Electrophysiological Properties and Responses to Simulated Ischemia in Cat Ventricular Myocytes of Endocardial and Epicardial Origin

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In multicellular preparations, there are differences in action potential configuration between endocardium and epicardium, and electrophysiological alterations induced by ischemia are more drastic in epicardium than in endocardium. The present study was designed to examine electrophysiological properties of single cardiac myocytes enzymatically isolated from the endocardial and epicardial surfaces of the cat left ventricle and to determine whether the differential responses to ischemia of intact tissue occur in single cells. Action potentials recorded from the isolated single cells of epicardial surface had lower action potential amplitude and a prominent notch between phase 1 and phase 2, compared with those of the cells isolated from the endocardial surface; these findings are similar to those in intact endocardial and epicardial preparations. Resting membrane potentials recorded from both endocardial and epicardial single cells were sensitive to the change in extracellular K⁺ concentration and had properties of a K⁺ electrode. Action potential duration was frequency dependent in both cell types and was shorter in epicardial cells than in endocardial cells at a stimulation rate of 3 Hz. When the cells were superfused with Tyrode's solution that was altered to mimic an ischemic environment in vivo (Po₂, 30–40 mm Hg; pH 6.8; [K⁺], 10 mM; and glucose free), resting membrane potential, action potential amplitude, and action potential duration were reduced, and the refractory period was shortened in both endocardial and epicardial single cells, but there were no differences in the degree of changes in action potentials and refractory periods induced between the two cell types. Action potential changes induced by L-α-lysophosphatidylcholine (5–40 mg/l) were also similar in endocardial and epicardial single cells. These results lead us to suggest that the differences in action potential configuration between endocardial and epicardial cells are due to intrinsic electrophysiological properties but that their different responses to ischemia in intact cardiac tissues may be due to the extracellular and electrotonic influences of the neighboring cells. (Circulation Research 1990;66:469–477)

Transmembrane action potentials recorded from epicardial cells have lower amplitude, a prominent notch between phases 1 and 2, and shorter action potential duration (APD), compared with those from endocardial cells in various species.¹⁻⁸ These differences have also been observed during simultaneous recordings of monophasic action potentials from the endocardial and epicardial surfaces of the canine heart in vivo.⁹,¹⁰ Differences in action potential configuration between ventricular endocardial and epicardial cells may result from the electrotonic interaction between ventricular muscle cells and Purkinje fibers¹¹ or from intrinsic electrophysiological differences between cells from the two sites.

Different electrophysiological characteristics of endocardial and epicardial cells are also observed under pathological conditions. Our previous studies⁴,⁵,¹² and those of others³,¹³,¹⁴ have demonstrated that epicardial cells are more susceptible to ischemia or simulated ischemia than are endocardial

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cells. Action potential changes and the prolongation of conduction and refractoriness are more prominent in epicardial tissues than in endocardial tissues during acute ischemia, and these differential responses to ischemia produce electrophysiological inhomogeneities that may facilitate reentrant arrhythmias. However, it is not known whether the electrophysiological differences between endocardial and epicardial cells during ischemia are due to inherent cellular electrophysiological differences at the two sites, changes in extracellular ionic concentrations, or the presence or absence of Purkinje fibers.

Recently, enzymatic procedures for dissociating cardiac tissue have been introduced. These procedures allow experiments to be done without the extracellular and electronic influences encountered in intact cardiac preparations. The objectives of the present study were to examine the electrophysiological properties of single cardiac myocytes isolated from the endocardial and epicardial surfaces and to test the hypothesis that the differences in electrophysiological properties between the cells from the two regions, under both normal and ischemic conditions, are due to their intrinsic electrophysiological properties.

Materials and Methods

Preparations and Perfusing Solutions

Domestic cats of either sex, weighing 2.0–3.5 kg, were anesthetized with sodium pentobarbital (30 mg/kg i.p.), and heparin sodium (400 IU/kg) was injected intravenously. The heart was excised and mounted on a Langendorff apparatus and was perfused via the aorta with a modified Tyrode’s solution (37°C) containing (mM) NaCl 143, KCl 4, CaCl₂ 1.8, MgCl₂ 0.5, NaHPO₄ 0.33, glucose 5.5, and N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES) 5.5 (pH 7.4 with NaOH) and gassed with 100% O₂. After a 10-minute equilibration period, the preparation was perfused with Ca²⁺-free Tyrode’s solution (otherwise identical to above) for 5 minutes, followed by perfusion with 0.04% collagenase (type 1, Sigma Chemical, St. Louis, Missouri) dissolved in Tyrode’s solution with 50 μM Ca²⁺. Exposure to the enzyme was continued until the solution flowed freely (15–20 minutes), after which the collagenase was washed out with 100 ml of a solution containing (mM) KOH 70, KCl 40, glutamic acid 50, tauroine 20, KH₂PO₄ 10, MgCl₂ 0.5, glucose 11, EGTA 0.5, and HEPES 10 (pH 7.4 with KOH). Small pieces of the left ventricular tissues were dissected from the endocardial and epicardial surfaces (to a depth not exceeding 20% of the thickness of the ventricular wall) with fine scissors and blades. After the tissues had been minced, single cells were separated from tissue pieces by passing them through nylon mesh.

Electrical Stimulation and Recording

Isolated cells were introduced into a superfusion chamber (1 ml in volume) on the stage of an inverted microscope and were superfused with Tyrode’s solution (37°C) at a rate of 3 ml/min. Transmembrane action potentials were recorded with conventional microelectrode techniques. The cells were impaled with glass microelectrodes filled with 3 M KCl (DC resistance, 40–60 MΩ). The microelectrode was advanced by using a three-dimensional hydraulic micromanipulator (model MO-103N, Narishige, Tokyo, Japan). When the electrode reached the surface of the cell, a relatively strong current (100–200 nA) was applied extracellularly to induce contraction. Impalement was easily achieved by such contraction without damage to the cell. The microelectrode was connected through a Ag-AgCl junction to a high-input impedance electrometer with input capacity neutralization (model KS 700, World Precision Instruments, New Haven, Connecticut). The cell was stimulated by intracellular current pulses through the recording microelectrode at a stimulation frequency of 1 Hz. Pulse duration was 0.5 msec, and current strength was twice late-diastolic threshold (approximately 10 nA). The amplified output was displayed on an oscilloscope (Tektronix, Beaverton, Oregon) and stored in a videocassette recorder for later analysis. Action potentials were photographed directly from the oscilloscope and also recorded on a chart recorder (Gould, Cleveland, Ohio).

Protocol

Experiments were carried out after a 15–30-minute equilibration period. In the first series of experiments, action potentials recorded from the cells of endocardial and epicardial origin were characterized. The cells were stimulated at different stimulation rates (0.25, 0.5, 1, 2, and 3 Hz) to determine the frequency dependence of action potential parameters. We also determined the relation between membrane potential and extracellular K⁺ concentration. Solutions were made by substituting KCl and NaCl to attain final K⁺ concentrations of 2, 4, 6, 8, 10, 20, and 40 mM. The cells were sequentially exposed to each test solution for 10 minutes.

In the second series of experiments, we examined the changes in action potentials and refractory periods during superfusion with Tyrode’s solution that was altered to mimic an ischemic environment, that is, low Po₂, low pH, high K⁺, and glucose free (test solution). The test solution had almost the same composition as normal Tyrode’s solution, but it contained 10 mM KCl and no glucose. It was gassed with 100% N₂ to produce a Po₂ of 30–40 mm Hg and a pH of 6.80 (adjusted with HCl). The changes in oxygen, KCl, and hydrogen contents in test solution are similar to those reported to occur during the early phase of myocardial ischemia. After an equilibration period, the cells, stimulated at 1 Hz, were superfused with test solution for 30 minutes, during which action potential changes were monitored. Then, the test solution was washed out with normal Tyrode’s solution. Changes in refractory periods were also measured with another group of cells.
during superfusion with test solution and washout in this series of experiments. Refractory periods were measured by the extrastimulus method. Premature stimuli (S\textsubscript{2}) were delivered by every seventh drive stimulus (S\textsubscript{1}) through the recording microelectrode (both S\textsubscript{1} and S\textsubscript{2}). The stimulus current strengths of S\textsubscript{1} and S\textsubscript{2} were twice late-diastolic threshold determined before superfusion with test solution and were kept constant during the entire period of the protocol. The initial S\textsubscript{1}-S\textsubscript{2} interval was 250–300 msec, and it was shortened progressively in 10-msec decrements until the cells failed to respond to S\textsubscript{2}. The scanning was then repeated with 2-msec decrements from an interval 20 msec longer than the refractory period. The refractory period was defined as the longest S\textsubscript{1}-S\textsubscript{2} interval that resulted in no response at all to S\textsubscript{2}.

In the third series of experiments, we examined the effects of lysophosphatidylcholine, one of the major lysophosphoglycerides, on action potentials recorded from endocardial and epicardial single cells. Lysophosphoglycerides have been shown to accumulate in ischemic myocardium to an extent sufficient to produce electrophysiological abnormalities, and an arrhythmogenic role during ischemia has been suggested.\textsuperscript{19–22} L-\alpha-Lysophosphatidylcholine (LPC) from egg yolk (Sigma Chemical), containing mainly palmitoyl and stearoyl LPC, was dissolved in chloroform, and the solvent was evaporated. Tyrode’s solution was added to make an LPC concentration of 40 mg/l. After an equilibration period, the cells, stimulated at 1 Hz, were sequentially superfused with normal Tyrode’s solutions containing different concentrations of LPC for 15 minutes to construct concentration-response curves. We also examined the time courses of changes in transmembrane action potentials during superfusion with 40 mg/l LPC.

**Statistical Analysis**

All data are expressed as mean±SEM and evaluated for statistical significance by analysis of variance with repeated measurements or by Student’s unpaired \( t \) test. Differences with \( p < 0.05 \) were considered significant.

**Results**

When the isolated cells were superfused with Tyrode’s solution containing 1.8 mM Ca\textsuperscript{2+}, approximately 40% of the cells obtained from the endocardial surface and 20% of the cells obtained from the epicardial surface were Ca\textsuperscript{2+} tolerant and rod-shaped. Single rod-shaped cells having smooth surfaces with clear striations were selected for the electrical measurements. Immediately after impalement, the cells had low resting membrane potentials (RMPs) of −20 mV to −60 mV or high RMPs of −80 mV to −88 mV. Some of the cells with low RMPs became spontaneously active, then hypercontracted, and died. The remaining cells, with or without spontaneous activity, hyperpolarized to approximately −80 mV within a few minutes and became quiescent. Successful impalements were judged by high RMPs (approximately −80 mV) and the presence of quiescence. When the successfully impaled cells were stimulated by intracellular current pulses through the recording microelectrodes at a stimulation frequency of 1 Hz, some cells had abnormal action potentials, characterized by short APD without the plateau phase. Data from these cells were not used for further analyses, and the cells were not studied during interventions.

**Action Potential Characteristics**

Figure 1 shows representative action potential recordings from successfully impaled endocardial and epicardial myocytes. Notice that these cells exhibit normal RMP, action potential amplitude, and APD. Action potentials recorded from epicardial single cells, when compared with those in endocardial cells, had lower amplitude and a prominent notch between phases 1 and 2, showing a “spike-and-dome” morphology. These action potential characteristics are similar to those in intact epicardial muscle cells.\textsuperscript{3–6} Action potentials with a spike-and-dome morphology were recorded in about 80% of the epicardial cells. The remaining cells had action potentials similar to those recorded from the endocardial single cells or with morphology between endocardial and epicardial action potentials. We are unable to determine whether the cells having action potentials without a spike-and-dome morphology represent true epicardial cells or reflect contamination with midmyocardial cells. Only epicardial cells having action potentials with a spike-and-dome morphology (similar to those in the intact epicardium) were used for the comparisons with endocardial cells. The cells obtained from the endocardial surface appeared to be a single population, based on their action potential characteristics, which were uniformly similar to those in the intact endocardial preparations. APD at 50% repolarization (APD\textsubscript{50})
and 90% repolarization (APD90) varied from cell to cell (APD90 ranged from 130 to 200 msec) in both endocardial and epicardial cells when the cells were stimulated at 1 Hz; therefore, there were no significant differences in APD between the cells from the two sites. Watanabe et al also noted a great variation in APD in isolated guinea pig myocytes. Action potential characteristics of the cells of endocardial and epicardial origin at a stimulation of 1 Hz are summarized in Table 1.

As has been observed in intact cardiac tissue, APD was dependent on stimulation frequency in both endocardial and epicardial single cells. Figure 2 shows the effects of stimulation frequency on APD90. Although there was no difference in APD90 at lower stimulation rates, probably due to great variation as described above, the variability of APD90 declined as the stimulation rate was increased, and at 3 Hz, APD90 was significantly shorter in epicardial cells than in endocardial cells. The changes in APD25 and APD50 were similar to those in APD90.

When the cells were superfused with solutions having different concentrations of K+, the membrane potential changed rapidly to a new level.

Figure 3 shows the relation between the membrane potential and extracellular K+ concentration. The membrane potentials in both endocardial and epicardial single cells were sensitive to the change in extracellular K+ concentration, indicating that their membrane properties as a K+ electrode were preserved. The slopes of the linear parts of the curves were 57 mV/decade in endocardial cells and 56 mV/decade in epicardial cells.

Effect of Ischemia-Simulated Solution

Figure 4 depicts a representative experiment showing the changes in action potentials recorded from single cells of endocardial (left panel) and epicardial (right panel) origin during superfusion with test solution (PO2, 30–40 mm Hg; pH 6.8; [K+]0, 10 mM; and glucose free) at a stimulation of 1 Hz. Figure 5 summarizes such data obtained from 10 endocardial cells and 10 epicardial cells. When the superfusate was switched from normal Tyrode’s solution to test solution, RMP, action potential amplitude, APD25, and APD50 were progressively reduced for the first 15 minutes. The changes in action potential characteristics stabilized after 15 minutes of superfusion with test solution. The magnitude of changes in RMP, action potential amplitude, APD25, and APD50 induced by test solution was almost identical between endocardial and epicardial cells. Even when the cells were stimulated at a higher rate (2 Hz) during 30 minutes of superfusion with test solution, there were no differences in the magnitude of action potential changes (n=3, data not shown). Two of 10 epicardial cells became inexcitable after 30 minutes of superfusion with test solution.

### Table 1. Action Potential Characteristics in Ventricular Myocytes Isolated From the Endocardial and Epicardial Surfaces

<table>
<thead>
<tr>
<th></th>
<th>RMP (mV)</th>
<th>APA (mV)</th>
<th>APD50 (msec)</th>
<th>APD90 (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endo (n=40)</td>
<td>84.6±0.4</td>
<td>121.6±1.6</td>
<td>164.1±4.9</td>
<td>188.7±5.4</td>
</tr>
<tr>
<td>Epi (n=40)</td>
<td>83.5±0.4</td>
<td>106.5±1.8</td>
<td>154.8±4.1</td>
<td>180.2±4.8</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM. RMP, resting membrane potential; APA, action potential amplitude; APD50 and APD90, action potential durations at 50% and 90% repolarization, respectively; Endo, myocytes isolated from the endocardial surface; Epi, myocytes isolated from the epicardial surface.

*p<0.01 Endo vs. Epi.

![Figure 2](image-url)  
**Figure 2.** Graph showing frequency dependence of action potential duration at 90% repolarization (APD90) in endocardial (endo) and epicardial (epi) single cells (n=10). *p<0.01 endo vs. epi.

![Figure 3](image-url)  
**Figure 3.** Graph showing relation between membrane potential and extracellular K+ concentration in endocardial (endo) and epicardial (epi) single cells. The steepness of the linear part of the curve is 57 mV/decade in endo cells and 56 mV/decade in epi cells.
Effect of LPC

In the final series of experiments, we examined the effects of LPC on action potentials recorded from single cells of endocardial and epicardial origin. Figure 8 shows action potential characteristics after 15-minute superfusion with various concentrations of LPC; Figure 9 shows the time course of action potential changes induced by 40 mg/l LPC. LPC reduced RMP and action potential amplitude in a concentration-dependent manner in both endocardial and epicardial cells. APD90 was slightly prolonged at an LPC concentration of 10 mM or 20 mM and began to shorten as the concentration was increased. Superfusion with 40 mg/l LPC reduced RMP and action potential amplitude progressively and prolonged APD90 and APD95 during the first 5–10 minutes; thereafter, APD90 and APD95 were shortened in both endocardial and epicardial cells. Both endocardial and epicardial single cells became inexcitable after 25 minutes of superfusion with 40 mg/l LPC. Neither the effective concentration of LPC nor the extent of action potential changes induced by LPC were different between endocardial and epicardial single cells. The time course of the changes also was not different between the two cell types. Also, the combination of 20 mg/l LPC with the test solution did not result in significant differences in action potential changes between endocardial and epicardial cells (n=3, data not shown).

Discussion

The development of techniques for enzymatic isolation of single cardiac cells provides an additional dimension for cellular electrophysiological studies.15,16

In contrast to multicellular preparations, experiments using single cells, in which the influences of extracellular ionic concentrations and electrotonic interactions can be excluded, provide more direct information on intrinsic membrane properties of the cells. Using this technique, we demonstrated that characteristic action potentials observed in intact endocardial and epicardial cells result from their intrinsic electrophysiological properties.

Differences in Action Potential Characteristics

It has long been recognized that electrophysiological properties are not uniform in the mammalian ventricle. APD has been reported to be longer in endocardial cells than in epicardial cells. There are also differences in action potential configurations between endocardial and epicardial cells in hearts from various species.1–8 Epicardial action potentials have lower amplitude and a prominent notch between phase 1 and phase 2, making a spike-and-dome configuration. However, in these multicellular preparations, there are extracellular and electrotonic interactions and influences by discontinuity in conduction that may modify the action potential configuration. The present study revealed that these action potential characteristics exist in even single myocytes isolated from the epicardial surfaces. Although APD was not different between the two cell types at stimulation frequency of 1 Hz, it was shorter in epicardial single cells than in endocardial single cells at a stimulation frequency of 3 Hz. These
findings suggest that action potential configuration can be mainly accredited to the cell's inherent membrane properties rather than the extracellular and electrotonic influences or geometrical differences.

The transient outward current has been shown to contribute the notch between phases 1 and 2 of the action potential in cardiac tissues. In addition, this current may modulate APD. Thus, it is quite possible that the transient outward current is involved in the difference in action potential characteristics between endocardial and epicardial cells.

Litovsky and Antzelevitch studied the action potentials recorded from intact canine endocardial and epicardial cells and showed indirectly that the transient outward current is prominent in epicardial cells but not in endocardial cells. Tseng and Hoffman also noted in their recent report that in half of the canine ventricular myocytes there was a large transient outward current that causes the notch between phase 1 and the plateau. These findings lead us to speculate that the absence and presence of this current in endocardial and epicardial cells may be responsible for their different action potential configuration. Voltage-clamp experiments are now under way to test this hypothesis.

Response to Ischemia-Simulated Solution

Electrophysiological disturbances are more remarkable in epicardial cells than in endocardial cells during ischemia or simulated ischemia in intact cardiac preparations. Action potentials, conduction, and excitability are more deteriorated in the epicardial site. The mechanisms of such enhanced susceptibility of epicardial cells to ischemia are unknown. When the dynamics of the coronary circulation and the susceptibility of the subendocardium to the development of ischemia after coronary artery occlusion are considered, this enhanced susceptibility of epicardial cells to ischemia is puzzling. Subendocardial ATP levels decrease more than those in subepicardium during ischemia. Also, coronary artery ligation reduces tissue pH to a greater degree.
in endocardial cells than in epicardial cells. These findings may exclude the idea that nutrition from the cavity blood and different capacity for anaerobic metabolism are involved in the preservation of endocardial electrical activity during ischemia. Enhanced electrophysiological disturbances in epicardial cells are also observed in isolated intact endocardial and epicardial preparations when they are superfused with ischemia-simulated solution. From these observations, Gilmour and Zipes proposed the hypothesis that the presence of Purkinje fibers, which are resistant to hypoxic and ischemic insults, preserves electrical activity of the endocardial surface cells. Our data support this concept. In the present study, the electrophysiological changes induced by superfusion with ischemia-simulated solution or LPC were almost identical in isolated single cells from the endocardial and epicardial surfaces, in which there were no extracellular and electrotonic influences of neighboring cells such as Purkinje fibers. These findings indicate that electrophysiological responses to ischemia may be intrinsically the same between the two cell types but may be modified by extracellular and electrotonic interactions.

It is possible that the “ischemic” condition used in the present study was not severe enough for single cardiac cells (compared with intact tissues) so that differences in electrophysiological responses between endocardial and epicardial single cells could not be detected. Action potentials are more severely deteriorated in whole hearts or in the multicellular preparations during ischemia. In particular, epicardial cells become inexcitable within 15 minutes of superfusion with ischemia-simulated solution that contains the same PO2, K+ concentration, and pH as in the present study. Less severe changes in action potentials in single cells may be ascribed to the nature of the preparations. Single cells are entirely surrounded by the solution; thus, it is difficult to deprive the cells of O2 and substrates, although multicellular preparations can be hypoxic under the same condition. Hayashi et al reported a moderate reduction of APD during superfusion with a hypoxic solution in guinea pig myocytes, and they speculated that contaminant oxygen from the room air resulted in the moderate change in single cells. We used LPC as a different approach to further examine electrophysiological changes in endocardial and epicardial single cells. Lysophosphoglycerides have been shown to accumulate in ischemic myocardium to an extent sufficient to produce electrophysiological abnormalities. However, in our comparison of endocardial and epicardial single myocytes, there were no differences in the concentration of LPC required to produce action potential changes or in the extent and

**Figure 7.** Tracing of burst of ectopic activity developed during washout after superfusion with test solution in an epicardial myocyte. Electrically driven action potentials.

**Figure 8.** Graphs showing action potential changes induced by 15-minute superfusion with various concentrations of L-α-lysophosphatidylcholine (LPC) in endocardial (endo) and epicardial (epi) single cells. LPC at a concentration of 5 mg/l reduced resting membrane potential (RMP) and action potential amplitude (APA) (p<0.01). Action potential duration (APD) shortened at a concentration of 40 mg/l (p<0.05). APD50 and APD90 are APDs at 50% and 90% repolarization, respectively.
time course of action potential changes induced by LPC. Nevertheless, we cannot entirely exclude the possibility that the nature of ischemic insults produced by our intervention in single myocytes is different from those occurring in vivo and in intact cardiac preparations. A different approach may be needed to further explore the mechanisms of enhanced susceptibility of epicardial cells to ischemia.

Finally, it is noteworthy that ectopic activity, probably resulting from spontaneous automaticity and triggered activity, developed during washout in single ventricular myocytes. Hayashi et al. have also observed delayed afterdepolarizations, triggered activity, and abnormal automaticity in intact and single ventricular cells of the guinea pig during reoxygenation. These findings suggest that abnormal automaticity and triggered activity could be an underlying mechanism of reperfusion arrhythmias and that ventricular muscle cells as well as Purkinje fibers might be involved in the development of abnormal automaticity and triggered activity during reperfusion.

In conclusion, the results of the present study lead us to suggest that the different configurations of transmembrane action potentials observed in intact endocardial and epicardial preparations are ascribed to the cells’ intrinsic membrane properties and that electrophysiological responses to ischemia may be intrinsically the same between the two cell types but may be modified by extracellular and electrotonic interactions.

References


27. Hiraoka M, Kawano S: Mechanism of increased amplitude and duration of the plateau with sudden shortening of diastolic intervals in rabbit ventricular cells. Circ Res 1987;60:14–26


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