Phase I and phase II of short-term mechanical restitution in perfused rat left ventricles

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Mechanical restitution and other aspects of the myocardial strength-interval relation (1, 24, 33) may reflect 1) changes in the gating mode of the L-type Ca2+ channels (dihydropyridine receptors, DHPRs) (29, 33); 2) changes in the extent to which Ca2+ release channels (ryanodine receptors, RyRs) of the sarcoplasmic reticulum (SR) recover the ability to release Ca2+ (34); 3) alterations in the amount of Ca2+ contained in the SR (6); and 4) cross-talk between the RyRs and the DHPRs wherein Ca2+ influx via the L-type Ca2+ channels triggers SR Ca2+ release and Ca2+ released from the SR inactivates the DHPRs (6). Each of these processes appears to be highly regulated and offers a potential target for therapeutic modalities designed to improve electromechanical function in diseased hearts.

The present study examined mechanical restitution in perfused rat left ventricles subjected to a variety of pharmacological perturbations. The biphasic nature of the mechanical restitution curves was abrogated by Ca2+ channel agonists, whereas phase II was eliminated by thapsigargin plus ryanodine. The results suggest that phase I of electromechanical restitution is caused by a transient L-type Ca2+ current facilitation, whereas phase II represents the recovery of the ability of the sarcoplasmic reticulum to release Ca2+. L-type calcium channels; sarcoplasmic reticulum; ryanodine receptors; myocardium; calmodulin.

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MATERIALS AND METHODS

Isolated Langendorff Perfused Hearts

Hearts from young (300–400 g) male Sprague-Dawley rats purchased from Zivic Miller (Portersville, PA) or Harlan (Indianapolis, IN) were used in this study. All animals were maintained on a 12:12-h light-dark cycle in a fully accredited facility. For in vitro studies of left ventricular performance, rats were injected intraperitoneally with 155 mg pentobarbital/kg body wt. An abdominal incision was made, and the thorax was opened, and hearts were rapidly excised and heparin (0.7 mg) was injected into the inferior vena cava. The heart was placed in ice-cold modified Krebs-Henseleit buffer, pH 7.4, saturated with 100% O2, with 115 mM sodium for the excitation and emission spectrum, Girouard et al. (18) described in a tapering wedge of tissue. The precise depth of optical recordings in preparations stained by perfusion has been subject to debate. Model calculations by Salama (32), based on the depth of field of the optical system, predicted a depth of 144 μm. Measurements by Knisley et al. (23) in a tapering wedge of tissue showed that the intensity of optical action potentials ceased to increase if the thickness of the tissue was >300 μM. From the measurements of the absorption coefficient of myocardium for the excitation and emission spectrum, Girouard et al. (15) predicted that 95% of the signal originates from a tissue depth of 500 μM or less.

A major limitation of current techniques of potential imaging is the lack of absolute calibration. Unlike many ratio-metric fluorescent probes for calcium imaging, voltage-sensitive dyes can provide only relative information about changes in transmembrane voltage. Although the changes in the absolute amount of fluorescence excited at one wavelength linearly depends on transmembrane voltage of the viewed cells, accurate calibration has so far been impossible because the number of cells contributing to the signal is unknown.

To measure action potential (AP) amplitudes and areas at 50% repolarization, we chose to average fluorescence signals that originated from the surface of the central part of the left ventricle, where motion artifacts are minimal. For each ESI, we averaged signals coming from at least four adjacent photodiodes facing the central region of the epicardium. We measured AP amplitudes, and we calculated by integration the area under the AP from the origin of the upstroke up to the point corresponding to 50% repolarization. Because of the lack of absolute calibration, both areas under AP and AP amplitudes are normalized to the F0 areas or amplitudes.

Most studies of electrical activity and mechanical restitution were done separately because the presence of an intraventricular balloon increased movement artifacts in the fluorescence traces. Agents such as butane dione monoxime (2) or cytochalasin D (38) that directly interfere with force development were not used in any of these studies. Instead, only those fluorescence traces showing a clear absence of movement artifacts, usually from the central region of the preparation, were analyzed.
Experimental Design

Pacing protocols. After a priming period of 100 beats at 3 Hz, test beats (extrasystoles) were introduced at varying time intervals, from as early as 40 ms to as late as 600 ms. Beyond 600 ms the hearts generally exhibited spontaneous activity. In experiments where high concentrations of thapsigargin and ryanodine were employed to disable the SR, the pacing rate was reduced to 2 Hz to allow for more complete relaxation of the steady-state beats. All extrasystoles were followed by an immediate return to the basic cycle length. That is, when the steady-state pacing frequency was 3 Hz, there was a standard 333-ms interval between the extrasystolic and the first postextrasystolic stimulus. Likewise, when the steady-state pacing rate was 2 Hz, there was a standard 500-ms interval between the extrasystolic and the first postextrasystolic stimulus. Each pacing protocol was repeated two to three times for each heart, and the F1/F0 data were averaged to obtain a single set of values for each heart. The average standard error for triplicate F1/F0 values was ~2% of the F1/F0 ratio.

Reagents. —BAY K 8644 and ryanodine were purchased from Calbiochem (LaJolla, CA). KB-R7943 (KBR) was the generous gift of Kanebo Pharmaceutical Laboratories (Osaka, Japan). All other reagents were purchased from Sigma (St. Louis, MO).

Data Analysis

Extrasystoles partially fused with control contractions were separated using a program for graphical analysis (Origin), by subtracting from the fused contraction the control contraction just preceding it (Fig. 1). The maximum developed pressure of the resultant derived extrasystolic contraction was measured (F1), and restitution curves were drawn by plotting the percentage of mechanical restitution, F1/F0 × 100%, where F0 is the steady-state value of LVDP against the ESI. A similar computerized subtraction method was used to separate overlapping APs, and the extrasystolic amplitude F1 was divided by the amplitude of the steady-state AP F0. F1/F0 values were again plotted as a function of ESI. Similar calculations were done using areas under the APs at 50% repolarization.

Phase II of mechanical restitution was curve fit using Origin software and the equation $Y = Y_{\text{max}} \cdot (1 - \exp[-(X - T_{0})/B])$, where $X$ is the ESI, $T_{0}$ is the X-axis intercept, and $B = \tau \ln 2$, where $\tau$ is the time constant for mechanical restitution. The values for the reversal point (nadir) and the data point immediately following it (e.g., ESI of 230 and 250 ms in Fig. 2) were not used in the curve fitting because of the apparent overlap with phase I.

Comparisons between complete restitution curves (phase I plus phase II) were done using Origin software and a resident statistical package for repeated measures one-way analysis of variance (ANOVA). When the complete curves were significantly different from each other we used a Newman-Keuls post hoc test to determine which time points were significantly different between the two curves. We also used one-way ANOVA to determine which F1/F0 values were significantly different from 100%. A $P$ value <0.05 was considered to be statistically significant.
RESULTS

Mechanical Restitution

Figure 2 summarizes restitution data for a group of untreated normal hearts. Note the early increase in $F_1/F_0$, which peaked at an ESI of $150 \text{ ms}$ and thereafter declined, reaching the nadir or reversal point at $230 \text{ ms}$. This phase I reached its peak value 30 ms after the steady-state beat developed maximum pressure, and the reversal point occurred before complete relaxation of the control beat, when about 35% of LVDP still persisted. Phase II of mechanical restitution was characterized by an exponential increase in $F_1/F_0$ with a $\tau$ of $98 \pm 8 \text{ ms}$, $T_0$ of $196 \pm 4 \text{ ms}$, and $Y_{\text{max}}$ of $158 \pm 4\%$. Also shown in Fig. 2 are data for the first beat following the extrasystole, i.e., the postextrasystolic beat, $F_2$. None of these postextrasystoles were fused to an adjacent beat, so the data are simple ratios of the directly measured LVDPs of the $F_2$ and $F_0$ beats. Phase I of mechanical restitution paralleled changes in AP area at 50% repolarization (Fig. 3), which is related to the strength and duration of the L-type Ca$^{2+}$ current (33); there were no significant time-dependent changes in APs coinciding with phase II of mechanical restitution.

Figure 4 illustrates the effects of isoproterenol and the Ca$^{2+}$ channel agonists –BAY K 8644 and FPL-64176 on mechanical restitution. Effects of these compounds on steady-state left ventricular contractile performance are summarized in Table 1. Both $\beta$-adrenergic stimulation and L-type Ca$^{2+}$ channel activation significantly accelerated phase II of mechanical restitution, thereby decreasing the $F_1/F_0$ at the plateau. The Ca$^{2+}$ channel agonists also eliminated the decline in $F_1/F_0$ between 150 and 230 ms. Isoproterenol did not alter the biphasic nature of the restitution curve but it shifted the reversal point leftward from 230 to 150 ms. Effects of the Ca$^{2+}$ channel and $\beta$-adrenoceptor agonists on phase II of mechanical restitution were partially reversed by the Ca$^{2+}$-calmodulin (CaM) kinase inhibitor KN-93 (1 $\mu$M, e.g., Fig. 5). KN-93 had no significant effect on steady-state contractile parameters ($n = 6$). KBR, a putative inhibitor of the Na$^+$/Ca$^{2+}$ exchanger (21), slightly reduced the amplitude of the early phase I ratios, but the effect failed to reach statistical significance (Fig. 6). KBR had no statistically significant effects on steady-state LVDP or time to peak pressure ($n = 4$), but there was a significant

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**Fig. 3.** Restitution of action potential (AP) amplitudes (open triangles) and AP areas (filled circles) at 50% repolarization. Data are means ± SE for 5 hearts. AP area ratios ($F_1/F_0 \times 100\%$) were significantly different from 100% at extrasystolic intervals (ESI) from 120 to 170 ms.

**Fig. 4.** Effects of isoproterenol (1 $\mu$M, $A$, $n = 3$), FPL-64176 (150 nM, $B$, open squares, $n = 4$) and –BAY K 8644 (100 nM, $A$, filled circles, $n = 6$) on mechanical restitution.
decline in maximal rates of pressure increase \( (+\frac{dP}{dt_{\text{max}}}) \) (2,040 ± 230 vs. 2,520 ± 160 mmHg/s) and decrease \( (-\frac{dP}{dt_{\text{max}}}) \) (1,260 ± 170 vs. 1,630 ± 90 mmHg/s).

To assess the involvement of the SR in phase I of mechanical restitution, hearts were perfused with thapsigargin, a specific inhibitor of the SR Ca\(^{2+}\) pump (22), plus ryanodine, a compound that renders the SR leaky to Ca\(^{2+}\) (17). This reduced peak systolic left ventricular pressure (LVP) to 88 ± 22 mmHg, increased end-diastolic pressure to 48 ± 14 mmHg, decreased \( +\frac{dP}{dt_{\text{max}}} \) to 410 mmHg/s, and decreased \( -\frac{dP}{dt_{\text{max}}} \) to 235 ± 62 mmHg/s (n = 3). Figure 7 is a typical pressure recording. Note the greatly enhanced relative amplitude of the extrasystole at 170 ms (basic cycle length, 500 ms) and the absence of postextrasystolic potentiation. Figure 8 summarizes the mechanical restitution data for a group of hearts perfused with thapsigargin plus ryanodine. With the SR unable to participate in excitation-contraction coupling, phase I of mechanical restitution was greatly exaggerated, whereas phase II was nearly flat. KN-93 (3 μM) had no statistically significant effect on mechanical restitution in the presence of thapsigargin plus ryanodine (Fig. 8).

**DISCUSSION**

The present study demonstrates that the electromechanical restitution of perfused rat left ventricles consists of two distinct phases: phase I, which is completed before the full relaxation of the steady-state beats, and phase II, which occurs later. Aside from the classic 1975 papers by Bass (3, 4), which described the two phases of electromechanical restitution in cat papillary muscles, most investigations have focused on phase II, specifically on that portion of phase II that follows complete relaxation of the steady-state beat (1, 36, 40). In such studies, there typically are mathematical extrapolations to zero developed force or zero intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) transient amplitude as the ESI is shortened; fused waveforms are not dealt with (36). Phase II appears to be a monoexponential process that results from the recovery of the ability of the RyRs of the SR to release calcium (34, 36). This may result from the recovery of the RyRs from inactivation (34) or adaptation (16). There could also be a time-dependent refilling of highly localized SR Ca\(^{2+}\) stores (8).

The characterization of phase I of mechanical restitution at first appears to be problematic in that it requires the computerized subtraction of a steady-state

<table>
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<th>Predrug Steady-State LVDP, mmHg</th>
<th>Drug Treatment</th>
<th>%Increase in LVDP</th>
<th>%Increase in ( +\frac{dP}{dt_{\text{max}}} )</th>
<th>%Increase in ( -\frac{dP}{dt_{\text{max}}} )</th>
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<tr>
<td>4</td>
<td>132 ± 16</td>
<td>FPL-64176 (150 nM)</td>
<td>48 ± 7</td>
<td>78 ± 11</td>
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<tr>
<td>6</td>
<td>132 ± 25</td>
<td>–BAY K 8644 (100 nM)</td>
<td>52 ± 5</td>
<td>71 ± 10</td>
<td>57 ± 10</td>
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<td>9</td>
<td>123 ± 16</td>
<td>Isoproterenol (1 μM)</td>
<td>95 ± 13</td>
<td>195 ± 25</td>
<td>169 ± 20</td>
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Values are means ± SE; n, numbers of rats. LVDP, left ventricular developed pressure; \( dP/dt_{\text{max}} \), maximal rates of pressure.

**Table 1. Drug effects on steady-state contractile parameters**

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Fig. 5. Effect of 1 μM KN-93 on mechanical restitution in hearts perfused with 150 nM FPL-64176. Open circles, +KN-93; closed squares, –KN-93. The two curves are significantly different at ESIs between 230 and 290 ms. (FPL-64176, n = 5; FPL + KN-93, n = 4.)

Fig. 6. There is no statistically significant effect of 10 μM KB-R7943, a putative inhibitor of Na\(^+\)/Ca\(^{2+}\) exchange on mechanical restitution. Filled squares, control; open circles, +KB-R7943 (n = 5).

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beat to separate out the amplitude and configuration of very premature extrasystoles. This raises questions about whether force development, APs, and Ca\(^{2+}\)/H\(_{11001}\) transients can realistically be thought of as additive for fused responses that result from two closely spaced stimuli. The curve describing the relative strength of the first postextrasystolic beat at short ESIs, which is similar in shape to the F\(_1/F_0\) curve, suggests that the computerized subtractions do provide realistic data. Extra calcium entering the cell in response to a premature stimulus is thought to be taken up by the SR, becoming available for release during the subsequent beat (5, 28). When the premature stimulus occurs while the RyRs are refractory, the extra contractile response of the postextrasystolic beat to this extra Ca\(^{2+}\) is roughly proportional to the amount of Ca\(^{2+}\) entering the cell during the extrasystole. Thus the time course and magnitude of changes in F\(_1/F_0\) as a function of the ESI for both the extrasystoles and the associated first postextrasystolic beats (F\(_2/F_0\)) were similar during phase I. Moreover, rat ventricular APs, because of their brief duration, are less fused than the corresponding mechanical traces at short ESIs, and their AP areas at 50% repolarization also paralleled the computer-resolved LVDPs for early extrasystoles.

The present study demonstrated that phase I of electromechanical restitution is independent of SR Ca\(^{2+}\) release and can be observed when the SR is chemically disabled with thapsigargin plus ryanodine and is unable to accumulate or retain significant amounts of Ca\(^{2+}\). Phase II, on the other hand, appears to be almost entirely attributable to recovery of the SR Ca\(^{2+}\) release process because this phase is flat when the SR can no longer accumulate or retain Ca\(^{2+}\).

Interestingly, neither phase was significantly affected by the putative cardiac Na\(^+\)/Ca\(^{2+}\) exchange inhibitor KBR (19, 25). We used 10 \(\mu\)M KBR because of a reported IC\(_{50}\) of 1.2–2.4 \(\mu\)M for reverse mode Na\(^+\)/Ca\(^{2+}\) exchange inhibition. Concentrations higher than 10 \(\mu\)M might have significantly affected other ion channels (19), which would have confounded interpretation of the data. Ladilov et al. (25) found 20 \(\mu\)M KBR to be the maximally effective dose in studies of perfused rat hearts and isolated adult rat cardiomyocytes, but significant inhibition was obtained with 10 \(\mu\)M KBR. Because there was a trend toward a decrease in the maximum F\(_1/F_0\) ratios during phase I in hearts perfused with 10 \(\mu\)M KBR, a portion of the increase in F\(_1/F_0\) during phase I in the absence of drug could have been due to Ca\(^{2+}\) influx via reverse mode Na\(^+\)/Ca\(^{2+}\) exchange.

Phase I of mechanical restitution was markedly exaggerated by perfusion with thapsigargin plus ryanodine, which were used to limit respectively SR Ca\(^{2+}\) uptake and retention. On the other hand, phase II of mechanical restitution and postextrasystolic potentiation were abolished by the SR poisons. (Identical effects were obtained with thapsigargin alone, data not shown.) Thapsigargin is a potent and selective inhibitor of the SR Ca-ATPase (22), and it prevents Ca\(^{2+}\) uptake by the SR. Ryanodine leaves the Ca\(^{2+}\) release channels (RyRs) of the SR in an open but subconducting state (17), precluding significant retention of any SR Ca\(^{2+}\) that could have been accumulated despite the presence of a maximally effective concentration of thapsigargin (22).
Janczewski and Lakatta (20) demonstrated that thapsigargin increases [Ca\(^{2+}\)], transients in guinea pig ventricular cardiomyocytes after SR Ca\(^{2+}\) depletion with caffeine, indicating that some Ca\(^{2+}\) entering the cell via the L-type Ca\(^{2+}\) channels is immediately sequestered by the SR and does not contribute to the global [Ca\(^{2+}\)] transient. This effect of thapsigargin may have contributed to the amplitude of the steady-state beats shown in Fig. 7, which are larger than what would be predicted from data showing that SR Ca\(^{2+}\) release contributes >90% of myofibrillar activator Ca\(^{2+}\) in response to a normal rat heart AP (5) (also see Ref. 22). Our results are consistent with early studies of the effects of caffeine on cat papillary muscles (3), but thapsigargin and ryanodine are more specific inhibitors of SR function, leaving the data less open to alternative explanations.

A study of mechanical restitution in ferret papillary muscles failed to detect an overshoot in Ca\(^{2+}\)-dependent aequorin luminescence for early fused beats in the presence of ryanodine (36), as might have been predicted by our data. Unfortunately, in that study, fused mechanical responses were not resolved, leaving it unclear whether such muscles exhibited an exaggerated mechanical phase I. It is also unclear whether ryanodine alone is as effective as ryanodine plus thapsigargin in eliminating the contribution of the SR to excitation-contraction coupling. The present study did not examine the effects of ryanodine alone on electromechanical restitution.

It should be emphasized that the exaggeration of phase I of mechanical restitution in the presence of thapsigargin and ryanodine was associated with a ~70% reduction in steady-state LVDP. LVDP averaged only 40 mmHg compared with ~130 mmHg for control steady-state beats. Because the peak F\(_I\)/F\(_0\) shown in Fig. 8 is 190%, it follows that the mean peak F\(_I\) amplitude is only ~75 mmHg, or about half that of typical control steady-state beats at 3 Hz. Nevertheless, perfusion with thapsigargin plus ryanodine allowed a near doubling of LVDP for very early extrasystoles, over and above that which could be attributed to the absence of L-type Ca\(^{2+}\) current inactivation by SR Ca\(^{2+}\) release (6) and the lack of SR sequestration of Ca\(^{2+}\) entering through the L-type Ca\(^{2+}\) channels (20). It has been estimated that L-type Ca\(^{2+}\) current can double when Ca\(^{2+}\)-dependent current inactivation by Ca\(^{2+}\) released from the SR is eliminated (6). Our data suggest that a further doubling of Ca\(^{2+}\) influx is possible for very premature beats in the intact rat heart and emphasize the importance of Ca\(^{2+}\)-dependent L-type Ca\(^{2+}\) current inactivation and facilitation as mechanisms for grading cardiac contractility.

As shown in the companion paper (9), electromechanical restitution kinetics are altered significantly in failing spontaneously hypertensive, heart failure. Phase II could be accelerated dramatically by positive inotropic agents such as the β-adrenergic agonist isoproterenol, and by the dihydropyridine Ca\(^{2+}\) channel agonist –BAY K 8644 and the nondihydropyridine Ca\(^{2+}\) channel agonist FPL-64176. All three of these compounds would be predicted to increase the amount of Ca\(^{2+}\) contained in the SR due to the increase in L-type Ca\(^{2+}\) current. Isoproterenol should have the additional effect of causing phosphorylation of phospholamban, the endogenous inhibitor of the SR Ca-ATPase (7). In vivo studies of mechanical restitution in transgenic mice with varying amounts of myocardial phospholamban (18) indicated that restitution was fastest in phospholamban knockout mice, intermediate in wild-type mice, and slowest in mice overexpressing phospholamban in the heart. These mouse data lend support to the concept that SR Ca\(^{2+}\) content is a major determinant of the rate of phase II mechanical restitution.

The acceleration of phase II of mechanical restitution by isoproterenol and the Ca\(^{2+}\) channel agonists was retarded slightly by the CaM kinase II inhibitor, KN-93. Thus CaM kinase-dependent phosphorylation of RyR2 (37) or an accessory protein (27) may have contributed somewhat to the acceleration of phase II of mechanical restitution. However, the pronounced increase in SR Ca\(^{2+}\) load caused by these positive inotropic agents was probably responsible for most of the acceleration of mechanical restitution (6). All three drugs induced spontaneously reversible periods of visibly disorganized contractile behavior and zero LVDP at the initiation of pacing. This behavior was suggestive of ventricular fibrillation, which is commonly associated with an SR Ca\(^{2+}\) overload (6, 26). Under conditions where β-adrenoceptor stimulation does not increase the SR Ca\(^{2+}\) load, it has been reported that there is no effect of β-agonists on the recovery of the ability of cardiac RyRs to release Ca\(^{2+}\) (35).

Isoproterenol had little effect on phase I of mechanical restitution, except to abbreviate it, owing for the most part to a greater overlap with the accelerated phase II and a shift of the reversal point from 230 to 150 ms. The two chemically distinct Ca\(^{2+}\) channel agonists, on the other hand, abrogated the reversal point separating phase I and phase II. This would be explained if the Ca\(^{2+}\) channel agonists elicited maximum activation of the L-type Ca\(^{2+}\) channels and if the rise in F\(_I\)/F\(_0\) during phase I with Ca\(^{2+}\) channel agonists absent were due to transient voltage and/or Ca\(^{2+}\) dependent L-type Ca\(^{2+}\) channel facilitation coupled with the time-dependent recovery of electrical excitability. Facilitation involves an increase in the type II L-type Ca\(^{2+}\) channel gating mode that is characterized by very prolonged openings (29), Ca\(^{2+}\) channel agonists also act to increase type II openings (13). The subsequent decline in F\(_I\)/F\(_0\) in the absence of the Ca\(^{2+}\) channel agonists could then be due to the time-dependent decay of facilitation, which would not occur with the Ca\(^{2+}\) channel agonists present. Isoproterenol also increases type II openings (29), but increases in SR Ca\(^{2+}\) uptake as a consequence of phospholamban phos-
phorylation (11) account for a large fraction of the resulting positive inotropic effect. As shown in Table 1, the maximally effective dose of isoproterenol (1 μM) had much larger effects on dP/dt max than did the Ca²⁺ channel agonists, suggesting a more pronounced effect on the kinetics of SR Ca²⁺ accumulation and possibly a lesser effect on the L-type Ca²⁺ channels.

Surprisingly, phase I of mechanical restitution was not affected by CaM kinase inhibition with KN-93, despite good evidence that Ca²⁺ current facilitation in single cardiomyocytes results from CaM kinase activation (10, 39). It should be noted that the concentration of KN-93 used in the experiments summarized in Fig. 8 (3 μM) was threefold higher than that used for the experiments summarized in Fig. 5, where a small but statistically significant effect of KN-93 was observed. Thus we should have seen an effect of KN-93 on phase I of mechanical restitution in the presence of thapsigargin plus ryanodine if CaM kinase II were responsible for the observed increase in F/F₀ at short ESIs. That we did not raises the possibility that cardiac Ca²⁺ current facilitation is not a single process in the intact heart but is instead several processes that together regulate frequency-dependent changes in Ca²⁺ influx across the sarcolemma. The changes observed in the present study peaked at ~150 ms at a slightly hypothermic temperature of 32°C and relatively slow pacing frequencies for rat hearts of 2–3 Hz. Most evidence for CaM kinase-dependent Ca²⁺ channel facilitation has dealt with longer time scales, as might be more appropriate for a phosphorylation-dependent mechanism.

Both Ca²⁺-dependent facilitation and inactivation of L-type Ca²⁺ channels require the presence of calmodulin bound to an isoleucine glutamate (IQ) motif in the carboxy tail of the α₁-subunit (41). Replacement of the native isoleucine of the IQ domain with alanine removes Ca²⁺-dependent channel inactivation and unmask a strong facilitation, whereas conversion of the isoleucine to glutamate eliminates both facilitation and inactivation (41). A peptide representing another region in the carboxy-terminal sequence of the L-type Ca²⁺ channel, the CB peptide, binds Ca²⁺ and CaM and enhances Ca²⁺-dependent Ca²⁺ current (I_Ca) facilitation when injected into cardiac myocytes, again suggesting a direct effect of Ca²⁺/CaM on this process (30).

**Limitations of the Present Study**

In the present study, there were no direct measurements of [Ca²⁺], L-type Ca²⁺ current density, or SR Ca²⁺ content or release. It was therefore necessary to interpret the results in light of published data on more carefully controlled systems and to employ pharmacological maneuvers in an attempt to address mechanistic issues. On the other hand, intact multicellular preparations are stable and can be subjected to conditions that more closely resemble the in vivo situation in terms of stimulation frequency, temperature, and intracellular environment (33). Moreover, the perfusion of intact ventricles reduces concerns about core ischec-

mia in thick trabeculae or papillary muscles. No isolated system can replicate the in vivo heart, however, and the entire range of systems from single channel recordings to unanesthetized animals must be investigated to complete our understanding of the critically important cardiac force-interval relation and its changes with cardiac pathology.

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