Role of Cardiac ATP-Regulated Potassium Channels in Differential Responses of Endocardial and Epicardial Cells to Ischemia

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Epicardial cells are more susceptible to the electrophysiological effects of ischemia than are endocardial cells. To explore the ionic basis for the differential electrophysiological responses to ischemia at the two sites, we used patch-clamp techniques to study the effects of ATP depletion on action potential duration and the ability of ATP-regulated K+ channels in single cells isolated from feline left ventricular endocardial and epicardial surfaces. During ATP depletion by treatment with 1 mM cyanide (CN−), shortening of action potential durations was significantly greater in epicardial cells than in endocardial cells. Thirty minutes after initiating exposure to 1 mM CN−, action potential duration at 90% repolarization was reduced to 0.70±0.12 of the control value for endocardial cells versus 0.39±0.18 for epicardial cells (p<0.01), and action potential duration at 20% repolarization was reduced to 0.72±0.13 for endocardial cells versus 0.12±0.09 for epicardial cells (p<0.01). In both endocardial and epicardial cells, the shortening of action potential by CN− treatment was partially reversed by 0.3 μM glibenclamide; the magnitude of reversal, however, was much greater in epicardial cells. After exposure to 1 mM CN−, the activity of ATP-regulated K+ channels in cell-attached membrane patches was significantly greater in epicardial cells than in endocardial cells. To study the dose–response relation between ATP concentration and open-state probability of the channels, intracellular surfaces of inside-out membrane patches containing ATP-regulated K+ channels were exposed to various concentrations of ATP (10−1,000 μM). The concentration of ATP that produced half-maximal inhibition of the channel was 23.6±21.9 μM in endocardial cells and 97.6±48.1 μM in epicardial cells (p<0.01). These data indicate that ATP-regulated K+ channels are activated by a smaller reduction in intracellular ATP in epicardial cells than in endocardial cells. The differential ATP sensitivity of ATP-regulated K+ channels in endocardial and epicardial cells may be responsible for the differential shortening in action potentials during ischemia at the two sites. (Circulation Research 1991;68:1693–1702)

Despite the greater susceptibility of endocardium to metabolic effects of ischemia,1−4 electrophysiological changes evoked by ischemia are greater in epicardial cells.5−9 Previous reports from our laboratory9 and others5−8 have demonstrated with in vivo studies or with multicellular ventricular muscle preparations that changes in action potential and the prolongation of conduction time and refractory periods are more prominent in epicardial tissue than in endocardial tissue during acute ischemia. These differential responses to ischemia produce electrophysiological inhomogeneities that may facilitate reentrant arrhythmias. However, the underlying ionic basis for the differential electrophysiological response to ischemia at the two sites remains unknown.

In 1983, Noma10 demonstrated that decreased intracellular concentration of adenosine triphosphate ([ATP]) brought about by exposure to cyanide ions (CN−) evoked outward currents through an ATP-regulated K+ channel. Several lines of experimental evidence have implicated this current in the shortening of action potential duration in ischemic myocardium.10−13 In the present study, we compared the sensitivity of ATP-regulated K+ channels to the reduction of [ATP], in single myocytes isolated from endocardial and epicardial surfaces and tested the

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Supported by grants HL-21735 and HL-19044 from the National Heart, Lung, and Blood Institute and by a grant-in-aid from the American Heart Association, Florida Affiliate (S.K., A.L.B.).

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Received January 5, 1990; accepted March 5, 1991.
hypothesis that the differential sensitivity of the channel to [ATP], between the two cell types contributes to the differential shortening in action potential duration during ischemia.

Materials and Methods

Preparation of Single Cardiac Myocytes

Single endocardial and epicardial myocytes from feline left ventricles were isolated by an enzymatic dissociation procedure.14-16 Domestic cats of either sex, weighing 2.0–3.5 kg, were anesthetized with pentobarbital sodium (30 mg/kg i.p.) and were anticoagulated with heparin sodium (400 IU/kg i.v.). The heart was rapidly excised and mounted on a Langendorff perfusion apparatus (60-cm height). It was perfused retrogradely via the aorta for 10 minutes with Tyrode’s solution equilibrated with 100% O2 at 37°C at a rate of 10–15 ml/min, followed by perfusion for 5 minutes with nominally calcium-free Tyrode’s solution. The Tyrode’s solution contained (mM) NaCl 143.0, KCl 4.0, CaCl2 1.8, MgCl2 0.5, NaHPO4, 0.33, glucose 5.5, and HEPES 5.5 (pH 7.4 with NaOH). The preparation was then perfused with 0.04% collagenase (type I, Sigma Chemical Co., St. Louis) dissolved in Tyrode’s solution containing 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 150 ml “Kraftbrühe” (KB) solution containing (mM) KCl 40.0, glutamic acid 50.0, taurine 20.0, KH2PO4 10.0, MgCl2 0.5, glucose 11.0, EGTA 0.5, and HEPES 10 (pH 7.4 with KOH). Small pieces of the left ventricular free wall tissues were dissected from the endocardial and epicardial surfaces (to a depth not exceeding 20% of the thickness of the ventricular wall) using fine scissors. After the tissues had been gently agitated in a beaker containing KB solution, single cells were separated from tissue pieces by passing them through 140-μm mesh nylon gauze. Isolated cells were stored in the KB solution at 4°C for a minimum of 60 minutes and were studied within 24 hours after the isolation. Single cells harvested from endocardial and epicardial surfaces were studied at random, with respect to the order of experiments.

Action Potential Recordings

A few drops of the solution containing isolated single myocytes were introduced into a recording chamber (1 ml in volume) mounted on the stage of an inverted microscope (Olympus Co., Tokyo); the cells were allowed to settle to the bottom (5 minutes). The bath was then continuously superfused with filtered Tyrode’s solution at a temperature of 37°C at a rate of 3 ml/min. When the isolated cells were superfused with Tyrode’s solution containing 1.8 mM Ca2+, 30–40% of the cells obtained from the endocardial or epicardial surface were Ca2+ tolerant and rod-shaped. Single rod-shaped cells having smooth surfaces with clear striations were selected for the electrical measurements. Membrane potentials were recorded by the single-pipette, whole-cell clamp technique.17 Pipettes were fabricated from 1.5–1.8-mm o.d. borosilicate glass (Kimble Products, Toledo, Ohio) by use of a two-stage pull with a vertical puller (model 720, David Kopf Instruments, Tujunga, Calif.). The pipette had an internal tip diameter of 2–4 μm and a resistance of 2–5 MΩ after heat polishing of the pipette tip and filling the pipette with solution containing (mM) KCl 140.0, MgCl2 10.0, and HEPES 5.0 (pH 7.2 with KOH). The pipettes were coated with Sylgard (Dow Corning Co., Midland, Mich.) before use. Electrical connection to the pipette and to the bath were made through Ag/AgCl half-cell electrodes. The electrode potential was adjusted to zero current between the pipette solution and the bath solution immediately before each cell was attached. Single cells were drawn onto the tip of the pipette by gently pressing the pipette tip onto the cell surface. A low amplitude square-voltage step was applied repetitively to the pipette to monitor cell sealing, and an appropriate negative pressure (10–30 cm H2O) was applied to the inside of the pipette to establish a seal resistance of 5–100 GΩ. The cell membrane under the pipette pore was broken by the application of a negative pressure transient, resulting in a whole-cell clamp configuration.

Action potentials were recorded from single cells in the current-clamp mode17 and by passing depolarizing currents of suprathreshold intensity (<2 msec in duration) through the pipette at a rate of 1 Hz. The general criteria used to select electrically sound and stable cells for the measurements of membrane potentials were as follows: 1) The resting membrane potential exceeded −80 mV, and its value did not vary more than 1 mV during a 10-minute observation period. 2) The configuration of action potential exhibited a distinct plateau similar to those recorded from multicellular ventricular preparations. 3) Total action potential duration was >150 msec at a constant stimulation rate of 1 Hz. 4) Action potential duration at 90% repolarization (APD90) and at 20% repolarization (APD20) did not vary by more than 5% during a 10-minute observation period.18 Cells that did not meet all of these criteria were discarded.

After a 10-minute equilibration period, the effect of cyanide (CN−) treatment on action potential duration was studied in cells isolated from endocardial (n = 10) and epicardial (n = 11) surfaces. The bath was superfused with Tyrode’s solution containing 1 mM CN−, and action potentials were continuously recorded for 30 minutes. CN−-Tyrode’s solution was prepared by omitting glucose and adding 1 mM NaCN to the Tyrode’s solution, and pH was adjusted to 7.4 with HCl.12 In three endocardial and three epicardial cells, we studied the effects of glibenclamide (Sigma Chemical), a specific blocker of an ATP-regulated K+ channel,19,20 on the shortening of action potential duration by CN− treatment. After treatment with 1 mM CN− for 30 minutes, the bath was superfused with CN−-Tyrode’s solution containing 0.3 μM glibenclamide, and action potentials were
continuously recorded for 15 minutes. Glibenclamide was solubilized in dimethylsulfoxide (DMSO, solvent concentration of 0.005% [vol/vol], Sigma Chemical), and the drug was added to the CN\(^{-}\)-Tyrode's solution immediately before use.

**Single-Channel Recordings**

Single-channel currents were recorded from a cell-attached membrane patch or an inside-out membrane patch with the voltage-clamp technique\(^{17}\) at a room temperature of 21--22°C, or in several experiments at 37°C. The procedures to establish a gigaseal were identical to those of experiments for membrane potential recordings described above, but the cell membrane under the pipette pore was not broken. In order to obtain cell-free, inside-out membrane patches, the pipette tip was slowly withdrawn from the cell surface.\(^{17}\) On withdrawal of the pipette from the cell, a vesicle was formed at the pipette tip opening, without a decrease in seal resistance. Then the pipette tip was briefly passed out and in through the solution-air interface. These procedures usually did not disrupt the gigaseal and resulted in inside-out membrane patches, in which the cytoplasmic face of the cell membrane was exposed to the bath solution and its extracellular face was exposed to the pipette solution.\(^{17}\)

The effect of CN\(^{-}\) treatment on single-channel currents in the cell-attached patch mode was tested in endocardial (\(n=5\)) and epicardial (\(n=6\)) cells. Tyrode's solution was used for both the bath solution and the pipette solution. Membrane potential was clamped for 60 seconds from the resting membrane potential to a test potential between −100 and 100 mV in 20-mV increments at 60-second intervals, and unitary currents were recorded before (control data) and 10 minutes after superfusion with Tyrode's solution containing 1 mM CN\(^{-}\). Recordings of current tracings were done with perfusion was momentarily stopped.

The characteristics of the ATP-regulated K\(^{+}\) channel and its sensitivity to [ATP], were compared in the inside-out patch mode between cells isolated from endocardial (\(n=17\)) and epicardial (\(n=24\)) surfaces. The membrane patch containing ATP-regulated K\(^{+}\) channels was bilaterally exposed to the high K\(^{+}\) solution containing (mM) KCl 140.0, HEPES 5.0, and EGTA 1.0 (pH 7.4 with KOH). Single-channel current was obtained by clamping membrane potential for 60 seconds from its reversal potential to a test potential between −100 and 100 mV in 20-mV increments at 60-second intervals. The control data were obtained >5 minutes after isolating the patch from the cell membrane, because the channel activity decays with time and reaches a relatively stable level 5 minutes after isolating the patch.\(^{21}\) After control data were obtained, the intracellular surface of the inside-out membrane patch was superfused with the high K\(^{+}\) solution containing 10, 25, 50, 100, 250, 500, and 1,000 \(\mu M\) ATP at a rate of 3 ml/min. Two minutes after superfusion with each concentration of ATP, perfusion was momentarily stopped, and single-channel currents were recorded. The time required to test the range of ATP concentration (10--1,000 \(\mu M\)) was ~20 minutes. ATP solution was prepared by adding ATP (as dipotassium salt, Sigma Chemical) immediately before use to the final concentration in the high K\(^{+}\) solution; pH was readjusted to 7.4 with KOH. The sensitivity of the ATP-regulated K\(^{+}\) channel to [ATP], was also compared at a temperature of 37°C between endocardial patches (\(n=4\)) and epicardial patches (\(n=4\)). To test whether the inactivation of the channel with time in inside-out patch mode was different in endocardial and epicardial cells, the activity of the channels was monitored for 30 minutes without exposure to ATP in three endocardial and three epicardial cells.

**Data Analysis**

Membrane potentials and single-channel currents were recorded through a patch-clamp amplifier (model 8900, Dagan Corp., Minneapolis, Minn.) and were stored on a videocassette recorder (model SL-HF900, Sony, Tokyo) through an analog-to-digital converter (model PCM-1, Medical Systems Corp., Greenvale, N.Y.) at a conversion rate of 40 kHz. The recorder signals for single-channel currents were filtered off-line through an eight-pole low-pass Bessel filter (48 dB/octave, model 902-LPF, Frequency Devices Inc., Haverhill, Mass.) with cutoff frequency of 1--5 kHz at −3 dB, digitized at a conversion rate of 30 kHz with a 12-bit Labmaster analog-to-digital converter (TecMar Scientific Solutions, Burlingame, Calif.) and analyzed using a software program pClamp (Axon Instruments, Inc., Burlingame, Calif.) on an IBM-AT computer. The stored signals for membrane potentials were played back on the chart recorder (Gould Inc., Altamonte Springs, Fla.) at a paper speed of 125 mm/sec for analysis.

All data are expressed as mean±SD. Statistical significance was evaluated by analysis of variance with repeated measurements or by Student's unpaired \(t\) test, where appropriate. Values of \(p<0.05\) were considered significant.

**Results**

**Changes in Action Potential Duration After CN\(^{-}\) Treatment**

Figure 1 shows representative action potential recordings from an endocardial and an epicardial myocyte in the control state and at 10 and 30 minutes after initiating superfusion with Tyrode's solution containing 1 mM CN\(^{-}\). In the control state, action potentials recorded from epicardial single cells, in contrast to those recorded from endocardial cells, demonstrated a prominent notch between phases 1 and 2, showing a "spike-and-dome" configuration. APD\(_{90}\) was longer in endocardial cells than in epicardial cells, whereas APD\(_{90}\) was not significantly different between endocardial and epicardial cells (APD\(_{90}\): 205±13 versus 181±9 msec, respectively,
**Figure 1.** Representative recordings showing action potential changes induced by CN⁻ treatment in an endocardial (left) and epicardial (right) single cell. Action potentials were elicited by stimulation at 1 Hz. Action potential tracings were recorded on a chart recorder at a paper speed of 125 mm/sec.

$\rho<0.01$; $\text{APD}_{20}$: 115±10 versus 113±9 msec, respectively, $\rho=\text{NS}$). The resting membrane potential was the same in endocardial cells (89±5 mV) and in epicardial cells (87±4 mV), whereas the action potential amplitude was higher in endocardial cells (124±6 mV) than in epicardial cells (113±5 mV) ($\rho<0.01$). These action potential characteristics are similar to those recorded in multicellular ventricular muscle preparations.7-9

Figure 2 lists $\text{APD}_{20}$ and $\text{APD}_{90}$ in the control state and at 10, 20, and 30 minutes after exposure to CN⁻ (expressed as a fraction of the control value). Exposure to CN⁻ evoked shortening in action potential duration of both endocardial and epicardial cells. The magnitude of shortening in $\text{APD}_{20}$ and $\text{APD}_{90}$ was significantly greater in epicardial cells than in endocardial cells at 10, 20, and 30 minutes after exposure to CN⁻. Neither resting membrane potentials nor action potential amplitudes were significantly changed by CN⁻ treatment.

Figure 3 shows representative recordings demonstrating the reversal effect of glibenclamide on CN⁻-induced action potential shortening. In both an endocardial and an epicardial cell, superfusion with CN⁻-Tyrode’s solution containing 0.3 $\mu$M glibenclamide partially reversed CN⁻-induced action potential shortening; the magnitude of reversal, however, was much greater in the epicardial cell. This was also observed in two other endocardial and two epicardial cells.

**ATP-Regulated K⁺ Channel in Cell-Attached Membrane Patches After Exposure to CN⁻**

In the cell-attached patch mode, the activity of ATP-regulated K⁺ channels was compared for endocardial ($n=5$) and epicardial ($n=6$) patches after treatment with 1 mM CN⁻. Single-channel currents were recorded by applying voltage steps of −100 to 100 mV for 60 seconds from the resting membrane potential in 20-mV increments. No significant current was evident before CN⁻ treatment. After CN⁻ treatment, single-channel currents were observed in both endocardial and epicardial membrane patches, which had current-to-voltage relations similar to those reported for ATP-regulated K⁺ channels.10

Figure 4 shows representative tracings of single-channel currents in the control state and after superfusion with CN⁻-Tyrode’s solution. Superfusion with 1 mM CN⁻ evoked marked single-channel openings in the epicardial membrane patches, which were not evident before superfusion, but it evoked much less activity in the endocardial membrane patches.

**ATP-Regulated K⁺ Channels in Inside-Out Membrane Patches in the Absence of ATP**

To explore the mechanisms of differential CN⁻ activation of ATP-regulated K⁺ channels in the endocardial and the epicardial membrane patches, the characteristics of the ATP-regulated K⁺ channel were studied in the inside-out membrane patch mode. Figure 5A shows representative tracings of single-channel currents in the absence of ATP at the intracellular membrane surface in an endocardial and an epicardial membrane patch. To measure the unitary current amplitude and open-state probability of ATP-regulated K⁺ channel, an amplitude histogram was formed for all the data points over recording periods of 60 seconds (Figure 5B).22 A “50%
In minutes.

In mM after msec

Similar to 42

The open-state probability \( P_{\text{open}} \) was calculated by measuring the average current as \( P_{\text{open}} = I/(N \cdot i) \), where \( I \) equals total current through open channels given as total area under the amplitude histogram, \( N \) is the number of channels, and \( i \) is the unitary current amplitude.\(^{22} \) \( P_{\text{open}} \) was calculated using data obtained at a membrane potential positive to the reversal potential by 80 mV; at potentials negative to the reversal potential, the openings of the inward rectifier \( K^+ \) channel interfere with the measurement.\(^{24} \)

Currents flowing through \( K^+ \) channels (\( I_{\text{KATP}} \)) had a reversal potential near 0 mV in both endocardial and epicardial membrane patches with symmetrical transmembrane [\( K^+ \)]. During hyperpolarizing pulses ranging from 20 to 100 mV more negative than the reversal potential, the current-to-voltage relation for \( I_{\text{KATP}} \) was linear with a slope conductance of 67.3±6.7 pS for endocardial cells and 70.5±3.9 pS for epicardial cells (\( p=NS \)) (Figure 5C). During rectangular depolarizing pulses (positive to the reversal potential) of the same magnitude, the current-to-voltage curve displayed moderate inward rectification, with slope conductances becoming nonlinear at strong depolarizations (>40 mV) in both endocardial and epicardial cells (Figures 5A and 5C). There was no significant difference in the degree of inward rectification between endocardial and epicardial cells (Figure 5C). The basal mean open-state probability at 80 mV in endocardial membrane patches was 42.0±13.6%, and that in epicardial membrane patches was 49.8±14.9% (\( p=NS \)).

To compare the gating kinetics of the channels in endocardial and epicardial membrane patches, the open-time and the closed-time histograms were calculated at a membrane potential 80 mV positive to the reversal potential. Open and closed times were measured from recordings showing no overlap of the

**Figure 3.** Recordings showing effect of 0.3 μM glibenclamide on the shortening of action potential evoked by \( CN^- \) treatment. In an endocardial cell, the action potential duration at 90% repolarization (APD\(_{90}\)) was shortened from 321 to 242 msec after superfusion with Tyrode’s solution containing 1 mM \( CN^- \) for 30 minutes; APD\(_{90}\) increased to 284 msec when the myocyte was subsequently superfused with Tyrode’s solution containing 1 mM \( CN^- \) and 0.3 μM glibenclamide for 15 minutes. In an epicardial cell, APD\(_{90}\) was shortened from 289 to 42 msec after superfusion with Tyrode’s solution containing 1 mM \( CN^- \). Superfusion with Tyrode’s solution containing 1 mM \( CN^- \) and 1 μM glibenclamide increased APD\(_{90}\) to 237 msec. Similar findings were also observed in two other endocardial and two epicardial cells. Action potential was elicited by stimulation at 1 Hz. Action potential tracings were recorded on a chart recorder at a paper speed of 125 mm/sec.

**Figure 4.** Representative tracings demonstrating effects of \( CN^- \) treatment on unitary currents in cell-attached membrane patches of an endocardial (panel A) and an epicardial (panel B) cell. Membrane potentials were held at 80 mV positive to the resting membrane potential. Solid lines indicate the closed state of channels; currents flowing from the internal to the external side (outward currents) are shown as upward deflections. The current was displayed through a low-pass filter of 1 kHz.
unitary current through the entire period of recording (Figure 6A). This indicated that the membrane patch contained only one ATP-regulated K+ channel. Each distribution histogram of open or closed times was formed from a continuous recording of 60-second duration. Measurement of the open and closed times was performed after setting the cutoff frequency of the filter at 5 kHz to increase the resolution. A nonlinear, least-squares method was used to fit a probability density function to open or closed times with a form of single or double exponential. Distribution of the open times could not be fitted with single-exponential functions in either endocardial or epicardial membrane patches; at least two components were necessary (fast and slow components) (Figure 6B). To describe the distribution of the closed times, at least two components of exponential function were also needed in both endocardial and epicardial membrane patches (Figure 6C).

There was no difference in the gating kinetics of the channels between endocardial and epicardial ATP-regulated K+ channels.

**ATP Sensitivity of the ATP-Regulated K+ Channel**

The results in the previous section demonstrated that the current-to-voltage relation and the gating properties of the ATP-regulated K+ channel were essentially identical between endocardial and epicardial membrane patches. Thus, we further examined the sensitivity of the ATP-regulated K+ channel to ATP. The intracellular surface of the inside-out membrane patch was superfused with the high K+ solution containing 10, 25, 50, 100, 250, 500, and 1,000 μM ATP, and single-channel currents at a test potential of +80 mV were recorded for 60 seconds. When the intracellular faces of endocardial and epicardial membrane patches were exposed to ATP, open-state probability of the channels was reduced in
a dose-dependent fashion, whereas unit current through a single channel was not affected (Figure 7). However, the magnitude of the reduction in the open-state probability by exposure to low concentrations of ATP was greater in endocardial membrane patches than in epicardial membrane patches. The dose–response relations between ATP concentration and the open-state probability of the channel (expressed as fractions of the value in absence of ATP) from endocardial and epicardial membrane patches are shown in Figure 8. The dose–response relation between ATP concentration and the open-state probability of the channel was fitted to the four-parameter logistic equation:

$$y = A + \frac{B-A}{1+(10^y/10^x)^D}$$

where $A$ is the bottom plateau, $B$ is the top plateau, $C$ is the log($EC_{50}$), and $D$ is the slope factor (the Hill coefficient). The concentration of ATP that produced half-maximal inhibition of the channel was $23.6 \pm 21.9 \mu M$ for endocardial patches and $97.6 \pm 48.1 \mu M$ for epicardial patches ($p<0.01$). Several experiments were carried out at a temperature of 37°C (four for endocardial patches and four for epicardial patches). At 37°C, we observed similar differential suppression of the channel by ATP; the concentration of ATP that produced half-maximal inhibition of the channel at 37°C was $31.8 \pm 30.2 \mu M$ for endocardial patches and $101.6 \pm 54.8 \mu M$ for epicardial patches ($p<0.01$).
inhibition of the channel was 28.9±23.3 μM for endocardial patches and 101.9±38.1 μM for epicardial patches (p<0.05).

In the experiments studying inactivation of channel activity with time in the absence of ATP, open-state probability was reduced to 66±12% of the initial value in 30 minutes for endocardial cells and 63±10% for epicardial cells (p=NS). This indicated there was no significant difference in time-dependent inactivation of the channel activity between the two cell types.

Discussion

Many studies have demonstrated that the subendocardium is significantly more sensitive to metabolic effects of ischemia than is the subepicardium. Ali et al reported that creatine phosphate and ATP content were reduced to a greater extent in subendocardium than in subepicardium during coronary artery occlusion. Watson et al reported that after coronary artery occlusion, tissue pH was significantly more reduced in the subendocardium than in the subepicardium. Despite the greater metabolic sensitivity of the subendocardium to ischemia, several experimental models have indicated that ischemia-induced electrophysiological changes are greater in epicardial cells than in endocardial cells. Boineau and Cox and Okumura et al reported in their in vivo studies that prolongation of conduction time and refractory period during coronary artery occlusion was greater in epicardium than in endocardium. Gilmour and Zipes and Kimura et al reported that, in multicellular ventricular muscle preparations, changes in action potential characteristics and prolongation of conduction time and refractory period by ischemia or superfusion with "simulated ischemic solution" were greater in epicardial cells than in endocardial cells. They suggested that these ischemia-induced electrophysiological inhomogeneities might facilitate reentrant arrhythmias. Indeed, Kimura et al demonstrated that premature ventricular contractions and ventricular tachycardias were more frequently recorded during the period of ischemia at which differences in action potential durations and refractoriness between endocardial and epicardial cells were greatest.

It is impossible to derive an understanding of inherent cellular electrophysiological properties from
data obtained from in vivo studies or from multicellular ventricular muscle preparations, which have extracellular ionic and electrotonic influences. Under isolated single myocytes, we demonstrated that epicardial cells are inherently more susceptible to CN−-induced metabolic inhibition than are endocardial cells. Action potential shortening was significantly greater in epicardial cells than in endocardial cells during exposure to CN−. We also found that the rapid activation of ATP-regulated K+ channels by CN− treatment in epicardial cells appeared to produce a greater shortening of the epicardial action potentials. Our data indicate that the differential sensitivity of the channels to [ATP], is partly responsible for the differences in activation of ATP-regulated K+ channel and shortening of action potential by CN− treatment between the two cell types. The physiological basis for the site-related differential sensitivity of ATP-regulated K+ channels to [ATP], is currently unknown.

The characteristics of ATP-regulated K+ channels observed in the present study are similar to those reported previously.10,21,24,26–31 Linear slope conductance during hyperpolarization pulses was 67.3±6.7 pS for endocardial cells and 70.5±3.9 pS for epicardial cells; values similar to those previously reported in various mammalian myocytes were 63–80 pS.24,26–28 Inward rectification was found in both endocardial and epicardial cells. In both cell types, distributions of open time and closed time have at least two components, although the time constants of open- and closed-time histograms were slightly smaller in our study compared with those noted by Kakei et al.24 The slight differences in time constants may be due to differences in species and/or experimental methodology. Kakei et al.24 studied guinea pig ventricular myocytes in the open-cell attached-patch mode using 50 mM extracellular K+ and 0.5 mM intracellular ATP, whereas we studied canine myocytes in the inside-out patch mode using 140 mM extracellular K+ and no intracellular ATP. To study ATP sensitivity of the channel under reduced influence of inactivation of the channel activity with time,21 we measured control data 5 minutes after isolating the patch. As a result, open-state probability in our study (42% for endocardial cells and 50% for epicardial cells) was relatively lower than the values reported in other studies (~80%),24,26–28 which were measured immediately after isolating the patch. However, the value in our study is similar to that measured 5 minutes after isolating the patch (34%) in a previous report.21

The activation of the channel by reduction of [ATP], was clearly more rapid in epicardial cells than in endocardial cells. The reported [ATP], that produces half-maximal inhibition of the channel in inside-out patch mode in other studies is 25–100 μM.10,13,28,31 In our study, it was 97.6±48.1 μM for epicardial cells, which was significantly greater than that for endocardial cells (23.6±21.9 μM). Trube and Hescheler21 reported that activity of ATP-regulated K+ channels in inside-out membrane patches diminished with time. However, it is unlikely that inactivation of channel activity with time substantially affected the comparison of ATP sensitivity of the channels between endocardial and epicardial patches. We used an experimental protocol with an identical time course to study the sensitivity of channels to [ATP], in endocardial and epicardial membranes. Furthermore, the time course of inactivation of the channels was not significantly different between the two cell types.

In a previous report, we demonstrated that single cells isolated from endocardial and epicardial surfaces showed the same magnitude of action potential shortening when superfused with simulated ischemic solution, that is, low PO2, low pH, and high K+ in glucose-free Tyrode’s solution.32 However, compared with data obtained in multicellular ventricular muscle preparations, the magnitude of the action potential shortening during exposure to the simulated ischemic solution was much smaller in the isolated single cells. Single cells are entirely surrounded by superfusate, making it difficult to deprive the cells of O2 and substrates, whereas multicellular preparations can be hypoxic under the same condition. In the present study, we used treatment with CN− to compare the responses of action potentials to “hypoxia” in single isolated endocardial and epicardial cells. CN− treatment has been used by several investigators 10–12,33–35 as a method to reduce hypoxia in isolated cells. The primary effect of hypoxia on cardiac myocytes is to decrease high-energy phosphates, such as ATP, and CN− treatment causes depletion of [ATP], inhibiting oxidative phosphorylation. Extrapolation of the present data to explain in vivo ischemia should be done with some caution, because the in vivo ischemic condition is obviously more complex and cannot be reproduced simply by exposure to CN−. Nonetheless, our data provide new information on specific differences between responses of endocardial and epicardial cells to the loss of oxidative phosphorylation that occurs during ischemia.

In conclusion, the reduction in [ATP], evokes currents through ATP-regulated K+ channel to a greater magnitude in epicardial patches than in endocardial patches. The rapid activation of ATP-regulated K+ channels in epicardial patches appears to be responsible for the greater action potential shortening in epicardial cells during ischemia. It is possible, however, that other membrane currents, such as slow inward current and transient outward current, also may contribute to the differential changes in action potential durations. The finding that glibenclamide, a specific blocker of ATP-regulated K+ channel, could only partially reverse CN−-induced action potential shortening indeed suggests the contribution of different classes of membrane currents. Further studies are now under way to test this hypothesis.

Acknowledgment

We gratefully acknowledge Mr. Marcel J. Smets for preparation of the manuscript and illustrations.
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Key words: adenosine triphosphate • endocardium • epicardium • single channels
Role of cardiac ATP-regulated potassium channels in differential responses of endocardial and epicardial cells to ischemia.
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Circ Res. 1991;68:1693-1702
doi: 10.1161/01.RES.68.6.1693

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