Mechanism of Selective Epicardial Activation Delay During Acute Myocardial Ischemia in Dogs

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Background. Previous studies have shown that acute myocardial ischemia in the dog results in much greater activation delays in epicardial than endocardial tissue. These results have been interpreted to indicate enhanced sensitivity of epicardial conduction properties to acute ischemia. This study was designed to test the hypothesis that the ischemic epicardial activation delay during supraventricular rhythms is due to slow conduction across the ischemic myocardial wall prior to epicardial activation and not to enhanced epicardial conduction slowing per se.

Methods and Results. Changes in epicardial and endocardial activation were measured with transmural decapolar needle electrodes during successive 5-minute left anterior descending coronary artery (LAD) occlusions separated by 30-minute reperfusion periods. Occlusions were performed during left atrial pacing, right ventricular pacing with a stimulating electrode located along the longitudinal axis of epicardial fiber orientation in the ischemic zone, or left ventricular pacing in nonischemic tissue located on a line transverse to fiber orientation in the ischemic zone. During both atrial and left ventricular pacing, activation in the ischemic zone began in the endocardium. Epicardial activation resulted from transmural conduction and was markedly delayed compared with endocardial activation during acute myocardial ischemia. During right ventricular stimulation, the ischemic zone epicardium was activated via longitudinal epicardial conduction, and its activation was only slightly delayed by acute ischemia. Epicardial activation mapping was used to assess ischemia-induced changes in longitudinal epicardial conduction velocity and to compare them with changes in transmural velocity during atrial or left ventricular pacing. Longitudinal conduction in the ischemic epicardium was slowed by 13±4% (mean±SE) relative to preischemic control values in contrast to transmural conduction, which was slowed 50±4% by LAD occlusion during atrial pacing and 49±5% during left ventricular pacing (both P<.001 versus longitudinal epicardial conduction). Transmural activation studies showed that the midmyocardium is the site of most of the ischemic activation delay during transmural propagation.

Conclusions. Epicardial activation is more delayed than endocardial by acute ischemia during supraventricular rhythms in dogs because of slowed conduction across the myocardial wall, not because of enhanced sensitivity of epicardial conduction to depression by acute ischemia. (Circulation. 1993;88[part 1]:2381-2388.)

Key Words • infarction • ischemia • arrhythmias • epicardium

Ventricular conduction slowing plays a major role in the pathogenesis of tachyarrhythmias caused by acute myocardial ischemia.1-4 Ischemic conduction delay in vivo is greater in the epicardium than in the endocardium,2-6 despite evidence that epicardial blood flow is significantly better preserved than endocardial after acute coronary artery occlusion in the dog.7 Gilmour and Zipes8 showed that epicardial muscle is much more depressed than endocardial muscle on superfusion in vitro with solutions mimicking the extracellular fluid composition during acute myocardial ischemia. They found, however, that endocardial cells of papillary muscle preparations, lacking in subjacent Purkinje fibers, were as susceptible to conditions of ischemia as epicardial tissue, and they therefore suggested that the resistance to ischemia of endocardial tissue is due to electrotonic interaction with Purkinje fibers.8 Kimura et al9 found that isolated canine epicardial and endocardial myocytes responded similarly to conditions of acute ischemia; however, in isolated, perfused cat ventricles paced via the left bundle branch and exposed to acute ischemia, conduction slowing was greater in the epicardium than the endocardium.10

There are several discrepancies in action potential properties between epicardial and endocardial tissues.11-13 Difference in transient outward (Iₒ)14 and rectifying (Iₖ₁ and Iₖ₉)15 potassium currents may account for the shorter epicardial action potential. Metabolic inhibition causes greater action potential shortening in epicardial cells16 due to greater sensitivity of ATP-regulated potassium17 and/or calcium16 currents.

Thus, there are intrinsic electrophysiologic differences between epicardial and endocardial cells, but the
work of Gilmour and Zipes\(^8\) showed that endocardial tissue devoid of overlying Purkinje fibers (papillary muscle) responded to ischemia like epicardial tissue. During supraventricular rhythms, the ventricles are activated via the conducting system, with propagation proceeding from the subendocardial Purkinje network to the endocardium and then to the epicardium. The activation of endocardial muscle depends on the conducting system, which is relatively resistant to acute ischemia.\(^5\) In contrast, to reach the epicardium, the propagating impulse has to traverse the full thickness of the ischemic myocardium. This offers an alternative explanation of the greater ischemic conduction delay in epicardial tissues— that it is due to slowed conduction across the ischemic myocardial wall. The present study was designed to assess whether the greater ischemic activation delay in canine epicardial tissue, compared with endocardial muscle, is due to intrinsic electrophysiological differences or whether it can be accounted for on the basis of propagation delay across the myocardial wall.

**Methods**

**General**

Experiments were performed on 21 mongrel dogs of either sex ranging in weight from 17 to 22 kg. Dogs were anesthetized with IV sodium pentobarbital (30 mg/kg), and additional doses of pentobarbital were given as necessary. Animals were intubated and ventilated mechanically, with a tidal volume obtained from a nomogram, using a mixture of oxygen and room air to maintain arterial pH between 7.38 and 7.44 and Sa\(_2\) >90%. Arterial blood gases were verified at approximately 1-hour intervals and prior to each coronary artery occlusion. One femoral vein was cannulated for drug administration, and a femoral artery was cannulated for arterial pressure recording and blood gas analysis.

The ECG was recorded using a Bloom Recording System (Bloom Associates Ltd, Narberth, Pa) and an MT95000 paper recorder (Astomed Inc, Toronto, Ontario). ECG signals were filtered (0.05 to 250 Hz) and amplified to produce a 1-mV/cm signal on the recording paper. A heating blanket was used to maintain normothermia (37 to 39°C).

A left thoracotomy was performed through the fifth intercostal space, and a pericardial cradle was created. The left anterior descending coronary artery (LAD) was exposed by blunt dissection approximately 1 cm below the origin of the first diagonal. A silk ligature was placed around the artery, and both ends were passed through polyethylene tubing to act as a snare. The chest cavity was closed with plastic to prevent epicardial cooling and drying.

The heart was stimulated via a bipolar, Teflon-coated stainless-steel hook electrode inserted in the left atrial appendage or via either of two bipolar Teflon-coated stainless-steel electrodes placed into the epicardium of the right and left ventricle, outside the ischemic area (Fig 1). The right ventricular pacing electrode was positioned at least 2 cm from the LAD, in a line parallel to fiber orientation to two decapolar electrodes in the ischemic zone. The left ventricular electrode was inserted at a site transverse to longitudinal epicardial fiber orientation in the ischemic zone. All stimuli were 2 milliseconds in duration and twice-diastolic threshold current. Recordings from left ventricular epicardium and endocardium were obtained by inserting two decapolar needle electrodes (J. Cassel, Durham, NC) in the region between the second diagonal branch and the LAD (ischemic zone) and one in the basal anterior left ventricular wall (normal zone). All electrograms were recorded as differential bipolar signals between two adjacent electrode sites. The decapolar electrode contains 10 electrode sites with 1-mm interelectrode distance, allowing for the recording of five bipolar electrograms including subendocardial, subepicardial, and three intramyocardial locations. Electrograms were filtered at 40 to 400 Hz and recorded at 200-mm/s paper speed. The time of activation for each electrogram was defined by the peak of the largest rapid deflection and was expressed relative to the onset of ventricular activation during atrial stimulation or to the stimulus artifact during right and left ventricular stimulation (after verifying constant latency). Ischemic conduction slowing was defined as the difference between activation time prior to LAD occlusion and activation time after occlusion.

**Activation Mapping**

An epicardial electrode array containing 56 bipolar electrodes with 2-mm-interpolar and 6-mm-interelectrode distance was sewn in place parallel to the LAD.
Fig 2. Activation in the left anterior descending coronary artery (LAD) territory, as recorded in the endocardium (IZ-END) and epicardium (IZ-EPI) under control conditions and after 5 minutes of ischemia. Epicardial activation was much more delayed by ischemia than endocardial during atrial and left ventricular (LV) stimulation. During right ventricular (RV) stimulation, ischemia delayed epicardial activation less than endocardial activation. Activation time was measured relative to the onset of ventricular activation during atrial pacing and relative to the stimulus artifact during ventricular pacing (after ensuring that latency from stimulus to nonischemic ventricular response was constant). (II indicates lead II ECG; SA, stimulus artifact; and NZ, normal zone electrogram.)

and below the diagonal branch (Fig 1). Each signal was filtered (30 to 400 Hz), digitized with 12-bit resolution and a 1-KHz sampling rate, and transmitted via duplex fiberoptic cables into a microcomputer (Compaq 286). Software routines were used to amplify, display, and analyze each electrogram signal, as well as to generate activation maps, as previously described. Each activation time was measured with computer-determined peak-amplitude criteria and reviewed manually to exclude low-amplitude signals. The data were downloaded on high-density diskettes for off-line analysis. Activation maps were based on data obtained before and 5 minutes after each occlusion.

Experimental Protocols

In all except 4 dogs, four 5-minute occlusions of the LAD were performed, separated by 30-minute reperfusion periods. The first occlusion was treated as a conditioning occlusion since electrophysiologic changes during a first brief coronary artery occlusion may differ from those of subsequent occlusions, whereas the latter tend to be quite similar. During each of the test occlusions (occlusions 2 through 4), the heart was paced at an identical cycle length (mean, 378±21 milliseconds), permitting consistent capture via the left atrial, right ventricular, or left ventricular electrode. Stimulation at one site was maintained throughout each occlusion, and the sequence of sites stimulated was varied from dog to dog to avoid bias related to time or to the occlusion number. In 4 dogs, a single 15-minute occlusion was performed, with atrial pacing during the first 30 seconds of every minute and right ventricular pacing during the last 30 seconds of each minute.

In 6 dogs, changes in activation time due to myocardial ischemia were studied at one epicardial and an adjacent endocardial site in the ischemic zone through the use of two decapolar needle electrodes situated within 1 mm of each other. A normal zone electrogram was also recorded as a control. In 7 additional dogs, 4 to 7 decapolar electrodes were inserted in the ischemic zone and the mapping system used to record bipolar electrograms at five levels from each decapolar electrode, spanning sites from the endocardium to the epicardium. These electrograms were designated 1 through 5, with level 1 corresponding to the endocardium, level 5 the epicardium, and levels 2 through 4 sequential sites within the myocardial wall. In the final series of experiments, bipolar electrograms were recorded with the mapping system from 56 epicardial sites in 4 dogs. Needle electrodes were used to record epicardial and endocardial activation just medial to the epicardial electrode array. Changes in transmural conduction velocity were inferred from differences in epicardial and endocardial activation time. Epicardial conduction velocity in the longitudinal direction was determined from maps obtained during right ventricular stimulation, from the conduction time and distance between adjacent bipolar electrodes in a direction parallel to fiber orientation and located next to the decapolar electrodes.

Arenal et al Epicardial Activation in Acute Ischemia 2383
Epicardial Conduction Delay

Conduction delay (msec)

Time (min)

Endocardial Conduction Delay

Conduction delay (msec)

Time (min)

Fig 3. Top, Epicardial conduction delay caused by acute ischemia, during atrial (AP), right ventricular (RV), and left ventricular (LV) pacing. *P<.001 compared with conduction delay during RV pacing. Bottom, Endocardial conduction delay as a function of ischemic time. No significant differences were observed between results during pacing at different sites.

Statistical Analysis

Group data are presented as mean±SE. Comparisons between only two sets of paired data were performed with Student’s t test, while comparisons between multiple groups were performed with ANOVA and Scheffé contrasts. An F test was used to test the significance of interaction effects. A probability of P<.05 was taken to indicate statistical significance.

Results

Activation Sequence and the Effects of Acute Ischemia

Representative recordings of endocardial and epicardial electrograms are shown in Fig 2. During atrial and left ventricular stimulation, activation of the endocardium always preceded that of the epicardium. In contrast, the epicardium was always activated prior to the endocardium during right ventricular pacing. In 6 dogs, the delay from endocardial to epicardial activation averaged 12±5 milliseconds in the absence of ischemia during atrial pacing and 8±4 milliseconds during left ventricular pacing. Epicardial activation preceded endocardial by 9±7 milliseconds during right ventricular stimulation.

Right Ventricular Pacing

Conduction delay (msec)

Time (min)

Fig 4. Plots of endocardial and epicardial activation delay recorded during single 15-minute left anterior descending coronary artery occlusions in four dogs, in which atrial pacing was performed for the first 30 seconds of each minute and right ventricular pacing at the same cycle length for the last 30 seconds of each minute. During atrial pacing (bottom), substantial epicardial activation delays occurred (*P<.05 versus endocardium). On the other hand, during right ventricular stimulation (top) epicardial activation delays were much smaller and not significantly different from endocardial.

During atrial stimulation, ischemia delayed epicardial activation, with epicardial activation time increasing from 22.5 milliseconds (control) to 40 milliseconds after 5 minutes of ischemia in the example shown in Fig 2. The epicardial conduction delay due to ischemia during atrial pacing in this dog was thus 17.5 milliseconds, substantially greater than the 2.5-millisecond delay caused by ischemia at the endocardial site. During left ventricular stimulation, ischemia delayed epicardial activation by 22 milliseconds, while leaving endocardial activation time virtually unchanged. In contrast, epicardial activation time was only slightly prolonged (5 milliseconds) by ischemia during right ventricular stimulation, to an extent less than the delay (10 milliseconds) in endocardial activation.

Conduction delays during atrial, right ventricular, and left ventricular pacing are shown as a function of time following coronary artery occlusion in Fig 3. Epicardial conduction slowing was substantially greater during atrial and left ventricular pacing than during right ventricular stimulation, and results during atrial and left ventricular pacing were not significantly different from each other. Endocardial conduction changes were much smaller and not significantly determined by pacing site. Fig 4 shows results obtained during a single 15-minute occlusion in 4 dogs, with conduction delays measured during successive 30-second pacing periods at atrial and right ventricular sites. There were no significant differences between epicardial and endocardial activation delays during right ventricular stimulation, but during atrial stimulation epicardial conduction delay increased to a significantly greater extent than endocardial.
Changes in Epicardial Activation During Acute Ischemia

Fig 6 shows epicardial activation in the LAD territory, during atrial (top) and right ventricular (bottom) pacing. The LAD runs parallel and to the left of each array, next to sites A1 to G1. Under control conditions (left), atrial pacing results in nearly synchronous activation, with activation times ranging from 22 to 39 milliseconds. During right ventricular pacing (bottom left), activation proceeds parallel to epicardial fiber orientation, with activation proceeding in a parallel fashion from the sites closest to the LAD. Five minutes after LAD occlusion, activation during atrial pacing is strongly slowed (top right), with a region of block or extremely slow conduction (hatched lines). Within the ischemic zone (indicated by the dotted outline), activation time increased by an average of 74±8% at 27 sites outside the hatched area. During right ventricular pacing, conduction was much less altered by ischemia, with a mean activation time increase at the same 27 sites of 12±3% (P<.001 versus atrial pacing). Similar results were obtained in three other experiments.

Ischemia-Induced Changes in Conduction Velocity

During both atrial and left ventricular pacing conduction proceeded from endocardium to epicardium. Changes in conduction velocity could therefore be calculated from changes in endocardial to epicardial conduction time. Activation maps of the type shown in Fig 6 (bottom) were used to calculate changes in longitudinal conduction velocity during right ventricular pacing. As shown in Fig 7, acute ischemia reduced transmural conduction velocity by about 50%, during both left atrial and left ventricular pacing. In contrast, ischemia reduced longitudinal epicardial conduction during right ventricular pacing by only 13% (P<.001 versus transmural conduction).

Discussion

We have shown that the magnitude of epicardial activation delay during acute myocardial ischemia depends on the propagation pathway by which the impulse reaches the epicardium. When the epicardium is activated via transmural propagation, during supraventricular rhythms, ischemia causes significant epicardial conduction delays due to impulse slowing in the ischemic myocardial wall. When the impulse propagates along the epicardial surface, without being forced to traverse the ischemic myocardial wall, epicardial activation is much less affected by ischemia.

Comparison With Previous Studies of the Response of Epicardial Tissue to Acute Ischemia

Like a variety of previous investigators, we observed that epicardial zone activation delay exceeds endocardial during supraventricular rhythms. We were unable to identify previous studies assessing epicardial activation in the absence of transmural conduction. Our results are consistent with the conclusions of Gilmour and Zipes, that differences in the response to ischemia of endocardial and epicardial tissues are due to coupling of endocardial tissue to the specialized conducting system. Since our experiments were performed in vivo, we cannot comment on intrinsic sensitivity to ischemic
conditions. While Kimura et al.\textsuperscript{10} noted greater epicardial than endocardial activation delay during acute ischemia of isolated, perfused cat left ventricles, their

preparations were paced via the left bundle branch, and activation would have proceeded transmurally from the Purkinje system to the epicardium. Their results are therefore compatible with ours. Cox et al.\textsuperscript{20} studied transmural activation during acute myocardial ischemia in dogs. After 30 minutes of ischemia, the earliest time for which they provide results, 75% of the transmural conduction delay measured by eight electrodes from the endocardial (electrode 1) to the epicardial (electrode 8) surface was due to slowing between electrodes 4 and 6, consistent with our finding that most of the transmural conduction delay caused by ischemia occurs in the midmyocardium.

**Determinants of Conduction Slowing at Various Levels Across the Myocardial Wall**

The His-Purkinje system is relatively resistant to the effects of acute ischemia because of intrinsic resistance to ischemic conditions and perhaps also because of
contact with adjacent fully oxygenated left ventricular cavity blood. Since the endocardium is the first layer activated by the conducting system, its activation is determined by conduction time in the His-Purkinje system rather than the myocardium per se. A number of mechanisms may contribute to limiting conduction slowing in the ischemic epicardium relative to the midmyocardium. ATP-regulated potassium current (I_{KATP}) limits ischemic damage and \( V_{\text{max}} \) depression during brief periods of markedly reduced coronary flow. This action may be mediated by action potential abbreviation, which limits calcium entry, force generation, and metabolic requirements. There is evidence for greater sensitivity of I_{KATP} to metabolic inhibition in epicardial tissue compared with endocardial. Furthermore, calcium current (I_{Ca}) is more depressed by metabolic inhibition in epicardial than endocardial cells. Since I_{Ca} inhibition preserves longitudinal conduction during simulated ischemia, the enhanced sensitivity of epicardial I_{Ca} to ischemia may contribute to maintaining conduction velocity. Finally, epicardial blood flow is greater in the ischemic dog heart compared with mid-myocardial or endocardial flow. This combination of better-preserved blood flow and ionic properties favoring tissue survival may explain the epicardial sparing from necrosis after LAD occlusion in the dog and account for the relative preservation of epicardial conduction during acute ischemia.

Potential Significance of Our Findings

There are considerable differences in cellular electrophysiologic properties as a function of location within the myocardium. It has commonly been assumed that the marked epicardial conduction delays observed during acute myocardial ischemia in the dog reflect greater intrinsic sensitivity of the epicardium to ischemic conduction slowing. If this were the case, identification of factors causing differential epicardial ischemic conduction slowing might lead to new targets for antiarrhythmic intervention against acute ischemic arrhythmias. Our results suggest that epicardial conduction is not overly sensitive to acute ischemia. The transmural heterogeneity in ischemic conduction slowing that we observed may combine with the now well-characterized transmurally heterogeneous response of refractoriness in leading to ventricular arrhythmogenesis during acute myocardial ischemia.

Consideration of the Model

Ventricular pacing may alter the consequences of acute ischemia in comparison with supraventricular pacing via mechanisms independent of activation pattern. For this reason, we included a comparison between responses during right and left ventricular pacing. Since conduction is relatively slow transverse to fiber orientation, epicardial propagation from the left ventricular pacing site toward the ischemic zone proceeds more slowly than activation via the His-Purkinje system, resulting in transmural activation (Fig 5). This contrasts with the initial epicardial activation (Fig 5) and epicardial impulse propagation (Fig 6) during right ventricular stimulation. Consequently, we were able to contrast epicardial activation via transmural conduction during left ventricular stimulation with epicardial activation not requiring transmural propagation during right ventricular stimulation.

In most of our experiments, we studied the consequences of 5 minutes of ischemia. A brief period of ischemia was selected to avoid irreversible damage and carry-over effects from one occlusion to another. We obtained similar results during 15 minutes of ischemia, and other authors have noted similar phenomena during more prolonged ischemia. The dog has an important coronary collateral circulation, which may contribute to epicardial sparing from necrosis after coronary artery occlusion. It would be interesting to evaluate ischemic changes in longitudinal epicardial compared with transmural conduction in an animal such as the pig, which has a relatively poor coronary collateral blood supply.

Conclusions

We have shown that prominent ischemic epicardial conduction delays occur when the impulse must traverse the myocardial wall to activate the epicardium. During longitudinal epicardial conduction through the ischemic zone, the conduction delay is much less. Our findings indicate that previously observed differences between endocardial and epicardial activation during acute ischemia in the dog are due not to intrinsic differences in sensitivity to ischemia but rather to the need for transmural propagation to the epicardium through the ischemic myocardial wall.

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