Cardiac Myocyte Function and Left Ventricular Strains After Brief Ischemia and Reperfusion in Rabbits

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Background After a brief episode of ischemia, myocardial function may be depressed for prolonged periods despite reperfusion. The mechanisms of postischemic dysfunction differ depending on the experimental model. Regional ischemia and reperfusion in the intact animal provide a clinically relevant model, but experimental variables are difficult to control. Experimental conditions can be well controlled in isolated cardiac muscle and myocyte preparations, but these models are limited by the assumptions used to mimic ischemia and reperfusion. This study combines the unique advantages of both preparations. We characterized in vivo alterations in regional two-dimensional finite strains with ischemia and reperfusion produced in the intact animal, then isolated cardiac myocytes from the region with postischemic dysfunction to characterize in vitro function of postischemic myocytes.

Methods and Results In seven anesthetized rabbits, three piezoelectric crystals were inserted in a triangular array to measure two-dimensional finite strains around the large coronary artery in the left ventricular anterior free wall. After 15 minutes of ischemia and reperfusion, strains were depressed at a stable level of 30% to 40% below control values between 1 and 6 hours after reperfusion. The direction of maximal shortening deformations was midway between circumferential and longitudinal directions during control and did not shift after reperfusion. In a second group of five rabbits, cardiac myocytes were isolated from the region with postischemic dysfunction after 15 minutes of ischemia and 45 minutes of reperfusion. We compared in vitro function in 45 postischemic myocytes with 48 cardiac myocytes isolated from five normal rabbits. Each rabbit (postischemic and control) contributed 9±1 (SD) myocytes to the study. All myocytes were studied within 1 hour after myocyte isolation (=3 to 5 hours after reperfusion for postischemic myocytes). Myocytes were stimulated at 0.5 Hz and perfused with 2 mmol/L [Ca2+] Tyrode's solution to measure unloaded cell shortening. There was significantly less shortening in postischemic myocytes (12.4±2.1%) than control myocytes (16.2±1.2%). Maximal cell length (Lmax) was significantly longer in postischemic (134±7 μm) than control myocytes (122±7 μm), as was minimum cell length (Lmin) (118±8 versus 103±9 μm, respectively). The duration of shortening (time from stimulation to Lmax) was significantly shorter in postischemic (279±56 milliseconds) than control myocytes (405±44 milliseconds). Peak rates of cell shortening (−dL/dt) and lengthening (+dL/dt) did not differ.

Conclusions In rabbits, 15 minutes of ischemia produced a stable depression in finite strains for 1 to 6 hours after reperfusion, with shortening deformations reduced by =30% to 40% without a shift in direction. Cardiac myocytes isolated from postischemic myocardium display functional impairments in vitro similar to those measured in vivo, with an ≈25% reduction in unloaded myocyte shortening and decreased contraction duration. This indicates that ischemia and reperfusion induce intrinsic impairments in contractility independently of external loading conditions. This model may be useful for examining cellular mechanisms of postischemic myocardial dysfunction. (Circulation. 1994;90:1942-1950.)

Key Words contractility ischemia reperfusion cells

Brief episodes of ischemia may produce a prolonged but reversible myocardial depression without causing irreversible cell injury. This phenomenon, called myocardial stunning, may be clinically important with coronary reperfusion early during acute myocardial infarction. The mechanisms of postischemic dysfunction are not entirely clear but may involve damage by oxygen-derived free radicals, transient calcium overload, impaired excitation-contraction (E-C) coupling, and/or damage to the extracellular collagen matrix. The mechanisms may differ depending on the experimental model. Postischemic dysfunction has been produced in several experimental models, including the intact animal (with single or repeated episodes of regional ischemia and reperfusion), isolated heart (global ischemia and reperfusion), and isolated cardiac muscle and myocytes (reversible hypoxia or anoxia, with or without metabolic inhibition). Although intact animal models of ischemia and reperfusion are more relevant for clinical conditions, the experimental variables are difficult to control. In contrast, the experimental variables are well controlled in isolated cardiac muscle and myocyte preparations, but major assumptions are required about how to “mimic” myocardial ischemia and reperfusion.

The goal of this study was to characterize an isolated myocyte model using the intact animal to produce postischemic dysfunction. We occluded the coronary artery in anesthetized rabbits for 15 minutes followed by reperfusion to produce postischemic dysfunction. Regional function was evaluated by measuring two-dimensional finite strains by techniques developed in this laboratory. After producing postischemic dysfunction...
in vivo, we isolated cardiac myocytes from the region subjected to ischemia and reperfusion to measure in vitro function in postischemic myocytes. This was compared with in vitro function in normal myocytes isolated from normal rabbits. This model has the advantage of producing postischemic dysfunction in the complex milieu of the intact animal, while cardiac myocytes are studied under well-controlled in vitro conditions with matched ionic and loading conditions (zero external load). This in vivo model of postischemic dysfunction represents the consequences of exposing myocardium to the complex mechanical and metabolic alterations produced by regional ischemia with potential exposure to oxygen-derived free radicals and calcium overload after reperfusion.

This study demonstrates that myocytes isolated from myocardium with postischemic dysfunction display functional impairments in vitro similar to those measured in vivo, indicating intrinsic contractile abnormalities that are independent of altered external loading conditions. This isolated myocyte model may be useful for studying cellular mechanisms of postischemic dysfunction.

**Methods**

**Intact Animal Studies**

**Experimental Preparation**

New Zealand White rabbits of either sex were sedated with ketamine (33 mg/kg IM) and xylazine (3.3 mg/kg IM). The chest, neck, and femoral regions were shaved. A tracheostomy was performed to intubate the animal and place it on a small-animal respirator. An auricular vein and femoral artery and vein were cannulated. Anesthesia was maintained with ketamine (33 mg/kg IV every hour) and pentobarbital (25 mg/kg IV initially, then 3.3 mg/kg as needed). Arterial blood samples were obtained every 15 to 30 minutes. Arterial pH was maintained between 7.35 and 7.45 by varying respiratory rate and/or with intravenous bicarbonate, and arterial Po2 was maintained at >80 mm Hg with supplemental oxygen. A rectal thermometer was placed to monitor core body temperature, which was maintained with a circulating water heating pad. The heart was exposed with a midline sternotomy. A 2.5F high-fidelity micromanometer (Millar) was inserted through a stab wound in the left ventricular (LV) apex to measure LV pressure. A Transonic 4SB flow probe was placed around the ascending aorta to measure stroke volume and to time end ejection. Leads for a surface ECG were placed.

Regional two-dimensional finite strains were measured with three ultrasonic crystals placed in a triangular array by methods developed in this laboratory.5 Arrow-shaped crystals (≈1 mm wide and 2 mm long) were inserted through small epicardial stab wounds until the base of the crystal was submerged 1 mm below the epicardial surface. Since the LV wall thickness is ≈3 to 5 mm (in 3- to 4-kg rabbits), the main body of the crystal was positioned in the midmyocardium. The crystal triangle straddled the large coronary artery along the LV anterior free wall. Each crystal focused on the other two crystals to measure all three segment lengths (≈6 to 9 mm per side). One crystal pair was aligned along the LV long axis, defined by the aortic root and LV apex. To measure the orientation of the crystal triangle relative to the LV long axis, a photograph of the crystal entry (and wire exit) sites was obtained with the camera lens parallel to the epicardial surface.

**Experimental Protocol**

A suture was placed around the large coronary artery that courses along the anterolateral surface of the left ventricle. Baseline measurements were made with respirations suspended at end expiration. The coronary artery was ligated to produce acute ischemia of the anterolateral wall, then released after 15 minutes to allow reperfusion. We measured LV function at 10 and 30 minutes and 1, 2, 3, 4, 5, and 6 hours after reperfusion. Fig 1A summarizes the experimental protocol.

**Data Analysis**

Data (ECG, LV pressure, and three segment lengths) were converted from analog to digital on-line at 500 Hz on a 386 computer with a Data Translation A/D board and AR Convert software (Dataq Instruments). A customized software program was used to calculate two-dimensional finite strains from the three lengths of the crystal triangle and their orientation relative to the LV long axis. Finite strains were calculated as described previously.5 We used an equation that defines a symmetric strain tensor, \( \varepsilon_{ij} \), where strains are related to small but finite distances before (\( \Delta s_i \)) and after (\( \Delta s_{i'} \)) deformations by

\[
\Delta s^2 = \Delta s_{i'}^2 = \sum_{i=1}^{3} \sum_{j=1}^{3} 2E_{ij} \Delta s_i \Delta s_j
\]

where \( \Delta s_i \) values are the coordinate components of the triangle edges in the diastolic configuration with references to a cartesian coordinate system. This equation was applied to the three line segments with end diastole used for the undeformed reference configuration and end systole (timed by the aortic flow tracing) for the deformed configuration. The three algebraic equations were solved to calculate the independent components of the symmetric strain tensor. The three strains consist of the normal circumferential (\( \varepsilon_n \)) and longitudinal (\( \varepsilon_l \)) strains and an in-plane shear strain (\( \varepsilon_{s} \)). An eigenvalue problem was solved to calculate the two principal strains, \( \varepsilon_n \) and \( \varepsilon_l \), and the direction of the principal axes with respect to the reference coordinate system where the circumferential direction (perpendicular to the LV long axis) was defined as 0°. Positive angles denote a counterclockwise direction from circumferential. The magnitudes of the principal strains are independent of the coordinate system, and the principal axes are mutually orthogonal. In this coordinate system, all deformations are extensional (ie, shortening or lengthening) without in-plane shears, and the principal angles define the orientation of the principal axes. The principal strains were ranked arbitrarily as \( \varepsilon_n \), \( \varepsilon_l \), or \( \varepsilon_{s} \), so that the end-systolic first principal strain (\( \varepsilon_n \)) was the more negative of the two principal strains and represents the greatest shortening.

**Isolated Myocyte Studies**

**Myocyte Isolation**

Myocytes were isolated from hearts after postischemic dysfunction produced by 15 minutes of coronary artery occlusion and 45 minutes of reperfusion. We confirmed that the ultrasonic crystals were within the ischemic bed by the presence of wall motion abnormalities during coronary artery occlusion and inclusion of all crystals within the region with color changes during ischemia. Postischemic dysfunction was confirmed by a significant decrease (>25%) in shortening strains 45 minutes after reperfusion. The crystals were removed, and the heart was excised, rinsed free of blood, and mounted in a Langendorff perfusion apparatus. The aorta was perfused for 6 to 8 minutes with a Ca2+-free Tyrode's solution containing (in mmol/L): NaCl 140, KCl 5.4, MgCl2 1, Na2HPO4 0.33, glucose 10, and HEPES 5, pH 7.4 at 37°C. The perfusate was changed to 50 μmol/L Ca2+ Tyrode's solution with 0.75 mg/mL type II collagenase (Boehringer Mannheim Corp) and 0.16 mg/mL protease (Sigma Chemical Co) for 25 to 30 minutes. Oxygen was bubbled through all perfusate solutions to avoid hypoxia during the digestion process.

Three small pieces of suture material were placed through the stab wounds to mark the sites of the three crystals. The left
ventricle was dissected free and placed in a Petri dish filled with the same solution. A stainless steel biopsy punch (6 mm diameter) connected to a solution-filled syringe was inserted through the center of the crystal triangle site and advanced until it contacted the Petri dish. This mechanically isolated the myocardium where function had been measured. The myocardial sample was aspirated and placed in a separate Petri dish, where the tissue was gently teased apart to disperse myocytes. The myocyte-containing solution was placed in a conical tube to allow viable myocytes to settle by gravity, while nonviable myocytes and fibroblasts remained suspended in solution. Serial washes were used to remove nonviable myocytes and digestive enzymes, while increasing from a 50 μmol/L to 2 mmol/L [Ca2+] solution of minimum essential medium (Gibco). Myocytes were stored at room temperature in minimum essential medium. Control myocytes were isolated in a similar manner from hearts excised from normal rabbits after an intravenous overdose of 125 mg/kg sodium pentobarbital.

**Evaluation of Myocyte Function**

Myocytes were isolated from the left ventricle of normal rabbits. Fig 1B shows representative data from one rabbit with LV pressure, three segment lengths from the crystal triangle (A, B, and C), and aortic flow. Data were obtained before (solid lines) and after (dotted lines) 15 minutes of ischemia and 4 hours of reperfusion. Finite strains calculated from these data are shown in Fig 3. Postischemic dysfunction was evident by a 20% to 25% decrease in shortening deformations in the circumferential direction (end-systolic $E_{1s}$ decreased from $-0.20$ to $-0.16$) and longitudinal direction (end-systolic $E_{2l}$ decreased from $-0.16$ to $-0.12$). In-plane shear strains by an unpaired, two-tailed $t$ test. We correlated myocyte shortening with peak $+dL/dt$ for all myocytes by linear regression. We determined whether the slope of this linear regression differed between postischemic and control myocytes by calculating $t$ values (difference in regression slopes divided by standard error of difference of regression slopes) and using two-tailed critical values of $t$. In all cases, statistical significance was defined at a level of $P<.05$. All results are presented as mean±SD except in the Table, where the SEM of the slope and intercept data for the linear regressions are given.

**Results**

Fig 1 summarizes the in vivo and in vitro experimental protocols. In Fig 1A, seven rabbits underwent 15 minutes of ischemia and 6 hours of reperfusion, with finite strains measured throughout. In Fig 1B, five rabbits underwent 15 minutes of ischemia and 45 minutes of reperfusion, with regional function measured. The heart was removed to isolate cardiac myocytes, a process generally requiring 2.5 to 3 hours. Isolated myocyte function was measured within 1 hour after myocyte isolation, which corresponded in time to 3 to 5 hours after reperfusion. Cardiac myocytes were isolated from a third group of control rabbits (not shown) and also were studied within 1 hour after myocyte isolation.

**Postischemic Dysfunction in the Intact Left Ventricle**

Fig 2 shows representative data from one rabbit with LV pressure, three segment lengths from the crystal triangle (A, B, and C), and aortic flow. Data were obtained before (solid lines) and after (dotted lines) 15 minutes of ischemia and 4 hours of reperfusion. Finite strains calculated from these data are shown in Fig 3. Postischemic dysfunction was evident by a 20% to 25% decrease in shortening deformations in the circumferential direction (end-systolic $E_{1s}$ decreased from $-0.20$ to $-0.16$) and longitudinal direction (end-systolic $E_{2l}$ decreased from $-0.16$ to $-0.12$).

**Statistical Analysis**

For intact animal studies, a one-way repeated-measures ANOVA was used to determine whether postischemic dysfunction altered finite strains ($E_{1s}$, $E_{2s}$, $E_{1l}$, or $E_{2l}$) or principal axes. When significant changes were found, post hoc analysis with Newman-Keuls test was performed on data from four time periods: control and 1, 2, and 4 hours after reperfusion. For isolated myocyte studies, myocyte shortening, peak $±dL/dt$, and time to peak $+dL/dt$ were measured. The mean value of all myocytes from each rabbit was used to represent that individual rabbit. The myocyte data for the five rabbits undergoing ischemia and reperfusion were compared with the myocyte data from the five control rabbits measured as indices of contraction duration. Data from 5 to 10 consecutive beats were averaged.

![Fig 1. Schematic of experimental protocols. A. The in vivo protocol with 15 minutes of ischemia followed by 6 hours of reperfusion in the intact rabbit, with measurements of two-dimensional finite strains in the anterior left ventricle. B. The in vitro protocol. Rabbits underwent 15 minutes of ischemia and 45 minutes of reperfusion with measurements of regional function. The heart was excised to isolate cardiac myocytes from the postischemic myocardium. Postischemic myocytes were studied within 1 hour after myocyte isolation, which was 3 to 5 hours after reperfusion. A third group of rabbits (not shown) underwent myocyte isolation without prior ischemia and reperfusion. These normal myocytes also were studied within 1 hour after myocyte isolation.](image-url)
(E_{12}) were small and did not change. The first principal strain (E_1) describes maximal deformations regardless of direction. In this example, maximal deformations occurred at -15° during control and at -10° after ischemia and reperfusion. Since this first principal angle was close to 0° and shear strains were small, maximal deformations were similar to circumferential strains (E_{11}=E_{1}) during control and after postischemic dysfunction. Similarly, the second principal strains (E_2), which describe deformations perpendicular to E_1, were similar to longitudinal strains (E_{2}=E_{2}). Since postischemic dysfunction did not alter the principal angle or in-plane shear strains, there were similar decrements in strains regardless of orientation.

Group data for the seven rabbits (mean±SD) are presented in Figs 4 and 5. Data are shown before (control) and at multiple time points (10 and 30 minutes and 1, 2, 3, 4, 5, and 6 hours) of reperfusion after 15 minutes of ischemia (n=7 except at 5 and 6 hours, n=6). Fig 4 shows that there was no significant change in heart rate or peak or end-diastolic LV pressure from 10 minutes to 6 hours after postischemic reperfusion. Fig 5 shows the end-systolic two-dimensional finite strain data. After ischemia, there was a significant decrease in circumferential (E_{11}), longitudinal (E_{22}), first principal (E_1), and second principal (E_2) strains. The most severe depression occurred 10 and 30 minutes after reperfusion, then strains were stable after the first hour. Post hoc analyses of the 1-, 2-, and 4-hour reperfusion data showed that each strain was significantly depressed from control (except for E_{11} at 1 hour), with no significant difference between the three reperfusion time periods.

In-plane shear strains (E_{12}) were small and did not change significantly with postischemic dysfunction. The first principal angle was 47±46° during the control period and did not change significantly with postischemic dysfunction. From 1 to 6 hours after reperfusion, the first principal angle was 47±37°, with an absolute change in angle from control of 21±17°. Since the first principal angle was midway between

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**Fig 2.** Representative traces from a rabbit showing (from top to bottom) left ventricular pressure (LVP), three segment lengths (A, B, and C), and aortic flow (Q) before (solid lines) and after (dotted lines) 15 minutes of ischemia and 4 hours of reperfusion. The three segment lengths composed a triangle, with segment A oriented 14° counterclockwise from the longitudinal direction and segments B and C completing the crystal triangle (in a clockwise sequence). Postischemic dysfunction did not alter LVP at end diastole or peak systole but decreased shortening in all segments and decreased aortic flow.

**Fig 3.** Plots showing two-dimensional finite strains calculated from Fig 2, beginning just before end diastole. As in Fig 2, data are shown before (solid lines) and after (dotted lines) 15 minutes of ischemia and 4 hours of reperfusion. Negative values indicate shortening deformations. E_{11} indicates circumferential strain (at 0°); E_{22}, longitudinal strain (at 90°); E_{12}, in-plane shear; E_1, first principal strain (maximal shortening deformation regardless of orientation); and E_2, second principal strain (deformation perpendicular to E_1). In this example, the first principal angle (orientation of E_1) was -15° before and -10° after ischemia and reperfusion. See text for additional discussion.

**Fig 4.** Plots showing heart rate (HR, solid circles) and peak (open diamonds) and end-diastolic (solid triangles) left ventricular pressure (LVP) before (control, to the left of the break on the x axis) and at several time points after reperfusion following 15 minutes of ischemia. Data are presented as mean±SD, with n=7 for all time periods except 5 and 6 hours (n=6). There was no significant change in heart rate or LVP compared with control.
circumferential and longitudinal directions, both circumferential and longitudinal strains underestimated maximal deformations (E_{1l} and E_{22}<E_l) by 21±20% during the control period and by 20% to 50% after the first hour of reperfusion. Nevertheless, postischemic dysfunction depressed all three strains (E_{1l}, E_{22}, and E_l) to a similar extent of 30% to 40% below their control values.

Fig 6 shows a linear relation between normalized circumferential (E_{1l}, top) or longitudinal (E_{22}, bottom) strain and normalized maximal shortening (E_l). Data are from all time periods 1 or more hours after reperfusion, with strains normalized to their control value. Although the slope was 1.0 with a zero intercept for the correlation between normalized E_{1l} and E_l, the r value was .63. A significant number of points fell below the 95% confidence limit of the regression, indicating a greater decrement in circumferential than maximal shortening. This was due to a shift in the first principal angle away from the circumferential direction after reperfusion, causing E_{1l} to overestimate the severity of functional impairments. There were also a number of points above the 95% confidence limit due to a shift in the first principal angle closer to the circumferential direction after reperfusion, causing E_{1l} to underestimate the severity of functional impairments. There are values of normalized E_{1l}>1.0 that indicate higher circumferential shortening in the postischemic compared with the control period. However, maximal shortening never exceeded control (normalized E_{i}≤1.0). Thus, an E_{i}>1.0 does not indicate postischemic "hyperfunction" but merely reflects a shift in the first principal angle closer to the circumferential direction. Although there was a tighter correlation between the decrease in E_{22} and E_l with an r value of .73, the slope was steeper (1.61) with a nonzero y intercept (~0.53). Thus, longitudinal shortening decreased to zero when maximal shortening was still 30% to 40% of control values.

Postischemic Dysfunction in Isolated Cardiac Myocytes

We studied 45 myocytes isolated from the postischemic myocardium of five rabbits that underwent 15 minutes of ischemia and 45 minutes of reperfusion ("postischemic" myocytes) and 48 myocytes isolated from five normal rabbits (control myocytes). Immediately after cell isolation, 70% to 90% of the myocytes were quiescent and rod-shaped, which was similar for both postischemic and control myocytes. In all cases, myocytes were stimulated at 0.5 Hz. Each rabbit is represented by the mean value of 9±1 myocytes studied from that individual rabbit within the first hour after myocyte isolation. Fig 7 shows data (mean±SD) for control myocytes (n=5 control rabbits) and postischemic myocytes (n=5 rabbits undergoing ischemia and reperfusion). Myocyte shortening (top) was significantly lower (by ~25%) in postischemic myocytes (12.4±2.1%) compared with control myocytes (16.2±1.2%). The bottom panel of Fig 7 shows that the resting myocyte length (L_{max}) and minimal myocyte length (L_{min}) were significantly longer (by ~10%) in postischemic compared with control myocytes.

The top panel of Fig 8 shows that there was no significant difference in peak rate of cell shortening
Discussion

This study characterizes functional impairments in cardiac myocytes isolated from rabbits with postischemic dysfunction. Myocyte shortening was depressed in postischemic myocytes to an extent similar to the decrease in shortening deformations measured in the intact ventricle after ischemia and reperfusion. Since myocytes were evaluated in the absence of external loads, decreased shortening in postischemic myocytes represents intrinsic impairments in contractility that occur independently of the altered external loading conditions that exist with postischemic dysfunction in the intact animal. Myocyte contraction duration was

Regression Data for Myocyte Shortening vs Peak Lengthening Rate

<table>
<thead>
<tr>
<th>No.</th>
<th>Slope</th>
<th>Intercept</th>
<th>Multiple</th>
<th>r</th>
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<tbody>
<tr>
<td>Control myocytes</td>
<td>48</td>
<td>8.4±1.5</td>
<td>11.2±24.7</td>
<td>.64</td>
</tr>
<tr>
<td>Postischemic myocytes</td>
<td>45</td>
<td>13.9±2.0*</td>
<td>-0.4±13.8</td>
<td>.73</td>
</tr>
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Regression of percent myocyte shortening vs peak lengthening rate (+dL/dt) data from Fig 9. Slope (μm·s⁻¹·%⁻¹) and intercept (μm/s) values are given as mean±SEM. *P<.05, postischemic vs control myocytes.
also depressed. This isolated myocyte model may be useful for studying cellular mechanisms of postischemic dysfunction using the clinically relevant model of regional ischemia and reperfusion in the intact animal.

The experimental model is an important determinant of the mechanisms for postischemic dysfunction. Several isolated muscle and myocyte models of “postischemic” dysfunction have used transient exposure to hypoxia or anoxia, acidosis, and metabolic inhibitors (eg, cyanide and 2-deoxyglucose). Although these models provide valuable insight into cellular alterations induced by reversible hypoxia or metabolic inhibition, these models do not replicate the complexities found in the intact animal, in which neutrophils and platelets may contribute to postischemic dysfunction. The absence of blood components may also affect the results in isolated, buffer-perfused hearts exposed to global ischemia and reperfusion. Finally, in isolated muscle and heart models, potentially important mechanical factors are minimized or eliminated. With regional myocardial ischemia, marked stretching of ischemic segments (“creep”) may contribute to systolic abnormalities. With global ischemia, ventricular pressures are low and the ischemic ventricle is not exposed to the same high wall stress with each heartbeat that contributes to repeated cycles of mechanical overdistension. Thus, the mechanisms of “postischemic” dysfunction in isolated myocyte, muscle, and heart models depend to some extent on assumptions used to mimic myocardial ischemia and reperfusion. In the present study, we used regional ischemia and reperfusion in the intact animal as a clinically relevant model for producing the complex changes associated with postischemic dysfunction.

The first issue addressed in this study was whether 15 minutes of ischemia followed by reperfusion produces stable myocardial depression in rabbits. The rabbit, like humans, lacks significant coronary collaterals. The relation between the duration of ischemia and infarction is sigmoidal in the rabbit, with little damage produced when ischemia lasts for <15 minutes. Although segment shortening decreases with ischemia and reperfusion, we were concerned that unidimensional measurements might not accurately characterize impairments with postischemic dysfunction. This concern was based on our prior finding that under physiological conditions, circumferential segment measurements underestimated maximal shortening by 30% to 40%, depending on the ventricular volume and region. This was caused by a shift in the direction of maximal deformations away from the circumferential direction. We also found that myocardial ischemia markedly shifted the direction of deformation, so that unidirectional measurements underestimated the severity of functional impairments. Therefore, we adapted our ultrasonic technique to measure two-dimensional finite strains in rabbits to determine whether postischemic dysfunction alters the direction, as well as magnitude, of systolic deformations.

We found that maximal deformations in the anterolateral wall of the rabbit ventricle occur midway between the circumferential and longitudinal directions. As a consequence, both circumferential and longitudinal strains underestimated maximal shortening deformations by ≈20%. Postischemic dysfunction did not significantly alter the direction of maximal shortening deformations. In some cases, however, small shifts in the first principal angle closer toward (or away from) the circumferential direction did cause circumferential strain to underestimate (or overestimate) the severity of functional impairments. Regional function was depressed at a stable level for several hours after the first hour of reperfusion. Compared with the control period, there was no shift in the direction of maximal shortening deformations, and in-plane shear strains remained small from 1 to 6 hours after reperfusion. Therefore, the relation between circumferential or longitudinal strain and maximal shortening deformations did not differ significantly between the stable phase of postischemic dysfunction compared with control. There was a linear relation between normalized circumferential or longitudinal strains and normalized maximal shortening deformations during stable postischemic dysfunction (each strain normalized to its control value before ischemia). Even though circumferential and longitudinal strains underestimated maximal shortening deformations, there was a similar (=25%) decrease in all three strains compared with their control values. From a practical standpoint, a unidirectional measurement such as circumferential or longitudinal segment shortening may underestimate the absolute value of maximal shortening, but a significant decrease in segment shortening would accurately reflect a similar percent decrease in maximal shortening during stable postischemic dysfunction (from 1 to 6 hours after reperfusion).

In the intact heart, a decrease in segment shortening or wall thickening may not necessarily indicate a decrease in contractility if there are concomitant changes in loading conditions. Both diastolic and systolic loads are altered by postischemic dysfunction. Myocardial ischemia increases passive segment lengths at zero transmural pressure (L0) by 16%. This phenomenon, called diastolic creep, persists for 4 hours after reperfusion following 15 minutes of ischemia. The recovery of diastolic creep correlates with the recovery of systolic function. The mechanisms of diastolic creep are not known but may involve disruption of the extracellular collagen matrix. Extensive damage of the extracellular collagen matrix occurs in myocardial postischemic dysfunction models produced by repeated10,11 but not single12 episodes of ischemia and reperfusion. Increased wall stress at the border of postischemic regions would also decrease segment shortening independently of changes in contractility. Stress amplification is predicted in models of myocardial infarction, where wall stress is twofold to fourfold higher in nonischemic regions directly adjacent to the ischemic zone. If diastolic creep or stress amplification is present with myocardial postischemic dysfunction, it would be difficult to distinguish whether decreased shortening in the intact heart is related to altered loading conditions or due to a decrease in contractility.

An advantage of studying isolated myocytes is the ability to compare function under matched ionic and external loading conditions. Unloaded shortening was measured so that external loads were largely eliminated in both control and postischemic myocytes. The glass coverslips were not coated (eg, with laminin) to minimize myocyte adhesion, since shortening is lower in adherent myocytes compared with unattached myocytes. Thus, depression in postischemic myocyte
function could be attributed to intrinsic impairments in contractility unrelated to alterations in external load, such as occur with postischemic dysfunction in the intact ventricle.

The postischemic myocardial depression documented in vivo was present in vitro as well. Cardiac myocytes isolated from the postischemic myocardium had ≈25% lower myocyte shortening than control myocytes during the first hour after myocyte isolation. Since hearts were excised 45 minutes after reperfusion and it required ≈2.5 to 3 hours to isolate cardiac myocytes, the first hour after myocyte isolation was ≈3 to 5 hours after reperfusion in the intact animal (Fig 1B). In the intact-animal protocol, maximal shortening deformations (first principal strain, E,) were reduced by ≈30% at 3 to 5 hours after reperfusion. Thus, functional impairments in the postischemic myocardium in vivo were comparable to the impairments found in postischemic myocytes studied in vitro. This indicates that the alterations in external loading conditions that occur with ischemia and reperfusion in the intact animal are not the major cause for postischemic dysfunction.

Postischemic myocytes had ≈10% longer resting cell lengths than control myocytes. Since there are no external loads in the resting myocyte, this is analogous to the diastolic creep described in the intact heart.6 These changes may be related to the repeated episodes of systolic stretch during regional ischemia in vivo. Mechanical overdistension is not present in intact-heart models with global ischemia or in isolated myocyte or muscle models with transient hypoxia, acidosis, and/or metabolic inhibition.

The duration of contraction was shorter in postischemic than control myocytes. However, there were no significant differences in peak rates of myocyte shortening (−dL/dt) or shortening (+dL/dt). Since postischemic myocytes were ≈10% longer than control myocytes, this may be associated with longer sarcomere lengths in postischemic myocytes as well. If so, this would increase peak shortening rates, thus obscuring any difference between postischemic myocytes and control myocytes.

Peak velocity of muscle lengthening is linearly related to the extent of muscle shortening.16 We found a linear relation between peak +dL/dt and myocyte shortening when all myocytes were combined. This relation was steeper for postischemic than control myocytes. Peak +dL/dt was higher in postischemic than control myocytes when compared at the same myocyte shortening.

Alterations in lengthening rates may be related to sarcoplasmic reticulum (SR) impairments with postischemic dysfunction. Although postischemic dysfunction decreases SR Ca2+ uptake and maximal SR Ca2+,Mg2+ ATPase activity by ≈20% in some studies,17-19 others report normal SR function or even a slight increase in SR Ca2+ uptake.20,21 Our finding of higher peak lengthening rates in postischemic myocytes suggests that SR impairments, if they occur, may not be of major functional importance. Differences in peak lengthening rates may also reflect alterations in myofilament binding to calcium. Postischemic dysfunction is associated with an increase in myofilament sensitivity to calcium in some22,23 but not all24 studies. Decreased binding of calcium to troponin C may allow more rapid dissociation and cell lengthening.

Peak rates of myocyte lengthening reflect not only the rate of SR Ca2+ uptake but also intracellular restoring forces.25 Although we largely eliminated external loads with our protocol, we cannot rule out differences in internal loads. It is conceivable that postischemic myocytes had higher +dL/dt because of lower internal loads and/or enhanced intracellular restoring forces. There is indirect evidence of structural alterations in postischemic myocytes, which had ≈10% longer resting myocyte lengths compared with control myocytes. There are several possible mechanisms for these findings. Ischemia and reperfusion may alter sarcolemmal properties, impairing normal homeostatic functions that maintain osmotic gradients. This could result in myocyte swelling and lengthening of postischemic myocytes. Ischemia and reperfusion can disrupt microtubular structures, with recovery after several days.26 This may be related to a transient calcium overload. Cytoskeleton changes can contribute to functional alterations in cardiac myocytes.27 Colchicine and cold temperatures depolymerize microtubules and increase the extent and velocity of sarcomere shortening in hypertrophied cardiac myocytes.27 It is possible that disruption of microtubules in postischemic myocytes may decrease internal loads, leading to more rapid rates of myocyte shortening and lengthening.

Although isolated cardiac myocytes provide several advantages, there are potential limitations with the cell isolation procedure. We cannot rule out the possibility that exposure to in vivo ischemia and reperfusion damaged the cardiac myocytes, making them more susceptible to further damage during the cell isolation procedure. However, if postischemic cells are more susceptible to damage, they are not likely to survive the myocyte isolation procedure. We had similar yields of 70% to 90% quiescent, rod-shaped myocytes immediately after isolation of both postischemic and control myocytes. Since serial washes selectively remove damaged myocytes that form myoballs that remain suspended in solution (which are decanted and discarded), there is a selective enrichment process whereby only healthier myocytes remain to be studied. Thus, selective damage to postischemic myocytes would bias our results against finding functional impairments compared with control myocytes. However, we did clearly identify a distinct population of postischemic myocytes with significantly longer cell lengths and depressed cell shortening and shortening duration.

Another potential limitation with this model exists if postischemic dysfunction is nonuniform. We minimized this problem by using a punch biopsy to remove a sample from the center of the region that had been exposed to ischemia and reperfusion. This mechanically separated the sample from the rest of the heart and ensured that we isolated myocytes from a region in which postischemic dysfunction had been confirmed. It is possible that postischemic dysfunction is nonuniform transmurally. It was not practical to separate our samples into transmural layers because the ventricular wall thickness was only ≈3 to 5 mm and samples were taken after the myocyte digestion was nearly complete. Although this may decrease selectivity, inclusion of any normal myocytes without postischemic dysfunction would have biased the results against our hypothesis.
The results of this study should not be extrapolated to later time periods without further evaluation. We documented stable depression in ventricular function in vivo from 1 to 6 hours after reperfusion, which was comparable to the time period used for evaluating cardiac myocyte function in vitro (≈3 to 5 hours after reperfusion). Comparisons at later time periods should take into account the potential for functional recovery in vivo (ie, after several more hours of reperfusion) as well as decrements in myocyte function in vitro under normal culture conditions. Furthermore, the time course for changes in function as well as cell viability may differ between control and postischemic myocytes.

In summary, we measured two-dimensional finite strains in the intact rabbit heart to document that 15 minutes of ischemia followed by reperfusion produced a stable depression in ventricular function. Maximal systolic shortening was depressed by ≈25% to 30% for 6 hours after reperfusion. This depression was similar regardless of the orientation of the strain measurement because postischemic dysfunction did not alter the orientation of systolic deformations. Cardiac myocytes isolated from the postischemic myocardium demonstrated a myocardial depression in vitro (≈25% decrease in myocyte shortening compared with normal myocytes), which was similar to the functional impairments measured in vivo. Since myocyte function was measured in the absence of external loads, this indicates an intrinsic depression in cardiac contractility unrelated to alterations in external loading conditions, as occurs with postischemic dysfunction in the intact heart. Postischemic myocytes had a shorter contraction duration, but peak rates of shortening and lengthening did not differ from control myocytes. When compared at a matched shortening extent, peak lengthening rates were significantly higher in postischemic than control myocytes. This, along with the ≈10% increase in resting myocyte length, suggests possible changes in intracellular structure and/or internal loading conditions in postischemic myocytes. This isolated myocyte model may be useful for evaluating cellular mechanisms of postischemic dysfunction.

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