Intracellular Calcium Handling in Isolated Ventricular Myocytes From Patients With Terminal Heart Failure

Dirk J. Beuckelmann, MD; Michael Nabauer, MD; and Erland Erdmann, MD

Background. Experiments were performed in human ventricular myocytes to investigate properties of excitation–contraction coupling in patients with terminal heart failure. Myocytes were isolated from left ventricular myocardium of patients with cardiac failure caused by dilated or ischemic cardiomyopathy undergoing transplantation. These results were compared with those obtained from cells of healthy donor hearts that for technical reasons were not suitable for transplantation.

Methods and Results. [Ca\(^{2+}\)]\(_i\), transients and Ca\(^{2+}\) currents were recorded from isolated cells under voltage clamp perfused internally with the Ca\(^{2+}\) indicator fura 2. In cells that were stimulated externally, the cell-permeant form of the indicator, fura 2-AM, was used. When action potentials were to be recorded, cells were stimulated in current clamp mode. Unstimulated Ca\(^{2+}\) current densities were not significantly different in myopathic and control cells. In diseased myocytes, resting [Ca\(^{2+}\)]\(_i\), levels were 165±61 nmol/l, compared with 95±47 nmol/l in normal cells. With 5 mmol/l Na\(^+\) in the pipette, peak [Ca\(^{2+}\)]\(_i\), transients were 367±109 and 746±249 nmol/l, respectively. The decline of [Ca\(^{2+}\)]\(_i\), during diastole was significantly slower in myopathic cells than in control cells. This was a result of a prolongation of the action potential and of a reduced Ca\(^{2+}\) sequestration by the sarcoplasmic reticulum.

Conclusions. These results may partly explain the alterations of contractility in vivo in patients with heart failure. (Circulation 1992;85:1046–1055)

KEY WORDS • calcium, intracellular • myocytes • fura 2 • heart failure

The pathophysiological basis of heart failure in humans that is not related to extramyocardial disease (constrictive pericarditis) or mechanical disease (valvular heart disease) remains obscure. A significant increase of interstitial fibrous tissue with cell death of myocytes has not been found regularly in these hearts. Therefore, it seems plausible that pathophysiological changes occur on a cellular level. Maximal force developed by electrically driven papillary muscles of patients with terminal heart failure undergoing transplantation was not significantly different from that of normal controls.\(^1\)\(^,\)\(^2\) Other authors have found that the Ca\(^{2+}\) sensitivity of the myofilaments in chemically skinned fibers of these hearts was comparable to that of healthy controls.\(^3\) Gwathmey et al\(^4\) were the first to describe an abnormal intracellular [Ca\(^{2+}\)]\(_i\), handling in human cardiac contractile failure. Their experiments were performed in papillary muscles from patients with terminal heart failure, using the bioluminescent photo-protein aequorin as a [Ca\(^{2+}\)] indicator. They found an additional [Ca\(^{2+}\)] signal (L\(_2\)) in this tissue compared with healthy controls, indicating a slowed diastolic decline of [Ca\(^{2+}\)], but systolic [Ca\(^{2+}\)], was unaltered.\(^5\) From their experiments, these authors postulated a combined defect of [Ca\(^{2+}\)], handling by the sarcolemma and sarcoplasmic reticulum (SR). Other authors found an unaffected number of dihydropyridine binding sites in these hearts,\(^6\) indicating that the number of Ca\(^{2+}\) channels in heart failure is unchanged. However, the correlation between the number of dihydropyridine binding sites, active Ca\(^{2+}\) channels, and Ca\(^{2+}\) current densities under physiological conditions and in certain pathological states in heart is still unknown.

Isolated myocytes under voltage clamp may provide several advantages over multicellular preparations in the study of excitation–contraction coupling: Membrane potential can be adequately controlled, and ionic currents can be recorded reliably. Novel fluorescent Ca\(^{2+}\) indicators (fura 2) can be calibrated in vivo to obtain quantitative [Ca\(^{2+}\)] signals from single cells. The extracellular environment is known, and access to the cytoplasm can be obtained by intracellular perfusion via the micropipette electrode to modify intracellular ionic concentrations or to perform pharmacological interventions.

We have shown previously that the basic characteristics of L-type Ca\(^{2+}\) currents in patients with end-stage heart failure are qualitatively similar to those described in human atrial cells and other mammalian species.\(^7\) The purpose of the present study was to test the hypothesis that steps between the excitation of the cell membrane and contraction are affected in cardiac failure.

Methods

Cell Isolation

The isolation procedure was a modification of the method described for isolation of ventricular myocytes

---

From the Department of Medicine I, University of Munich, Klinikum Grosshadern, Munich, FRG.

Supported by grant Be 1113/2-1 from the Deutsche Forschungsgemeinschaft.

Address for correspondence: D.J. Beuckelmann, Marchionini- str. 15, D-8000 Munich 70, FRG.

Received April 22, 1991; revision accepted November 5, 1991.
in small vertebrates.6,9 Cells were prepared from hearts of patients with end-stage heart failure caused by dilated cardiomyopathy or ischemic heart disease who were undergoing transplantation. Immediately upon tissue removal, samples were placed in cardioplegic solution (4°C), taken to the laboratory, and processed within 15 minutes. All patients received digoxin and diuretics and were under vasodilator therapy. No catecholamines or β-adrenoceptor blocking drugs were given during 48 hours before the operation. Informed consent was obtained before organ explantation.

A part of the left ventricular wall was excised together with its artery branch. The wall segment was then perfused via this artery branch: 30 minutes with a nominally Ca2+-free modified Tyrode’s solution (138 mmol/l NaCl, 4 mmol/l KCl, 1 mmol/l MgCl2, 10 mmol/l glucose, 0.33 mmol/l Na2HPO4, and 10 mmol/l HEPES [sodium salt]; pH was 7.3 with addition of NaOH, 37°C) followed by 40 minutes with the same solution added with collagenase (type I, 70 mg/50 ml, Worthington) and protease (type XIV, 6 mg/50 ml, Sigma Chemical Co.). Enzyme solution was recycled during this time. Finally, the enzyme was washed out for 15 minutes with modified Tyrode’s solution that contained 200 μmol/l Ca2+. Ventricular cells were then disaggregated by gentle mechanical agitation and, after filtering through a nylon mesh, were stored in Tyrode’s solution containing 2.0 mmol/l Ca2+ at room temperature.

Solutions

Cells were superfused at 35°C with a modified Tyrode’s solution containing 2.0 mmol/l CaCl2, 138 mmol/l NaCl, 10 mmol/l CsCl, 1 mmol/l MgCl2, 10 mmol/l glucose, and 10 mmol/l HEPES (sodium salt); pH was 7.3 with addition of NaOH. Cs+ was included to block K+ currents that might interfere with the measurement of Ca2+ currents.

For recordings of action potentials, solutions did not include Cs+ but did contain 4 mmol/l KCl.

Loading of Cells With Fura 2 and Internal Perfusion

Cells were either loaded with fura 2 (pentapotassium salt) by internal perfusion through diffusion from the micropipette electrode over 5 to 15 minutes or, for protocols that required extracellular stimulation through the use of the membrane-permeant form, fura 2-AM.10

In all voltage clamp experiments, cells were loaded with fura 2 salt. Electrodes had resistances of 2.0–3.0 MΩ and were filled with 0.050 mmol/l fura 2 (Molecular Probes), 120 mmol/l Cs glutamate, 10 mmol/l CsCl, 1 mmol/l MgCl2, 5 mmol/l NaCl, 10 mmol/l HEPES (cesium salt), and 2 mmol/l Mg-ATP; pH was 7.2 with addition of CsOH. For recordings of action potentials, 140 mmol/l KCl was substituted for CsCl and Cs glutamate. Positive pressure to the electrode was never applied to avoid potential disintegration of intracellular structures during such a procedure.

In all experiments in which external field stimulation was used, cells were loaded with fura 2-AM. The method of loading cells with the membrane-permeant form was a modification of the method that has been described by Barcenas-Ruiz and Wier.11 A mixture was made of 10 μl of 1 mmol/l fura 2-AM (Molecular Probes) in dimethyl sulfoxide, 2.5 μl of 25% (wt/wt) Pluronic F-127 (Molecular Probes), and 75 μl of fetal calf serum. To load the cells, this solution was added to 2 ml cell suspension and 40 μl fetal calf serum. Loading proceeded for 6 minutes (23°C) on an orbital shaker to slowly agitate the cells. A sample was then transferred to the experimental chamber, and cells were washed for at least 15 minutes with Tyrode’s solution to allow for complete deesterification of the trapped dye.

Recording Technique

The experimental apparatus was constructed around a Zeiss Axiovert 35 microscope with an MPM 201 photometer attachment similar to that described previously.9 Briefly, UV light emitted from a 75-W xenon arc lamp passed through 10-nm interference filters (340- or 380-nm wavelength) and was reflected by a dichroic mirror centered at 405 nm into the objective for excitation of the Ca2+ indicator in the cell. Fluorescence emitted from the cell passed through the objective and a 510–540-nm bandpass filter and was directed into a photomultiplier tube. An aperture at the image plane in front of the photomultiplier tube was reflected onto the cell image to precisely select a circular region of the cell for fluorescence recordings. Currents were recorded with a patch clamp amplifier (model EPC-7, List Instruments) with a 100-MΩ feedback resistor. Microelectrodes were pulled from borosilicate glass capillaries and had resistances of 2.0–3.0 MΩ. Series resistance was compensated for as much as possible. Fluorescence recordings were filtered with a cutoff frequency of 120 Hz (Bessel) and, together with the current or voltage recordings from the patch clamp amplifier, were digitized (1–5 kHz, Indec IDA interface) and stored for off-line analysis.

In Vivo and In Vitro Calibration of Fura 2 Fluorescence

Rmin and Rmax (the fluorescence ratio 340/380 nm in 0 mmol/l Ca2+ and in a saturating concentration of Ca2+) were determined intracellularly by metabolic inhibition with the metabolic poisons carbonyl cyanide m-chlorophenylhydrazone (5.0 μmol/l) (inhibition of oxidative phosphorylation) and rotenone (2.0 μmol/l) (inhibition of mitochondrial electron transport) and subsequent extracellular exposure to 1 mmol/l CaCl2 for determination of Rmax or 5 mmol/l EGTA (for determination of Rmin) in the presence of the Ca2+ ionophore bromo A 23187 (10 μmol/l). The average value for Rmin was 0.06 and for Rmax was 1.8. By analysis of thin solutions in the microscope, the dissociation constant for the reaction Ca2++fura 2↔Ca2+:fura 2 was 210 nmol/l; β10 for 380 nm was 13. These values could not be determined by in vivo calibration because [Ca2+] could not be “clamped” to a defined concentration by this method. Rmin and Rmax could be determined only with an excess of extracellular EGTA or CaCl2.

Background fluorescence was always measured at both wavelengths after establishing a gigaohm resistance seal but before “breaking in” to the cell and establishing whole-cell recording. (For details of calibration, see Reference 9.)

Calibration of fluorescence signals from cells loaded with fura 2-AM has been shown to be difficult in heart
cells. Therefore, this method has been used only when extracellular field stimulation was used. \([Ca^{2+}]\), values obtained with this method have not been included in the quantitative analysis of \([Ca^{2+}]\), data.

**Statistical Analysis**

Values are mean±SD. The t test for unpaired data was used for statistical analysis; values of \(p<0.05\) were considered significant.

**Results**

**Isolated Ventricular Myocytes**

Cells were prepared from 14 hearts of patients with end-stage heart failure caused by dilated cardiomyopathy (\(n=9\)) or ischemic cardiomyopathy (\(n=5\)) undergoing transplantation. The age of the patients (11 men and three women) was 51.8±11.6 years; cardiac index was 2.4±0.7 l/min/m\(^2\), and ejection fraction was 26±10%. Results were compared with cells isolated from three normal human hearts without cardiac disease that could not be transplanted for technical reasons.

The living cell yield was approximately 5%, although some preparations gave slightly better or worse results. Only cells with clear cross striation, without significant granulation and without spontaneous contraction, were selected for experiments. Such cells could not be stained with trypan blue. Experiments were carried out at 35°C.

Figure 1A shows a typical example of such a cell (objective \(×100\)). The average cell surface area was 240±115×10\(^{-6}\) cm\(^2\), assuming a surface area of 10\(^{-6}\) cm\(^2\)/pF membrane capacity. To illustrate the cell yield, Figure 1B shows a low-power micrograph of a typical preparation (objective \(×10\)).

A total of 52 cells yielded results for these experiments.

**Change of the \([Ca^{2+}]\), Transient With Time**

Because fura 2 acts as a \(Ca^{2+}\) chelator, quantitative analysis of fluorescence signals requires that changes of the indicator concentration in the cell through continuous diffusion from the micropipette electrode during the time course of an experiment do not affect kinetics of the \([Ca^{2+}]\), transient. Therefore, repetitive depolarizations to +10 mV (duration, 300 msec) were given in a human ventricular myocyte over 15 minutes. This cell was isolated from a heart with terminal heart failure resulting from dilated cardiomyopathy. Figure 2A shows fluorescence recordings at both wavelengths after 5 and 15 minutes of perfusion. The horizontal lines indicate the background fluorescence at the two wavelengths. \([Ca^{2+}]\), transients calculated from these records are shown in Figure 2B. With 50 μmol/l fura 2 in the pipette, \([Ca^{2+}]\), transients were superimposable, indicating that significant buffering of \([Ca^{2+}]\), by the indicator under these conditions does not occur.

**\([Ca^{2+}]\), Transients During Extracellular Stimulation**

Cells that were loaded with the indicator through the use of the cell-permeant form, fura 2-AM, were stimulated externally at a frequency of 0.5 Hz. As pointed out above, \([Ca^{2+}]\), transients can only be estimated with this method.

Figure 3 shows an example record from a cell of a patient with dilated cardiomyopathy and from a control cell. In the cell isolated from the heart with cardiac failure, the resting \([Ca^{2+}]\), level was significantly higher (220 nmol/l versus 140 nmol/l) and the peak \([Ca^{2+}]\), transient was smaller (480 nmol/l versus 710 nmol/l). Furthermore, the decay of \([Ca^{2+}]\), was markedly prolonged. In all, 11 cells isolated from four hearts with terminal heart failure and from six cells isolated from undiseased myocardium (two hearts) were investigated with this protocol; the transients shown in Figure 3 depict representative examples.

**Action Potentials**

Prolongation of the action potential may theoretically cause a slowed diastolic decline of \([Ca^{2+}]\), through its effect on \(Ca^{2+}\) extrusion via \(Na^{+}–Ca^{2+}\) exchange. To investigate whether such prolongation of the action potential can be found in single cells, membrane potentials were recorded in current clamp mode in cells stimulated at a frequency of 0.5 Hz. Figure 4 depicts typical examples of action potentials recorded from normal cells and cells isolated from organs with severe cardiac failure. Under our conditions, duration of the action potential in myocytes from patients with heart failure was markedly prolonged (AP\(_{\text{max}}\), 1,038±223 msec \([n=7]\) versus 649±101 msec \([n=4]\); \(p<0.05\)).

**\([Ca^{2+}]\), Transients During Voltage Clamp Pulses**

To investigate whether factors other than prolongation of the action potential may additionally account for the slow diastolic decay of \([Ca^{2+}]\), cells were stimulated in voltage clamp mode. The dependence of \(Ca^{2+}\) currents and \([Ca^{2+}]\), transients on clamp pulse potential over the range from -30 to +70 mV is shown in a cell isolated from a control heart (Figure 5) and from myocardium of a patient with ischemic cardiomyopathy (Figure 6). In all cases, test pulses were preceded by a train of six conditioning pulses to +10 mV (300 msec) to ensure that the SR was loaded to the same extent before each test pulse. From a holding potential of -80 mV, a 400-msec prepulse to -40 mV was used to inactivate the \(Na^{+}\) current, followed by a 300-msec test pulse from -30 to +80 mV. Only the last 100 msec of the prepulse to -40 mV is shown in Figures 5 and 6.

Under normal conditions, there was a fast rise of \([Ca^{2+}]\), from a resting level of 60 nmol/l to a maximum of 810 nmol/l. Peak \([Ca^{2+}]\), transients were elicted at the same voltage at which the maximal amplitude of the \(Ca^{2+}\) current was triggered (+10 mV). Decay of \([Ca^{2+}]\), started already during depolarization. The time necessary for \([Ca^{2+}]\), to decay to its half-maximum concentration (t\(_{\text{1/2}}\)) was 270 msec. Upon repolarization from +70 mV, a tail transient was elicited.

When the same protocol was applied to a cell from a patient with terminal heart failure, the \([Ca^{2+}]\), resting level was 180 nmol/l. Although the maximal amplitude of the \(Ca^{2+}\) current was comparable to the undiseased cell, the peak \([Ca^{2+}]\), transient was only 360 nmol/l, and the decline was markedly slower than the control cell (t\(_{\text{1/2}}\), 640 msec). No differences could be found between different types of heart failure (dilated cardiomyopathy or ischemic cardiomyopathy). Table 1 summarizes these results of intracellular \([Ca^{2+}]\), handling; Table 2 shows that there was no significant difference in \(Ca^{2+}\) current densities in cells isolated from hearts with cardiac failure compared with controls.
FIGURE 1. Photomicrographs of isolated human ventricular myocytes (dilated cardiomyopathy). Panel A: ×100; panel B: ×10 (original magnification). Only cells with clear cross striation, without significant granulation and without spontaneous contraction, were used for experiments.

Effect of cAMP

Some authors postulate that a significant reduction of cAMP may play a critical role in the development of heart failure in patients with dilated cardiomyopathy. If there were a causal relation between intracellular levels of cAMP and changes in $[Ca^{2+}]_i$ regulation, intracellular application of cAMP might partly reverse these alterations. The effect of incubation of myopathic cells with a cell-permeant form of cAMP (dibutyryl cAMP) is shown in Figure 7. The cell was stimulated externally at a frequency of 0.5 Hz. After exposure of the cell with $5 \times 10^{-4}$ mol/l dibutyryl cAMP over 15 minutes, only a minimal effect of cAMP on the rate of diastolic decay of $[Ca^{2+}]_i$ could be observed. Resting $[Ca^{2+}]_i$ levels and peak $[Ca^{2+}]_i$ transients were unaffected. This result was found in five cells investigated with this protocol.

Discussion

The purpose of this study was to study intracellular $[Ca^{2+}]_i$ handling in isolated human ventricular myocytes. $Ca^{2+}$ currents, $[Ca^{2+}]_i$ transients, and action potentials were recorded in cardiac myocytes, comparing normal
myocardium with tissue from patients with severe heart failure. Specimens were obtained from explanted hearts of patients with end-stage heart failure caused by dilated or ischemic cardiomyopathy undergoing transplantation or from organ donors without cardiac disease whose hearts could not be transplanted for technical reasons.

Several conclusions can be drawn from our experiments. Some are in agreement and others are in contrast with results published by other authors using different methods. For the interpretation of our data, it is important to keep advantages and limitations of the methods used in mind.

Single isolated ventricular myocytes have been widely used in recent years to study excitation-contraction coupling in heart. The yield of viable cardiac cells in a typical preparation of human ventricular myocytes used for our experiments was approximately 5% (see Figure 1B). This cell yield is significantly smaller than what can usually be expected in a cell preparation from hearts of rats or other mammals and may be a potential limitation of this study. With our method of cell isolation, however, a lower yield than in rat or guinea pig has to be expected, because only part of the ventricular wall is perfused via a branch of the coronary artery, and cells located in areas that are not perfused or only marginally perfused through this artery branch of course cannot be expected to be viable. Cells that fulfilled our selection criteria (see “Methods”) did contract during stimulation, as judged by visual control. Therefore, we feel that these cells are indeed representative of single cells in the intact heart in vivo.

The basic characteristics of 
$[Ca^{2+}]_i$, handling were found to be similar in diseased hearts and in healthy controls. $[Ca^{2+}]_i$ transients were maximal upon depolarization to $+10 \text{ mV}$, as was the $Ca^{2+}$ current. Depolarization to more negative or more positive potentials elicited a smaller transient and a smaller $Ca^{2+}$ current. Furthermore, upon repolarization from very positive potentials, a “tail transient” could be elicited. These tail transients have been shown to be triggered by “tails” of the $Ca^{2+}$ current during the time of deactivation of the...
Ca\(^{2+}\) channel and strongly support the concept of Ca\(^{2+}\)-induced Ca\(^{2+}\) release in heart.\(^{11}\) These results are very similar to those obtained in normal guinea pig and rat ventricular myocytes.\(^{9,14,15}\) On the other hand, significant differences between myopathic and control cells could be observed. In cells isolated from myocardium of patients with terminal heart failure, resting [Ca\(^{2+}\)], levels were higher, peak [Ca\(^{2+}\)], transients were significantly smaller, and the rate of diastolic decay of [Ca\(^{2+}\)] was markedly slowed. Furthermore, action potentials recorded in myopathic cells were significantly longer than under control conditions. These phenomena were independent of whether or not ATP was included in the pipette solution, and cAMP did not significantly affect these alterations in [Ca\(^{2+}\)] handling.

Gwathmey et al.\(^{14,15}\) found that systolic [Ca\(^{2+}\)], transients in papillary muscle from patients with terminal heart failure were similar to or higher than in undiseased myocardium. In their experiments, the photoprotein aequorin injected into multiple cells was used as a [Ca\(^{2+}\)], indicator. They found an additional light signal (L\(_2\)), indicating a prolongation of the diastolic [Ca\(^{2+}\)], decay. Through the use of pharmacological agents (ryanodine and verapamil), they postulated a reduced rate of [Ca\(^{2+}\)], reuptake by the SR and an increase in transsarcolemmal Ca\(^{2+}\) influx via Ca\(^{2+}\) channels.

Recent experiments in certain animal models provided evidence for alterations of Ca\(^{2+}\) channels in cardiac failure. Kuo et al.\(^{16}\) found the number of dihydropyridine receptors to be increased in hamsters with hereditary cardiomyopathy; others\(^{17}\) presented evidence for a reduced number of dihydropyridine binding sites in myocardium of rats with heart failure. When cardiac dihydropyridine binding sites were investigated in human heart failure, however, the number of Ca\(^{2+}\) channels was found to be unchanged.\(^{6}\) In our experiments, Ca\(^{2+}\) currents were recorded directly in isolated myocytes. We could not find any significant difference in the maximal amplitude of the Ca\(^{2+}\) current between diseased and control cells, thus supporting the indirect results of Rasmussen et al.\(^{6}\) We therefore conclude that the trigger signal for the release of Ca\(^{2+}\) from the SR seems to be unaltered in severe heart failure.

Our results in single cells are in agreement with findings in papillary muscle that systolic [Ca\(^{2+}\)], levels in diseased cells are higher than in control cells and that the rate of diastolic [Ca\(^{2+}\)], decay is markedly slowed.\(^{4,5}\) Under pathological conditions, however, peak systolic [Ca\(^{2+}\)], transients were significantly smaller than in normal cells. A similar finding of smaller peak [Ca\(^{2+}\)], transients in heart failure has also been described in guinea pigs with heart failure and hypertrophy caused...
by pressure overload (aortic banding\(^{18}\)). These authors also used fura 2 as a [Ca\(^{2+}\)] indicator. In this respect, our finding is in contrast to results obtained in papillary muscles and using aequorin as a [Ca\(^{2+}\)] indicator.\(^{4,5}\) Several possibilities may account for this difference. A possible explanation for the different results may be that in our study, isotonic contractions of single cells were investigated, whereas Gwathmey et al measured [Ca\(^{2+}\)] transients during isometric contractions. Under physiological conditions in vivo, the heart does not contract isometrically, nor does it contract isotonically. The contraction during a cardiac cycle is a combination of isometric tension development and isotonic cell shortening, so with each model, only part of the contractile process can be investigated. Because the Ca\(^{2+}\) affinity of the myofilaments is known to be tension-dependent, the different experimental conditions may partly explain this striking difference.

In the past, usually only relative changes in light levels in an individual experiment were given when the aequorin method was used.\(^{4,19,20}\) Attempts have been made in recent years to calibrate aequorin signals quantitatively.\(^{21,22}\) Through these calibration procedures, difficulties in calibrating aequorin light signals in papillary muscles have been partly overcome.\(^{23}\)

Although fluorescent indicators such as fura 2 or indo 1 are at present the only suitable dyes for measurement of [Ca\(^{2+}\)], in single cardiac cells, problems in the quantification of [Ca\(^{2+}\)] may also occur. Kao and Tsien\(^{24}\)

---

**TABLE 1. Intracellular [Ca\(^{2+}\)], Regulation**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Resting [Ca(^{2+})] (nmol/l)</th>
<th>Peak [Ca(^{2+})] (nmol/l)</th>
<th>(t_{1/2}) (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart failure ((n=31))</td>
<td>165±61</td>
<td>367±109</td>
<td>692±166</td>
</tr>
<tr>
<td>Control ((n=8))</td>
<td>96±47</td>
<td>746±249</td>
<td>320±68</td>
</tr>
</tbody>
</table>

\(p<0.01\) for all values.

Peak [Ca\(^{2+}\)] was measured during depolarization to +10 mV.

\(t_{1/2}\) Time for decay of [Ca\(^{2+}\)], to its half-maximal value. Myocytes were perfused with 5 mmol/l Na\(^+\) and 50 \(\mu\)mol/l fura 2. All values are listed as mean±SD.

---

**TABLE 2. Ca\(^{2+}\) Current Density**

<table>
<thead>
<tr>
<th>Condition</th>
<th>(I_{Ca}) density ((\mu\text{A/cm}^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart failure ((n=22))</td>
<td>-4.7±2.4</td>
</tr>
<tr>
<td>Control ((n=8))</td>
<td>-3.8±1.2</td>
</tr>
</tbody>
</table>

\(p=\text{NS.}\)

\(I_{Ca}\) density was taken as the peak \(Ca^{2+}\) current per square centimeter of cell surface, assuming a surface area of 10\(^{-8}\) cm\(^2\)/pF membrane capacity.
have shown in in vitro experiments that the kinetic characteristics of fura 2 are such that the dye is well suited to monitoring rapid changes in intracellular [Ca^{2+}]. However, fura 2 acts as a Ca^{2+} buffer through its effect as a Ca^{2+} chelating agent and thereby may alter intracellular [Ca^{2+}] kinetics. We have shown that under our experimental conditions (see “Methods”), during the time course of an experiment (5-15 minutes after the start of intracellular perfusion), significant differences in buffering do not occur (Figure 2). Various authors have shown that the membrane-permeant form fura 2-AM may enter subcellular compartments, which may hamper the quantification of cytosolic [Ca^{2+}]. Spurgeon et al. estimated that approximately 50% of indo 1 may be trapped in intracellular compartments in myocytes, which is why we did not include our [Ca^{2+}] measurement with fura 2-AM in the quantitative analysis. However, Barcenas-Ruiz and Wier have shown that these estimates of [Ca^{2+}] are closely related to values obtained with the cell-impermeant form of fura 2 injected directly into the cell. Therefore, the method is perfectly suitable for the analysis of qualitative changes of [Ca^{2+}] kinetics (Figure 3) or for investigating the effects of pharmacological interventions (Figure 7). Although lack of compartmentalization with the cell-impermeant form of the dye has not yet been verified experimentally, we know of no experimental evidence to support such entering of fura 2 (salt) into intracellular organelles.

The dissociation constant $K_d$ of the reaction fura 2+Ca^{2+}$\leftrightarrow$fura 2:Ca^{2+} and $\beta$ (see “Methods”) could only be measured in vitro. Therefore, these values may be slightly different within the myocyte. Furthermore, Blatter and Wier have shown that only 30-35% of the diffusible fura 2 is free in the myoplasm. The rest seems to bind reversibly to nonsaturable binding sites; this may alter the binding kinetics of the dye, resulting in a distortion of the [Ca^{2+}], transient. Although the ratio method does not exclude miscalculations of [Ca^{2+}], because of morphological changes of the cell, analysis of the fluorescence signal at an excitation wavelength of 360 nm (isosbestic wavelength) did not reveal any significant motion artifact (result not shown).

All possible difficulties in the quantification of [Ca^{2+}], cannot account for the striking difference in the resting level of [Ca^{2+}], in the reduction of the peak of the [Ca^{2+}], transient, and in the prolonged diastolic decay of [Ca^{2+}] in cells isolated from diseased hearts compared with controls. Two possibilities can be postulated to account for the reduced systolic [Ca^{2+}], transient. A reduction of [Ca^{2+}], reuptake by the SR may cause a reduced availability of Ca^{2+} to be released by the SR during the next beat. The other possibility would be that a trigger signal (the Ca^{2+} current being unchanged) is not adequately answered by the SR Ca^{2+} release channel, indicating a partial electromechanical uncoupling. Recordings of currents through the SR Ca^{2+} release channel, however, did not reveal any significant alteration on a single-channel level.

The delayed diastolic decay of [Ca^{2+}], in heart failure was more pronounced when cells were stimulated externally than when under voltage clamp conditions. The Ca^{2+} ATPases of the SR and of the sarcolemmal membrane are thought to act independently of membrane voltage. Prolongation of the action potential in hypertrophy and heart failure has been described in a variety of animal models. Gwathmey et al. have shown that in isometrically contracting human papillary muscle from patients with heart failure, the action potential duration is also prolonged. Our results show that this can be confirmed in isolated single ventricular myocytes. Action potential duration was markedly longer in diseased myocytes. Therefore, reduction of Ca^{2+} efflux through the Na^{+}-Ca^{2+} exchange system or even Ca^{2+} influx via this system during the prolonged time of depolarization is most likely the reason for the difference in diastolic decay of [Ca^{2+}], under external stimulation and voltage clamp conditions. Until the intracellular Na^{+} concentrations under normal and pathological conditions are known, however, no quantitative analysis of the Na^{+}-Ca^{2+} exchange system can be done reliably.

When cells were perfused internally with the electrode solution containing 5 mmol/l Na^{+}, a marked difference in [Ca^{2+}], relaxation could still be observed between normal and heart failure myocytes. By the skinned fiber method, Ca^{2+} sensitivity of the ventricular contractile proteins has been found to be unchanged in heart failure. Because differences in Na^{+}-Ca^{2+} exchange cannot play a role under these conditions, differences in [Ca^{2+}], relaxation most likely result from a reduced Ca^{2+} reuptake rate of the SR, as has been found by others. This reduction of Ca^{2+} reuptake by the SR seems to be a widely found alteration in heart failure in a variety of species. Our measurements are supported by the recent finding of Mercadier et al., who demonstrated that the expression of messenger RNA encoding for SR ATPase is markedly reduced in terminal heart failure. Other authors who measured Ca^{2+} uptake of isolated SR, however, could not find any difference in the Ca^{2+} uptake rate.

We were unable to demonstrate any significant difference in resting [Ca^{2+}], peak [Ca^{2+}], transients, or prolonged diastolic [Ca^{2+}] decay between cells isolated from hearts with dilated or ischemic cardiomyopathy. This may indicate that alterations in intracellular [Ca^{2+}], handling may not be a feature of a specific cause of heart failure and may not be limited to the failing human heart. Important alterations of the $\beta$-adrenergic pathway in human papillary muscles of patients with heart failure, namely increase of G protein and reduction of $\beta$-adrenoceptors, have
Further results in myocardium of patients with hypertrophic cardiomyopathy have recently been reported. The authors investigated the role of drugs that increase cAMP on diastolic relaxation and of diastolic decline of [Ca2+]i. At high enough pacing rates in their specimen, diastolic [Ca2+]i was increased and systolic active tension development was reduced because of incomplete relaxation. These alterations could be partially reversed by cAMP-increasing agents. Whether these differences in the effect of cAMP can be explained by different stages of heart failure (hypertrophy versus terminal heart failure) needs further clarification.

The results of this study have several important clinical implications. Our results would indicate that at least parts of the clinical features in heart failure are caused by a reduction in force development as a result of a reduction in systolic [Ca2+]i. The question of whether this is caused by a partial electromechanical uncoupling or by a reduced availability of SR Ca2+ to be released still needs to be resolved. If a reduced Ca2+-loading of the SR is the cause, which seems likely, then inotropic agents that primarily enhance Ca2+ reuptake of the SR would be beneficial. Although catecholamines are known to enhance SR Ca2+ uptake, they cannot act sufficiently because of the pronounced downregulation of β-adrenoceptors in heart failure. Furthermore, this may explain the beneficial effect of slowing the heart rate in these patients. As diastole is primarily shortened during tachycardia, a slow heart rate would provide sufficient time to refill the SR even when the SR Ca2+-ATPase had a reduced reuptake rate.

Isolated human ventricular myocytes have several advantages for studying excitation–contraction coupling in human heart failure and may provide additional and complementary information to results obtained with multicellular preparations.

Acknowledgments

The expert technical assistance of Johanna Nußer is gratefully acknowledged. Our special thanks to Professor B. Reichart and his colleagues (Department of Cardiac Surgery, University of Munich) for providing the myocardial tissue.

References


35. Mullins LJ: The generation of electric currents in cardiac fibers by Na/Ca exchange. A J Physiol 1979;236:C103–C110
Intracellular calcium handling in isolated ventricular myocytes from patients with terminal heart failure.
D J Beuckelmann, M Näbauer and E Erdmann

Circulation. 1992;85:1046-1055
doi: 10.1161/01.CIR.85.3.1046

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/85/3/1046