Isometric Relaxation of Rat Myocardium at End-Systolic Fiber Length

ALLEN W. WIEGNER AND OSCAR H.L. BING

SUMMARY In a "physiologically sequenced" contraction (PSC), which loads the isolated muscle preparation in a manner which approximates that of the intact heart, isometric relaxation precedes isotonic relaxation and occurs at minimum ("end-systolic") length. We studied the effects of initial muscle length, load, temperature, calcium, and isoproterenol on the isometric relaxation phase of physiologically sequenced contractions to define the determinants of the rate of isometric relaxation of rat left ventricular myocardium. At the baseline temperature (28°C), relaxation was found to be nonexponential, and the maximum rate of decline of force (−dF/dtmax) was used to evaluate changes in relaxation. Three factors, shortening, end-systolic length, and total load, were examined as possible mechanical determinants of −dF/dtmax. We found that −dF/dtmax is linearly related to end-systolic muscle length for lengths below 94% of Lmax; −dF/dtmax is also strongly related to total load for lightly loaded contractions, but peaks at loads of approximately 80% of peak developed force and declines thereafter. Shortening is poorly correlated with −dF/dtmax. The slope of the linear portion of the relation between −dF/dtmax and end-systolic length appears to be independent of muscle-loading conditions, and sensitive to factors known to alter relaxation.

FOLLOWING ejection, the ventricle remains isovolumic while intraventricular pressure declines. The decline of ventricular wall stress begins at the approximate length to which the muscle fibers have just shortened, that is, end-systolic length. Two additional mechanical parameters normally characterize the ventricle at the onset of isovolumic relaxation: the total load borne (ventricular wall stress) and the amount of fiber shortening which has just occurred. It seems reasonable that the mechanical determinant(s) of the subsequent isometric relaxation should be found among these three parameters. Indeed, in studies of relaxation in the intact heart, different investigators have labeled each of these three parameters (load,1 shortening,2 and end-systolic length3) as the primary hemodynamic determinant of relaxation rate.

The diversity of these findings may be attributed in part to differences in preparations and in the parameters used as measures of relaxation. The simplicity and controlled milieu of the isolated muscle preparation seem ideal for elucidating the physiological factors which underlie the phenomenological descriptions of relaxation reported in studies of the intact heart. Relaxation of isolated cardiac muscle has previously been studied in afterloaded isotonic contractions4 in which isometric relaxation occurs at the precontraction length. However, it has been shown5 that the rate of isometric relaxation is depressed during periods of afterloaded isotonic contractions, when compared to periods of "physiologically sequenced"6 contractions, in which isometric relaxation precedes lengthening. Thus, although relaxation can be studied under a variety of loading conditions, results obtained from physiologically sequenced contractions may be particularly relevant to the understanding of relaxation in the in situ heart.

A recent study by Tamiya et al.7 focused on isometric relaxation of canine papillary muscle at end-systolic fiber length. However, their study did not evaluate muscle shortening in detail, or systematically report on the relation between −dF/dtmax and end-systolic length. Additional ambiguities in their study led us to believe that more complete information was needed.

The present study was undertaken to consider systematically the effects of the three mechanical parameters on isometric relaxation.

Methods

Trabeculae carneae were rapidly dissected from the left ventricles of freshly decapitated rats and mounted vertically in a 100-ml Plexiglas chamber containing Krebs-Henseleit solution8 at 28°C, gassed with 95% O2 and 5% CO2. The preparations were stimulated at a rate of 12 per minute by parallel platinum plate electrodes delivering 5-msec pulses at voltages 10% above threshold. The upper end of the muscle was held by a spring clip and
connected via a length of 30-gauge stainless steel tubing to the lever arm of a low-inertia DC motor (General Scanning model G100PD) above the chamber; the lower end of the muscle was attached via a spring clip directly to a semiconductor strain gauge transducer (Kistler-Morse Corp., model DSC-3) immersed in the bath.

Either the force or the length of the preparation could be specified at will by means of an electronic servosystem6 controlled by a hybrid computer.9 The precision of force and length settings was 10 mg and 5 μm, respectively. The system step response to a length step input was 90% complete in 1 msec. Equipment compliance was less than 1 μm/g.

Muscles first were allowed to contract isotonically at a preload of approximately 0.5 g/mm² for 45 minutes. They then were stretched gradually to the peak of their length-tension curves (Lmax) while contracting isometrically. Finally, the muscles equilibrated for 30 minutes at Lmax while performing physiologically sequenced contractions with an afterload equal to 25% of peak developed tension.

The effects of changes in preload, total load, temperature, and calcium concentration, and the addition of isoproterenol were studied in five muscles. The order of the interventions in the protocol was varied, although the isoproterenol experiment always was performed last. Muscles were studied at three levels of preload: the control level, 1.0 g/mm² (Lmax); 0.3 g/mm² (95.5 ± 0.7% of Lmax); and 0.05 g/mm² (89.9 ± 1.0% of Lmax). The control calcium concentration was 1.26 mM; the concentration was reduced to 0.63 and increased to 2.52 and 5.04 mM by the removal or addition of calcium chloride. Temperature effects were studied at 23°, 28° (control), and 33° C. Isoproterenol was added to bring its concentration in the bath to 10⁻⁵ M.

In the control state and with each new intervention, the muscles were allowed to equilibrate while at Lmax by performing physiologically sequenced contractions with an afterload equal to 25% of maximum developed tension. A series of contractions then was recorded, at afterloads ranging from 0 (isotonic) to maximum (isometric) with step size selected to obtain an average of five or six steps per series. Two minutes passed between each recording at a new afterload; during this time, the afterload was maintained at 25% of maximum developed tension. Thus only one contraction every 2 minutes differed from the steady state load. This procedure was used to minimize changes in performance as a consequence of changes in afterload.10,11 A typical series of contractions is shown in Figure 1.

Contractions were recorded on a Tektronix model D15 oscilloscope equipped with a model C-5 camera, and data were measured directly from the photographic recordings. Maximum rate of decline of force (−dF/dtmax) was calculated by drawing a tangent line to the most steeply declining portion of the relaxation curve and measuring the angle between the tangent line and a zero-force baseline on each photograph with a precision protractor by guest on June 24, 2017 http://circres.ahajournals.org/ Downloaded from
shortening alone, as the relation is preload-dependent. We then repeated this procedure, first looking at end-systolic length values of 82, 84, 86, 88, 90, 92, 94, and 96% of L_max and then looking at total loads of 1, 2, 3, and 4 g/mm². No significant differences in $-\frac{dF}{dt_{\text{max}}}$ with variations in preload were found at any values of end-systolic length or total load. These results are shown in Figures 2 and 3.

The relation between $-\frac{dF}{dt_{\text{max}}}$ and muscle length at the onset of relaxation is shown in Figure 2. This relation is linear for muscle lengths less than 94% of L_max and is independent of preload. If total load as a percentage of peak-developed tension at L_max is plotted on the abscissa instead of muscle length (Fig. 3), relaxation rate is again independent of preload. As total load is increased toward a level such that contraction becomes isometric (4.8 ± 0.6 g/mm² in this set of muscles), $-\frac{dF}{dt_{\text{max}}}$ begins to decrease, so that the maximum rate of decline of force occurs for loads around 80% of isometric force. Since the curves in Figure 2 are more linear than those in Figure 3, muscle length was chosen as the independent (abscissa) variable in subsequent figures.

Figure 4 shows the relation between $-\frac{dF}{dt_{\text{max}}}$ and muscle length as a function of the concentration of calcium in the bath. The curve for Ca²⁺ = 5.04 mM is not included in the figure because it overlaps the curve at 2.52 mM. Again, a linear relation is seen between length and relaxation rate for muscle lengths below 94% of L_max except for the 2.52 mM curve which is linear below 90%. It appears that $-\frac{dF}{dt_{\text{max}}}$ increases with bath calcium at a given muscle length. This increase is explained by the fact that a muscle that shortens to a given length in a high-calcium bath (top curve) is more heavily afterloaded than a muscle that shortens to the same length in a low-calcium bath (bottom curve). Thus, the larger developed force is responsible for the...

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**Figure 2** The relation between isometric relaxation rate and muscle end-systolic length at three levels of preload. The relation is linear below 94% of L_max and is independent of preload. Relaxation rate of each muscle was normalized by dividing by its peak isometric force. The right-most point of each curve represents isometric contraction at that preload, whereas points farther to the left represent more lightly loaded contractions. The number adjacent to each curve is the slope (per % muscle length) of the curve for lightly loaded contractions. Brackets indicate ±1 SEM.

**Figure 3** The relation between isometric relaxation rate and total load is more curvilinear than the relation shown in Figure 2, although still independent of preload. Load has been expressed as a percentage of isometric total load at L_max. Only points for which data were available from all muscles have been plotted.

**Figure 4** The effect of bath calcium concentration on the relation between isometric relaxation rate and muscle end-systolic length. The curve for Ca²⁺ = 5.04 mM overlapped the curve for 2.52 mM and was not drawn. The right-most point on each curve represents isometric contraction; points to the left correspond to contractions that were lightly loaded, and thus shortened more. Numbers represent the slopes of the curves along their linear portions. The control curve (filled circles) is redrawn from Figure 2.
is redrawn from Figure 2. Numbers represent slopes of the linear portions of the curves. The control curve (filled circles) is redrawn from Figure 2.

**Table 1** Effect of Calcium on $-\frac{dF}{dt_{\text{max}}}$ (g/sec)

<table>
<thead>
<tr>
<th>Bath $[\text{Ca}^{2+}]$</th>
<th>1 g</th>
<th>2 g</th>
<th>3 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.63 mM</td>
<td>14.3 ± 1.2</td>
<td>17.7 ± 1.7</td>
<td>19.1 ± 2.1</td>
</tr>
<tr>
<td>1.26 mM</td>
<td>16.3 ± 1.2*</td>
<td>20.1 ± 1.6*</td>
<td>22.5 ± 2.2*</td>
</tr>
<tr>
<td>2.52 mM</td>
<td>17.4 ± 1.2†</td>
<td>21.8 ± 1.8†</td>
<td>24.9 ± 2.5†</td>
</tr>
<tr>
<td>5.04 mM</td>
<td>16.9 ± 1.3‡</td>
<td>21.7 ± 1.7‡</td>
<td>24.5 ± 2.2‡</td>
</tr>
</tbody>
</table>

Data are means ± SE (n = 5).

* $P < 0.05$ vs. 0.63 mM Ca$^{2+}$.
† $P < 0.05$ vs. 1.26 mM Ca$^{2+}$.
‡ Not significant vs. 2.52 mM Ca$^{2+}$.

Increased rate of relaxation. To correct for the effect of load on $-\frac{dF}{dt_{\text{max}}}$, the relation between $-\frac{dF}{dt_{\text{max}}}$ and bath calcium at a constant load is shown in Table 1. In comparison to 1.26 mM Ca$^{2+}$, $-\frac{dF}{dt_{\text{max}}}$ is slightly but significantly smaller at 0.63 mM and larger at 2.52 mM Ca$^{2+}$, and it does not increase further at 5.04 mM Ca$^{2+}$.

Decreasing the bath temperature to 23°C results in slowed relaxation at a given length, whereas increasing the temperature to 33°C increases the relaxation rate, as shown in Figure 5. The point at which the curves become linear appears to be temperature-dependent: at 23°C there is no strictly linear portion, whereas at 33°C linearity begins at 98% of $L_{\text{max}}$. One might therefore predict a linear relation at in vivo temperatures.

**Discussion**

Parameters used as measures of relaxation rate fall into two groups: those using maximum slopes ($-\frac{dP}{dt_{\text{max}}}$, $-\frac{dF}{dt_{\text{max}}}$) which can be applied to any declining force or pressure curve, and the exponential time constants ($\tau$, $t_{\frac{1}{2}}$) which apply when relaxation is exponential. In the intact heart, Weiss et al. found that ventricular pressure declined exponentially, and studied $\tau$, whereas Cohn et al. found only a brief period of exponential decay, and chose maximum negative $dP/dt$ as a measure of relaxation, as did Weisfeldt et al. In isolated muscle experiments, afterloaded isotonic contractions yield an exponential decline in force following an initial rapid decline, whereas physiologically sequenced contractions, in which isometric relaxation occurs at minimum (end-systolic) length, demonstrate a more linear isometric relaxation phase. Neither skeletal nor cardiac muscle appears to relax exponentially following an isometric contraction.

Although relaxation is substantially exponential for nonisometric physiologically sequenced contractions near 37°C, only the terminal portion of isometric relaxation is exponential at 28°C. Since the conditions under which relaxation of cardiac muscle may be considered to be exponential are not yet well defined, we chose $-\frac{dF}{dt_{\text{max}}}$ as our measure of relaxation rate.

We found that both end-systolic length and total load were related to $-\frac{dF}{dt_{\text{max}}}$, but muscle shortening was not. Tamiya et al., studying physiologically sequenced contractions of blood-perfused canine papillary muscles, found a linear relation between maximum rate of decline of force and total load and used the ratio of these two as an index of relaxation. In the present study, the relation in Figure 3 may be a straight line but does not pass through the origin. Thus, the ratio used by Tamiya et al. is not constant and cannot be used as an index of relaxation. The reasons for the difference between the results of the present study and those of Tamiya et al. are not clear. Since the temperature at which their studies were performed was omitted, it is not known what effect temperature may have had on their results; one might assume that the preparations were maintained near 37°C by the warm blood from the donor dog. It might also be pointed out that their blood-perfused papillary muscles developed a mean isometric force of only 1.06 g/mm². In the present study, mean isometric force was 4.8 ± 0.6 g/mm², which corresponds favorably with the 5.1 ± 0.5 g/mm² reported by Suga et al. in nonexcised canine papillary muscle preparations at 37°C.

Tamiya et al. reported that $-\frac{dF}{dt_{\text{max}}}$ (which they referred to as $-\frac{dt}{dt_{\text{max}}}$) was independent of end-systolic fiber length at various preloads under
conditions of constant total load and contractility. The argument leading to this conclusion is, however, unclear. According to their Figure 4, a slight increase in $-dF/dt_{\text{max}}$ accompanies an increase in preload at constant total load, yet at point A in their Figure 6, a large decrease in $-dF/dt_{\text{max}}$ accompanies an increase in preload at constant total load, contradicting Figure 4. Unless Figure 6 is anomalous, this casts doubt on their claim that $-dF/dt_{\text{max}}$ can be regarded as a function of total load alone under conditions of constant contractility.

Tamiya et al. also found that end-systolic length increases with preload at constant total load. However, it has been reported elsewhere that when preload is varied while total load remains constant, end-systolic length also remains approximately constant.

The change in end-systolic length reported by Tamiya et al. may derive from the large ratio of preload to total load (approximately 0.8) after point A of their Figure 6. In any case, the magnitude of the change in end-systolic length—around 10%—is comparable to the change in $-dF/dt_{\text{max}}$ with preload at constant total load, shown in Figure 4, which the authors chose to ignore. Since $-dF/dt_{\text{max}}$ appears somewhat affected by both preload (at constant total load) and end-systolic length, it would seem that neither total load nor end-systolic length appears to be a better determinant of relaxation than the other.

A possible explanation for the increase in $-dF/dt_{\text{max}}$ with increasing calcium shown in Table 1 is that increased calcium results in increased shortening, which enhances the rate of relaxation through the mechanism of shortening deactivation. However, the slowing of relaxation at 5.04 mM Ca$^{2+}$, in spite of maximal shortening at that concentration, demonstrates a decrease in relaxation rate which cannot be explained by shortening deactivation and might be attributed to either the beginning of saturation of the relaxing system or another influence of an abundance of calcium on intracellular calcium movements.

The slope of the linear portion of the relation between $-dF/dt_{\text{max}}$ and end-systolic length appears to be independent of loading conditions, only slightly sensitive to the calcium concentration in the bath, and sensitive to factors known to alter relaxation. In Figure 2, a linear relation is seen between $-dF/dt_{\text{max}}$ and end-systolic length below 94% of $L_{\text{max}}$: the slope of this relation is independent of preload. In Figure 4, the slope of the linear portion of the curves increases only slightly as the bath calcium is increased from 0.63 to 2.52 mM. By altering temperature, the rate of many biochemical processes, including calcium sequestration, can be changed. In Figure 5, the slope of the linear portion is a function of temperature. In fact, the slopes at 23°, 28°, and 33°C, doubling with each 5° increase, form a geometric progression which suggests an underlying temperature-dependent rate constant for relaxation. Inserting these values for temperature and activity into the Arrhenius equation yields an apparent calcium pump activation energy of 25 kcal/mol. In a study of the uptake of Ca$^{2+}$ in vitro by sarcoplasmic reticulum vesicles from rabbit white skeletal muscle, activation energy has been calculated to be 28 kcal/mol between 5° and 20°C, and 17 kcal/mol between 20° and 40°C. Isoproterenol is thought to augment myocardial relaxation by stimulating calcium transport by the sequestering system, and indeed, a large increase in the slope of the linear portion of the isoproterenol curve is seen in Figure 5.

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