Abnormal $\text{Ca}^{2+}$ release from cardiac sarcoplasmic reticulum in tachycardia-induced heart failure

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Abstract

Objective: In heart failure, little information is available as to the $\text{Ca}^{2+}$ release function of sarcoplasmic reticulum (SR), which plays a major role in cardiac contractile function. Here, we assessed the rapid kinetics of drug-induced $\text{Ca}^{2+}$ release from cardiac SR in combination with a measurement of ryanodine binding in heart failure.

Methods: The SR vesicles were isolated from dog left ventricular (LV) muscles (normal (N), $n=10$; pacing induced heart failure (HF), $n=10$). The time course of SR $\text{Ca}^{2+}$ release was continuously monitored by a stopped-flow apparatus using arsenazo III as a $\text{Ca}^{2+}$ indicator, and $\text{Ca}^{2+}$ uptake and $[\text{H}]$ryanodine binding assays were done using a filtration method.

Results: The amount of $\text{Ca}^{2+}$ uptake was reduced in HF to 55% of N ($P<0.05$). Even the more marked and earlier appeared decrease was seen in the rate constant and the initial rate of polylysine (PL; a specific release trigger)-induced $\text{Ca}^{2+}$ release ($P<0.05$). However, the PL concentration dependency of the initial rate shifted towards lower concentrations of PL in HF than in N ([PL] at half maximum stimulation $=0.13$ vs. $0.35\mu\text{M}$). The $[\text{H}]$ryanodine binding assay revealed a lower $B_{\text{max}}$ (pmol/mg) in HF than in N ($0.91\pm0.19$ vs. $2.64\pm0.59$, $P<0.05$), but no difference in $K_{d}$ (nM) ($0.95\pm0.29$ vs. $0.90\pm0.11$, $P=\text{n.s.}$). The [PL] dependency on the enhancement of $[\text{H}]$ryanodine binding again showed a shift towards lower [PL] in HF than in N.

Conclusions: In pacing-induced heart failure, the $\text{Ca}^{2+}$ releasing function of SR is disturbed, which may result in an intra-cellular $\text{Ca}^{2+}$ transient that was slowed down.

Keywords: Calcium; E-C coupling; Heart failure; Ion channels; SR

1. Introduction

In cardiac muscle, the majority of the $\text{Ca}^{2+}$ transient, triggering contraction, is provided by a large amount of $\text{Ca}^{2+}$ release from the sarcoplasmic reticulum (SR) following the small influx of $\text{Ca}^{2+}$ through L-type $\text{Ca}^{2+}$ channels, that is $\text{Ca}^{2+}$-induced $\text{Ca}^{2+}$ release [1,2]. The available $\text{Ca}^{2+}$ for cardiac contraction is released from the SR’s $\text{Ca}^{2+}$-release channel, which is also referred to as the ryanodine receptor [3,4]. Using canine failing heart produced by rapid ventricular-pacing, Vatner et al. [5] reported that the number of ryanodine receptors was decreased even one day after rapid ventricular pacing, in association with the decrease in left ventricular (LV) contractility. In contrast, the number of ryanodine receptors was increased in SR from prehypertrophic cardiomyopathic hamster heart [6]. We also demonstrated that the density of ryanodine receptors was increased in mild pressure-overload rat hypertrophied heart [7], whereas it was decreased in volume-overloaded rat heart [8]. Thus, it has been suggested that the quantitative or qualitative alteration of ryanodine receptor might affect the change in contractile function during the development of various types of cardiac hypertrophy and/or failure. However, it remained to be elucidated whether the contractile dysfunction in cardiac failure is related to the altered kinetics of SR $\text{Ca}^{2+}$ release in association with the functional abnormality of...
the SR Ca\textsuperscript{2+}-release channel, the ryanodine receptor.

In skeletal muscle, nanomolar concentrations of polylysine bind specifically to ryanodine receptors and stimulate Ca\textsuperscript{2+} release from the SR [9,10] and, recently, we demonstrated that nanomolar concentrations of polylysine stimulate ryanodine binding and induce rapid Ca\textsuperscript{2+} release from cardiac SR also [11]. Since polylysine exhibits specificity towards the ryanodine receptor in terms of Ca\textsuperscript{2+} release and ryanodine binding, it might be a useful drug for analysis of the complex mechanism of excitation–contraction (E–C) coupling in cardiac muscle. In this regard, isolated SR vesicles are useful in elucidating the molecular mechanism of E–C coupling because of their simplified protein composition, in which major components required for E–C coupling are involved.

In the present study, we have focused on the function of the ryanodine receptor and the rapid kinetics of Ca\textsuperscript{2+} release from cardiac SR in tachycardia-induced heart failure.

2. Methods

The investigation conformed with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.1. Experimental model and instrumentation

Heart failure was induced in conscious, chronically instrumented beagle dogs of either sex by 21 days of rapid right ventricular (RV) pacing at a rate of 250 bpm using an externally programmable miniature pacemaker (Medtronic Inc., Minneapolis, USA). The specific details of the chronic instrumentation were as follows. Beagle dogs were sedated with morphine sulfate (15 mg s.c.) and cromazine maleate (10 mg s.c.). Then, they were anesthetized with isoflurane (2%, 1.5 l/min) and a mixture of nitrous oxide and oxygen (50:50), intubated with a cuffed endotracheal tube, and ventilated at a tidal volume of 22 ml/kg and a respiratory rate of 15 breaths/min. A bipolar pacing lead was fixed to the endocardial RV surface, and the distal lead was tunneled to a subcutaneous pocket that was constructed on the animal’s back, where they were connected to a pacemaker (Medtronic Inc.), modified to pace at 250 bpm. The thoracotomy was closed in layers. Cefazolin (1 g i.v.) was administered before and after surgery.

After allowing one week for recovery, the pacemaker was programmed to 250 bpm. Dogs were monitored daily for clinical signs and symptoms of heart failure. With the pacing turned off one or three weeks after rapid RV pacing, LV pressure was measured using a 7-F micromanometer (Millar, Texas, USA), inserted percutaneously via the carotid artery, and two-dimensional short axis echocardiograms were obtained at the level of the head of the papillary muscle.

The care of the animals and the protocols used were in accord with guidelines laid down by the Animal Ethics Committee of Yamaguchi University School of Medicine.

2.2. Preparation of SR vesicles

The SR vesicles were prepared as described previously [7,8,11], according to the method of Kranias et al. [12]. Left ventricles were homogenized in a solution containing 30 mM Tris–maleate, 0.3 M sucrose, 5 mg/l leupeptin and 0.1 mM PMSF, at pH 7.0 (solution I). The homogenate was centrifuged at 5500 g for 10 min and the resultant supernatant was filtered through four layers of cheesecloth before centrifugation at 12 000 g for 20 min. The supernatant was again filtered through cheesecloth and centrifuged at 143 000 g for 30 min. The pellet was resuspended in a solution containing 0.6 M KCl, 30 mM Tris–maleate, 0.3 M sucrose, 5 mg/l leupeptin, 0.1 mM PMSF, at pH 7.0 (solution II). This suspension was centrifuged at 143 000 g for 45 min. The pellet was resuspended again in solution II, homogenized and centrifuged at 143 000 g as described above. The pellet was suspended in solution I and centrifuged at 143 000 g. The resultant pellet represents the microsomal fraction that is rich in SR vesicles, and it was suspended in a solution containing 0.1 M KCl, 20 mM Tris–maleate, 0.3 M sucrose, 5 mg/l leupeptin, 0.1 mM PMSF, at pH 7.0, to give a final concentration of about 10–20 mg protein/ml. This fraction was rapidly frozen in liquid nitrogen and stored at −80°C. An aliquot was retained for determination of protein concentration by the method of Lowry et al. [13].

2.3. Ca\textsuperscript{2+} release assay

SR vesicles (0.5 mg/ml) were incubated in a solution containing 0.15 M KCl, 10 mM NaN\textsubscript{3}, 2.5 mM Mg\textsubscript{ATP} and 20 mM MES, pH 6.8 (adjusted using KOH) (Solution A) for 5 min, to load the SR moiety with Ca\textsuperscript{2+}. Then, one volume of solution A was mixed with one volume of Solution B containing 0.15 M KCl and 20 mM MES, pH 6.8 (adjusted using KOH), and various concentration of polylysine (MW 27,000). The Ca\textsuperscript{2+} concentration in both solutions was buffered at 3 \textmu M using an EGTA–calcium buffer (0.212 mM CaCl\textsubscript{2}, 0.25 mM EGTA, pH 6.8). The time course of SR Ca\textsuperscript{2+} release was monitored in a stopped-flow apparatus (Unisoku RSP-601S, Osaka) using 5 \textmu M arsenazo\textsuperscript{III} as a Ca\textsuperscript{2+} indicator [10,11,14,15]. All of the reactions mentioned above were carried out at 22°C. Twenty to twenty-five traces (each representing 1000 data points) of the arsenazo\textsuperscript{III} signal were averaged for each experiment. The arsenazo\textsuperscript{III} signal was converted to nanomol of Ca\textsuperscript{2+} released per mg of protein by determining the Δ arsenazo\textsuperscript{III} signal/Δ [Ca\textsuperscript{2+}] coefficient from a
Ca\textsuperscript{2+} uptake and release assays were determined at different polylysine concentrations. Curves were fitted by a single exponential function, 
\[ y = A(1 - e^{-kt}) \]
where \( y \) is the amount of Ca\textsuperscript{2+} released at time \( t \), \( A \) is the final amount of Ca\textsuperscript{2+} released at an infinite time, and \( k \) is the rate constant of release.

2.4. Ryanodine binding assay

To determine the number of ryanodine receptors in normal and failing SR vesicles, \[^{3}H\]ryanodine binding assays were carried out as described previously [7,8]. Briefly, the cardiac microsomes (0.1 mg/ml) were incubated for 120 min at 36°C in 25 mM imidazol, 1 M KCl, 0.95 mM EGTA, 1.103 mM CaCl\textsubscript{2} (free Ca\textsuperscript{2+}= 20 \mu M), at pH 7.4, each with a concentration of \[^{3}H\]ryanodine (68.3 Ci/ml, DuPont NEN) in the range of 0.3 to 10 nM. The incubated reaction mixture was filtered through Millipore filters (type HA; pore size, 0.45 \mu m) and washed twice with 5 ml of the same reaction solution that was devoid of microsomes and \[^{3}H\]ryanodine. The specific binding was calculated as the difference between the binding in the absence (total binding) and in the presence (nonspecific binding) of 10 \mu M unlabeled ryanodine. Experiments were carried out in duplicate; each datum point was obtained by averaging the duplicates.

For assessment of the enhancement of \[^{3}H\]ryanodine binding to the SR moiety by polylysine, the binding assays were carried out according to the method by Lu and Meissner [16]. The cardiac microsomes (0.1 mg/ml) were incubated in 1.0 ml of a reaction solution containing 5 nM \[^{3}H\]ryanodine, 0.1 M NaCl, 20 mM Na–Hepes, pH 7.2, at [Ca\textsuperscript{2+}]= 3 \mu M, using an EGTA–calcium buffer (0.974 mM MES, pH 6.8 (adjusted using KOH), 0.3 mM MgCl\textsubscript{2}, 20 mM Hepes, pH 7.2) for 120 min at 36°C in the absence of polylysine or various concentrations of polylysine. The incubated reaction mixture was filtered through Millipore filters (type HA; pore size, 0.45 \mu m) and washed twice with 5 ml of the same reaction solution that was devoid of microsomes and \[^{3}H\]ryanodine. The specific binding was calculated as the difference between the binding in the absence (total binding) and in the presence (nonspecific binding) of 10 \mu M unlabeled ryanodine. Experiments were carried out in duplicate; each datum point was obtained by averaging the duplicates.

2.5. \(^{45}\text{Ca}^2+\) uptake and release assays

Ca\textsuperscript{2+} uptake was measured by the filtration technique using \(^{45}\text{Ca}^2+\) [17] under similar conditions to those used for the Ca\textsuperscript{2+} release assay with a stopped-flow fluorometric system. The SR vesicles (0.2 mg/ml) were incubated in 2 ml of a solution containing 0.15 M KCl, 1 mM MgCl\textsubscript{2}, 30 \mu M \(^{45}\text{Ca}^2+\), (1 mM Cl/ml), 10 mM NaN\textsubscript{3} and 20 mM MOPS, pH 7.1. Ca\textsuperscript{2+} uptake was initiated by the addition of 1 mM ATP and was determined at varying time intervals by placing a 2-ml aliquot on a 0.45-\mu m Millipore filter, and rinsing it with 5 ml of washing buffer (0.15 M KCl, 20 mM MOPS, pH 7.1, containing 30 mM EGTA and 15 \mu M ruthenium red). The radioactivity retained on the filters was determined by liquid scintillation counting. Experiments were carried out in duplicate; each datum point was obtained by averaging the duplicates. All of the reactions mentioned above were carried out at 22°C.

The % fraction of Ca\textsuperscript{2+} released by polylysine to Ca\textsuperscript{2+} sequestered during Ca\textsuperscript{2+} uptake was also measured following the active loading of SR vesicles with \(^{45}\text{Ca}^2+\), as described above. After actively loading SR vesicles with \(^{45}\text{Ca}^2+\) for 5 min, the Ca\textsuperscript{2+} release was induced by the addition of 1.11 \mu M polylysine into the above solution containing 0.15 M KCl, 1 mM MgCl\textsubscript{2}, 30 \mu M \(^{45}\text{Ca}^2+\), 10 mM NaN\textsubscript{3} and 20 mM MOPS, pH 7.1. An aliquot containing 2 ml of reaction solution was placed onto a 0.45-\mu m Millipore filter followed by a rinse with 5 ml of washing buffer (0.15 M KCl, 20 mM MOPS, pH 7.1, containing 30 mM EGTA and 15 \mu M ruthenium red). The radioactivity retained on the filters was determined by liquid scintillation counting. To assess the amount of Ca\textsuperscript{2+} released by polylysine, the difference between the radioactivity on the filters with or without polylysine was calculated.

2.6. Ca\textsuperscript{2+}–ATPase activity assay

The Ca\textsuperscript{2+}–ATPase activities in SR vesicles were obtained by measuring the amount of P\textsubscript{i} released during the reaction after adding ATP [18,19]. The assay mixture, in a total assay volume of 500 \mu l, contained 0.15 M KCl, 20 mM MES, pH6.8 (adjusted using KOH), 0.3 mM MgCl\textsubscript{2}, 10 mM NaN\textsubscript{3}, 6 \mu M ionophore A-23187, 0.212 mM CaCl\textsubscript{2}, 0.25 mM EGTA (free Ca\textsuperscript{2+}= 3 \mu M) and SR vesicles (0.1 mg). To start the reaction, 1.0 mM ATP was added to the above priming solution in the presence or absence of 3.7 \mu M polylysine. The amount of P\textsubscript{i} reacted was calculated by converting the absorbance of 0.1% malachite green (in nm) to nmol by means of a standard linear line.

2.7. Statistics

The unpaired \( t \)-test was used to compare the hemodynamic data between control conditions and conditions of cardiac failure. In addition, two-way analysis of variance was employed to compare the changes of the parameters for Ca\textsuperscript{2+} release and the amount of Ca\textsuperscript{2+} uptake between control conditions and conditions of cardiac failure. When a significant trend was identified by the \( F \) test, Scheffe’s post hoc test was used to compare the data. For comparisons of the Ca\textsuperscript{2+}–release fraction, the [\[^{3}H\]ryanodine binding (\( B_{\text{max}} \), \( K_d \)], and the polylysine-induced change in [\[^{3}H\]ryanodine binding between control and failure, the
unpaired t-test was used. A P value of less than 0.05 was accepted as being statistically significant.

3. Results

3.1. Morphological data

At three weeks after rapid RV pacing, we observed the clinical signs of heart failure, e.g. anorexia, fatigue, tachypnea and pleural effusion. Table 1 summarizes the morphological data. There was no significant difference in each parameter among all three groups.

3.2. Hemodynamic data

Hemodynamics are summarized in Table 2. Although there was no significant difference in peak LV pressure between the control group and the failure group, LV end-diastolic pressure was significantly elevated in the failure group compared with the control group (P<0.05). Both the peak +dP/dt of LV pressure and the fractional shortening were significantly decreased in the failure group compared with the control group (P<0.05), indicating contractile dysfunction. The time constant of LV pressure decay during the isovolumic relaxation period (Tau) was also prolonged in the failure group compared with the control group (P<0.05), indicating relaxation disturbance in the failure group.

3.3. Polylysine-induced Ca²⁺ release from normal and failing cardiac SR vesicles

In the absence of polylysine, the Ca²⁺ release from normal or failing SR vesicles was negligible because there was virtually no change in [Ca²⁺] after mixing solution A with solution B (not shown). Hence, the induced Ca²⁺ release was solely dependent on the polylysine concentrations. Fig. 1 shows the time course of Ca²⁺ release induced by different concentrations of polylysine. Various parameters characterizing the kinetics of Ca²⁺ release in both normal and failing SR vesicles (A, magnitude; k, rate constant; Ak, initial rate of Ca²⁺ release) are summarized in Table 3. The amount of Ca²⁺ released, which was expressed as A, tended to decrease in failing SR vesicles, however, there was no significant difference between these two groups. In contrast, both the rate constant (k) and the initial rate of Ca²⁺ release (Ak) were significantly decreased in failing SR vesicles, suggesting alteration of the gating function of the ryanodine receptor. Moreover, there is a clear difference in the polylysine dose-dependence of these kinetic parameters between normal and failing SR vesicles. Namely, the Ak reached a peak at a lower concentration of polylysine in failing SR vesicles than in normal SR vesicles; the polylysine concentration, at which half maximum stimulation was obtained, was 0.35 μM in normal vs. 0.13 μM in failing SR vesicles.

3.4. Ca²⁺ uptake and release by normal and failing cardiac SR vesicles

Fig. 2 shows the time course of Ca²⁺ uptake during active loading of SR with Ca²⁺. In failing SR vesicles, the amount of Ca²⁺ uptake was significantly decreased compared with normal SR vesicles. In contrast, as shown in Fig. 3, the releasable fraction of sequestered Ca²⁺ upon induction by 1.11 μM polylysine was significantly larger in failing SR vesicles than in normal SR vesicles (P<0.05).

Table 1
Morphological data

<table>
<thead>
<tr>
<th></th>
<th>BW (kg)</th>
<th>BV wt./BW (g/kg)</th>
<th>LVwt./BW (g/kg)</th>
<th>SR yield (mg protein/g LV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=10)</td>
<td>11±1</td>
<td>7.6±0.87</td>
<td>5.2±0.6</td>
<td>1.3±0.3</td>
</tr>
<tr>
<td>One week of pacing (n=6)</td>
<td>11±2</td>
<td>7.1±0.82</td>
<td>5.3±0.8</td>
<td>1.1±0.2</td>
</tr>
<tr>
<td>Three weeks of pacing (n=10)</td>
<td>11±2</td>
<td>8.8±2.3</td>
<td>6.0±1.4</td>
<td>1.2±0.2</td>
</tr>
</tbody>
</table>

* Data represent means±SD. BW, body weight; BV wt., biventricular weight; LV wt., left ventricular weight.

Table 2
Hemodynamic data

<table>
<thead>
<tr>
<th></th>
<th>HR (bpm)</th>
<th>LVSP (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>(+) dP/dt (mmHg/s)</th>
<th>Tau (ms)</th>
<th>LVDd (mm)</th>
<th>LVDs (mm)</th>
<th>FS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=10)</td>
<td>86±9</td>
<td>124±28</td>
<td>5.1±3.4</td>
<td>2358±764</td>
<td>28±4</td>
<td>27±3</td>
<td>19±2</td>
<td>31±3</td>
</tr>
<tr>
<td>One week of pacing (n=6)</td>
<td>88±19</td>
<td>121±17</td>
<td>9.1±4.9*</td>
<td>1463±323*</td>
<td>36±14*</td>
<td>30±2*</td>
<td>26±4*</td>
<td>14±7*</td>
</tr>
<tr>
<td>Three weeks of pacing (n=10)</td>
<td>111±22*#</td>
<td>109±24</td>
<td>18.0±8.5*#</td>
<td>1312±503*</td>
<td>47±6*</td>
<td>31±3*</td>
<td>28±3*</td>
<td>10±3*</td>
</tr>
</tbody>
</table>

*HR, heart rate; LVSP, left ventricular peak systolic pressure; LVEDP, left ventricular end-diastolic pressure; (+) dP/dt, peak (+) dP/dt of LV pressure; Tau, time constant of left ventricular pressure decay during isovolumic relaxation period; LVDd, left ventricular end-diastolic diameter; LVDs, left ventricular end-systolic diameter; FS, fractional shortening; (LVDD−LVDs)/LVDD×100). Data represent means±SD. *P<0.05 vs. control; #P<0.05 vs. one week of pacing.
regression for the bound ligand versus bound/free ligand. The mean values for the number of binding sites ($B_{\text{max}}$) and the dissociation constants ($K_d$) for all of the hearts are shown in Table 4. The $B_{\text{max}}$ for SR vesicles in failing hearts was significantly lower than that in normal hearts. However, there was no significant difference in the $K_d$ values of $[^3H]$ryanodine binding between these two groups. Because ryanodine binds to the ryanodine receptor when it is in an open state, the increase in ryanodine binding upon addition of Ca²⁺-release triggers like polylysine leads to activation of the receptor [3,16]. In failing SR vesicles, the concentration dependence of polylysine-induced stimulation of ryanodine binding was shifted towards the lower concentration of polylysine (Fig. 5), indicating the higher sensitivity of ryanodine binding to polylysine.

3.6. Effect of polylysine on Ca²⁺–ATPase activity

The Ca²⁺–ATPase activity was lower in three-week pacing-induced failing SR (0.25±0.01 μM P/ min/mg, $n=5$) than in normal SR (0.44±0.01 μM P/min/mg, $P<0.05$, $n=4$). Polylysine (3.7 μM) decreased the Ca²⁺–ATPase activity by 30.1±2.2% in normal SR and by 26.3±7.7% in failing SR following three weeks of pacing. There was no significant difference in the extent of the inhibition between normal and failing SRs.

4. Discussion

The major findings of this study are as follows. First, the time course of rapid Ca²⁺ release triggered by polylysine revealed that the rate of Ca²⁺ release was decreased in cardiac SR vesicles taken from failing heart. Second, compared with normal SR vesicles, the polylysine concentration dependency in terms of the initial rate of Ca³⁺ release and also $[^3H]$ryanodine binding shifted towards the lower concentration of polylysine in failing SR vesicles, suggesting the hypersensitized gating function of the SR Ca²⁺-channel by a release trigger, i.e., polylysine, in pacing-induced heart failure. Third, in failing SR vesicles, the amount of Ca²⁺ uptake was significantly decreased compared with normal SR vesicles, however, the releasable fraction of sequestered Ca²⁺ upon addition of polylysine was significantly larger in failing SR vesicles than in normal SR vesicles.

4.1. Altered Ca²⁺ regulation in heart failure

The abnormal regulation of intracellular Ca²⁺ by SR has been shown to be involved in the mechanism of contractile and relaxation dysfunction in heart failure. Several investigators have demonstrated that Ca²⁺ uptake by SR was decreased in association with the decreased density of Ca²⁺–ATPase in cardiac hypertrophy and/or failure [20–
Table 3
The amount (A), the rate constant (k) and the initial rate of release (Ak) induced by various concentrations of polylysine

<table>
<thead>
<tr>
<th>Polylysine (μM)</th>
<th>0.037</th>
<th>0.111</th>
<th>0.37</th>
<th>1.11</th>
<th>3.70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (nmol/mg)</td>
<td>0.55±0.20</td>
<td>1.64±0.67</td>
<td>3.33±0.36</td>
<td>4.71±0.83</td>
<td>5.78±0.45</td>
</tr>
<tr>
<td>k (s⁻¹)</td>
<td>0.81±0.47</td>
<td>2.14±1.09</td>
<td>3.73±0.75</td>
<td>3.80±0.89</td>
<td>3.44±0.88</td>
</tr>
<tr>
<td>Ak (nmol/mg/s)</td>
<td>0.37±0.17</td>
<td>3.22±2.44</td>
<td>12.5±2.75</td>
<td>17.8±5.39</td>
<td>20.1±5.91</td>
</tr>
<tr>
<td>One week of pacing (n=6)</td>
<td></td>
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</tr>
<tr>
<td>A (nmol/mg)</td>
<td>2.34±0.60</td>
<td>1.43±0.75</td>
<td>3.26±0.98</td>
<td>4.01±1.24</td>
<td>4.62±1.23</td>
</tr>
<tr>
<td>k (s⁻¹)</td>
<td>0.20±0.07*</td>
<td>2.23±0.59</td>
<td>2.41±0.28*</td>
<td>1.67±0.34*</td>
<td>1.99±0.29*</td>
</tr>
<tr>
<td>Ak (nmol/mg/s)</td>
<td>0.41±0.15</td>
<td>2.85±1.04</td>
<td>7.60±1.75*</td>
<td>6.59±2.63*</td>
<td>9.05±2.33*</td>
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<tr>
<td>Three weeks of pacing (n=10)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (nmol/mg)</td>
<td>1.82±1.73</td>
<td>1.14±0.72</td>
<td>2.18±0.74</td>
<td>3.35±1.90</td>
<td>4.12±2.08</td>
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<tr>
<td>k (s⁻¹)</td>
<td>0.40±0.25*</td>
<td>1.68±0.57</td>
<td>2.43±0.59*</td>
<td>1.25±0.37*</td>
<td>1.23±0.47*</td>
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<tr>
<td>Ak (nmol/mg/s)</td>
<td>0.42±0.15</td>
<td>1.76±0.83*</td>
<td>4.80±0.61*</td>
<td>3.46±0.70*</td>
<td>4.20±0.14*</td>
</tr>
</tbody>
</table>

* Kinetic parameters shown were calculated by fitting a single exponential model, y = A(1−e⁻ᵏᵗ) to the Ca²⁺ release time courses shown in Fig. 1. Data represent means±SD. *P<0.05 vs. control.

27]. However, no direct evidence is available as to the abnormal Ca²⁺ release function of the SR’s Ca²⁺-release channel in cardiac failure, although alteration of SR Ca²⁺ release is considered to be a cause of the slowed Ca²⁺ transient that is frequently observed in failing hearts [28–30]. To the best of our knowledge, this is the first study to demonstrate directly the abnormal kinetics of Ca²⁺ release from SR in cardiac failure.

Although this model of tachycardia-induced heart failure causes well defined, predictable and progressive LV dilatation, contractile dysfunction and neurohumoral activation [36], several limitations should be addressed. First, this model lacks a hypertrophic compensatory phase [37]. Second, heart failure disappears if pacing is discontinued after three weeks of pacing [38]. Therefore, this model does not resemble a chronic model of heart failure. Clearly, more work using other models of heart failure are needed to determine if there is really an abnormality in the kinetics of the ryanodine receptor in chronic heart failure.

In view of the central role that the ryanodine receptor plays in the Ca²⁺-release mechanism, the possible abnormality in the rapid Ca²⁺-release kinetics in cardiac hy-
pertrophy and/or failure described above seems to be due to an abnormality within this protein. In this regard, polylsine, at nanomolar concentrations, binds specifically to the ryanodine receptor in skeletal SR vesicles [9], then induces a rapid conformational change of the ryanodine receptor, followed by Ca release [10]. In a previous report [11], we demonstrated that polylsine also induced rapid Ca release from cardiac SR vesicles, which was mediated through the ryanodine receptor. Therefore, polylsine might be useful in analyzing the functional alteration of cardiac E-C coupling that is responsible for the abnormal Ca transient and contractile dysfunction in cardiac failure.

According to Xu and Kirchberger [39], polylsine (50 μM) inhibits the SR’s Ca^{2+} ATPase activity by about 80% of that in controls. Therefore, we should address whether or not Ca^{2+} release is influenced by this inhibitory effect of polylsine on Ca^{2+} re-uptake, which probably occurs simultaneously during Ca^{2+} release. In this study, polylsine (3.7 μM) inhibited the Ca^{2+} ATPase activity to a similar extent in both SRs (normal SR: 30%, failing (three weeks of pacing) SR: 26%, P=ns). To eliminate Ca^{2+} uptake during Ca^{2+} release, polylsine-induced Ca^{2+} release was evaluated in the presence of 1 μM thapsigargin (SR Ca^{2+} ATPase blockade) in the Ca^{2+}-release solution. However, both in control and in failure, the time course of Ca^{2+} release was not affected by the addition of thapsigargin (not shown). Taken together, it is unlikely that the inhibitory effect of polylsine on Ca^{2+}—ATPase activity leads to artifacts in the analysis of Ca^{2+} release.

4.2. Rate constant and the initial rate of Ca^{2+} release from SR are decreased in failing heart

Consistent with the large body of literature, we observed...
significantly larger in failing SR vesicles than in normal SR vesicles. This may partly contribute to maintenance of the available Ca\(^{2+}\) required for muscle contraction. Using skeletal SR vesicles, Ikemoto et al. [31] demonstrated that most of the Ca\(^{2+}\) sequestered by Ca\(^{2+}\)-ATPase first binds the luminal Ca\(^{2+}\)-buffering protein, calsequestrin. Thereafter, when a Ca\(^{2+}\) release trigger is applied, the triggering signal is transmitted from the Ca\(^{2+}\)-release channel to calsequestrin and some fraction of the bound Ca\(^{2+}\) is dissociated from calsequestrin and released through the Ca\(^{2+}\)-release channel. One can speculate that the type of mechanism by which luminal Ca\(^{2+}\) is regulated might be altered in cardiac failure.

### Table 4

<table>
<thead>
<tr>
<th></th>
<th>(B_{\text{max}}) (pmol/mg)</th>
<th>(K_d) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ((n=10))</td>
<td>2.64±0.59</td>
<td>0.90±0.11</td>
</tr>
<tr>
<td>One week of pacing ((n=6))</td>
<td>1.34±0.28*</td>
<td>1.22±0.37</td>
</tr>
<tr>
<td>Three weeks of pacing ((n=10))</td>
<td>0.91±0.19*#</td>
<td>0.95±0.29</td>
</tr>
</tbody>
</table>

The cardiac microsomes (0.1 mg/ml) were incubated for 120 min at 36°C in 25 mM imidazole, 1 M KCl, 0.95 mM EGTA, 1.103 mM CaCl\(_2\) (free Ca\(^{2+}\)=20 \(\mu\)M), at pH 7.4, each with a concentration of \([^{3}H]ryanodine\) in the range of 0.3 to 10 nM. Data represent specific \([^{3}H]ryanodine\) binding. Data represent means±SD. \(B_{\text{max}}\), maximal number of binding sites; \(K_d\), dissociation constant. *\(P<0.05\) vs. control; #\(P<0.05\) vs. after one week of pacing.

Fig. 5. Enhancement of \([^{3}H]ryanodine\) binding by various concentrations of polylysine in SR vesicles taken from normal (—□—, \(n=10\)) and from failing dog hearts after one week of pacing (—△—, \(n=6\)) and three weeks of pacing (—○—, \(n=10\)). For assessment of the enhancement of \([^{3}H]ryanodine\) binding to the SR moiety by polylysine, cardiac microsomes (0.1 mg/ml) were incubated in 1.0 ml of a reaction solution containing 5 nM \([^{3}H]ryanodine\), 0.1 M NaCl, 20 mM Na±Hepes, pH 7.2, at a \([Ca^{2+}]\) of 3 \(\mu\)M using an EGTA±calcium buffer (0.974 mM CaCl\(_2\), 1 mM EGTA, 20 mM Hepes, pH 7.2) for 120 min at 36°C in the absence or presence of various concentrations of polylysine. The change in specific \([^{3}H]ryanodine\) binding after the addition of polylysine was expressed as the ratio to the maximum \([^{3}H]ryanodine\) binding (\(B_{\text{max}}\)). Data represent means±SD. *\(P<0.05\) vs. control; #\(P<0.05\) vs. one week of pacing.

### 4.3. Hypersensitization of the ryanodine receptor upon induction of Ca\(^{2+}\) release by polylysine

We demonstrated that the \(B_{\text{max}}\) for ryanodine binding was reduced by three weeks of pacing-induced heart failure, to 35% of control value. This is particularly striking in light of the observation by Vatner et al. [5] that the \(B_{\text{max}}\) was reduced only to 77% of the control value in this model, for a similar degree of heart failure. This discrepancy may be partly explained by the difference in the membrane preparations. They used a cruder membrane preparation than ours. According to Kranias et al. [12], our method for the purification of SR minimizes cytosolic contamination, as judged from the levels of the specific activity of lactate dehydrogenase in SR (less than 0.2% of the cell homogenate). Also, the enzyme activities of
mitochondrial membranes (cytochrome c oxidase and sarclemma (Na\(^{+}\),K\(^{-}\)-ATPase, \(^{3}H\)ouabain binding, adenylate cyclase) were less than 10% of the levels present in isolated sarcolemmal membranes. However, we cannot exclude the possibility that the loss of SR fractions during our extensive purification may be larger in failing SR than in normal SR.

In the present study, we observed that, upon addition of polylysine to SR vesicles, the concentration dependence of either the initial rate of Ca\(^{2+}\) release or the ryanodine binding shifted towards the lower concentration of polylysine. This finding strongly suggests that the gating mechanism by which Ca\(^{2+}\) is released is hypersensitized in failing SR vesicles. Kim et al. [32] also demonstrated that the Ca\(^{2+}\)-release channels in hypertrophied left ventricles showed a significantly increased sensitivity to Ca\(^{2+}\)-release agonists (e.g. caffeine and doxorubicin), as characterized by the effects of these agonists on ryanodine binding to whole homogenates and on Ca\(^{2+}\) release from isolated SR vesicles, although the density of Ca\(^{2+}\)-release channels was decreased. It is probable that hypertrophied heart and pacing-induced heart share a common alteration in the gating mechanism by which Ca\(^{2+}\) is released.

The changes in agonist sensitivity associated with cardiac failure could be caused by expression of abnormal Ca\(^{2+}\)-release channels [33], by altered expression of putative regulatory protein and/or by an alteration in the phosphorylation mechanism of the ryanodine receptor. Although the mechanism for the altered Ca\(^{2+}\)-releasing function in cardiac failure remains to be elucidated, the present findings suggest that the contractile disturbance in cardiac failure might be due, in part, to the decreased density of ryanodine receptors and/or the decreased rate of Ca\(^{2+}\) release from SR vesicles.

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References


