Relationship between Changes in Left Ventricular Bipolar Electrograms and Regional Myocardial Blood Flow during Acute Coronary Artery Occlusion in the Dog

RUDOLPHE RUFFY, D. EUGENE LOVELACE, THOMAS M. MUELLER, SUZANNE B. KNOEBEL, AND DOUGLAS P. ZIPES

SUMMARY The purpose of this study was to determine whether a quantitative relationship existed between a reduction in regional myocardial blood flow, measured by radiolabeled microspheres, and the degree and type of changes in myocardial activation recorded in bipolar left ventricular subepicardial and subendocardial electrograms, in open-chest dogs following acute coronary artery occlusion. We found that the degree of regional myocardial ischemia was related quantitatively to the reduction in amplitude recorded with bipolar electrograms in the subepicardium and subendocardium, and to the increase in duration of subepicardial electrograms. Other characteristics measured in electrograms did not relate to the degree of ischemia. Despite a comparable reduction in regional myocardial blood flow, subepicardial conduction delay exceeded that recorded in the subendocardium, which often exhibited accelerated conduction. Circ Res 45: 764-770, 1979

ACUTE myocardial ischemia, produced by coronary artery occlusion, results in electrophysiological alterations manifested by changes in myocardial activation. Durrer et al (1961) qualitatively described the changes recorded in extracellular bipolar electrograms following coronary artery occlusion, and since then several studies have provided quantitative data relating the extent of electrogram alterations to the development of ventricular arrhythmias (Scherlag et al., 1970; Waldo and Kaiser, 1973; Boineau and Cox, 1973; Cox et al., 1973; Hope et al., 1974; Williams et al., 1974). However, no information exists on whether the degree of myocardial ischemia influences the magnitude of disturbance in activation or the specific type of electrogram change. To this end, the present study was undertaken. Its purpose was to determine whether a quantitative relationship existed between a reduction in regional myocardial blood flow, measured by radiolabeled microspheres, and the degree and type of changes in myocardial activation recorded in bipolar left ventricular subepicardial and subendocardial electrograms.

Methods

Nineteen mongrel dogs weighing 10-27 kg were anesthetized with intravenous secobarbital (30 mg/kg), or morphine sulfate (2.25 mg/kg) and α-chloralose (50-100 mg/kg), and artificially ventilated with room air. Administration of anesthetics was repeated as necessary; no experimental measurements were obtained for at least 15 minutes after drug administration. The body temperature was kept normal with a heating blanket. A carotid artery was cannulated to monitor the systemic arterial pressure, and a jugular vein was used to administer the anesthetic and 0.9% normal saline solution (150 ml/hour) to replace fluid lost during the procedure. Lead II of the electrocardiogram was monitored constantly. The brachial artery and a femoral artery were cannulated to collect arterial blood during regional myocardial blood flow measurements.

The chest was opened via a left thoracotomy or midline sternotomy. The heart was exposed and suspended in a pericardial cradle. The sinus node was crushed to slow its spontaneous discharge rate. The left atrium was cannulated to inject radiolabeled microspheres. The epicardial surface of the right ventricular outflow tract was paced constantly at a cycle length between 300 and 500 msec with a bipolar hook electrode that had a pole separation of 1-2 mm. Stimuli, delivered through an isolation transformer, were rectangular pulses 1-2 msec in duration, at an intensity twice the diastolic threshold.

Electrogram Recordings and Analysis

In each dog, three 22-gauge decapolar plunge needle electrodes were placed at least 1.5 cm apart...
near the center of the potentially ischemic zone of the anterior left ventricular wall, and a fourth plunge electrode was inserted in the high lateral left ventricular wall, well outside of the potential ischemic zone. The recording electrodes on the needle were 0.05 mm in diameter and 1 mm apart. Needle electrodes were inserted perpendicular to the epicardium. Two adjacent electrodes, located close to the epicardium and endocardium, were selected on each needle to record bipolar subepicardial and subendocardial activity. The signals were amplified (Medical Electronics Consulting Associates), filtered between 12 and 500 Hz, displayed on a storage oscilloscope (Tektronix 5 A 18 N), and simultaneously recorded on a Honeywell strip chart recorder at a paper speed of 400 mm/sec.

Electrograms from one cardiac cycle before occlusion, and during occlusion at the beginning of the microsphere injection, were selected from each recording site. Only electrograms that showed measurable changes of less than 5% throughout the entire control period, and for at least five consecutive cardiac cycles at the beginning of the microsphere injection, were selected. In the absence of coronary artery occlusion, the electrograms were stable and the electrogram characteristics that were measured varied by less than 5% over several hours. The data were rejected if the plunge needle electrode became dislodged during the coronary artery occlusion, or if frequent premature complexes occurred.

The changes in amplitude, time-to-onset, time-to-major peak, time-to-end (total time), and duration of each electrogram were measured (Fig. 1), using a computerized data acquisition system consisting of a Tektronix 4010-1 graphics terminal and tablet, and associated software. All points to be measured were chosen by the same investigator. Electrograms then were traced, and the various fiducial points were marked. For measurements of the time intervals, the stimulation artifact served as the reference point. Individual points were measured with an accuracy of 0.25 mm (0.6 msec). Repeated measurements by the same investigator varied by less than 1%.

The data were normalized by expressing the ischemia-induced electrogram changes as a percent of the control values measured immediately prior to coronary artery occlusion.

**Measurement of Myocardial Perfusion**

Myocardial perfusion was measured by carbonized microspheres (7–10 µm in diameter) labeled with 51 Cr or 141 Ce. For each flow determination, between 2 × 10^6 and 3 × 10^6 microspheres were injected over a 10-second period, followed by a 5-ml saline flush. The microspheres were obtained from Minnesota Mining and Manufacturing Company as 1 mCi of nuclide suspended in 10 ml of 10% dextran to which 1 drop of Tween-80 had been added. Starting at least 10 seconds before injection and continuing for at least 60 seconds after injection, blood was withdrawn at the rate of 10 ml/min with a Harvard infusion-withdrawal pump (Harvard Apparatus Company, model 940) from the brachial and femoral arterial cannulae. Prior to injection, the vial containing the microspheres was vigorously agitated ultrasonically and mechanically for approximately 10 minutes. Microscopic examination of spheres treated in this manner showed that they were completely dispersed.

After the dog had been killed, the heart was excised and washed free of blood. Each epicardial and endocardial recording site was located precisely and labeled for later identification. The ventricular cavities were stuffed with gauze to preserve shape, and the heart was stored in formalin for 48 hours to facilitate sectioning. At this time, epicardial fat, major blood vessels, and the atria were removed from the heart. The heart was weighed and then sliced into six slices of equal thickness perpendicular to its long axis. In each slice, the right ventricular wall was separated from the left ventricle and interventricular septum. The slices of the left ventricle, including the interventricular septum, were divided into seven sections, and each of these sections was divided into epicardial and endocardial halves (Fig. 2). Each numbered piece of the left ventricle sectioned in such a fashion had a constant anatomic position so that pieces could be compared from one study to the next. The samples were placed in preweighed plastic tubes, weighed, and counted for at least 5 minutes in a well-type γ scintillation counter (Packard Instrument Company). Reference blood samples were divided and counted in a similar manner. Standard techniques were used for isotope separation (Heymann et al., 1977).

Myocardial perfusion was calculated using the formula, \( BF_m = \left( \frac{C_m \times 100 \ BF_r}{C_r} \right) \), in which \( BF_m \) = myocardial blood flow (ml/min X 100 g), \( C_m \) =...
counts/g of myocardium, \( BF \) = reference blood flow (the rate of withdrawal from the reference artery), and \( C_r \) = the total counts in the reference blood (Heymann et al., 1977). For each flow measurement, a normal zone (core area) value was derived from the mean flow measurement in eight sections (16 halves) of the posterior left ventricular wall. The core area was defined as the endocardial and epicardial halves of sections 4 and 5 from slices 2, 3, 4, and 5 of the left ventricle. Blood flow outside of the core area was expressed as a percentage of the core area flow and then grouped into four categories of normalized regional blood flow: category I, 0-25% core area flow; category II, >25-50% core area flow; category III, >50-75% core area flow; and category IV, >75% core area flow. Only data from myocardial pieces in which electrogram recordings were made were used for correlations between electrogram parameters and blood flow. To determine whether pieces from category I represented a region in the center of the ischemic zone, blood flow in the nearest neighbors of category I pieces also was analyzed. “Nearest neighbor” is defined as all myocardial pieces that have a common adjacent surface to the piece being referenced (Marcus et al., 1975).

Study Protocol

After heparin (300 U/kg) administration, acute myocardial ischemia was produced by complete occlusion of the left anterior descending (LAD) coronary artery 1–2 cm distal to the origin of the anterior septal artery. The first occlusion served to determine the time needed to develop alterations in the bipolar electrograms and to establish the approximate boundaries of the ischemic zone. The occlusion then was released. If ventricular fibrillation occurred, direct current defibrillation was performed within 15 seconds, using 10–20 joules. A recovery period of at least 30 minutes was allowed between LAD occlusions. All initial occlusions lasted less than 6 minutes.

Just before the second coronary artery occlusion, a control recording of the electrograms was obtained. The LAD then was occluded for 2–5 minutes and electrograms were monitored continuously. When marked electrogram alterations had taken place, or 1 minute before the expected onset of ventricular fibrillation (as determined by the first LAD occlusion), electrograms were recorded and microspheres were injected simultaneously. The arterial occlusion was maintained during the entire microsphere sampling period, and the data were excluded if ventricular fibrillation occurred before completion of the arterial blood withdrawal. After completion of the blood flow determination, the dog was killed by inducing ventricular fibrillation electrically, and the heart was removed and studied as described above.

Data Analysis

The extent of changes in bipolar electrograms at each recording site was compared to the regional blood flow measured at that site. The data were analyzed statistically using the analysis of variance, the Mann-Whitney U-test, and the Spearman rank order correlation coefficient. The analysis of variance was used to define the significance of difference between the mean values of groups of observations. Because of the heterogeneity of the data, in some cases, the analysis of variance was replaced with the nonparametric equivalent, the Mann-Whitney U-test. The Mann-Whitney U-test was used whenever the data groupings resulted in populations that grossly violated the homogeneity of variance assumption as shown using Bartlett’s test and Cochran’s test. The Spearman rank order correlation coefficient was used, since the relationship between the electrophysiological variables and the regional myocardial blood flow may not be linear.

Results

Myocardial Blood Flow

Myocardial blood flow in the core areas was 96.5 ± 11.9 ml/min × 100 g. The number of segments at each level of flow for all recording electrode sites (Fig. 3), as well as the data for subepicardial (Fig. 4) and subendocardial (Fig. 5) recordings sites, separately analyzed, are presented. The ratio of endocardial to epicardial flow in the core area was 1.07 ± 0.12. Of the nearest neighbors of the endocardial pieces in category I, 66.7% also had 0–25% core area flow. An additional 11.1% of the nearest neighbors were in category II. For epicardial pieces of category I, 68.1% of the nearest neighbors had 0–25% core area flow.
Subendocardial Vs. Subepicardial Observations

When data from subepicardial (Fig. 4) and subendocardial (Fig. 5) electrograms were analyzed separately, it was found that the electrograms recorded at the subepicardium demonstrated progressive loss of amplitude and increase in duration as the regional blood flow decreased. Of the other intervals measured, only total time showed a significant increase in severely underperfused epicardial zones. The subendocardial electrograms also exhibited a progressive decrease in amplitude with reduction in regional blood flow. However, no significant increases in other subendocardial electrogram measurements were found, although a trend toward shortening of the time intervals was apparent in most low flow categories. Statistical significance of the various changes is given in each figure.

Since both quantitative and directional differences existed between changes in subepicardial and subendocardial electrograms within the same categories of reduction in blood flow, the subepicardial
recordings were pooled and compared to the pooled subendocardial recordings (Fig. 6). It was seen that the increase in time-to-peak, total time, and electrogram duration at the subepicardium exceeded those changes at the subendocardium. Overall reduction in regional myocardial blood flow and electrogram amplitude was similar at the subendocardium and subepicardium.

Discussion

The purpose of this study was to determine whether a quantitative relationship existed between the severity of regional ischemia and the magnitude and type of local activation changes that occurred in the canine ventricular myocardium after coronary artery occlusion. We found that a reduction in the amplitude of subepicardial and subendocardial electrograms and increase in the duration of the subepicardial electrograms were related to the degree of ischemia, but other measured electrogram parameters were not.

Comparison of Epicardial Vs. Endocardial Changes

For a comparable reduction in regional myocardial blood flow and loss of electrogram amplitude, the magnitude of the activation delay measured at the subepicardial surface significantly exceeded that measured at the subendocardium. Such differences have been observed before (Cox et al., 1973; Lazzara et al., 1973; Hope et al., 1974; Scherlag et al., 1974; Friedman et al., 1973b; Corr et al., 1978; Elharrar et al., 1977), and indicate a spatial and temporal dissociation in the development of ischemia-induced conduction delay between the subepicardium, where slow conduction has developed, and the subendocardium, which exhibits much less conduction delay or even accelerated conduction. This dissociation may be one of the factors leading to the occurrence of ventricular arrhythmias within the first few minutes following coronary artery occlusion.

At present, it is not certain why similar degrees of regional ischemia exert different effects on conduction in the subepicardium and subendocardium. The late development of subendocardial conduction delay appears somewhat paradoxical, since the subendocardium is generally damaged first and most severely following coronary artery occlusion (Jennings et al., 1960; Rivas et al., 1976). Various explanations have been offered. These include the nearby presence of oxygenated cavity blood that protects superficial Purkinje fibers and subendocardial muscle from the effects of ischemia (Friedman et al., 1973a, 1973b; Lazzara et al., 1973), blood flow from luminal collaterals that may nourish the subendocardium (Fixler et al., 1974), an increased capacity...
of the subendocardium for anaerobic glycolysis compared to the subepicardium (Lundsgaard-Hansen et al., 1967), and the resistance of Purkinje fibers to the effects of hypoxia and ischemia (Bagdonas et al., 1961; Friedman et al., 1973a; Lazzara et al., 1973).

Accelerated impulse propagation has been observed in canine (Gambetta and Childers, 1969) and porcine (Holland and Brooks, 1976) myocardium, transiently and very early after coronary artery occlusion, and may relate to a transient reduction in the excitability threshold of the ischemic myocardium (Elharrar et al., 1977). Cellular excitability is one of the determinants of conduction velocity (Peon et al., 1978), and a reduction in excitability threshold, perhaps caused by the initial increase in extracellular potassium (Harris, 1966; Hill and Gettes, 1977), could be responsible for an initial increase in conduction velocity. Further cellular depolarization and elevation of excitability threshold from an additional increase in extracellular potassium concentration could result in the subsequent development of slowed conduction. However, this reasoning cannot explain the persistence of accelerated impulse propagation in the subendocardium as long as 5 minutes after the onset of coronary artery occlusion (Figs. 5 and 6), since the dip in excitability threshold is quite transient (Elharrar et al., 1977). It is possible that the increase in extracellular potassium concentration is balanced in certain superficial areas of the subendocardium by washout into the left ventricular cavity, for example, resulting in persistence of enhanced conduction at a time when slowed conduction has developed in the subepicardium. Changes in activation sequence cannot be eliminated entirely as a cause of the accelerated conduction.

Changes in Electrograms

The time intervals recorded in the bipolar electrograms appear to measure different phenomena. Assuming that the actual sequence of myocardial activation did not change during ischemia, alterations in electrogram amplitude and duration might be expected to reflect primarily changes in local activation, whereas time-to-onset, time-to-peak, and total time would also be influenced by conduction from the stimulation site, traveling through normal and ischemic myocardium, to the recording site. Changes in these values might be influenced by the distance of the stimulation site from the ischemic zone and, therefore, by how much normal myocardium is traversed before reaching the ischemic zone.

From this reasoning, one might predict that changes in electrogram amplitude and duration would relate best to changes in regional myocardial blood flow, and that alterations in total time, time-to-onset, and time-to-peak would be less influenced by the degree of regional ischemia. It is of interest that, at the subepicardium, both a decrease in electrogram amplitude and an increase in electrogram duration were related to changes in regional perfusion, the former better than the latter. At the subendocardium, only electrogram amplitude demonstrated a significant relationship with the reduction in regional blood flow.

Regional Myocardial Blood Flow

Since segments of myocardium of the size used in this experiment (about 1 g) can contain heterogeneous regions of ischemic and well-perfused tissue (Jennings et al., 1957), it is possible that the bipolar electrodes were situated in a well-perfused area masked by surrounding ischemia. To minimize the effects of such heterogeneous blood flow that might obscure the correlation of electrogram changes with blood flow, we attempted to place the electrodes in the center of the potentially ischemic zone. The central region of an ischemic zone is more likely to be uniformly ischemic than are more peripheral portions, (Hirzel et al., 1976). Since we found that blood flow was diminished to <50% of core area flow in about 80% of the segments neighboring on those segments designated as category I (0-25% core area flow), it is probable that the markedly reduced levels of blood flow in category I segments accurately reflected the perfusion in the region surrounding the recording electrodes. Furthermore, since the degree of ischemia was very similar in the subendocardial segments compared to the subepicardial segments, it is unlikely that the differences in subendocardial and subepicardial electrogram parameters could be explained by an artifact of the myocardial perfusion measurements falsely giving a low estimate of subendocardial blood flow in the region of subendocardial electrodes. It is possible that electrograms recorded in segments with lesser degrees of ischemia (i.e., greater than 25% mean core flow) are more likely to reflect regions of greater heterogeneity of blood flow.

Considerations of the Model

We chose to study occlusions of less than 5 minutes in duration for several reasons. First, ventricular fibrillation generally occurs in this early period after coronary artery occlusion, making it an important period to investigate. The electrophysiological alterations that occur early after coronary artery occlusion are unstable, rapidly changing, and seem to play a key role in the genesis of ventricular fibrillation resulting from ischemia. Later on, when the changes stabilize, or even begin to revert toward normal, ventricular fibrillation occurs less often. Second, we wished to create a rather large area of ischemia to produce extensive electrogram and blood flow changes, but we had to obtain measurements prior to the onset of ventricular fibrillation.
that often occurred within 5 minutes of occlusion. Finally, we chose to measure blood flow at a time when the influence of collateral blood flow would be relatively small.

A determination of the relationship between the degree of regional ischemia and the extent of changes in local myocardial activation is complicated by the temporal dissociation of the events. Although a decrease in blood flow immediately follows coronary artery occlusion, electrogram changes are delayed in onset for several minutes and then evolve rapidly after the occlusion. This latency in the development of ischemia-induced alterations in electrograms contrasts with the changes in contractility observed almost immediately after coronary artery occlusion (Theroux et al., 1976), and appear concomitant with the conversion of the myocardium to anaerobic metabolism (Pirzada et al., 1975).

Finally, since the ischemic state results from an imbalance between the energy requirements of the myocardium and its metabolic supply, measurement of regional blood flow provides only a partial assessment of the magnitude of myocardial ischemia. Thus, at a given level of blood flow reduction, various degrees of ischemia can be present, depending on the instantaneous metabolic requirements.

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R Ruffy, D E Lovelace, T M Mueller, S B Knoebel and D P Zipes

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