Differences in the Effect of Metabolic Inhibition on Action Potentials and Calcium Currents in Endocardial and Epicardial Cells

Shinichi Kimura, MD; Arthur L. Bassett, PhD; Tetsushi Furukawa, MD; Nanako Furukawa, DDS; and Robert J. Myerburg, MD

**Background.** Ischemia-induced electrophysiological changes are more prominent in epicardial cells than in endocardial cells. Epicardial action potentials shorten more than endocardial action potentials during ischemia. Since the L-type Ca**2+** current plays an important role in the maintenance of action potential duration, we hypothesized that the Ca**2+** current is affected more in epicardial cells than in endocardial cells during ischemia.

**Methods and Results.** To test this hypothesis, we examined the effect of metabolic inhibition, a major component of ischemia, on action potentials and the Ca**2+** current in single cells isolated from the endocardial and epicardial layers of the feline left ventricle. The membrane voltage and current were measured by using the whole-cell mode of the patch-clamp technique. During control periods, action potentials recorded from epicardial myocytes had lower amplitude, a prominent notch between phases 1 and 2, and shorter action potential duration compared with those recorded from endocardial myocytes. However, the amplitude and current–voltage relation of the Ca**2+** current were similar in endocardial and epicardial cells at test potentials of −30 to 60 mV elicited from a holding potential of −40 mV. The time course of inactivation of the Ca**2+** current also was identical in the two cell types. After 15 minutes of superfusion with glucose-free Tyrode's solution containing 1 mM CN−, action potential duration was reduced by 13±7% in endocardial cells and by 80±9% in epicardial cells (p<0.01). The peak Ca**2+** current was reduced by 21±9% in endocardial cells and by 37±6% in epicardial cells (p<0.01).

**Conclusions.** We conclude that enhanced depression of the Ca**2+** current may account in part for the greater action potential shortening in epicardial cells during ischemia and metabolic inhibition. (*Circulation* 1991;84:768–777)

Electrophysiological disturbances are more remarkable in the epicardium than in the endocardium during ischemia\(^4\)–\(^3\) despite the greater propensity of the endocardium to metabolic effects of ischemia induced by coronary artery ligation.\(^4\)–\(^6\) Our previous studies\(^7\)–\(^9\) and those of others\(^10\) have shown that action potentials shorten to a greater extent in epicardial cells than in endocardial cells during simulated ischemia in isolated ventricular preparations independent of the influences of hemodynamics of regional differences in myocardial blood flow. However, the underlying ionic basis for the differential electrophysiological responses to ischemia between endocardial and epicardial cells remains unsettled. We have recently shown a greater sensitivity to lack of ATP in ATP-regulated K⁺ channels in epicardial cells.\(^11\) However, several ionic currents contribute to action potential duration. In particular, the L-type Ca**2+** current plays an important role in maintaining action potential duration at the plateau level, and this current has been shown to be modulated by intracellular ATP concentration.\(^12\),\(^13\) Thus, we hypothesized that the differential responses to ischemia between endocardial and epicardial cells may also reflect differential susceptibility of the Ca**2+** channels in the two cell types to ischemic insults. To test this hypothesis, we examined the effect of intracellular ATP depletion with metabolic inhibition, a major component of ischemia, on action potentials and Ca**2+** current recorded from single...
myocytes isolated from the endocardial and epicardial layers of the left ventricle.

**Methods**

**Cell Isolation**

Domestic cats of either sex weighing 2.5–4.0 kg were anesthetized with sodium pentobarbital (30 mg/kg i.p.), and heparin sodium (400 units/kg) was injected intravenously. The heart was excised and mounted on a Langendorff apparatus and 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 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 I, Sigma Chemical Co., St. Louis, Mo.) 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 a solution containing (mM): KOH 70, KCl 40, glutamic acid 50, taurine 20, KH₂PO₄ 10, MgCl₂ 0.5, glucose 11, EGTA 0.5, and HEPES 10 (pH 7.4 with KOH). Small pieces of left ventricular tissue 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. Voltage- and current-clamp studies were performed using low resistance (2–4 MΩ) suction pipettes in the whole-cell recording mode. The pipettes were pulled by a two-stage puller (David Kopf, Tujunga, Calif.) and heat-polished before use. The electrode resistance was adjusted to give zero current between the pipette solution and bath solution immediately before each cell was attached. After a gigaseal (>5 GΩ) was formed by gentle suction, the cell membrane under the electrode tip was broken by further application of negative pressure.

Action potentials were recorded in the current-clamp mode. The pipette solution contained 140 mM KCl, 4 mM MgCl₂, and 10 mM HEPES (pH 7.2 with KOH). The cells were stimulated by passing depolarizing currents (twice diastolic threshold) through the pipette at a rate of 1 Hz. After a 10-minute equilibration period, cells were superfused with glucose-free Tyrode’s solution containing 1 mM NaCN for 15 minutes, during which action potentials were monitored.

The slow inward Ca²⁺ current (Iₛ) was recorded in the voltage-clamp mode. The pipette solution contained 120 mM CsCl, 4 mM ATP, 4 mM MgCl₂, 10 mM HEPES, and 2 mM EGTA. In experiments with the effect of CN⁻ on Iₛ, ATP was not included in the pipette solution. The sodium current was inactivated by holding the membrane at −40 mV. Transient outward current, which is dominant in epicardial cells but not in endocardial cells, overlaps Iₛ in the plateau range of membrane potentials. This current was minimized by substitution of 120 mM CsCl for KCl in the pipette solution. The Ca²⁺ current was activated by clamping the membrane voltage for 500 msec from a holding potential of −40 mV to a test voltage (−30–60 mV); the clamp step was induced every 2 seconds. The amplitude of Iₛ was measured as the difference between the peak of the inward current and the holding current level. The effect of CN⁻ on Iₛ was examined by monitoring the currents every 5 minutes during superfusion with glucose-free Tyrode’s solution containing 1 mM NaCN.

Although the involvement of transient outward current (Iₒ) was reduced by substitution of CsCl for KCl in the pipette solution, it may be that the reduction of Iₛ during exposure to CN⁻ is due to some change in Iₒ. Our recent study demonstrates that a significant level of Iₒ is recorded only in epicardial cells of feline hearts; therefore, we examined the effect of CN⁻ on Iₒ in such cells. The pipette solution contained 130 mM KCl, 4 mM MgCl₂, and 10 mM HEPES (pH 7.2 with KOH). The cells were superfused with glucose-free Tyrode’s solution (37°C) containing 1 mM NaCN. Tetrodotoxin (15 μM) and CoCl₂ (2 mM) were added to the Tyrode’s solution to block Na⁺ and Ca²⁺ currents, respectively. Iₒ (4-aminopyridine-sensitive component) was activated by clamping the membrane voltage for 500 msec from a holding potential of −80 mV to a test potential (−40–60 mV); the clamp step was induced every 2 seconds. The amplitude of Iₒ was measured as the difference between the peak of Iₒ and the minimum current level during the depolarizing pulse after the peak Iₒ. We monitored Iₒ every 5 minutes during exposure to CN⁻.

**Data Acquisition and Analysis**

The whole-cell membrane currents and potentials were measured by an Axopatch-1B patch-clamp system (Axon Instruments, Inc., Burlingame, Calif.) or a Dagan 8900 patch-clamp amplifier (Dagan Co., Minneapolis, Minn.). The pipette capacitance and series resistance were compensated. Voltage and current data were displayed on an oscilloscope (Tektronix, Beaverton, Ore.). Analog waveforms for voltage-clamp command pulses and data acquisition and analysis were obtained with a set of software programs (pCLAMP, Axon Instruments) using a 12-bit resolution Labmaster A-D converter (Tecmar Inc., Cleveland, Ohio) and an IBM-AT personal computer. Current traces elicited by voltage steps were filtered at 10 kHz and stored on a hard disk for later analysis.
Data are presented as mean±SD. Two-way analysis of variance was used to test for significant differences in CN− response among the various groups. When significance was established, comparisons between specific means were made using the Newman-Keuls method. Differences with a probability value of less than 0.05 were considered significant.

Results

Effect of CN− on Action Potentials of Endocardial and Epicardial Myocytes

Figure 1 (upper panels) shows action potentials recorded from single myocytes isolated from the endocardial and epicardial layers of the left ventricle before and after 15 minutes of superfusion with glucose-free Tyrode’s solution containing 1 mM CN−. During the control periods, action potentials recorded from epicardial myocytes had lower amplitude, a prominent notch between phases 1 and 2, and slightly shorter action potential duration compared with those recorded from endocardial myocytes. These characteristics of action potentials in endocardial and epicardial myocytes are similar to those observed in intact endocardial and epicardial preparations.7−10,15−18 Action potential characteristics of the single cells of endocardial and epicardial origin at a stimulation of 1 Hz are provided in Table 1.

During superfusion with glucose-free Tyrode’s solution containing 1 mM CN−, action potential duration was shortened in both endocardial and epicardial cells. However, as shown in Figure 1 (upper panels), action potential duration was reduced to a greater extent in the epicardial cell than in the endocardial cell. Figure 1 (lower panels) shows the time course of CN−-induced changes in action potential duration measured at 50% and 90% of repolarization. There were significant differences in the extent of action potential shortening between the two cell types during exposure to 1 mM CN−. Resting membrane potential and action potential amplitude were not affected by exposure to CN−.
**TABLE 1. Action Potential Characteristics in Endocardial and Epicardial Myocytes**

<table>
<thead>
<tr>
<th></th>
<th>RMP  (-mV)</th>
<th>APA (mV)</th>
<th>APD20 (msec)</th>
<th>APD90 (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endo</td>
<td>88±6</td>
<td>123±6</td>
<td>114±9</td>
<td>207±14</td>
</tr>
<tr>
<td>Epi</td>
<td>87±5*</td>
<td>112±5</td>
<td>112±9</td>
<td>185±9*</td>
</tr>
</tbody>
</table>

Endo, endocardial myocytes (n=20); Epi, epicardial myocytes (n=20); RMP, resting membrane potential; APA, action potential amplitude; APD20 and APD90, action potential duration measured at 20% and 90% repolarization, respectively.

*p<0.01 endocardial vs. epicardial myocytes.

**Characteristics of ICa Under Control Conditions**

To study the activation of ICa, membrane potential was stepped from a holding potential of −40 mV to test potentials ranging from −40 to +60 mV for 500 msec with a stimulation frequency of 0.5 Hz. This holding potential was chosen to inactivate fast sodium channels. Figure 2 depicts representative current recordings from endocardial and epicardial cells elicited by depolarization steps from a holding potential of −40 mV. The voltage dependence of activation of peak ICa is shown in Figure 3. We calculated the capacitive surface area by integrating the area under the capacitive transient and dividing this area by the voltage step (10-mV hyperpolarizing step from 0 mV). The capacitive surface area was not different between endocardial cells (94±35 pF, n=25) and epicardial cells (89±31 pF, n=20). Therefore, the peak currents shown in Figure 3 were not corrected for capacitative surface area. The peak ICa occurred at similar test potentials for endocardial

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**Figure 2.** Superimposed Ca2+ current tracings from endocardial (ENDO) and epicardial (EPI) cells elicited by depolarization steps from a holding potential of −40 mV to mV indicated to left of current traces. Horizontal line represents the zero current level.
and epicardial cells, and the magnitude of $I_{Ca}$ did not differ between the two cell types. Also, $I_{Ca}$ was measured in the presence of 2 mM aminopyridine in the external solution to further exclude the involvement of $I_{to}$, and similar results were obtained as shown in Figure 3B.

The steady-state inactivation of $I_{Ca}$ for endocardial and epicardial cells was compared. Membrane potential was stepped for 500 msec from holding potentials ($-50$ to $0$ mV) to $0$ mV, at which $I_{Ca}$ was fully activated at a frequency of $0.5$ Hz. In Figure 4, the current elicited by the test pulse was expressed as a fraction of the maximum current obtained, and the normalized magnitude of $I_{Ca}$ was plotted against holding potentials. The data points were fitted to the Boltzmann distribution equation:

$$f = 1/[1 + \exp((V - V_h)/b)]$$

where $f$ is the extent of inactivation of $I_{Ca}$, $V$ is the holding potential, $V_h$ is the half-inactivation potential, and $b$ is a constant. The inactivation curve of $I_{Ca}$ for endocardial and epicardial cells was nearly identical, and $V_h$ was $-28$ mV in endocardial cells and $-29$ mV in epicardial cells.

We next compared the time course of inactivation of $I_{Ca}$ in endocardial and epicardial cells by stepping...
membrane potential from −40 mV to 0 mV for 500 msec. The inactivation of $I_{Ca}$ was fitted to a two-exponential function of the form:

$$A_0 + A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)$$

There were no differences in time constants of inactivation between endocardial and epicardial cells. The fast time constant was $11.6 \pm 2.8$ msec for endocardial $I_{Ca}$ ($n=12$) and $10.9 \pm 3.4$ msec for epicardial $I_{Ca}$ ($n=16$). The slow time constant was $50.4 \pm 15.2$ msec for endocardial $I_{Ca}$ and $51.3 \pm 11.5$ msec for epicardial $I_{Ca}$.

The results described above demonstrate that the characteristics of $I_{Ca}$ are fundamentally the same in endocardial and epicardial cells under physiological conditions.

**Effect of CN⁻ on $I_{Ca}$**

In the second series of experiments, we compared the changes in $I_{Ca}$ in endocardial and epicardial cells during superfusion with glucose-free Tyrode’s solution containing 1 mM CN⁻. Changes in $I_{Ca}$ during exposure to CN⁻ were examined by repeating 500-msec clamp pulses from −40 mV to test potentials ranging from −30 to +60 mV at 5-minute intervals. Figure 5 shows a representative experiment of changes in $I_{Ca}$ before and after 15 minutes of exposure to 1 mM CN⁻ in endocardial and epicardial cells. It is clearly demonstrated that the magnitude of reduction in $I_{Ca}$ after 15 minutes of exposure to CN⁻ was greater in the epicardial cell than in the endocardial cell. Figure 6 summarizes the percent change of peak $I_{Ca}$ from the value obtained before superfusion with CN⁻. Since $I_{Ca}$ may “run down,” we also examined the changes in $I_{Ca}$ during superfusion with normal Tyrode’s solution (without CN⁻) for both endocardial and epicardial cells; these control data are shown in Figure 6. The experimental conditions including the pipette solution were identical to those in the experiments with CN⁻. The peak $I_{Ca}$ decreased to the same extent in endocardial ($15 \pm 5\%$ reduction) and epicardial ($17 \pm 4\%$ reduction) cells after 15
minutes of superfusion with normal Tyrode's solution (without CN\(^{-}\)). However, the peak I\(_{\text{Ca}}\) decreased by 21\(\pm\)9\% in endocardial cells and by 37\(\pm\)6\% in epicardial cells after 15 minutes of superfusion with Tyrode's solution containing 1 mM CN\(^{-}\). The difference between I\(_{\text{Ca}}\) changes during superfusion with normal Tyrode’s solution and Tyrode’s solution with CN\(^{-}\) was statistically significant in epicardial cells (17\(\pm\)4\% versus 37\(\pm\)6\%; \(p<0.01\), \(n=10\)) but not in endocardial cells (15\(\pm\)5\% versus 21\(\pm\)9\%; \(p=\text{NS}\), \(n=10\)). The difference in I\(_{\text{Ca}}\) changes induced by CN\(^{-}\) between endocardial and epicardial cells was statistically significant (21\(\pm\)9\% versus 37\(\pm\)6\%; \(p<0.01\)). Although the peak I\(_{\text{Ca}}\) was reduced by CN\(^{-}\)-induced metabolic inhibition, the voltage dependence of I\(_{\text{Ca}}\) was not significantly affected, as shown in Figure 7.

**Effect of CN\(^{-}\) on I\(_{\text{Io}}\)**

Although the contribution of I\(_{\text{Io}}\) was minimized by substitution of 120 mM CsCl for KCl in the pipette solution, it is possible that the reduction of I\(_{\text{Ca}}\) during exposure to CN\(^{-}\) is due to changes in I\(_{\text{Io}}\). Thus, we directly examined the effect of 1 mM CN\(^{-}\) on I\(_{\text{Io}}\). Significant 4-aminopyridine-sensitive I\(_{\text{Io}}\) is present

**Figure 6.** Bar graph showing percent changes of peak slow inward Ca\(^{2+}\) current after 15 minutes of superfusion with normal Tyrode’s solution (Control) and glucose-free Tyrode’s solution containing 1 mM CN\(^{-}\) (CN) in endocardial (Endo) and epicardial (Epi) cells (\(n=10\) for each group). #\(p<0.01\) Epi–CN vs. Epi–Control; *\(p<0.01\) Epi–CN vs. Endo–CN.

**Figure 7.** Plot of current-voltage relation of slow inward Ca\(^{2+}\) current in endocardial (ENDO, \(n=10\)) and epicardial (EPI, \(n=10\)) cells before and after 15 minutes of superfusion with glucose-free Tyrode’s solution containing 1 mM CN\(^{-}\).
only in epicardial cells.\textsuperscript{15-19} Figure 8A shows current tracings of \( I_{\text{ps}} \) elicited by depolarizing pulses for 500 msec from a holding potential of \(-80\) mV to test potentials before and after 20 minutes of exposure to \( \text{CN}^- \) in an epicardial cell. The current–voltage relation before and after superfusion with glucose-free Tyrode’s solution containing 1 mM \( \text{CN}^- \) is shown in Figure 8B. The magnitude and kinetics of \( I_{\text{ps}} \) were not affected by 1 mM \( \text{CN}^- \).

**Discussion**

It has long been recognized that electrophysiological properties are not spatially uniform in mammalian ventricles. In particular, there are distinct differences in action potential configuration between endocardial and epicardial cells in various species.\textsuperscript{7-10,15-24} Action potentials recorded from epicardial cells have lower amplitude, a prominent notch between phase 1 and phase 2, and shorter action potential duration compared with those recorded from endocardial cells. Our previous\textsuperscript{19,25} and present studies have shown that these differences in action potential characteristics are observed in single myocytes isolated from the endocardial and epicardial surfaces, indicating that these electrophysiological differences are due to intrinsic properties of the cells from the two regions.

Action potential duration is determined by the balance between the inward and outward currents. Thus, differences in the magnitude and/or the kinetics of \( I_{\text{Ca}} \) could account for the differential action potential characteristics between endocardial and epicardial cells. However, the present study revealed that the voltage dependency of \( I_{\text{Ca}} \), its magnitude, and the time course of decay of \( I_{\text{ps}} \) were not different between the two cell types. The disparate action potential durations may result from differences in the properties of other currents such as \( I_{\text{ps}} \). Using intact canine tissues, Litovsky and Antzelevitch\textsuperscript{15-18} have shown that \( I_{\text{ps}} \) is prominent in epicardial cells but not in endocardial cells; this has been confirmed by our recent studies using the patch-clamp technique in isolated feline myocytes.\textsuperscript{19} The inward rectifier and delayed rectifier potassium currents also may contribute to the different action potential durations. Our preliminary data show location-related differences in the magnitude of delayed rectifier potassium current (unpublished observations).

Disparate location-related electrophysiological responsiveness of endocardial and epicardial cells is observed during ischemia. Our previous studies\textsuperscript{7-9} and those of others\textsuperscript{10} have demonstrated that action potential changes and the prolongation of conduction and refractoriness are more prominent in epicardial tissues during ischemia or simulated ischemia. In the present study, action potentials recorded from single isolated epicardial cells shortened to a greater extent than those from endocardial cells during \( \text{CN}^- \)-induced metabolic inhibition, a major component of ischemia. In a previous study\textsuperscript{26} using single cells, we did not find a difference in response to simulated ischemia (\( \text{PO}_2=30-40\) mm Hg, pH 6.8, KCl=10 mM, and glucose free) between endocardial and epicardial cells. This probably reflects the experimental conditions; hypoxia at \( \text{PO}_2 \) 30–40 mm Hg may not be sufficiently low to deprive the cells of \( \text{O}_2 \).

Other investigators\textsuperscript{26} also have found difficulty in
producing significant effects of hypoxia in single cell experiments.

Our data also showed that $I_{Ca}$ was reduced to a greater extent in epicardial cells than in endocardial cells during metabolic inhibition. These findings indicate that enhanced shortening of action potentials in epicardial cells compared with those in endocardial cells during ischemia and metabolic inhibition is partly attributed to greater depression of $I_{Ca}$ in epicardial cells.

The mechanisms by which exposure to $CN^-$ reduces $I_{Ca}$ to a greater extent in epicardial cells are unknown. It is unlikely that the cell isolation procedure preferentially damages the epicardial cells since there were no differences in the magnitude and kinetics of $I_{Ca}$ between endocardial and epicardial cells before exposure to $CN^-$. In addition, $I_{Ca}$ was reduced similarly in both cell types during control superfusion with normal Tyrode’s solution.

There are at least two possibilities to explain the difference in $I_{Ca}$ reduction induced by $CN^-$ between endocardial and epicardial cells: 1) ATP depletion is greater in epicardial cells during exposure to $CN^-; 2$) the $Ca^{2+}$ channels of epicardial cells are more sensitive to ATP depletion than are those of endocardial cells. The effects of superfusion with Tyrode’s solution containing $CN^-$ on action potentials and membrane currents of ventricular cells in the present study are probably due to a decrease in intracellular ATP with inhibition of oxidative phosphorylation. Intracellular ATP concentration has been demonstrated to influence the $Ca^{2+}$ current.\textsuperscript{12,13} Noma and Shibasaki\textsuperscript{13} have shown that the amplitude of the $Ca^{2+}$ current decreased in a concentration-dependent manner over the concentration range of $0–5$ mM ATP when guinea pig single ventricular cells were dialyzed with various ATP-deficient internal solutions. Thus, if intracellular ATP was reduced to a greater extent in epicardial cells during exposure to $CN^-$, it would enhance the reduction in $I_{Ca}$. However, it has been shown in in vivo experiments that electrophysiological disturbances are more severe in the subepicardium than in the subendocardium\textsuperscript{1–3} despite the fact that ATP levels and pH decrease to a greater extent in the subendocardium during coronary artery ligation.\textsuperscript{4–6} Thus, it seems unlikely that different electrophysiological responses to ischemia or $CN^-$-induced metabolic inhibition result from the extent of metabolic changes.

Another possibility is that even if intracellular ATP concentration is reduced to the same extent in endocardial and epicardial cells during exposure to $CN^-$, the sensitivity of the $Ca^{2+}$ channels to ATP might be different between the two cell types. That is, the $Ca^{2+}$ channels in epicardial cells might be more susceptible to ATP depletion than those in endocardial cells. Although our data show no differences in the fundamental characteristics of $I_{Ca}$ between endocardial and epicardial cells, the modulation of the channel activity by various factors including intracellular ATP concentration may differ between the two cell types.

It may also be possible that ATP depletion produces changes in intracellular ionic concentrations to a different extent in endocardial and epicardial cells during exposure to $CN^-$. Depletion of ATP may increase intracellular $Ca^{2+}$ concentration by suppressing $Ca^{2+}$ pump activity of the sarcoplasmic reticulum and the surface membrane; in turn, this could contribute to changes in $I_{Ca}$. Although we included EGTA in the pipette solution to minimize the influence of intracellular $Ca^{2+}$ on $I_{Ca}$ measurements, we cannot exclude the possibility that exposure to $CN^-$ increased intracellular $Ca^{2+}$ concentration to a different extent in endocardial and epicardial cells.

Finally, it should be noted that other ionic currents may be involved in differences in the sensitivity of the endocardial and epicardial cells to ischemia and metabolic inhibition. Although the present study showed that the magnitude of the 4-aminopyridine component of $I_{So}$ is not affected by exposure to $CN^-$, the presence of this current in epicardial cells but not endocardial cells could contribute to different responses to ischemia.\textsuperscript{18} Antzelevitch et al\textsuperscript{18} have demonstrated that application of 4-aminopyridine reverses ischemia-induced depression of epicardial action potentials. Furthermore, it is possible that the $Ca^{2+}$-sensitive component of $I_{So}$ may be increased by a rise in intracellular $Ca^{2+}$ during ischemia and metabolic inhibition, which may enhance action potential shortening. Also, we have shown that ATP-sensitive $K^+$ channels are activated during superfusion with $CN^-$ and we have suggested that the enhanced shortening of the action potential in epicardial cells during metabolic inhibition reflects activation of ATP-sensitive $K^+$ channels. The present study indicates that current through $Ca^{2+}$ channels as well as through $K^+$ channels are involved in the location-related electrophysiological responses to ischemia and metabolic inhibition.

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