pronase), 2 IU/ml (total volume 50 ml), and then 10 min with recirculating collagenase-containing buffer (Sigma type V, 0.5 mg/ml, total volume 50 ml). The composition of the low calcium buffer was (in mM): NaCl 120, KCl 5.4, MgSO₄ 5, pyruvate 5, glucose 20, taurine 20, HEPES 10, nitrilotriacetic acid (NTA) 5, pH 6.96, containing 10–15 μM calcium. All solutions were prewarmed and pre-oxygenated. After removal from the perfusion apparatus undigested tissue was trimmed away, and only digested epicardium was chopped and put into a boiling tube plus 10 ml of collagenase-containing solution. The medium was shaken gently in a water bath at 35°C and kept under an atmosphere of 100% O₂. After 5 min the first digest was taken: the content of the tube was filtered through a nylon mesh (300 μm) and centrifuged for 1 min at 400 revolutions/min. The resulting pellet was resuspended in fresh buffer without collagenase. Undigested tissue retained on the nylon mesh was resuspended in collagenase-containing solution, and after 10 min, the 2nd digest was prepared in the same way. The cells were washed once by centrifugation in low calcium buffer without enzymes or NTA and were resuspended in a similar solution. This procedure yielded between 10 and 50% elongated quiescent cells with clear cross-striations.

2.3. Experimental procedures

The cells were investigated in a Perspex chamber placed on the stage of an inverted microscope (Zeiss IM). The myocytes spontaneously attach to the glass coverslip which forms the floor of the bath. After allowing 5 min for cell attachment, the chamber was perfused at a rate of 1.5–2.5 ml/min with Krebs-Henseleit medium equilibrated to pH 7.4 with 95% O₂ and 5% CO₂. The temperature was kept constant at 32 ± 0.5°C by a heater placed just before the chamber inlet and by two thermocouples for feedback control of the heater. The cells were electrically stimulated at 50% above their voltage threshold by bipolar pulses through platinum electrodes placed along the side of the bath. The amplitude of contraction and rates of contraction and relaxation were monitored continuously with a video-camera/length detection system as previously described [23]. In order to ensure that the cell population used in this study displayed consistent functional properties, the following selection criteria were employed [23]: (1) the cells were rod-shaped, showed clear striations and had no blebs or granulations; (2) the mean sarcomere length calculated as cell length divided by number of sarcomeres was more than 1.7 μm; (3) the spontaneous contraction rate was lower than 1/min when unstimulated.
For measurement of resting cell dimensions, hard copies of the images of at least 30 cells from each dog were obtained immediately after isolation. The images were analysed by measuring maximum cell length, width and circumference on a digitizing tablet with associated software VIDS III. In order to account for the irregularities in shape of the cells, we noted both minimum and maximum length and width for each cell. The length-to-width ratio was estimated from a 'form factor' for each myocyte. This form factor assigns a value depending on how closely the cell shape approximates to a circle. Wide cells have a large form factor and thin cells have a small one [24].

For experimentation, cells were allowed to stabilise at 0.5 Hz in 2 mM calcium. Concentration-response curves to calcium were performed in a cumulative manner. Responses to variation in frequency (0.125 to 2 Hz) were obtained at different calcium concentrations (2–10 mM) that did not induce spontaneous activity or cause incomplete relaxation. Post-rest contractions were measured after 10, 30 and 180 s of transient interruption of basal stimulation (0.5 Hz) in both the absence and the presence of thapsigargin.

2.4. Materials

Salts were purchased from Merck (Merck House, Poole, Dorset, UK) and were AnalaR grade except for KCl, taurine and glucose which were AristaR. AnalaR water was used for the low Ca\(^{2+}\) solutions and double-distilled deionised (MiliQ system) for the remainder.

2.5. Statistics

Results from different cells of an individual dog were averaged, means ± s.e.m. were obtained from sham-operated controls and from dogs in heart failure. Statistics were calculated using data pooled by animals (i.e., n = number of dogs). Analysis of covariance (ANCOVA) was performed for frequency responses, with frequency as the covariate, and results were accepted as significant for P < 0.05.

3. Results

3.1. Hemodynamics

After 6 weeks of right ventricular pacing at 250 beats/min all dogs had developed clinical symptoms of dyspnea, peripheral edema and ascites. Spontaneous heart rate was measured while the dogs were in chloralose anesthesia for an experimental protocol not related to this study (effects of tilting on hemodynamics, no drugs involved). In sham-operated dogs, spontaneous heart rate was 113 ± 4 as compared to 107 ± 8 beats/min after weaning the dogs off the pacemaker. Following treatment with atropine (1.5 mg), heart rate significantly increased to 180 ± 13 beats/min in the control group and to 158 ± 5 beats/min in the failing hearts. The differences in heart rates between the two groups were not statistically significant either before or after atropine treatment. The following hemodynamic changes were observed. Left ventricular end diastolic pressure (LVEDP) increased from 5.6 ± 1.1 in control to 17.2 ± 4.4 mmHg in failing hearts; left ventricular dp/dt\(_{\text{max}}\) had dropped from 4713 ± 304 to 1613 ± 149 mmHg/s; and left ventricular volume was increased from 30–35 to 60.5 ± 6.2 ml. These findings are consistent with previous reports, that pacing-induced tachycardia produces dilated cardiomyopathy [1,8,21].

3.2. Cell dimensions

Cell length and cell width varied over a wide range of values in control and paced dogs. When averaging dimensions for 30 to 40 cells in each dog, mean cell length in 5 control hearts was 139.9 ± 4.8 \(\mu\text{m}\) and in 6 failing hearts 155.6 ± 6.5 \(\mu\text{m}\) (not significant, n.s.); mean cell width was 34.5 ± 1.7 \(\mu\text{m}\) in control and 29.8 ± 1.5 \(\mu\text{m}\) in failing hearts (n.s.). There were no statistically significant differences between control and paced dog hearts in cell area (4192 ± 290 vs. 4705 ± 310 \(\mu\text{m}^2\)), and cell perimeter (3347 ± 108 vs. 3628 ± 132 \(\mu\text{m}\), respectively); however, the form factor (see Methods) was significantly reduced from 0.47 ± 0.01 in sham operated to 0.40 ± 0.01 in paced animals (P < 0.001), indicating that cells from failing hearts were longer and more slender than cells from control hearts.

3.3. Responses to calcium

At basal stimulation rate (0.5 Hz) and a low extracellular calcium concentration (2 mM Ca\(^{2+}\)) some depression of contraction amplitude in myocytes from paced hearts was evident, though the difference was not statistically
significant. Contraction amplitude expressed as percent
cell shortening was 3.9 ± 0.4% in myocytes from sham-
operated controls (n = 8 cells/6 animals) and 2.4 ± 0.4%
(n = 10/5) in cells from failing hearts. In both groups of
cells contraction amplitudes increased with increasing ex-
tracellular [Ca\textsuperscript{2+}] (Fig. 1). However, the response to ele-
vated calcium was always smaller in cells from paced hearts, the differences being significant at 6 and 8 mM Ca^{2+}. Contraction amplitude was depressed by 35% in cells from failing hearts at 8 mM Ca^{2+}.

It should be noted that, while the average lengths of population of elongated, non-contracting cells was not significantly different between control and failing groups, the contracting myocytes used for the actual experiments were longer from the failing hearts. The mean length of the myocytes studied for contraction was 132 ± 6 μm (n = 18) from controls and 161 ± 11 μm (n = 19) from failing groups (P < 0.05). The mean sarcomere length of these cells was not significantly different (1.89 ± 0.02 vs. 1.87 ± 0.05 μm) between groups. The myocytes whose contraction amplitudes are included in the 8 mM Ca^{2+} point in the concentration–response curves (Fig. 1) had lengths of 122 ± 7 μm (n = 8 cells) in controls vs. 147 ± 26 μm (n = 6 cells) for failing. Thus the difference in contraction amplitude, when expressed as μm change in length rather than percent shortening, was less marked between groups. At 8 mM Ca^{2+} contraction amplitude in μm was 14.8 ± 1.0 (5 dogs) in controls vs. 11.4 ± 2.2 (4 dogs) in cells from failing hearts, representing a 23% depression. The significance of the difference was lost both because of the decreased size of the effect when contraction was expressed in this way and because of the increase in variability due to the scatter in myocyte size. Contraction and relaxation velocities changed in parallel with contraction amplitudes in myocytes from paced hearts, however, time-to-peak contraction (TTP) and to 50% (R50) and 90% of relaxation (R90) were not altered with increasing extracellular [Ca^{2+}] (data not shown).

3.4. Variation in stimulation rate

Since the positive force–frequency response in human myocardium is flattened in the failing heart [9–11], contraction amplitudes of canine myocytes from control and failing hearts were studied at various stimulation rates. Fig. 2 shows tracings from two individual experiments at a maximum [Ca^{2+}]. In the control cell, the contraction–frequency response was flat. Base-line cell length decreased at stimulation rates larger than 0.8 Hz and alternating contraction amplitudes were observed with 2 Hz. In contrast, in the myocyte from a failing heart a clear negative staircase was obtained in the range of low frequencies (0.125–0.5 Hz), but the frequency–response levelled with frequencies > 0.5 Hz. Myocytes from failing hearts ceased to follow electrical stimulation at lower rates than the control cells (1.0 vs. 2 Hz in the two cells of Fig. 2).

Flat or slightly negative frequency responses in control cells as compared to the distinctly negative frequency responses in myocytes from failing hearts were also found at lower extracellular [Ca^{2+}] (Fig. 3), with exception of 2 mM Ca^{2+}, where the frequency responses were flat in both groups of cells. At this calcium concentration, however, contraction amplitudes were least stable and spontaneous contractions occurred frequently in myocytes from both groups. Hence with 4–8 mM Ca^{2+} the difference in contractility between cells from non-failing and failing hearts was most prominent at high and smallest at low stimulation rate. Once again, cells from the failing hearts tended to be longer than controls; cell lengths for each group have been included in the legend to Fig. 3. It should be noted that these differences are not sufficient to explain the changes in contraction in the failing myocytes, since there is a 48% decrease in amplitude at 1 Hz and 8 mM Ca^{2+}, although cells from the control group are only 23% shorter than those from failing. We have previously shown that large and small myocytes from the same hypertrophied heart show equivalent decreases in percent shortening compared to control [24]. Nor is the difference between the groups explained by a diastolic contracture at high Ca^{2+} concentrations or high frequencies. At 8 mM Ca^{2+}, for example, the diastolic length of the cells decreased during the change from 0.125 to 1 Hz stimulation by only 1.47 ± 0.49% in control and 0.48 ± 0.28% in myocytes from failing hearts.

Dog myocytes responded with post-rest potentiation to intervals of increasing duration (i.e., after 10 s, 30 s, or 3 min of rest). Any difference in contraction amplitude at regular stimulation frequency (0.5 Hz) disappeared (Fig. 4); after 3 min of rest, cell shortening was 13.6 ± 2.3% in control cells (n = 7/6) and 14.1 ± 2.6% in myocytes from failing hearts (n = 8/6).

Increasing frequency of contraction tended to accelerate the beat. Table 1 shows paired experiments in 8 control myocytes (4 dogs) and 6 failing myocytes (4 dogs) at 4–6 mM Ca^{2+}, where TTP, R50 and R90 could be accurately measured at both 0.125 and 1 Hz in the same cell. TTP was significantly reduced at the higher frequency in con-
Table 1

<table>
<thead>
<tr>
<th></th>
<th>0.125 Hz</th>
<th>1 Hz</th>
<th>P &lt; n (cells/dogs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control TTP, s</td>
<td>0.18 ± 0.02</td>
<td>0.15 ± 0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>R50, s</td>
<td>0.07 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>NS</td>
</tr>
<tr>
<td>R90, s</td>
<td>0.17 ± 0.02</td>
<td>0.17 ± 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Failing TTP, s</td>
<td>0.26 ± 0.03</td>
<td>0.19 ± 0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>R50, s</td>
<td>0.13 ± 0.01</td>
<td>0.11 ± 0.01</td>
<td>NS</td>
</tr>
<tr>
<td>R90, s</td>
<td>0.25 ± 0.03</td>
<td>0.16 ± 0.02</td>
<td>0.01</td>
</tr>
</tbody>
</table>

TTP = time-to-peak contraction; R50 and R90 = time-to-50% and time-to-90% of relaxation, respectively. Mean values ± s.e.m., P for comparison of values at different frequencies (paired data); NS = not significant. [Ca²⁺]o: 4-6 mM.

3.5. Effects of thapsigargin

In myocytes from non-failing and failing dog hearts, thapsigargin reduced the contraction amplitude both during regular pacing and after a period of rest. Table 2 contains pooled data of TTP, R50 and R90 at various frequencies including post-rest beats. However, only at 0.1 Hz did we find a statistically significant prolongation in R50. Although the time-course of the post-rest beat was prolonged, there was no significant difference between myocytes from control and failing hearts.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>0.125 Hz</th>
<th>1 Hz</th>
<th>0.5 Hz</th>
<th>Post-rest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control n (dogs)</td>
<td>4</td>
<td></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Failing n (dogs)</td>
<td>4</td>
<td></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>P</td>
<td></td>
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Fig. 5. Contraction amplitude, percent of initial value, after treatment with increasing concentrations of thapsigargin in myocytes from control (solid line) and failing (dashed line) dog hearts. Means ± s.e.m., numbers of myocytes are indicated. Stimulation frequency 0.5 Hz.

4. Discussion

The purpose of this study was to characterize contractile abnormalities in left ventricular myocytes isolated from dogs with tachycardia-induced failure. We found moderate failure-induced changes in canine cell morphology, impaired contraction amplitudes in particular at high stimulation rates and high [Ca²⁺]o, and no changes in sensitivity to thapsigargin. These contractile abnormalities differ in several respects from those of human myocytes from failing hearts [17,25].

4.1. Hemodynamics

Prolonged tachycardic electrical pacing caused marked left ventricular dilation without signs of hypertrophy [21]. Left ventricular volume was almost doubled, and LVEDP...
increased and dP/dt max decreased substantially. These hemodynamic changes together with clinical symptoms demonstrate that the paced dogs were indeed in moderate to severe heart failure. We did not observe any increase in spontaneous heart rate after turning off the pacemaker, and control and paced dogs showed normal positive chronotropic responses to atropine. This is in contrast to the significant elevation of spontaneous heart rate after pacemaker disconnection in conscious dogs [5,8,21,26] and most likely due to the inhibitory effect of anesthesia on sympathetic tone.

4.2. Cell size

The significant increase in left ventricular volume of paced hearts indicates substantial left ventricular dilatation. Quiescent myocytes from failing hearts were longer and more slender as indicated by the significant decrease in form factor. However, we did not detect significant differences in either cell length or cell width when data were pooled for each dog. This contrasts with results for pacing-induced heart failure in pigs [27] and dogs [28]. It should be emphasized, however, that the myocytes chosen for investigation were longer from failing than from non-failing hearts (see above). We cannot exclude some experimental bias in choosing suitable cells as those with regular strong contractions were preferred. Apparently, longer cells from failing hearts were more likely to give the wanted response. Since individual sarcomere length does not appear to change in dilatative cardiomyopathy [29], elongation of myocytes must take place by series addition of sarcomeres [30].

4.3. Impairment of myocyte contractility

Contraction amplitude was attenuated in myocytes from the paced hearts as compared to controls. Potential mechanisms responsible for this depression include impairment of any step in excitation–contraction coupling. Thus failure-induced changes in Ca 2+ handling and Ca 2+ homeostasis may be involved as well as altered Ca 2+ sensitivity of the contractile proteins or changes at the level of cross-bridges. The impaired contractility in myocytes from failing hearts could not be overcome by elevating extracellular [Ca 2+] and was, rather, accentuated. Similar reduced responsiveness to Ca 2+ was also reported for pig myocytes from a similar model of dilated heart failure [27].

Reduced Ca 2+ influx via L-type Ca 2+ channels could contribute to impairment of contraction, but no data on L-type Ca 2+ current in canine myocytes from tachycardia-induced failing hearts are available. Results from other species, however, are conflicting: In human ventricular myocytes from end-stage failing hearts Ca 2+ current was not different from controls [31], whereas in myocytes from pigs with tachycardia-induced cardiomyopathy Ca 2+ current was significantly reduced [32]. Cory and co-workers have provided evidence in dog hearts that the Ca 2+ release channels [33] and the Ca 2+ pump of the sarcoplasmic reticulum (SR) are downregulated. Impaired Ca 2+ uptake into the SR would also account for the prolongation in TTP and relaxation, although it should be emphasized that with exception of time-to-50% of relaxation (R50) at 0.1 Hz, the differences between myocytes from control and failing hearts were not statistically significant in our study due to the large variability in parameters from individual cells. Other groups, however, have reported significant changes in TTP and relaxation times [27,34]. In those studies, heart failure may have been more severe than in our dogs. Lack of contracture even at high stimulation frequency and at high extracellular [Ca 2+] in the present study would further argue against a problem of impaired SR function.

Increased expression and activity of Na+/Ca 2+ exchange were reported to be associated with heart failure in human myocardium [35]. Hence altered function of the Na+/Ca 2+ exchanger could also contribute to reduced contractility. However, the Na+,K+-ATPase in failing myocardium was found to be downregulated in activity and density [36,37]. Such an effect would reduce the electrochemical gradient of Na + across the sarcolemma and by decreasing the driving force for the Na+/Ca 2+ exchange, would counteract enhanced expression and activity of exchanger protein.

4.4. Frequency responses

A positive force–frequency relationship is usually found in dog papillary muscles and trabeculae [38–40], and even in papillary muscles from failing hearts the frequency response remained positive [40]. There are, however, some reports where under in-vivo conditions the frequency response was flat in dog hearts [41,42]. The frequency response in the present study was flat or slightly negative in control myocytes and was converted into a clearly negative staircase, particularly in the low frequency range, in myocytes from failing hearts. The reason for this discrepancy is not clear; however, experimental conditions such as temperature, [Ca 2+] and frequency range must be identical for direct comparison of our findings with results reported in the literature.

During a positive staircase response, a greater percent of Ca 2+ entering the muscle cells during an action potential may directly contribute to contraction, and/or the processes of excitation–contraction coupling may become more efficient at higher frequencies [38]. The negative force–frequency response in rat heart was suggested to be due to diminished SR Ca 2+ release with increasing frequency [43]. As tachycardia-induced failure appears to impair SR function (see above), the pronounced negative
Failure of thapsigargin to substantially affect contraction amplitude in myocytes from failing human heart is in line with such an explanation.

4.5. Comparison of canine myocytes from paced hearts and human myocytes from patients with end-stage heart failure

One of the principle intentions of this study was to compare the characteristics of contraction and frequency response in myocytes from failing dog heart with those that we obtain from the ventricles of patients with end-stage failure. There is a strong frequency-dependent depression of contraction in myocytes from failing human heart, and this was reproduced in the dog failure model. However, in the dog it occurred as an exacerbation of the tendency to a negative staircase seen in control myocytes, while in human cells there is a conversion of the control positive staircase to a flat response [25]. There were corresponding differences in post-rest behaviour between control human and dog myocytes, with decay of contraction occurring during rest for human and an SR-dependent (thapsigargin-sensitive) accumulation of Ca$^{2+}$ in the dog. The amplitude of rested-state beats did not differ between control and failing dog myocytes, while there was significant attenuation of post-rest decay in myocytes from failing compared with non-failing human heart [20].

High Ca$^{2+}$ increased the difference between control and failing cells in the dog, but this did not occur in the human: the relative difference in contraction amplitude between myocytes from non-failing and failing human ventricle was similar in 2 mM or maximally inotropic Ca$^{2+}$ [25]. One important observation in human cells—the slowing of contraction and relaxation with heart failure [17]—was not reproduced in the dog. We have shown that this effect in the human is also frequency-dependent, being more marked at slower rates [25], but even rested-state contractions of dog myocytes did not show differences between control and failing groups. Velocities of contraction and relaxation were decreased in failing dog hearts, but only in proportion to the change in amplitude.

We have also shown that myocytes from failing human heart, although relaxing slowly, are less sensitive to the additional slowing effects of thapsigargin [20]. This may represent decreased SR function in the failing cells, as suggested by biochemical measurements in other studies [12,13]. Failing dog myocytes, however, do not appear to be markedly different from control in their sensitivity to thapsigargin, with contraction amplitude reduced by the same proportion as in control cells. However, it is interesting that SR removal with thapsigargin reproduces in control dog cells some of the effects of failure, in that contraction amplitude is depressed while contraction and relaxation speeds are not greatly affected. In myocytes from non-failing human heart thapsigargin has only a small effect on contraction amplitude but produces a 2–3-fold increase in relaxation time [20]. This implies that the functional consequences of compromised SR function are manifest in different ways in the two species.

Tachycardia-induced failure in dogs and human end-stage heart failure are also different with respect to the time scales of development and persistence of failure. Furthermore, in contrast to the dogs, most human subjects receive drug treatment. Overall, we consider that the differences in characteristics of non-failing dog and human myocytes limit the usefulness of this species for the investigation of SR function in heart failure, especially given the expense and difficulties involved with this model. This does not preclude its use for other aspects of human heart disease; for example, the distribution and percentage of $\beta_1$AR to $\beta_2$AR resembles the human more closely than most other species.

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References


