Effects of Coronary Artery Occlusion and Reperfusion on Cardiac Cycle-Dependent Variation of Myocardial Ultrasonic Backscatter

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SUMMARY. We have recently reported a systematic variation in integrated ultrasonic backscatter throughout the cardiac cycle in canine hearts. This study was performed to determine whether the pattern of such variation is modified systematically by ischemia. Measurements of integrated ultrasonic backscatter in selected regions of normal, ischemic, and reperfused hearts were compared in view of known differences in systolic function of myocardium in each of these regions. Integrated ultrasonic backscatter (3–7 MHz) gated to the first derivative of left ventricular pressure was measured at the apex, midwall, and base in 10 dogs and at the apex before and during transient ischemia and reperfusion in four dogs. Quantitative integrated ultrasonic backscatter was referenced to a steel reflector. Cyclic variation of integrated ultrasonic backscatter was greatest at the apex [peak to trough variation 5.5 ± 0.9 dB (mean ± SE)] with the maximum near end diastole (−52.9 ± 0.9 dB) and minimum near end systole (−58.4 ± 1.0 dB). Variation at the apex (5.5 ± 0.9 dB) and the midwall (4.3 ± 0.8 dB) was greater than at the base (0.5 ± 1.0 dB) (P < 0.01 for either region compared with base). Left anterior descending coronary occlusion for 10 minutes in four of 10 dogs reduced variation at the apex to 0.4 ± 1.5 dB (P < 0.02 compared with preocclusion). Reperfusion for 2 hours restored apical cyclic variation to 3.9 ± 1.7 dB, i.e., to values not significantly different from those before occlusion. Thus, regional differences in cyclic variation of integrated ultrasonic backscatter in the intact canine heart are blunted promptly by ischemia and restored substantially by reperfusion. These results suggest that quantitative ultrasonic interrogation may permit assessment with ultrasound of the intrinsic properties of myocardium modified by ischemia by analysis of physical properties of the tissue per se, rather than exclusively by assessment of geometrical changes. (Circ Res 56: 683–689, 1985)

ULTRASONIC tissue characterization has been undertaken to differentiate specific pathological entities by analysis of the interaction of ultrasound with myocardial tissue (Miller et al., 1976; Gramiak et al., 1979; Chivers, 1981). This approach differs from M-mode and two-dimensional echocardiography, which employ ultrasound to depict the shape and motion of cardiac structures (Feigenbaum, 1981). Approaches to ultrasonic characterization of cardiac tissue have included qualitative interpretation of the original (Wells and Halliwell, 1981; Bhandari and Nanda, 1981) or processed (Logan-Sinclair et al., 1981) two-dimensional image, stochastic analysis of A-mode amplitude (Green et al., 1981), and gray scale analysis (Skorton et al., 1983; Collins et al., 1983). Our laboratory has employed quantitative ultrasonic backscatter, which measures energy scattered by intramural tissue, and attenuation of transmitted ultrasound to characterize normal and diseased myocardium.

We have previously observed that ischemic injury in canine myocardium alters attenuation measured in vitro (Mimbs et al., 1977) and elevates backscatter measured both in vitro and in vivo in open and closed-chest preparations (Mimbs et al., 1981a; Cohen et al., 1982). Structural correlates of these findings include increased tissue collagen (Mimbs et al., 1980) and water content (Mimbs et al., 1981a). In addition, determination of quantitative backscatter and attenuation has detected doxorubicin-induced cardiomyopathic changes in rabbit heart (Mimbs et al., 1981b) and the naturally occurring cardiomyopathy of Syrian hamsters (Pérez et al., 1984).

We have recently demonstrated that myocardial backscatter varies systematically throughout the cardiac cycle in open and closed-chest dogs, with the highest values recorded at end diastole and the lowest at end systole (Barzilai et al., 1984). These and related observations suggested that quantitative ultrasonic backscatter can be used not only to detect changes in structural components of the heart but also to detect changes in its functional state. Accordingly, we undertook the present study to determine whether quantitative backscatter can detect normal regional gradations as well as serial changes in intrinsic properties of the tissue associated with functional abnormalities induced by myocardial ischemia and reperfusion.
Methods

Ultrasonic measurements were made with the electronic equipment represented in the block diagram (Fig. 1). The pulser-receiver (Metrotek MP 215 pulser and MR101 receiver) launched broadband ultrasonic impulses from a 5-MHz focused transducer (Panametrics model V309) at the rate of 1000/sec. The transducer was mounted in a water-filled plexiglass holder fitted with a highly distensible latex balloon tip to avoid compression of subjacent myocardium. The transducer was held static at each site of interrogation by a clamp and ringstand assembly. The sample volume was defined by the ultrasonic beam width and an electronic gate triggered on the specular reflection of the epicardial surface which selected a 4-μsec portion of the backscattered RF signal, corresponding to a 3-mm-long cylinder of intramural myocardium. Signals from the sample volume were fed to an analog spectrum analyzer (Hewlett-Packard 8553) whose peak output was recorded at a rate of 500/sec by a Biomation 8100 recorder. Values from the spectrum analyzer were acquired for 2 seconds at each frequency from 3-7 MHz in 1-MHz steps under the control of a Hewlett-Packard model 9825 desk-top computer. Data acquisition for each cardiac cycle was triggered by the positive deflection of left ventricular pressure (dP/dt), a physiological event corresponding to the onset of mechanical systole. After compensation for a 50-msec time shift introduced by an electronic filter used in the differentiator, the data collected during each cardiac cycle were time renormalized into 32 windows, and the data gathered within each window were averaged. Integrated backscatter for each window was calculated as described below in the section entitled Data Analysis.

Dog Preparations

Ten mongrel dogs (15-25 kg) were anesthetized with sodium pentobarbital, 25 mg/kg, iv, and ventilated with a Harvard respirator. A left thoracotomy was performed in the 5th intercostal space, the pericardium was opened, and the heart was suspended in a pericardial cradle to minimize respiratory motion. The left atrium was incised, and a fluid-filled catheter connected to a Statham P23dB pressure transducer was inserted across the mitral valve into the left ventricle. The pressure pulse was differentiated with respect to time with a Hewlett-Packard model 3825 desk-top computer. Data acquisition for each cardiac cycle was triggered by the positive deflection of the derivative of left ventricular pressure (dP/dt), a physiological event corresponding to the onset of mechanical systole. After compensation for a 50-msec time shift introduced by an electronic filter used in the differentiator, the data collected during each cardiac cycle was time renormalized into 32 windows, and the data gathered within each window were averaged. Integrated backscatter for each window was calculated as described below in the section entitled Data Analysis.

Three regions of the free left ventricular wall were identified, as indicated in Figure 2. The base was considered to be the region adjacent to the bifurcation of the circumflex and left anterior descending coronary arteries, the midwall was taken to be approximately half-way between the circumflex coronary artery and the tip of the apex, and the apical region was identified as the region distal to the origin of the last diagonal branch of the left anterior descending coronary artery (LAD).

Backscatter measurements were obtained at five adjacent sites within each of the three regions, and the results were averaged. A Honeywell E for M mechanical scanner with a 3.5-MHz transducer was applied directly to the epicardium to record baseline two-dimensional echocardiograms from the four dogs which were to undergo transient ischemia. Normal regional backscatter recordings were obtained in all 10 dogs. A reversible snare was then used to occlude the LAD completely, just proximal to the last major diagonal branch, in four dogs. Ischemia of the apical region was confirmed by an immediate appearance of cyanosis and by repeat two-dimensional echocardiography which demonstrated apical dyskinesis. Segmental systolic wall thickening was quantified off-line from recorded two-dimensional echocardiograms with an image analysis computer using electronic calipers (Easy View II, Microsonics). Backscatter measurements and two-dimensional echocardiograms were repeated immediately before release of the snare 10 minutes after occlusion, and at 15, 30, 60, 90, and 120 minutes after reperfusion.

FIGURE 2. Regions interrogated by ultrasonic backscatter in intact dog myocardium illustrating the site of temporary coronary occlusion.
Data Analysis

Data from systole and diastole for each cardiac cycle at every site and frequency were fit to a time-renormalized cycle to permit comparisons of data gathered from different animals and different conditions. The systolic and diastolic phases of each cycle were divided into 16 equal time windows. The onset of systole and the onset of diastole were defined as the start of the upstroke and the nadir of the dP/dt, respectively. The backscatter data recorded during each phase were allocated among the 16 windows assigned to that phase, and the data corresponding to each window were then averaged. Thus, 50% of the time-renormalized cycle comprised data from systole and 50% data from diastole. The magnitude of the energy backscattered from tissue at each frequency from 3-7 MHz was referenced to the magnitude received from a planar stainless steel reflector placed in a water bath at the same distance from the transducer as was the tissue to obtain the backscatter transfer function (BSTF). Integrated backscatter was then calculated by averaging the BSTF over the range of frequencies employed. To highlight the magnitude of the cyclic variations, the time-average value of backscatter was offset to zero to obtain the relative integrated backscatter, which was averaged for all animals (Fig. 3).

To quantify regional cyclic variation, Fourier transforms of the relative integrated backscatter over the cardiac cycle were performed. The fundamental component, or that spectral component which exhibits one complete cycle over the cardiac period, was the only spectral component of appreciable amplitude. Thus, the peak-to-peak amplitude of the fundamental spectral component was chosen as an index to quantify the magnitude of the cyclic variation of backscatter (Fig. 4). Higher frequency components are present in the spectra, but were not of substantial amplitude and could have been affected by the time-renormalization process, and so were not included in the analysis.

The standard errors of the amplitudes of variation were calculated from the amplitude of the fundamental of each animal's relative integrated backscatter. Statistical significance was determined with the use of t-tests modified for multiple comparisons (Bonferroni's procedure). (Wallenstein et al., 1980).

Results

Figure 3 depicts regional differences in relative integrated backscatter recorded throughout the cardiac cycle. At the apex and midwall, where a cyclic pattern was discernible, the lowest values for backscatter were recorded near end systole, and the highest near end diastole. The amplitude of the fundamental frequency for each region (Fig. 4) increased progressively from base (0.5 ± 1.0 dB; mean ± se) to midwall (4.3 ± 0.8 dB) to apex (5.5 ± 0.9 dB). The difference in the magnitude of cyclic variation between the apex or midwall and the base was significant (P < 0.01, with allowance for multiple comparisons).

Despite marked regional differences in the pattern of cyclic variation, the absolute value of backscatter averaged over the cardiac cycle was similar for each area of the heart (apex = -55.6 ± 0.7 dB, midwall = -53.6 ± 0.9 dB, base = -53.8 ± 0.7 dB, P = NS for comparison of any two regions.)

Figure 5 depicts relative integrated backscatter measurements recorded throughout the cardiac cycle from the apical region of four dogs under control conditions (upper panel), after 10 minutes of ischemia (middle panel), and after 2 hours of reperfusion (lower panel). The amplitude of peak-
to-trough variation, which was $5.6 \pm 1.4$ dB under control conditions, diminished to only $0.4 \pm 1.5$ dB after 10 minutes of ischemia ($P < 0.02$, with Bonferroni's procedure), and returned to $3.9 \pm 1.2$ dB after 2 hours of reperfusion ($P = \text{NS}$ compared with control) (Fig. 6, upper panel). Although early recovery of the amplitude of cyclic variation after transient ischemia was substantial, the course of improvement was gradual and still incomplete after two hours of reperfusion (Fig. 7). Furthermore, although there were small increases in absolute time-average values of integrated backscatter during ischemia, and subsequently decreases during reperfusion, these were not significant in contrast to the amplitude of the cyclic variation (Fig. 6, lower panel).

Systolic wall thickening values before coronary occlusion averaged $63.4 \pm 17.7\%$, compared with $2.2 \pm 11.5\%$ during coronary occlusion (SD; $P < 0.001$; $n = 20$). Two hours after reperfusion, wall thickening did not differ from control values (52.85 $\pm 26.8$; $P = \text{NS}$). Representative end-diastolic and end-systolic frames of apical four chamber echocardiograms are displayed in Figure 8. Recordings were obtained at times corresponding to the backscatter measurements presented in Figure 5. Normal symmetric contraction of the left ventricle under control conditions (panel A) was replaced by apical systolic bulging under conditions of ischemia (panel B). Substantial improvement in mechanical function was noted after 2 hours of reperfusion (panel C).

**Discussion**

The results obtained indicate that myocardial ischemia modifies the cardiac cycle-dependent variation of myocardial ultrasonic backscatter in intact canine hearts. Despite significant regional differences in cyclic variation, the absolute value of backscatter averaged over the cardiac cycle was similar throughout the heart. This suggests that the observed gradient in cyclic variation is due to regional heterogeneity in dynamic, not structural, scattering properties of the tissue. The progressive increase in the magnitude of cyclic variation from base to apex is parallel to a regional gradient in contractile function previously described by others in normal
hearts of dogs and humans. Measurements performed with implanted ultrasonic crystals (LeWinter et al., 1975; Badke and Covell, 1979), biplane cineangiography (Kong et al., 1971; Liedtke et al., 1973), digital intravenous ventriculography (Slutsky et al., 1982), and two-dimensional echocardiography (Haendchen et al., 1983) have demonstrated an increase in magnitude of contractile activity from base to apex in the intact heart. The parallel increases in cardiac cycle-dependent regional variation in ultrasonic backscatter from base to apex are consistent with the hypothesis that the ultrasonic alterations are expressions of differences in myocardial fiber orientation (Streeter et al., 1969).

The cardiac cycle-dependent variation of backscatter appears to be sensitive not only to the locus of the region within the heart, but also to changes in the myocardial properties induced by ischemia or reperfusion. Changes in the cardiac cycle-dependent variation of backscatter accompanied changes in local contractile function documented by simultaneous two-dimensional echocardiography. Ten minutes after LAD occlusion, apical four-chamber views of the apex demonstrated dyskinesis and loss of systolic thickening of the septum (Fig. 8B). At the same time, normal apical cyclic variation of backscatter was eliminated (Fig. 5). After release of the coronary occlusion, echocardiographically delineated apical function gradually improved, and was substantially, although not completely, restored 2 hours after the onset of reperfusion (Fig. 8C). Simultaneously, apical cyclic variation of ultrasonic backscatter returned to 70% of control values (Fig. 5, lower panel; Fig. 6, upper panel).

Based on the behavior of other indexes of intrinsic properties of myocardium, such as contractile performance, it is not surprising that the cyclic variation of backscatter was not fully restored 2 hours after release of a transient LAD occlusion. Indeed, the time course of observed changes in cyclic variation of backscatter are consistent with results of studies of the time course after transient ischemia of altered contractile function assessed with implanted ultrasonic crystals. Heyndrickx et al. (1975) found that velocity of myocardial shortening at the apex of conscious dogs was eliminated 5 minutes after LAD occlusion, and that it returned to approximately 50% of control velocity after 2 hours of reperfusion. Other investigators, using implanted ultrasonic crystals (Théroux et al., 1976; Kloner et al., 1981) and segment length gauges (Weiner et al., 1976), have found that the impairment of contractile function induced by brief ischemia exhibits prompt partial resolution, but that complete recovery requires more prolonged reperfusion.

Because the electronic gating arrangement employed in this study does not permit the precise tracking of the same sample volume of tissue, we cannot exclude possible displacement of the segment relative to the ultrasonic beam during a heart cycle. However, it is unlikely that cardiac wall motion per se was responsible for the cyclic variation of ultrasonic backscatter observed, in view of the absence of systematic differences in backscatter recorded at the apex when it was rendered ischemic in each animal, in spite of marked systolic bulging and diastolic retraction.

It is improbable that regional differences in myocardial perfusion account for the observed regional differences in cyclic variation of backscatter in nor-
normal hearts. Studies with radiolabeled microspheres have shown that blood flow is relatively evenly distributed throughout the normal left ventricle (Marcus et al., 1975, 1977). Radiotracer studies performed in isolated perfused canine hearts (Yipintsoi et al., 1973; Warltier et al., 1975; Mosca et al., 1981) and open-chest anesthetized dogs (Griggs and Nakamura, 1968) indicate that the endocardial-to-epicardial blood flow ratio in normal hearts does not exhibit significant regional differences. We cannot exclude the possibility that variations in myocardial blood flow changes throughout the cardiac cycle may have influenced our ultrasonic measurements. However, we have documented systematic changes in backscatter values in isolated, nonperfused skeletal amphibian muscle associated with contractile state (Glueck et al., 1984). Thus, changes in blood flow per se are not a necessary condition for the alteration in backscatter associated with a change in function.

With respect to transient ischemia and reperfusion, the discrepancy in time course between the gradual recovery of contractile activity and cyclic variation and the prompt restoration of perfusion after transient ischemia suggests that blood flow per se is not the primary determinant of the observed changes in cardiac cycle-dependent variation in backscatter. Using radiolabeled microspheres, Heyndrickx et al. (1978) found that transmural blood flow was 90% restored after 1 hour of reperfusion following 15 minutes of ischemia, but that systolic wall thickening recovered more slowly. Kerber and collaborators (1975), using a similar technique, demonstrated normal myocardial perfusion 30 minutes after release of a transient coronary occlusion, although mechanical function remained significantly impaired. Thus, the backscatter changes parallel those of myocardial systolic function more closely than they parallel changes in perfusion per se.

Our results do not define a relationship between the amplitude of the cyclic variation of ultrasonic backscatter and myocardial contractility. Although myocardial ischemia and reperfusion are characterized by disruption and restoration of segmental contractile function, these interventions are accompanied also by considerable mechanical alterations in the tissue that could influence the observed changes in backscatter. Nevertheless, it appears likely that both cyclic variation of backscatter and contractile function may reflect conformational changes of myocardial contractile elements. We have previously reported that measurements of ultrasonic backscatter and attenuation in excised canine myocardium are highly dependent on the angle between the interrogating beam and the predominant myofiber orientation (Mottley and Miller, 1982). Furthermore, cineangiographic and morphometric studies of the canine heart have demonstrated that myocardial fiber orientation varies systematically throughout the cardiac cycle, and that there are regional differences in the magnitude of this variation (Streeter et al., 1969; Meier et al., 1982).

In addition, cyclic changes in the attenuation of contracting cardiac muscle may account in part for the observed systematic changes in backscatter. Thus, changes in myocardial fiber orientation as a result of altered contractile activity may provide one ultrastructural basis for the changes in cardiac cycle-dependent variation in ultrasonic backscatter manifest in myocardium subjected to ischemia.

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