Early loss of postextrasystolic potentiation in acutely ischemic myocardium: evaluation by contrast two-dimensional echocardiography

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ABSTRACT  Studies in animals with acutely ischemic hearts have suggested that postextrasystolic potentiation (PESP) may predict the viability of dysfunctional myocardium. Most of these data have been obtained with sonomicrometers and therefore the presence and extent of PESP throughout the entire region at risk has not been defined. In this study we used contrast two-dimensional echocardiography (2DE) to define region at risk in vivo, and then with quantitative 2DE we examined the proportion of the region at risk that demonstrated PESP, the degree of the potentiation, and the time course of this response. The region at risk was divided into a central (inner 50%) and two peripheral (25% each) ischemic zones. Adjacent contrast-enhanced myocardium was divided into near and far border zones that were equal in size to the adjacent peripheral ischemic zone. Systolic thickening was analyzed within each zone along multiple radii at 5, 30, and 120 min after coronary occlusion. PESP was absent in the central ischemic zone at all three times. In the peripheral ischemic zone at 5 min, a small amount of PESP was detected (−4.1% vs +3.1% for nonpotentiated and potentiated thickening, respectively; p < .01). At 30 min after occlusion, no potentiation was seen in the region at risk and PESP was confined to the contrast-enhanced near and far border zones. These findings persisted at 120 min. These data indicate that the response to PESP is localized to perfused myocardium by 30 min after acute occlusion. PESP is therefore of limited value in predicting the presence of ischemic, potentially viable myocardium early in the course of acute infarction.


IN PATIENTS with coronary artery disease, revascularization of chronically ischemic tissue can improve systolic function of some dysynergic regions.1-3 This has led to a search for interventions that will predict viability of abnormally contracting segments. Dyke and Cohn and their colleagues4-5 and others6,7 have shown that in patients with chronic ischemia, the reversibility of regional wall motion abnormalities resulting from improved perfusion after coronary artery bypass grafting can be predicted before surgery by the response to postextrasystolic potentiation (PESP). The data regarding PESP during acute ischemia are far less conclusive.8 Several animal studies in which epicardial segment-length gauges9-11 and subendocardial sonomicrometers12,13 were used have indicated that, although unstimulated contractile function is severely impaired almost immediately after coronary occlusion, some portion of the region at risk of infarction shows persistent PESP even after 214 and 4 hr15 of acute ischemia. These data suggest that PESP might be used to predict the presence, if not the full extent, of ischemic myocardium that could be salvaged by reperfusion. The studies are limited, however, since they examine only a small portion of the ischemic region and thus leave unanswered critical questions regarding the fraction of the region at risk that demonstrates PESP and the time course of the potentiated response.

The present study was designed to determine the presence, extent, and temporal course of PESP in acutely ischemic myocardium within the region at risk and in adjacent normally perfused regions. To accomplish this we used contrast two-dimensional echocardiography (2DE) to delineate a region at risk in vivo and thus eliminate the need for retrospective matching of pathologic material with echocardiographic results. With these techniques we could determine the potential applicability of PESP to the problem of dif-
ferentiating reversibly from irreversibly ischemic myocardium.

Methods

Experimental preparation. Eight mongrel dogs weighing 18 to 27 kg were preselected for this study on the basis of their high-quality two-dimensional echocardiograms. They were premedicated with 1 mg/kg of intravenous morphine sulfate, anesthetized with 10 mg/kg iv pentobarbital, intubated, and placed on a Harvard respirator. Adequate oxygenation and appropriate ventilation were confirmed by determination of arterial blood gases at baseline and at least once after occlusion. The heart was exposed by a thoracotomy through the left fifth intercostal space and a pericardial suture was constructed. The right femoral artery and left atrial appendage were cannulated for pressure monitoring and the electrocardiogram (ECG), arterial pressure, and left atrial pressure were continuously recorded on a Grass polygraph. The left circumflex coronary artery distal to the first obtuse marginal artery was dissected free. Lidocaine was given in a dose of 1.5 mg/kg and the pericardial suture was released. Five minutes later the circumflex artery was occluded by tying a suture at the point where it had been dissected free and the chest was closed. A second dose of lidocaine (1 mg/kg) was given 5 min after occlusion. All animals were killed at 6 hr after occlusion.

Extrastimulus technique. Two epicardial pacing electrodes were sutured to the right ventricular free wall and were connected to a programmable stimulator (Bloom Associates Ltd.). Ventricular effective refractory period was determined before occlusion by delivering premature stimuli at twice the mid-diastolic threshold. Thereafter all single premature stimuli were delivered at 10 msec beyond the effective refractory period. Throughout the study no interventions were made to alter intrinsic heart rate.

Contrast echocardiography. The technique of supra-aortic H2O2 injection for delineation of region at risk of infarction has been outlined in previous communications. Briefly, a mixture of 2 ml of 0.3% H2O2 and 1 ml of autologous blood was flushed into the aortic root through a No. 8F pigtail catheter that had been advanced from the left femoral artery and positioned flush into the aortic root through a No. 8F pigtail catheter that had been advanced from the left femoral artery and positioned under ultrasound guidance. That myocardium which was not enhanced with ultrasonic contrast was the 2DE-defined region at risk. This technique has been shown in our laboratory to be accurate when compared with those in which region at risk is determined by monastral blue staining (r = .93 and SEE = 7.7%) or autoradiography (r = .89 and SEE = 4.5%).

Image acquisition. Animals were imaged from the right parasternal transducer position with a 90 degree mechanical sector scanner (ATL-ADR, Advanced Technology Labs, Inc.). A Sony Betamax videotape recorder was used to record all images for later analysis.

Serial imaging was performed at that short-axis level which included mitral chordae and was just superior to the papillary muscles. After control (preocclusion) 2DE studies in all animals with and without PESP, the circumflex coronary artery was ligated as outlined above. After occlusion, the examination sequence was as follows: (1) wall motion without PESP, (2) potentiating wall motion, and (3) supra-aortic H2O2 injection. All three steps were performed in a single imaging sequence without moving the transducer at 30 and 120 min after occlusion. Adequate quantitative studies of the effects of H2O2 on regional function very early after coronary occlusion have not been done. Since the clinically relevant time interval for the assessment of PESP as a potential predictor of reversibility of regional wall motion abnormalities was 30 min, we wished to avoid any possibility of an as yet undescribed persistent depression of contractility related to injection of contrast biasing the 30 min results. Thus, no contrast study was performed at 5 min.

Data analysis. Endocardial and epicardial outlines of the left ventricle in diastole (defined as peak of the R wave) and systole (defined as the smallest cavity area) were traced onto acetate overlays from stop-frame images by an observer unaware of the results of the pathologic analyses. The section traced was then played repeatedly at normal speed and adjustments were made in the tracings. Tracings of the unstimulated and postextrasystolic beats were done on separate days. After each tracing was completed, the tape was advanced to the supra-aortic H2O2 injection and region at risk was marked directly on the tracings for both diastole and systole. For the 5 min examination, the contrast-negative region was determined after the 30 min H2O2 injection by matching end-diastolic and end-systolic images and superimposing the 30 min region at risk onto the 5 min tracings.

The contrast-negative region of the short-axis section was divided into a central ischemic zone (inner 50% of the region at risk) and a peripheral ischemic zone (outer 25% of the region at risk on either side of the central ischemic zone) (figure 1). Near border and far border zones were defined as zones within the contrast-enhanced region, directly adjacent to the region at risk, that were equal in size to the peripheral ischemic zone. This was done for both the end-diastolic and end-systolic images.

Systolic thickening analysis was performed along radii placed every 4.5 degrees. To derive systolic thickening, diastolic and systolic radii were separately matched by their position relative to the contrast border. Thus, for example, the first near border radius was that radius in both systole and diastole that was immediately adjacent to the contrast border within the contrast-enhanced region. In this way, translation and rotation of the ventricle were completely corrected for by use of the contrast border during systole and diastole as an internal reference. A digitized tablet and light-pen system (Franklin Quantic 1200; Franklin Industries, Inc.) were used to generate percent systolic
thickening for each radius from the following formula: (thickness at end systole — thickness at end-diastole)/thickness at end diastole × 100%.

Detailed analysis was performed for the anterior half of the region at risk and border zones. This orientation places the region of interest in the near field of the transducer with endocardium and epicardium oriented axially to the transducer head, thus yielding maximal resolution of these structures as well as the anterior H2O2-defined border of region at risk.

Reproducibility of results for region at risk as determined by the supra-aortic H2O2 method has been examined previously in our laboratory.14,15 Correlation coefficients for both intraobserver and interobserver variability were greater than .94 and SEEs were 3.2%. Reproducibility of the tracings of parasternal short-axis end-diastolic and end-systolic images has also been examined,16,17 and the mean difference in infarct size between paired observations was 4.9 ± 1.4%.17

Pathologic analysis. Immediately before they were killed (at 6 hr after occlusion) the dogs' chests were reopened and monastral blue pigment (0.5 ml/kg) was injected via the left atrial line.18 Twenty seconds after the injection the animals were killed by a left atrial injection of potassium chloride solution. The heart was rapidly excised, frozen with liquid refrigerant, and cut into 1 cm thick slices, parallel to the atrioventricular groove, from apex to base. The pathologic slice corresponding to the 2DE section imaged was identified as the one immediately superior to the section containing the tips of the papillary muscles.

The pattern of monastral blue staining on both sides of the interest slice was traced on an acetate overlay and the slices were photographed. The slices were then incubated in triphenyltetrazolium-chloride (TTC) for determination of infarct size.19 Tracings were again made of both sides of the interest slice on acetate overlays. Extent of region at risk and infarct size were expressed as a percent of muscle area by taking the mean of the areas previously traced.

Statistical analysis. Significant differences between paired data were determined by Student's t test and those between sequential observations by analysis of variance. Correlation coefficients and estimation errors were determined for the regions at risk by comparison of results of contrast 2DE vs monastral blue staining. Data are mean ± SEM unless stated otherwise.

Results

The hemodynamic data obtained are listed in table 1. Mean blood pressure and heart rate were higher at baseline than at 5 min after occlusion. Thereafter, these did not change and were not significantly different at 5, 30, and 120 min after occlusion. Left atrial pressure rose from baseline to 5 min after occlusion but did not change significantly thereafter. Coupling interval, expressed as a percent of the RR interval, was not significantly different from control at the three time periods.

Infarct size in the target slice expressed as a percent of the region at risk was 75 ± 11.5% (range was 58% to 92%). Two of the eight animals had infarctions that included less than 70% of the region at risk.

Figure 2 is a plot of regions at risk as determined by 2DE contrast-negative region at 2 hr vs those determined by monastral blue staining. The correlation coefficient was .92 and the SEE was 3%.

The size of the region at risk as determined by contrast 2DE did not change over the course of the experiment. The mean difference in size of the region at risk among sequential observations at 30 min, 2 hr, and 6 hr was 2.5 ± 2.2% (mean ± SD). Correlation coefficients and SEEs for size of the echo contrast defect at 30 min, 2 hr, and 6 hr ranged from r = .90 with SEE = 2.5% to r = .95 with SEE = 1.8%.

Figure 3 shows results of the analysis of systolic thickening along individual radii in the four zones and along the radius at the border of the contrast-enhanced and contrast-negative regions at 5, 30, and 120 min after occlusion. Figure 4 shows the results for radii grouped by zone.

The mean percent circumferential extent of the echo contrast defect was 31 ± 6%. Thus, the average circumferential extent of the central ischemic zone was approximately 2.2 cm and those of each peripheral ischemic zone and of the near and far border zones were approximately 1.1 cm.

The normal zone demonstrated significant potentiation of thickening at all three examinations (figure 4). At 5 min after occlusion (figure 3, top), thinning occurred during unstimulated contraction along all radii in the central and peripheral ischemic zones. With PESP there was no improvement in thickening along any radius in the central ischemic zone (p = NS for all

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BP = mean arterial blood pressure; LAP = left atrial pressure.

ᵇp < .05 vs baseline.
radii, with PESP vs without PESP). In the peripheral ischemic zone, two of the five radii showed statistically significant potentiation. The amount of potentiation was minimal, however, and the increase in percent thickening with PESP averaged 7%. Taking all peripheral ischemic zone radii together (figure 4, top), potentiated thickening remained markedly depressed compared with the nonpotentiated preocclusion value (preocclusion, without PESP: 44 ± 17%; postocclusion with PESP: 3 ± 7%). In the contrast-enhanced near and far border zones, significant potentiation occurred along six of the nine radii (figure 3, top). Potentiated thickening remained depressed, however, and was 35% and 59% of the nonpotentiated preocclusion values for the near border and far border zones, respectively (figure 4, top).

At 30 min after occlusion (figures 3 and 4, middle) systolic thinning again occurred with nonpotentiated beats in both the central and peripheral ischemic zones. As opposed to the results at 5 min, however, significant potentiation was not seen along any radius in the peripheral ischemic zone. Significant potentiation was

FIGURE 2. Plot of tissue malperfusion (region at risk) as determined by monastral blue staining (MB) vs echocardiographic contrast defect (ECD) at 2 hr after occlusion. Regression line is solid and line of identity is dashed.

FIGURE 3. Plot of nonpotentiated thickening (open circles) and potentiated thickening (closed circles) along individual radii in the four zones at 5 min (top), 30 min (middle), and 120 min (bottom) after occlusion. B = radius at the border of contrast-negative and contrast-enhanced regions. Other abbreviations are as in figure 1.
limited to the contrast-enhanced near border and far border zones. PESP increased systolic thickening to 63% and 99% of control at 30 min in the near and far border zones, respectively (figure 4).

The results at 120 min after occlusion (figures 3 and 4, bottom) were very similar to those at 30 min. No significant potentiation was seen along any radius in the central or peripheral ischemic zones (figure 3). Systolic thinning occurred with both potentiated and nonpotentiated beats. Potentiation was limited to the near and far border zones where PESP increased systolic thickening to 78% and 126% of the nonpotentiated preocclusion values, respectively (figure 4).

Discussion

Early studies of PESP during acute ischemia demonstrated significant improvement in epicardial shortening. Boden et al.\textsuperscript{11} found potentiation in the center of the ischemic region as late as 4 hr after occlusion. Subsequent studies, however, have indicated that epicardial shortening bears an inconsistent relationship to blood flow and to systolic wall thickening.\textsuperscript{20-23} Gelagher et al.\textsuperscript{23} have concluded that epicardial shortening is probably of minimal importance to overall systolic function.

Crozatier et al.\textsuperscript{12} used sonomicrometers to examine subendocardial shortening, which is highly correlated with systolic wall thickening.\textsuperscript{21} They found a total loss of PESP in the center of the ischemic region after 5 min of occlusion. Potentiation of shortening persisted at 2 hr in sonomicrometers placed at the margin of the ischemic zone. In a subsequent study\textsuperscript{13} in which microsphere blood flow determinations were used they again observed complete loss of potentiation at 5 min in the most ischemic segments. Some PESP was again seen at 5 min in less severely ischemic segments. Later time intervals were not observed. While these sonomicrometric data demonstrate the presence of PESP in acutely ischemic tissue, they cannot evaluate the proportion of the ischemic region that shows this response since they examine only a small part of the region at risk.

In the present study, PESP was absent in the central 50% of the contrast-negative region after 5 min of ischemia. In the peripheral 50% of the region at risk, some PESP was present at 5 min. These data, obtained with 2DE, are compatible with Crozatier's sonomicrometric data.\textsuperscript{12, 13} After 30 min of ischemia, a clinically more relevant time, potentiation was not seen along any radius within the contrast-defined region at risk. From this point on, PESP was confined to the contrast-enhanced regions. Thus, it appears that the region described by Crozatier et al.\textsuperscript{12} that is at the margin of the ischemic region and that demonstrates persistent PESP is a very small fraction of the ischemic zone at the far lateral edges of the region at risk. Our data suggest that after 5 min it is too narrow to be detected by 2DE even when systolic thickening is examined every 4.5 degrees (or approximately 2.5 mm).

Our data are highly compatible with those of Marcus et al.\textsuperscript{24} and Hearse, Yellon, and their colleagues.\textsuperscript{25-27} The latter group measured several metabolic parameters and blood flow across the border zone between ischemic and normal tissue and found an extremely narrow transition zone between normal and severely depressed values. Blood flows, adenosine triphosphate content, and phosphocreatine content dropped from nonischemic levels to levels equal to those in the center of the infarct zone over a few millimeters. This indicates that the zone of marginally ischemic tissue (which is equivalent to that shown by Crozatier et al.\textsuperscript{12} to persistently potentiate) is very narrow. Since the vast majority of the region at risk is markedly ischemic, with little difference in blood flow from the...
center of the ischemic zone to very near the border of the region at risk, it is not surprising that we found early and complete loss of PESP throughout this region.

The duration of ischemia is the most important factor that determines the extent of the area at risk that becomes infarcted. Numerous studies in dogs have shown that with coronary occlusion times of 20 min, myocardial necrosis is virtually never seen and with occlusion times of 30 to 40 min, necrosis is either absent or limited to a small percentage of the region at risk. Since the eventual extent of infarction at 6 hr after occlusion in our series was similar to or less than that reported in other series, at the 30 min examination when PESP was absent the vast proportion of the region at risk, and in particular the peripheral ischemic zone, was undoubtedly viable. Just as with resting contraction, then, there is an extreme dissociation between the viability of the region at risk and systolic function in response to PESP. We conclude that the response to PESP cannot be used to predict viability of acutely ischemic tissue.

Our findings differ somewhat from those of Sakamaki et al. They performed serial 2DE examinations with and without PESP in dogs with left anterior descending coronary arterial (LAD) occlusions. The ischemic region was measured by glycogen depletion and infarct size was determined by triphenyl tetrazolium chloride staining at 3 hr after occlusion. They found marked potentiation of wall thickening at 20 min after occlusion (to greater than preocclusion values) in areas that were profoundly ischemic (greater than 80% transmural infarction at 3 hr after occlusion). In addition, segments that were within the ischemic region (as determined by glycogen depletion) but were not infarcted showed persistent PESP. They concluded that PESP could be used to detect dysfunctional myocardium that was ischemic but not yet infarcted.

We believe these disparities can be explained by differences in experimental methods. First, they studied LAD infarctions, which usually have an inverted V pattern with the greatest involvement at the apex. Consequently, small changes in transducer angulation on successive studies could produce significant changes in the arc subtended by the region at risk. This would complicate the interpretation of changes in thickening over time. Compounding this is the difficulty in precisely matching the echocardiographic section with the pathologic slice. Given the inverted V pattern of the infarctions in the LAD distribution, if the pathologic section were even slightly above or below the level of the echocardiographic section, smaller or larger infarct sizes, respectively, would be obtained and significantly different conclusions might be reached. We studied circumflex occlusions, which result in more rectilinear regions at risk. Thus, slight changes in transducer angulation should not lead to major changes in the size of the region at risk. Most importantly, however, contrast 2DE provided us with a means of determining the region at risk in vivo and of accounting for cardiac rotation, thus avoiding the problem of having to match pathologic slices and echocardiographic sections to interpret results of the systolic thickening analyses. Finally, to assess potentiation, Sakamaki et al. used an epicardial center of mass-floating axis system with which there are many difficulties when examining wall motion in the short-axis view. Schnittger et al. found that a floating system of wall motion analysis incorrectly localized regional wall motion abnormalities in over half of their subjects. With these inherent inaccuracies, the wall motion data of Sakamaki et al are difficult to interpret. Recently Armstrong et al. have demonstrated that such analyses may lead to the comparison of nonanalogous regions of the ventricle in systole and diastole not only in the examination of wall motion but even in the analysis of systolic thickening. The contrast defect provided us with an internal reference that allowed us to examine systolic function without having to correct for whole heart motion and ensured that analogous regions were examined in systole and diastole.

Limitations. The first potential limitation of our technique lies with the contrast 2DE determination of the region at risk since the conclusions reached here are critically dependent on this. The technique has, however, been shown to be both accurate and reproducible at defining region at risk by comparison with monastral blue or autoradiographic techniques in our laboratory and others. We found similar accuracy in this study. Another way of assessing the accuracy of boundary detection with contrast 2DE is to use our SEE for region at risk determined by contrast 2DE vs by monastral blue staining to determine the distance that might separate the contrast-estimated boundary of the region at risk from the pathologic boundary. Our SEEs suggest that contrast echocardiographic region at risk matches to within approximately 4 mm of the monastral blue boundary, assuming that perfectly accurate matching of the pathologic and echocardiographic slice was attained. Since the SEEs for the size of the contrast defect on successive studies were smaller (approximately 2%), the technique may well be more accurate (to within 2 to 3 mm). The slightly larger SEEs for echocardiographic vs monastral blue
region at risk may reflect the difficulty in achieving perfect matching of 2DE and pathologic slices.

The second potential limitation is that there was no injection of contrast at the time of the 5 min study. If PESP is to be of clinical value in predicting the reversibility of regional wall motion abnormalities, it certainly must be demonstrable for longer than 5 min after the onset of ischemia. Although this study evaluated the ability of 2DE to detect subtle degrees of PESP that had previously been demonstrated at 5 min with sonomicrometers, the examinations at 30 and 120 min after occlusion were the critical studies. Therefore, to avoid the possibility of attributing the results at 30 min to any persistent alteration in regional function resulting from $\text{H}_2\text{O}_2$ injection, no 5 min injection was performed. West et al. have shown that the contrast-defined region at risk does not change from immediately to 4 hr after occlusion. Furthermore, Judgutt et al. have shown that serially determined microsphere blood flow measured across the region at risk and border regions does not change between 5 min and 6 hr after occlusion and thus, presumably, neither does the region at risk. However, between 20 sec and 5 min after occlusion blood flow did increase in their study, suggesting that the region at risk could be somewhat larger very early after occlusion. If this were the case, the ischemic border radius in all likelihood would be located in our border zone. One can see from figure 3, top, that the results obtained in the 5 min study would not significantly change — i.e., the peripheral ischemic zone shows only slight PESP at this time.

In conclusion, canine myocardium demonstrates minimal PESP very early after the onset of acute ischemia, but loses this response quickly. After 30 min of acute ischemia, any regions demonstrating potentiation can be assumed to be outside the region at risk of infarction. Although our results cannot be directly extrapolated to the clinical setting, our data suggest that PESP may be of limited value in the prediction of the presence of acutely ischemic but viable myocardium that may be salvaged by reperfusion.

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