In Situ Measurements of Crossbridge Dynamics and Lattice Spacing in Rat Hearts by X-Ray Diffraction
Sensitivity to Regional Ischemia

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Background—Synchrotron radiation has been used to analyze crossbridge dynamics in isolated papillary muscle and excised perfused hearts with the use of x-ray diffraction techniques. We showed that these techniques can detect regional changes in rat left ventricle contractility and myosin lattice spacing in in situ ejecting hearts in real time. Furthermore, we examined the sensitivity of these indexes to regional ischemia.

Methods and Results—The left ventricular free wall of spontaneously beating rat hearts (heart rate, 290 to 404 bpm) was directly exposed to brief high-flux, low-emittance x-ray beams provided at SPring-8. Myosin mass transfer to actin filaments was determined as the decrease in reflection intensity ratio (intensity of 1,0 plane over the 1,1 plane) between end-diastole and end-systole. The distance between 1,0 reflections was converted to a lattice spacing between myosin filaments. We found that mass transfer (mean, 1.71 ± 0.09 SEM, n = 13 hearts) preceded significant increases in lattice spacing (2 to 5 nm) during systole in nonischemic pericardium. Left coronary occlusion eliminated increases in lattice spacing and severely reduced mass transfer (P < 0.01) in the ischemic region.

Conclusions—Our results suggest that x-ray diffraction techniques permit real-time in situ analysis of regional crossbridge dynamics at molecular and fiber levels that might also facilitate investigations of ventricular output regulation by the Frank-Starling mechanism. (Circulation. 2004;109:2976-2979.)

Key Words: ischemia ▪ myocardial contraction ▪ myosin ▪ radiography

Despite the history of studies on crossbridge dynamics, lower photon counts and poorer quality of diffraction patterns obtained from cardiac muscle than skeletal and insect flight muscles1–3 have limited progress with cardiac muscle until recently.4,5 Some of us used third-generation synchrotron radiation (SPring-8, Japan Synchrotron Radiation Research Institute) to determine x-ray diffraction patterns in excised, perfused rat hearts while moving systematically across the left ventricular (LV) equator from the epicardium through to the ventricular cavity.6

X-ray diffraction patterns of cardiac muscle produce 2 equatorial-position reflections from the lattice-like arrangement of its protein elements.2 Mass transfer of myosin heads to actin during contraction is inferred from a decrease in the integrated 1,0 reflection intensity (I_{1,0}, lattice plane containing only thick myosin filaments) and an increase in 1,1 reflection intensity (I_{1,1}, plane with thick myosin and thin actin filaments).7 The myocardial intensity ratio (defined as I_{1,0}/I_{1,1}) is minimal in the rigor state and maximal in a quiescent state.1,2,6,8

Furthermore, the distance between 1,0 reflection peaks (d_{1,0} spacing) represents the myosin lattice spacing, which is inversely related to sarcomere length in isolated fibers5 as static myocytes maintain a constant cell volume. Whether decreases in myofilament spacing contribute to increasing Ca^{2+} sensitivity and increased probability of crossbridge formation at longer sarcomere lengths has been actively debated.9 However, it is still not known if lattice spacing is regulated to maintain constant lattice volume (ie, if lattice cross-sectional area decreases with increasing sarcomere length, then interfilament spacing must decrease) during dynamic contractions in vivo.

Recently, it was shown that the intensity ratio derived from x-ray diffraction patterns of isolated whole hearts decreased during isovolumic contractions with a similar time course throughout the LV,6 implying that crossbridge cycling in fibers of different myocardial layers is similar despite differences in fiber orientation and rate of short-
ening. However, it was not possible to follow dynamic lattice spacing changes. In the present study, we used a fine-focused x-ray beam to record diffraction patterns of a localized region of the LV of ejecting rat hearts in situ and then determined crossbridge cycling and myosin lattice spacing.

**Methods**

**Animals and Surgical Preparation**

Anesthetized (50 mg/kg sodium pentobarbital IP) male Sprague-Dawley rats (Japan SLC, Hamamatsu, Japan), 9 to 10 weeks of age (350 to 400 g), were artificially ventilated and thoracotomized. Procedures were performed according to SPring-8 guidelines for the care and welfare of experimental animals. The heart was continuously irrigated while the apex was raised by a manipulator paddle and restrained by 2 superficial sutures in the LV to minimize vertical movements. Pressure-volume loops were recorded from an apically inserted 1.4F micromanometer (SPR-671 Millar Instruments) and a 1.5F conductance catheter (S-I Medico-tech Co Ltd, Osaka) to determine the temporal sequence of cardiac events and heart rate (determined from end-diastole [ED] interval).

**X-Ray Diffraction With Collimated Synchrotron Radiation**

Measurements were conducted at the 40XU beamline of SPring-8. A collimated quasimonochromatic beam (wavelength, 0.08 nm) with a beam flux of \( \sim 10^{12} \) photons per second (15 keV; ring current, 60 to 100 mA) and dimensions 0.2 \( \times \) 0.2 mm was focused at an oblique tangent to the myocardium (\( \sim 3 \) m from the detector). The ventilator was stopped at end-expiration to reduce heart movements during measurements (\( \sim 2.1 \) seconds). Images were digitally recorded at a 15-ms sampling interval with the use of an image intensifier and a fast CCD camera, simultaneous with pressure-volume analog signals (1000-Hz sampling frequency). The beam passed through the apical myocardium between the ends of the descending branch of the left coronary artery (LAD) and the posterior interventricular vein. Final burning of the recorded region (higher energy levels) confirmed that the beam only exposed fibers in the epicardium and part of the intermediate layer (histological inspection).

**Acute Ischemia Treatment**

Heart baseline recordings were established, permanent ligation of the proximal LAD was performed, and recordings were repeated 5 to 10 minutes later.

**Intensity Ratio Calculations and Analyses**

Integrated intensity of \( I_{1,0} \) and \( I_{1,1} \) was determined from the areas under the reflection peaks after background subtraction. Intensity ratio (\( I_{1,0}/I_{1,1} \)) was used rather than absolute reflection intensities of \( I_{1,0} \) and \( I_{1,1} \), which are influenced by changes in the quantity of fibers sampled during contractions. Myosin mass transfer index was defined as the difference in intensity ratio between ED and end systole (ES).

**Results**

**Mass Transfer and Lattice Spacing in Nonischemic Hearts**

Intensity ratio significantly decreased during systole (increase in LV pressure [LVP] and decrease in LV volume [LVV]) and conversely, increased during diastole under the baseline rhythm (Figure 1a). Averaging intensity ratio over multiple beats reduced variability during diastole in the otherwise sinusoidal patterns (black lines, Figure 1b). With regard to time, \( d_{1,0} \) spacing increased continuously during systole and then decreased during diastole, suggesting that considerable changes occur in the myofilament spacing (red line, Figure 1b).

Intensity ratio averaged \( 2.80 \pm 0.11 \) (SEM, \( n=13 \) hearts) at ED, and the average myosin mass transfer index was \( 1.71 \pm 0.09 \). In all hearts, the decrease in intensity ratio during crossbridge formation was completed before the full extent of the \( d_{1,0} \) spacing change (2 to 5 nm between hearts, Figure 2a). Furthermore, at any given LVV, the \( d_{1,0} \) spacing during systole was 1 to 2 nm larger than diastole (Figure 2b).

**Mass Transfer and Lattice Spacing During Regional Ischemia**

LAD occlusion reduced the intensity ratio change and prevented normal lattice spacing increase, consistent with reduced contractility of the ischemic region (Figure 1, c and d). Occlusion significantly increased intensity ratios at both ED (\( P<0.05 \)) and ES (\( P<0.001 \)) in the same LV region (\( n=6, \)
Figure 2c). Although mean heart rate was not depressed (341±16 bpm, 4% increase) and there was only a small decrease in mean LV ES pressure (−26.9±5.8 mm Hg SEM), regional ischemia severely depressed the mass transfer index (55% of baseline).

Discussion
Our data clearly demonstrate that current synchrotron technology can produce sufficient energy to obtain well-defined reflections from single exposures to enable calculation of intensity ratio and d_{1,0} spacing in situ rat hearts (Figure 1). The mean ED intensity ratio of this study is similar to 2.96 obtained from LV in arrested rat hearts under normoxic perfusion. Furthermore, in rat papillary muscles, the resting ratio was 3.07. The results presented here were restricted to epicardial recordings (within 0.8-mm depth), consisting of helically orientated fibers, to minimize the contributions from fibers with orientations that vary at greater depth. Nevertheless, it was recently established that neither diastolic intensity ratio nor mass transfer varies with depth of x-ray beam penetration in beating, perfused hearts (paced at 2 Hz).

In Situ Changes in Myofilament Spacing
We showed that significant lattice expansion occurs during contraction and that the relation between d_{1,0} spacing and intensity ratio is not linear but a loop in nonischemic hearts (black line, Figure 2a). The d_{1,0} spacing changes during contraction (2 to 5 nm) were larger than the 1-nm difference in d_{1,0} spacing reported between the epicardium and endocardium (at diastole). Therefore, the larger d_{1,0} spacing change found in ejecting hearts cannot be explained by shifts in the fiber layers exposed to the beam. The loop formed by these indexes might contain valuable information about how crossbridge axial and radial forces alter the dynamics of lattice spacing changes. Crossbridge projections from the myosin backbone produce radial force perpendicular to that of axial force in the filament direction. Release of isometric tension in intact skeletal myofibers during sarcomere shortening causes a brief and rapid lattice spacing increase, in excess of that predicted by fiber shortening in itself. We therefore conclude that lattice volume is not constant in the dynamic state because myosin lattice spacing is significantly larger (1 to 2 nm) during contraction than ventricular filling at the same LVV (d_{1,0} spacing during systole greater than diastole, Figure 2b). Crossbridge formation probably causes a brief lattice expansion in ejecting hearts mediated by radial forces.

Sensitivity of In Situ Indexes to Regional Ischemia
The relevance of our new findings is that although the intensity ratio of beating hearts in diastole was similar to that of relaxed papillary muscles, there is a very different response of the myocardium in beating hearts to ischemia in terms of crossbridge dynamics and lattice space changes. Higher intensity ratios and more variable intensity ratio changes during systole (Figure 1, c and d) occurred as the result of lower absolute I_{1,1} in systole and a lack of consistent increase in I_{1,1} when I_{1,0} decreased (data not shown). Thus, permanent regional ischemia severely attenuated mass transfer in the epicardium (Figure 2c). Furthermore, ischemia induced increases in ED intensity ratio in vivo, whereas other studies report maximal decreases in the intensity ratio under anoxic perfusion (isolated arrested hearts) or rigor. An increase in intensity ratio might be related to metabolite accumulation or pronounced passive stretching, because fiber shortening in infarcted regions progressively decreases until fibers eventually become passively stretched (bulging) by fiber shortening in the nonischemic region. In support of the bulging possibility, we found that d_{1,0} spacing no longer increases between ED and ES after occlusion.

The cellular basis of the Frank-Starling law of the heart involves increases in contractility caused by length-dependent increases in Ca^{2+} sensitivity associated with increased ventricular filling. However, it is still debated whether increased crossbridge activation results from increased probability of crossbridge formation with decreasing lattice spacing associated with fiber stretching (see review in Reference 9). In a future publication, we will examine how LV volume loading influences mass transfer in relation to myofilament spacing and length-dependent activation of contraction in situ.

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