Cellular Electrophysiological Changes During Ischemia in Isolated, Coronary-Perfused Cat Ventricle With Healed Myocardial Infarction

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Cellular electrophysiological consequences of acute ischemia superimposed on healed myocardial infarction were studied in isolated, coronary-perfused cat left ventricles 2–4 months after ligation of multiple distal tributaries of the left anterior descending and circumflex coronary arteries. Oxygenated Tyrode’s solution was perfused through the left anterior descending and circumflex coronary arteries, and the preparations were superfused with Tyrode’s solution gassed with 95% N₂-5% CO₂. Transmembrane action potentials were recorded from the endocardial cells in normal and infarcted zones. There were no significant differences in measured action potential variables and refractory periods between cells in the normal and infarcted zones before acute ischemia. When coronary perfusion was discontinued (“ischemia”), resting potential, action potential amplitude, and action potential duration were reduced, and the refractory period was shortened progressively in cells of the normal zone. However, the action potential changes were less prominent, and the refractory period was unchanged in cells in the infarcted zone. As a result, there were significant differences in resting membrane potential, action potential amplitude, action potential duration, and refractory period between cells in the normal and infarcted zones at 10 minutes of ischemia. These differences became larger as the ischemic period was prolonged. Spontaneous rapid ventricular activity was observed during the last 20–30 minutes of ischemia in four of eight preparations with healed myocardial infarction, whereas no spontaneous rapid ventricular activity was recorded in any of six normal heart preparations. Our data suggest that superimposition of acute ischemia on healed myocardial infarction produces electrophysiological inhomogeneities that may enhance arrhythmogenesis. (Circulation 1988;78:401–406)

Acute ischemia is a common clinical occurrence in hearts that have healed after a previous ischemic injury or infarction. The majority of survivors of out-of-hospital cardiac arrest have extensive coronary artery disease, usually with previous myocardial infarction.1–4 The high incidence of recurrent ventricular fibrillation and sudden cardiac death in this group4–6 suggests that ischemic events in the presence of preexisting healed myocardial infarction enhance the risk for the occurrence of fatal arrhythmias. Experimentally, the incidence of spontaneous ventricular tachycardia and fibrillation is higher when acute ischemia is superimposed on previous myocardial infarction than with acute ischemia alone.7,8 Our previous studies also have shown that cat hearts with acute infarction superimposed on healed myocardial infarction have a greater incidence of spontaneous and induced ventricular arrhythmias than do hearts with acute infarction alone.9 Dispersion of local refractoriness also is greater in the former.9 However, less information is available on the cellular electrophysiological changes that occur during transient acute ischemia superimposed on a healed myocardial infarction.

This study was designed to examine ischemia-induced changes in transmembrane action potentials and refractory periods that are simultaneously recorded from cells in the normal and infarcted zones of the cat left ventricle with healed myocardial infarction. The left ventricles were perfused through their coronary arteries, and "ischemia" was produced by cessation of coronary perfusion.10–12 This technique permitted
us to test the hypothesis that dynamic changes, induced by acute ischemia in hearts with healed myocardial infarction, exaggerate electrophysiological inhomogeneities and thus facilitate arrhythmias.

Materials and Methods
Preparation and Solution
Domestic cats, weighing 2.0–3.5 kg, were anesthetized with sodium pentobarbital (30 mg/kg i.p.). Acute myocardial infarction was created by single-stage ligation of multiple, distal tributaries of the left anterior descending and left circumflex coronary arteries as described previously. Acute myocardial infarction was created by single-stage ligation of multiple, distal tributaries of the left anterior descending and left circumflex coronary arteries as described previously.13 The surviving cats were maintained in a colony for 2–4 months. On the day of terminal studies, the cats with healed myocardial infarction were anesthetized with sodium pentobarbital as described above, and the heart was rapidly removed and immersed in cool, oxygenated Tyrode’s solution. The method of dissection, and the perfusion and superfusion techniques have been previously described in detail.10,11 In brief, after removal of both atria and the right ventricle, the left anterior descending and circumflex coronary arteries were cannulated with polyethylene cannulas (PE 10 or PE 50, Intramedic) through the left main coronary ostium in the aortic root. An interval of no more than 5 minutes elapsed from the time the heart was excised to cannulation. The cannulas were secured in place with 6–0 silk suture, and the preparations were perfused with Tyrode’s solution equilibrated with 95% O2-5% CO2. The perfused area, with the exception of the infarct itself, was distinctly delineated by its pale appearance after injection of Tyrode’s solution. The tissue beyond the perfused area was excised, and the major branches of arteries that were transected by the dissection were ligated with 6–0 silk suture. The cannulated preparations with healed myocardial infarction were placed with the endocardial surface side up in a superfusion chamber and were superfused with Tyrode’s solution gassed with 95% N2-5% CO2 at a flow rate of 30 ml/min. The preparations were simultaneously perfused through the coronary cannulas with Tyrode’s solution gassed with 95% O2-5% CO2 at a perfusion rate of 1.0 ml/g wet wt/min. The temperature of the solution was 37°C, and its pH was 7.30. The PO2 of the perfusate was greater than 500 mm Hg, whereas the PO2 of the superfusate was 30–40 mm Hg. The composition of Tyrode’s solution was (mM): NaCl 129, KCl 4, NaHCO3 20, NaH2PO4 1.8, MgCl2 0.5, CaCl2 2.7, and dextrose 5.5.

Electrical Stimulation and Recording
Driving stimuli were delivered to the left bundle branch at a cycle length of 800 msec through bipolar, Teflon-coated, silver-wire electrodes. Pulse duration was 3 msec, and current strength was twice late-diastolic threshold. Conventional microelectrode techniques were used to record transmembrane action potentials. Glass microelectrodes, filled with 3 M KCl (resistance, 10–25 MΩ), were connected through silver–silver chloride electrodes to a high-input impedance electrometer with input capacity neutralization (KS-700, World Precision Instruments, New Haven, Connecticut). Transmembrane action potentials were recorded simultaneously from the endocardial cells in the normal and infarcted zones. Bipolar electrogams were recorded from the normal and infarcted zones with fine silver-wire electrodes (diameter, 0.1 mm) placed close to the action potential recording electrodes. The distance between the stimulating electrodes and the recording electrodes was approximately 1.5 cm. The signals were displayed on oscilloscopes and recorded on Polaroid film and a polygraph. Action potential variables measured were resting membrane potential, action potential amplitude, and action potential duration at 50% and 90% repolarization. Conduction time was estimated as the interval between the upstroke of the stimulus artifact and the major deflection of the bipolar electrogams.

The local refractory periods in the normal and infarcted zones were measured by the extrastimulus method. The bipolar-stimulating electrodes were placed on the normal and infarcted zones 2 mm from the recording microelectrodes. Premature stimuli (S2) were delivered after every seventh drive stimulus (S1). The stimulus current strengths of S1 and S2 were twice late diastolic threshold determined before ischemia and were kept constant during 30 minutes of ischemia. The initial S1-S2 interval was 250 msec, and it was shortened progressively in 10-msec decrements until the preparations failed to respond to S2. Then, the scanning was repeated with 2-msec decrements from an interval 10–20 msec longer than the refractory period. The refractory period was defined as the longest S1-S2 interval that did not result in a response to S2.

Protocol
After a 45-minute equilibration period, myocardial ischemia was produced by discontinuing coronary perfusion while maintaining superfusion with Tyrode’s solution gassed with 95% N2-5% CO2. Transmembrane action potentials recorded from cells in the normal and infarcted zones were continuously displayed and recorded on film every 10 minutes during the period of ischemia. Rapid ventricular activity, defined as rapid runs of ectopic impulses that lasted longer than 5 seconds, was monitored during ischemia and reperfusion. In a second series of experiments, the local refractory periods in the normal and infarcted zones were measured before and during 30 minutes of ischemia.

Statistical Analysis
All data are expressed as mean ± SD and were evaluated for statistical significance by analysis of variance with repeated measurements. Differences with p<0.05 were considered significant.
### Table 1. Action Potential Variables in Cells of Normal and Infarcted Zones

<table>
<thead>
<tr>
<th>Zone</th>
<th>RMP (-mV)</th>
<th>APA (mV)</th>
<th>APD&lt;sub&gt;50&lt;/sub&gt; (msec)</th>
<th>APD&lt;sub&gt;90&lt;/sub&gt; (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>80.4 ± 1.2</td>
<td>110.4 ± 3.7</td>
<td>137.3 ± 8.5</td>
<td>178.3 ± 7.7</td>
</tr>
<tr>
<td>Infarct</td>
<td>80.0 ± 0.6</td>
<td>109.5 ± 3.0</td>
<td>138.5 ± 8.4</td>
<td>185.9 ± 6.6</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD; n = 8. RMP, resting membrane potential; APA, action potential amplitude; APD<sub>50</sub> and APD<sub>90</sub>, action potential duration measured at 50% and 90% repolarization, respectively. None of the comparisons between cells of the normal and infarcted zone show statistically significant differences.

### Results

#### Changes in Transmembrane Action Potentials During Ischemia

Table 1 summarizes transmembrane action potential measurements in cells of the normal and infarcted zones during control periods. Action potential duration at 90% repolarization was slightly longer in cells of the infarcted zone than in cells of the normal zone, but the difference did not reach statistical significance. Resting membrane potential and action potential amplitude did not differ between cells of the normal and infarcted zones.

When coronary perfusion was discontinued (ischemia), resting membrane potential, action potential amplitude, and action potential duration were reduced. Figure 1 shows ischemia-induced changes in transmembrane action potentials simultaneously recorded from cells of the normal and infarcted zones. During 30 minutes of ischemia, action potentials recorded from cells of the normal zone deteriorated progressively. In contrast, changes in transmembrane action potentials recorded from the infarcted zone were less prominent. As shown in Figure 1, there were significant differences in resting membrane potential, action potential amplitude, and action potential duration between cells of the normal and infarcted zones after 10 minutes of ischemia, and the differences became greater as the ischemic period was prolonged.

During 30 minutes of ischemia, conduction times were prolonged in both the normal zone (from 18.2 ± 3.2 to 23.8 ± 3.8 msec) and the infarcted zone (from 19.8 ± 3.8 to 25.0 ± 4.1 msec). There was no difference between the normal and infarcted zones in the magnitude of prolongation of conduction time induced by ischemia. Ischemia-induced changes in electrograms were not remarkable in either the normal or infarcted zone.

Another group of hearts was studied to determine whether the less-pronounced changes in action potentials during ischemia in cells of the infarcted zone are due to the dependence of these cells on the superfusate rather than on the perfusate. Three preparations were superfused with oxygenated Tyrode's solution during 30 minutes of perfusion with oxygenated Tyrode's solution. Then, the superfusate was changed to Tyrode's solution gassed with 95% N<sub>2</sub>-5% CO<sub>2</sub> while maintaining coronary perfusion with oxygenated Tyrode's solution. The action potential duration at 90% repolarization in cells of the infarcted zone was slightly reduced from 198.2 ± 8.3 msec during O<sub>2</sub> superfusion to 187.3 ± 6.5 msec during N<sub>2</sub> superfusion, whereas action potential duration at 90% repolarization in cells of the normal zone remained unchanged (from 185.8 ± 7.9 to 180.1 ± 7.5 msec).
Changes in Refractory Period During Ischemia

Additional experiments were performed to examine the change in refractory period in the normal and infarcted zones during 30 minutes of ischemia. Before ischemia, there was no significant difference in the refractory period between the normal and infarcted zones (194.4 ± 14.4 and 203.2 ± 15.3 msec in the normal and infarcted zones, respectively). As shown in Figure 3, the refractory period in the normal zone shortened progressively during 30 minutes of ischemia, whereas the refractory period in the infarcted zone remained unchanged. The refractory period in the infarcted zone was significantly longer than that in the normal zone at 10 minutes of ischemia, and the difference became greater as the ischemic period was prolonged.

Arrhythmias During Ischemia and Reperfusion

Table 2 summarizes the incidence of rapid ventricular activity during 30 minutes of ischemia and subsequent reperfusion. During the final 20–30-minute period of ischemia, spontaneously rapid ventricular activity was recorded in four of eight preparations with healed myocardial infarction. In a parallel series of experiments with normal hearts (n = 6), no rapid ventricular activity was recorded during 30-minute periods of ischemia. However, during reperfusion, rapid ventricular activity was recorded in all six normal heart preparations and in seven of eight preparations with healed myocardial infarction. During the experiments designed to measure refractory period, rapid ventricular activity was induced by extrastimuli in two of six preparations with healed myocardial infarction at 10 minutes of ischemia, in three preparations at 20 minutes, and in three preparations at 30 minutes. Rapid ventricular activity was induced only when the preparations were stimulated from the normal zone. In normal hearts, rapid ventricular activity could be induced in two of six preparations at 10 minutes of ischemia and in three preparations at 30 minutes of ischemia. However, rapid ventricular activity was not inducible at 20 minutes of ischemia in the normal heart preparation.

**Table 2. Incidence of Spontaneous, Rapid Ventricular Activity During Ischemia and Reperfusion in Normal Hearts and in Hearts With Healed Myocardial Infarction**

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Normal</th>
<th>Healed myocardial infarction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemia</td>
<td>0/6</td>
<td>4/8</td>
</tr>
<tr>
<td>Reperfusion</td>
<td>6/6</td>
<td>7/8</td>
</tr>
</tbody>
</table>

**Discussion**

The majority of survivors of potentially lethal episodes of ventricular tachycardia or fibrillation have extensive coronary artery disease with previously recognized or unrecognized myocardial infarction.1–6 These findings indicate that the superimposition of an acute ischemic event on preexisting myocardial infarction is a common pathophysiological substrate favoring the development of fatal arrhythmias. Indeed, it has been demonstrated experimentally in dogs that spontaneous ventricular tachycardia and fibrillation developed more frequently when acute ischemia is superimposed on previous myocardial infarction than with acute ischemia alone.7,8 Our previous studies with cat hearts also showed that the combination of acute infarction and earlier myocardial infarction scars was more arrhythmogenic than either acute infarction or healed infarction alone.9 However, no information is available on the dynamic cellular electrophysiological changes that occur during shorter periods of acute ischemia in hearts with previous, healed infarctions.

In the present study, there were no significant differences in action potential variables between cells of the normal and infarcted zones during control periods. This finding differs from our previous studies14,15 that showed that action potential duration was longer in cells of the infarcted zone than in cells of the normal zone. However, the difference in action potential duration between the present study and the previous observations is not in cells of the infarcted zone, but in cells of the normal zone. Action potential duration in cells of the normal zone in this study (approximately 180 msec) is much longer than that in the previous studies (approximately 130 msec). This discrepancy may be due to different experimental conditions. The preparations were perfused through the coronary arteries in this study, whereas the preparations were superfused in the previous studies. When left ventricular preparations are superfused, the cells in the midmyocardium may be hypoxic, and this may result in shortening of action potential duration in the superfused preparations.
The present study demonstrated that ischemia-induced changes in transmembrane action potentials were different between cells of the normal and infarcted zones of cat hearts with infarcts 2–4 months old. Resting membrane potential, action potential amplitude, and action potential duration were reduced to a lesser degree in cells of the infarcted zone than in cells of the normal zone, and there were significant differences in refractory periods, as well as action potential variables, between cells of the normal and infarcted zones during transient acute ischemia. Such electrical dispersion could facilitate the development of arrhythmias. Indeed, spontaneously rapid ventricular activity was recorded during ischemia in four of eight healed, infarcted heart preparations, whereas there was no spontaneously rapid ventricular activity during periods of ischemia in otherwise normal heart preparations. However, these data must be interpreted cautiously because the electrophysiological variables were monitored at only two sites. Ischemia-induced electrophysiological changes may be inhomogeneous, especially in the infarcted tissue. Furthermore, in this study, global ischemia was produced so that we were able to study electrophysiological effects of ischemia on cells of the infarcted zone without interference by unidentified collateral perfusion. Regional ischemia, which would mimic clinical events more closely, could produce more complex heterogeneities in electrophysiological properties between normal and acutely ischemic tissues as well as acutely ischemic and chronically infarcted tissues. Further studies are required to understand the contributions to arrhythmogenesis of the electrophysiological interactions of normal myocardium, regional ischemia, and chronically infarcted tissues. However, before such studies could be interpreted, it was necessary for us to clarify specific responses of normal and previously infarcted areas to the uniform effects produced by global ischemia.

Slow conduction in the infarcted myocardium may enhance arrhythmogenesis in healed, infarcted heart preparations. Slow conduction due to changes in membrane characteristics and axial resistance has been demonstrated in chronically infarcted myocardium. Although the results of the present study showed no difference in conduction time between the normal and infarcted zones before and during ischemia, the technique we used for the measurement of conduction time has limitations. It is impossible to detect local conduction abnormalities and changes in activation patterns during ischemia with a single bipolar electrode.

It is unclear from this study why ischemia-induced electrophysiological changes were less remarkable in cells of the infarcted zone. There are several possibilities. For example, the cells overlying the infarct may not be dependent on coronary perfusion to obtain oxygen and substrates. It has been shown in dog hearts with 4-day- or 2-week-old myocardial infarction that subendocardial blood flow in the infarcted zone is approximately 10% of that in the normal myocardium. Stenosis or occlusion of the coronary artery that provides collateral flow to the infarcted zone did not change endocardial flow although epicardial blood flow in the infarct zone was reduced. Thus, it is possible that the endocardial cells overlying the infarcted zone from which action potentials were recorded were not affected by cessation of coronary perfusion. The endocardial cells in the infarcted zone may obtain oxygen from the surface (cavity blood in vivo). This concept is further supported by additional experiments that showed that action potential duration in cells of the infarcted zone was slightly shortened, whereas action potential duration in cells of the normal zone did not change when superfusate was switched from oxygenated solution to hypoxic solution with maintenance of coronary perfusion.

There may also be other mechanisms for these differences. The diffusion of ions and metabolic products between the deep zone and the subendocardial tissue may play a role in the electrophysiological changes during ischemia. Such substances may diffuse from a large myocardial source to the endocardial boundary in the normal zone, whereas the source may be much smaller when the underlying tissue is heavily scarred. In addition, the reduced space constant of infarcted myocardium would make the infarcted myocardium less susceptible to electrotonic influence from the deeper layers of myocardium that may have more depressed action potentials because of a lack of washout of extracellular potassium and other products by the superfusate.

It is also possible that the cells in the infarcted zone that survived the previous ischemic injury may have increased resistance to the second ischemic insult. It has been shown that when preparations are subjected to repeated ischemic episodes, electrophysiological changes are less prominent during the second ischemic period than during the first. However, in these previous studies, electrophysiological changes are reversible because ischemic periods are short. Thus, it is unknown that this is the case after healing of myocardial infarction.

Kabel et al demonstrated that the subepicardium in the infarcted zone was more sensitive than normal myocardium to the reduction of coronary blood flow by stenosis of the circumflex coronary artery that served as a collateral vessel to the infarcted zone and that it was the site of most of the severe electrophysiological disturbances. We have previously shown that epicardial action potentials deteriorate more than endocardial action potentials during ischemia and that this difference in electrophysiological response to ischemia produces greater dispersion of refractoriness and arrhythmias. Although it was impossible in our experiments to record transmembrane action potentials from the epicardium in the infarcted zone because of adhe-
sion of the epicardium to the pericardium after surgery, these observations suggest that acute ischemia in healed myocardial infarction would likely cause large electrophysiological inhomogeneities between the endocardial and epicardial sites in the infarcted zone as well as between the normal and infarcted zones.

Finally, it is noteworthy that ischemia-induced, rapid ventricular activity was recorded more frequently in the preparations with healed myocardial infarction than in normal preparations, but reperfusion-induced, rapid ventricular activity was recorded with equal frequency in both normal and healed infarcted preparations. These findings suggest that previous myocardial infarction enhanced ischemic mechanisms of arrhythmias but that it was not required for the initiation of arrhythmias with mechanisms dependent upon reperfusion. This observation is consistent with data in the recent study by Pogwizd and Corr, demonstrating both reentrant and nonreentrant mechanisms of ischemic and reperfusion arrhythmias.

References

KEY WORDS: acute ischemia, healed myocardial infarction, action potential, refractory period
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