Differences in the Electrophysiological Response of Canine Ventricular Epicardium and Endocardium to Ischemia
Role of the Transient Outward Current

Anton Lukas, PhD; Charles Antzelevitch, PhD

Background. Acute ischemia is known to produce more severe electrophysiological disturbances in canine ventricular epicardium than endocardium, although the mechanism for the differential sensitivity is still unresolved. Recent studies have demonstrated the presence of a prominent transient outward current ($I_{\text{to}}$) in ventricular epicardium but not endocardium. The present study was designed to test the hypothesis that the differential sensitivity of these two tissues to ischemia results, at least in part, from a more prominent $I_{\text{to}}$ in epicardium than in endocardium.

Methods and Results. Isolated canine ventricular epicardial and endocardial tissues and myocytes were studied by standard microelectrode techniques. Simulated ischemia (hyperkalemia, hypoxia, and acidosis) abolished the action potential plateau and caused a 50% to 60% shortening of action potential duration in epicardium but only a 10% to 20% shortening in endocardium. 4-Aminopyridine, an $I_{\text{to}}$ inhibitor, restored the plateau in epicardium and reduced the dispersion of action potential duration between epicardium and endocardium. Stimulation protocols that minimized the contribution of $I_{\text{to}}$, such as acceleration of the stimulation rate or introduction of early premature beats, produced a paradoxical prolongation of the epicardial response caused by restoration of the action potential dome. Thus, ischemia-induced dispersion of repolarization was greatly diminished at rapid rates and after premature beats. Similar results were obtained in tissues and myocytes obtained from the same myocardial layers, suggesting that the differential sensitivities of epicardium and endocardium to ischemia are largely a result of inherent differences in cellular properties.

Conclusions. Our data suggest that the presence of a prominent $I_{\text{to}}$ in epicardium but not endocardium contributes importantly to the selective electrical depression of epicardium by simulated ischemia. The repolarizing influence of $I_{\text{to}}$ serves to amplify the ischemia-induced changes in inward ($I_{\text{in}}$ and $I_{\text{ax}}$) and outward (calcium-activated) currents. By facilitating loss of the dome in epicardium, $I_{\text{to}}$ contributes to the development of a marked dispersion of repolarization between normal and ischemic epicardium and between epicardium and endocardium, thereby providing the electrophysiological substrate for the genesis of reentrant arrhythmias. (Circulation. 1993;88:2903-2915.)

Key words • ventricles • myocardium • epicardium • endocardium • electrophysiology • ischemia

Numerous in vivo studies have established that acute myocardial ischemia elicits much more severe electrophysiological disturbances in epicardium than in endocardium. Epicardium exhibits more pronounced changes in monophasic action potentials\(^1\) and greater prolongation of conduction time and refractory period\(^2\) than does endocardium during acute ischemia. Various explanations have been offered, including contact of endocardium with cavity blood,\(^3\) thebesian blood flow,\(^4\) a greater capacity of endocardium for anerobic metabolism,\(^5\)\(^,\)\(^6\) and electrotonic interaction of endocardial muscle with subendocardial Purkinje fibers, which are much more resistant to the depressant effects of hypoxia and ischemia.\(^7\)\(^,\)\(^8\)\(^,\)\(^9\)\(^,\)\(^10\) Recent studies showing a selective depression of the epicardial response despite comparable depolarizations in resting membrane potential in epicardium and endocardium have questioned the first three proposed explanations.\(^11\)\(^,\)\(^12\) The hypothesis that the differential sensitivity is due to the presence of subendocardial Purkinje fibers has also been weakened by studies indicating that subendocardial Purkinje fibers are insulated from muscle and do not influence muscle electrotonically, except at the terminal Purkinje fiber–muscle junctions.\(^13\)\(^,\)\(^14\)\(^,\)\(^15\)\(^,\)\(^16\)

The available data suggest that the differential sensitivities of epicardium and endocardium to ischemia may reflect fundamental electrophysiological differences between the two tissue types. Notably, transmembrane and monophasic action potentials recorded from epicardium exhibit a spike-and-dome morphology that is absent in action potentials recorded from endocardium.\(^13\)\(^,\)\(^14\)\(^,\)\(^17\)\(^,\)\(^18\) Recent studies have shown that the differences in action potential configuration are in large part due to the presence of a prominent transient
outward current ($I_o$) in ventricular epicardium but not endocardium. However, the precise role of $I_o$ in epicardium is not clear, and it is uncertain how conditions of ischemia influence this current. The present study was therefore designed to test the hypothesis that the presence of an additional repolarizing current ($I_o$) in epicardium contributes to its selective depression during ischemia.

**Methods**

**Studies on Syncytial Tissue Preparations**

Hearts were removed from adult, male mongrel dogs (15 to 30 kg) anesthetized with sodium pentobarbital (30 mg/kg IV). Epicardial tissues (10×15×0.6 to 1.0 mm) were shaved from the epicardial surface of the right ventricle midway along the apico basal axis with a dermatome (Davol, Cranston, RI). Endocardial preparations consisted of trabeculae shaved from the base of the right ventricle. Only endocardial preparations that did not exhibit “Purkinje-like” action potentials (verified by roving microelectrode impalements) were used in the study to eliminate the influence of subendocardial Purkinje fibers. Epicardial and endocardial preparations from the same heart were pinned side by side in the same tissue bath (6-mL volume) and superfused with Tyrode’s solution containing (in mmol/L): NaCl 129.0, KCl 4.0, NaH2PO4 0.9, NaHCO3 20.0, CaCl2 1.8, MgSO4 0.5, and glucose 5.5. The solution was maintained at 37±0.5°C and had a final pH of 7.35±0.5. The Tyrode’s solution was bubbled with 95% O2/5% CO2 to produce a PO2 of 550 to 650 mm Hg in the tissue bath.

The tissues were stimulated at a basic cycle length (BCL) of 800 milliseconds through silver bipolar electrodes insulated except at the tips. Stimuli were pulses (2 milliseconds in duration and twice diastolic threshold intensity) generated by two Pulsar 6i stimulators (Frederick Haer & Co, Brunswick, Me). Transmembrane potentials were recorded by use of glass microelectrodes filled with 2.7 mol/L KCl (15 to 30 MΩ) connected to a model 750 dual microprobe amplifier (WPI, New Haven, Conn). The amplifier provides an A-B (differential) output by feeding the endocardial and epicardial channels into an internal differential amplifier. This output was used to generate a simulated ECG trace on-line. Signals were displayed on a storage oscilloscope (Tektronix, Beaverton, Ore), amplified with a programmable amplifier (model 1903A; Cambridge Electronic Design [C.E.D.], UK), and digitized with a model 1401 AD/DA system (C.E.D.). Data acquisition was controlled by a Dell System 310 personal computer (Austin, Tex), which was also used for analysis of data files with Spike2 software from C.E.D.

Tissues were equilibrated with the normal Tyrode’s solution until steady-state transmembrane activity was obtained (typically 3 to 4 hours). The stimulation frequency was then varied over a wide range of BCLs ranging between 250 and 2000 milliseconds, and a restitution of action potential characteristics was performed with single test pulses ($S_t$; 2 milliseconds in duration) delivered after every tenth basic beat ($S_b$). Tissues were then superfused with a solution modified to mimic conditions encountered during ischemia (hyperkalemia, hypoxia, and acidosis). The “ischemic” Tyrode’s solution contained (in mmol/L): NaCl 129.0, KCl 6.0, NaH2PO4 0.9, NaHCO3 20.0, CaCl2 1.8, MgSO4 0.5, and glucose 5.5. The pH was adjusted to 6.8 with 5N HCl. The glass reservoir containing the “ischemic” solution was sealed, and the solution was bubbled with 95% N2/5% CO2 for at least 3 hours. The PO2 of the “ischemic” solution was <45 mm Hg measured in the tissue bath (ISO2 Dissolved Oxygen Meter; WPI, New Haven, Conn). Tissues were superfused with the “ischemic” solution until the action potential dome in epicardium was abolished; frequency and restitution scans were then repeated. In most instances, tissues were then exposed to “ischemic” solution containing 4-aminoopyridine (4-AP; 1 to 2 mmol/L). This solution was prepared at least 15 minutes before use, since addition of 4-AP caused a transient pH change lasting 5 to 10 minutes. In some experiments, the normal and “ischemic” solutions contained propranolol (0.3 µg/mL), phenolamine (0.5 µg/mL), and atropine (1 µg/mL), since 4-AP has been reported to cause neurotransmitter release from adrenergic and cholinergic nerve endings. However, no differences in the effect of 4-AP were found in the presence or absence of these agents.

**Studies on Enzymatically Dissociated Ventricular Myocytes**

Ventricular myocytes were isolated as follows. Adult male mongrel dogs were anesthetized with sodium pentobarbital (30 mg/kg IV) containing heparin (220 IU/kg). The heart was removed, fibrillated, and submerged in bicarbonate Tyrode’s solution. A large wedge of left ventricle was excised around the left anterior descending coronary artery, and the artery was cannulated and flushed with 50 mL nominally calcium-free “Kreb’s buffer” containing (in mmol/L): NaCl 118.5, KCl 2.8, NaHCO3 14.5, KH2PO4 1.2, MgSO4 2.7, and glucose 11.1, with 0.1% bovine serum albumin (BSA). The wedge was then mounted on a recirculating pump and perfused at a rate of 12 mL/min with calcium-free Krebs buffer supplemented with 0.04% collagenase (at 37°C). After 12 to 17 minutes, the epicardial and endocardial layers (<1.5 mm thick) were shaved from the wedge with a dermatome and placed in 15 mL Krebs buffer supplemented with 1.5% BSA, 0.04% collagenase, 0.5 mmol/L MgSO4, and 0.3 mmol/L CaCl2. The minced tissues were bubbled with 95% O2/5% CO2 and agitated in a 37°C water bath. After 15 minutes, the epicardial and endocardial digests were passed through a nylon mesh (220 µm). Tissue fragments that did not pass through the mesh were returned to the water bath in fresh enzyme solution, whereas the filtrates were centrifuged at 400 rpm. Pellets were then resuspended in HEPES Tyrode’s solution containing (in mmol/L): NaCl 132.0, HEPES 20, MgSO4 3.2, glucose 11.1, CaCl2 0.5, and KCl 5.0, with 1.5% BSA. The incubation was repeated three or four times at 15-minute intervals. Yields of 20% to 50% (viable to nonviable cells) were typical for each myocardial layer.

Aliquots of cells were placed in a temperature-controlled superfusion chamber (PDMI-2, Medical Systems Corp, Greensville, NY) mounted on a Nikon Diaphot TMD inverted microscope. Cells were superfused with HEPES Tyrode’s solution at a rate of 3 mL/min. The “normal” HEPES Tyrode’s solution contained (in mmol/L): NaCl 132.0, KCl 4.0, CaCl2 1.8, MgSO4 1.2, HEPES 20.0, and glucose 11.1 (pH 7.4;
Results

Effects of Simulated Ischemia on Ventricular Action Potentials

Fig 1 shows representative tracings of transmembrane action potentials and simulated transmural ECGs recorded from canine endocardial and epicardial tissues under control conditions and after exposure of the preparations to simulated ischemic conditions. Under control conditions (panel A), action potentials recorded from epicardium display smaller phase 0 and phase 1 amplitudes but a larger notch than those recorded from endocardium. Typically, action potential duration (APD) was longer in endocardium than epicardium (see Table 1), resulting in a positive T wave in the simulated ECG. The ECG was obtained by differential recording of the endocardial and epicardial transmembrane activity. Exposure of the tissues to ischemic Tyrode’s solution (panel B) caused a decrease in the amplitude of phase 0 and marked changes in the plateau (dome) of the action potential recorded from epicardium. After 28 minutes of ischemia, the spike-and-dome morphology of the epicardial response was accentuated. One minute later, marked depression of the dome occurred and was followed by...
**TABLE 1. Effects of Simulated Ischemia and 4-Aminopyridine on Action Potential Parameters in Canine Ventricular Epicardial and Endocardial Tissues**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>n</th>
<th>Intervention</th>
<th>Phase 0 Amp, mV</th>
<th>Phase 1 Amp, mV</th>
<th>Notch, mV</th>
<th>Phase 2 Amp, mV</th>
<th>APD90, ms</th>
<th>MDP, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epicardium</td>
<td>20</td>
<td>Control</td>
<td>98.4±5.2</td>
<td>62.6±5.4</td>
<td>35.7±7.2</td>
<td>107.7±3.3</td>
<td>204.2±14.2</td>
<td>-84.8±1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>“Ischemia”</td>
<td>76.4±7.9</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>“I”+4-AP</td>
<td>78.1±4.9</td>
<td>73.3±3.8</td>
<td>6.9±3.2</td>
<td>74.6±3.1</td>
<td>133.2±11.7</td>
<td>-67.8±1.0</td>
</tr>
<tr>
<td>Endocardium</td>
<td>20</td>
<td>Control</td>
<td>115.5±3.4</td>
<td>102.2±4.1</td>
<td>8.7±3.9</td>
<td>102.7±4.6</td>
<td>213.4±16.1</td>
<td>-82.4±1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>“Ischemia”</td>
<td>97.8±3.4</td>
<td>89.9±2.2</td>
<td>3.6±2.8</td>
<td>88.9±2.3</td>
<td>174.5±14.6</td>
<td>-67.2±1.2</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>“I”+4-AP</td>
<td>97.4±4.8</td>
<td>91.9±3.7</td>
<td>2.2±2.2</td>
<td>89.2±3.7</td>
<td>191.7±21.2</td>
<td>-67.1±1.7</td>
</tr>
</tbody>
</table>

Amp indicates amplitude; Notch, phase 2 amplitude minus phase 1 amplitude; APD90, action potential duration at 90% repolarization; MDP, maximum diastolic potential; “I”, “ischemia”; and 4-AP, 4-aminopyridine (1 to 2 mmol/L, 15 minutes of exposure). All values are mean±SD obtained at a basic cycle length of 800 milliseconds.

*P<.05 compared with same tissue control; †P<.05 compared with same tissue “ischemia” value.

complete abolition of the dome after a total of 30 minutes of ischemia. As a result, APD at 90% repolarization (APD90) was markedly abbreviated in epicardium. APD90 decreased from 204.2±14.2 milliseconds in control to 90.4±23.3 milliseconds during ischemia (Table 1). In contrast, ischemia produced only a 10% to 20% abbreviation of APD90 in endocardium, despite a similar decrease in resting membrane potential in the two tissue types. The marked dispersion of repolarization that developed was manifested as an elevated ST segment in the ECG trace. As an initial test of the hypothesis that the presence of a prominent In in epicardium but not endocardium contributes to the differential sensitivities of the two tissues to “ischemia,” we exposed the preparations to the In inhibitor 4-AP. In panel C, 1 mmol/L 4-AP was introduced 10 minutes after the dome was abolished in epicardium. 4-AP restored the plateau phase of the epicardial action potential within 1 minute, and the records shown were obtained after 5 minutes of ischemia plus 4-AP. The ST segment and T wave also returned to near normal in the simulated ECG. With continued exposure to 4-AP, phase 0 and phase 1 amplitudes increased further as the size of the notch decreased (time course, ≈10 minutes; data not shown, but see Table 1). Thus, 4-AP greatly reduced the marked dispersion of repolarization between epicardium and endocardium caused by ischemia (Table 1).

**Ischemia-Induced Changes in Action Potential Duration**

The complete time course of changes in APD90 recorded in endocardium and epicardium during the experimental protocol is summarized in Fig 2. In epicardium, simulated ischemia produced a triphasic response characterized by an initial abbreviation of APD90, followed by a slight prolongation caused by accentuation of the notch, and finally a marked abbreviation caused by loss of the action potential dome. Introduction of 4-AP (1 mmol/L) resulted in restoration of the dome and prolongation of APD90. With continued exposure to 4-AP, APD90 decreased progressively as the spike-and-dome morphology (notch) of the epicardial response attenuated. The effects of ischemia and 4-AP were rapidly reversed upon washout (reperfusion).

In contrast, endocardium exhibited only slight changes in APD90 throughout the experimental protocol. Action potentials in endocardium were much more resistant to the depressant effects of ischemia. Major depression of action potentials in endocardium occurred only after several hours of ischemia. Similar results were obtained in 20 epicardial and endocardial preparations, as summarized in Table 1.

**Relation Between Phase 1 Amplitude and Susceptibility to Simulated Ischemia**

Ischemia led to loss of the dome in 20 of the 29 epicardial preparations studied. The duration of expo-
During ischemia (Fig 4). Under control conditions (panel A), the spike-and-dome configuration and size of the notch in epicardium are much more exaggerated at slow stimulation rates (BCL, 2000 milliseconds) compared with fast rates (BCL, 400 milliseconds). The rate-dependence of $I_o$ in dog is such that the current increases in amplitude with deceleration of the stimulation rate because of its slow reaction kinetics.20,21 In contrast, $I_{Ca}$ decreases in amplitude with deceleration.20,21 Thus, the accentuated spike-and-dome configuration in epicardium at slower rates probably reflects a larger $I_o$ opposing a smaller $I_{Ca}$. At fast stimulation rates, this relation would be reversed, with a smaller $I_o$ opposing a larger $I_{Ca}$. This shift in the balance of current is thought to account for attenuation and accentuation of the notch at fast and slow stimulation rates, respectively. The relation between these two opposing currents would be dramatically altered if ischemia inhibited $I_{Ca}$ to a greater extent than $I_o$. Under such conditions, action potential configuration would become more sensitive to rate-dependent changes in $I_o$.

Indeed, acceleration of the stimulation rate under “ischemic” conditions led to restoration of the dome and thus to a paradoxical prolongation of the action potential in epicardium, as shown in Fig 4B. In this case, the ischemia-induced loss of the dome was reversed by acceleration of the stimulation rate from a BCL of 2000 milliseconds to 400 milliseconds. The reduced availability of $I_o$ at the faster rate allows for reappearance of the action potential dome that was lost because of ischemia-induced changes in $I_{Ca}$ and other currents.

Fig 5 plots the complete APD/rate relations obtained in the experiment illustrated in Fig 4. Under control conditions (panel A), progressive acceleration of the stimulation rate from a BCL of 4000 to 200 milliseconds was attended by a progressive shortening of APD$_{90}$ in endocardium and epicardium. During ischemia (panel B), APD$_{90}$ in epicardium was markedly abbreviated at BCLs longer than 600 milliseconds because of absence of the action potential dome. In contrast, the rate dependence of APD$_{90}$ in endocardium during ischemia was similar to that of control. Ischemia thus gave rise to a 110- to 130-millisecond dispersion of APD$_{90}$ between epicardium and endocardium at BCLs ranging from 1000 to 4000 milliseconds. At faster BCLs (<600 milliseconds), the dispersion of APD was nearly eliminated because of restoration of the dome in the epicardial action potential.

Restitution of Action Potential Characteristics During Ischemia

To further characterize the role of $I_o$, we examined the restitution of action potential parameters in epicardium (Fig 6) and endocardium (Fig 7) under control and simulated ischemic conditions. Each panel is composed of 9 or 10 superimposed traces recorded from an epicardial or endocardial preparation. The first response in each panel is the last of a train of 10 basic beats. Subsequent beats are the responses elicited by premature stimuli delivered progressively later in diastole. Under control conditions in epicardium (Fig 6A), the spike-and-dome morphology, absent in early premature beats, develops and becomes progressively more pronounced as the S$_1$-S$_2$ interval prolongs. Also, the restitution of phase 0, phase 1, and phase 2 amplitude

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**Rate-Dependence of Action Potential Configuration During Ischemia**

As a second test of the hypothesis that the presence of an additional outward current renders epicardium more susceptible to the effects of ischemia, we examined the rate-dependence of transmembrane activity in endocardium and epicardium before and after exposure to ischemia (Fig 4). Under control conditions (panel A), the spike-and-dome configuration and size of the notch in epicardium are much more exaggerated at slow stimulation rates (BCL, 2000 milliseconds) compared with fast rates (BCL, 400 milliseconds). The rate-dependence of $I_o$ in dog is such that the current increases in amplitude with deceleration of the stimulation rate because of its slow reaction kinetics.20,21 In contrast, $I_{Ca}$ decreases in amplitude with deceleration.20,21 Thus, the accentuated spike-and-dome configuration in epicardium at slower rates probably reflects a larger $I_o$ opposing a smaller $I_{Ca}$. At fast stimulation rates, this relation would be reversed, with a smaller $I_o$ opposing a larger $I_{Ca}$. This shift in the balance of current is thought to account for attenuation and accentuation of the notch at fast and slow stimulation rates, respectively. The relation between these two opposing currents would be dramatically altered if ischemia inhibited $I_{Ca}$ to a greater extent than $I_o$. Under such conditions, action potential configuration would become more sensitive to rate-dependent changes in $I_o$.

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was biphasic in epicardium, since the amplitude of premature beats elicited early in diastole was greater than that of the basic beat. In contrast, the restitution of action potential parameters was monotonic in endocardium (Fig 7A); the amplitude of early premature beats was never greater than that of the basic beats.

In epicardial tissues exposed to ischemia (Fig 6B), premature beats elicited early in diastole exhibit an almost normal response in which the dome is restored. Responses elicited later in diastole exhibit a progressively more pronounced phase 1; the spike-and-dome morphology becomes more prominent until, at an S1-S2 interval of 450 milliseconds, the dome is abolished. Also noteworthy is the postrepolarization refractoriness that develops during ischemia in both epicardium (Fig 6B) and endocardium (Fig 7B). Fig 6C illustrates the effect of the I\textsubscript{Na} inhibitor 4-AP (1 mmol/L) to restore the dome of the basic beat and to greatly diminish time-dependent changes in the early phases of the action potential in epicardium. The restitution of action potential parameters in endocardium during "ischemia" in the presence of 4-AP is illustrated in Fig 7C.

Fig 8 graphically illustrates the restitution data obtained in the experiment pictured in Figs 6 and 7. Under control conditions (Fig 8A), APD\textsubscript{90} values in epicardium and endocardium were similar and relatively constant over the range of S1-S2 intervals tested. The effective refractory period (ERP) measured under control conditions at a BCL of 800 milliseconds was 185 milliseconds in epicardium and 195 milliseconds in endocardium (\Delta\text{ERP}\textsubscript{endo-epi}=10 milliseconds). During ischemia (Fig 8B), the dispersion of APD\textsubscript{90} between epicardium and endocardium was small at short coupling intervals but increased dramatically at S1-S2 intervals >400 milliseconds because of loss of the dome in epicardium. The latter would be expected if I\textsubscript{Na} availability is greater later in diastole. The ERP measured under ischemic conditions was 125 milliseconds in epicardium and 190 milliseconds in endocardium. Thus, \Delta\text{ERP}\textsubscript{endo-epi} during ischemia increased to 65 milliseconds versus 10 milliseconds in control, whereas \Delta\text{APD}\textsubscript{endo-epi} increased from 10 milliseconds in control to 100 milliseconds during ischemia (a BCL of 800 milliseconds).

The effects of 4-AP (1 mmol/L) on the restitution of APD\textsubscript{90} in epicardial and endocardial preparations exposed to ischemia are shown in Fig 8C. The addition of 4-AP led to restoration of the dome at all S1-S2 intervals, but APD\textsubscript{90} remained considerably briefer in epicardial responses compared with those recorded from endocardium. The ERPs measured during ischemia plus 4-AP were considerably longer than those measured during ischemia alone because of the effect of 4-AP to dramatically prolong APD in epicardium. The ERP was 165 milliseconds in epicardium and 195 milliseconds in endocardium (\Delta\text{ERP}\textsubscript{endo-epi}=30 milliseconds).
Loss of the Plateau in Epicardium as a Mechanism for Electrical Alternans

Under ischemic conditions, complete suppression of the dome in most epicardial preparations was preceded by a period of electrical alternans of varying duration. Fig 9 illustrates the two most common patterns of alternans observed. Each panel shows microelectrode recordings from an endocardial and epicardial preparation and the simulated ECG obtained by differential recording of the two voltage traces. Panel A illustrates a 2:1 pattern in which the action potential dome was alternately present and absent, resulting in alternating long and short action potentials. Since the endocardial APD remained constant during this period, the T-wave alternans seen in this example was due entirely to changes in APD of epicardium. This particular example of 2:1 alternans was stable for a period of approximately 2 minutes at a BCL of 800 milliseconds. Thereafter, all epicardial beats were devoid of a dome at a BCL of 800 milliseconds, but alternans could be observed at progressively faster stimulation rates.

Fig 9B illustrates another example of electrical alternans recorded in a different set of preparations. The 3:1 pattern shown is another one commonly encountered during ischemia. In this example, an epicardial action potential with a dome was followed by two beats lacking a dome. Alternans of the T-wave amplitude and polarity were a direct result of the changes in APD of epicardium.

In all cases, the pattern of alternans was found to be a sensitive function of the stimulation rate. At any...
Fig 7. Tracings showing restitution of action potential characteristics in an endocardial preparation under control conditions, during ischemia, and during ischemia plus 4-aminopyridine (4-AP). Traces are arranged as in Fig 6. Under control conditions (A), the restitution of action potential characteristics is monophasic in endocardium. During ischemia (B), the restitution is qualitatively similar to the control, although postpolarization refractoriness is evident in the endocardial traces. Addition of 4-AP to the ischemic solution (C) did not appreciably alter the restitution pattern elicited in endocardium. The traces in C were recorded after 15 minutes of exposure to 1 mmol/L 4-AP.

Fig 8. Plots showing effects of ischemia or ischemia plus 4-aminopyridine (4-AP) on the restitution of action potential duration at 90% repolarization (APD90) in canine endocardium (Endo) and epicardium (Epi). A, Restitution of APD90 in epicardium (○) and endocardium (●) under control conditions. The restitution protocol is the same as described in Fig 6. Under control conditions, progressive shortening of the S1-S2 interval resulted in premature responses with progressively shorter APD90 in both epicardium and endocardium. B, Restitution of APD90 obtained in the same preparations during ischemia. During ischemia, the restitution of APD90 in endocardium was qualitatively similar to control. In epicardium, however, the APD90 of premature beats introduced at S1-S2 intervals <350 milliseconds was greater than that of the basic beats because of restoration of the dome. Indeed, a premature beat introduced at an S1-S2 interval of 350 milliseconds elicited a response in epicardium that was longer than that obtained in endocardium. The dome was not present in premature beats introduced at S1-S2 intervals >400 milliseconds, and this is reflected by the very short APD90. C, Restitution of APD90 obtained after 15 minutes of exposure to 4-AP (1 mmol/L) in the presence of ischemia. All restitution scans were performed at a basic cycle length of 800 milliseconds.

Effects of Ischemia on Electrical Activity in Single Cardiac Myocytes

In another experimental series, we tested the hypothesis that the differential sensitivity of epicardium and endocardium to ischemia was a result of differences in intrinsic electrophysiological properties of the cells and not of extracellular properties of the two tissue types or electrotonic influences of neighboring cells. Myocytes isolated from discrete epicardial and endocardial sites were studied before and after exposure to ischemia.

Fig 10 shows the effects of ischemia on the electrical activity of myocytes dissociated from endocardial and epicardial layers of the left ventricular free wall. Under control conditions, transmembrane action potentials recorded from endocardial and epicardial myocytes showed distinctions similar to those observed in the respective syncytial tissue preparations. Epicardial cells exhibited a lower phase 0 and phase 1 amplitude but a larger notch than did cells isolated from endocardium. When the cells were exposed to simulated ischemia (panel B), the action potential dome in the epicardial instant in time during ischemia, small changes in rate were often sufficient to induce alternans, shift the pattern of alternans, or abolish alternans entirely. The window of rates at which alternans was observed varied from preparation to preparation (range, 600 to 1800 milliseconds).

Effects of Ischemia on Electrical Activity in Single Cardiac Myocytes

In another experimental series, we tested the hypothesis that the differential sensitivity of epicardium and endocardium to ischemia was a result of differences in intrinsic electrophysiological properties of the cells and not of extracellular properties of the two tissue types or electrotonic influences of neighboring cells. Myocytes isolated from discrete epicardial and endocardial sites were studied before and after exposure to ischemia.

Fig 10 shows the effects of ischemia on the electrical activity of myocytes dissociated from endocardial and epicardial layers of the left ventricular free wall. Under control conditions, transmembrane action potentials recorded from endocardial and epicardial myocytes showed distinctions similar to those observed in the respective syncytial tissue preparations. Epicardial cells exhibited a lower phase 0 and phase 1 amplitude but a larger notch than did cells isolated from endocardium. When the cells were exposed to simulated ischemia (panel B), the action potential dome in the epicardial instant in time during ischemia, small changes in rate were often sufficient to induce alternans, shift the pattern of alternans, or abolish alternans entirely. The window of rates at which alternans was observed varied from preparation to preparation (range, 600 to 1800 milliseconds).
Selective Depression of Epicardium by Ischemia

Fig 9. Typical patterns of electrical alternans obtained during simulated ischemia. In each panel, the upper and middle traces are microelectrode recordings from endocardium (Endo) and epicardium (Epi), respectively, and the lower trace is the simulated ECG obtained by differential recording of the endocardial and epicardial voltages. A, A 2:1 pattern of alternans obtained at a basic cycle length (BCL) of 800 milliseconds. The action potentials in epicardium show an alternating sequence of long and short durations caused by the alternate presence and absence of the dome. Action potential duration is relatively constant in endocardium, so that the changes in the T wave are due entirely to the changes occurring in epicardium. B, A 3:1 pattern of alternans obtained during ischemia in a different set of epicardial and endocardial preparations at a BCL of 800 milliseconds. In this example, an action potential with a dome in epicardium is followed by two action potentials where the dome is suppressed. Changes in both the amplitude and polarity of the T wave were obtained in the ECG under these conditions. Action potential duration remained constant in endocardium during the period over which the record was taken.

cell was abolished within 15 minutes, resulting in a marked abbreviation of APD. Ischemia-induced abolition of the dome occurred more rapidly in epicardial myocytes (10 to 15 minutes) than in the syncytial tissue preparations (15 to 60 minutes). Endocardial myocytes exhibited only a slight shortening of APD and depolarization during exposure to ischemia, paralleling the changes observed in syncytial preparations.

Addition of 4-AP (1 mmol/L) to the ischemic solution resulted in restoration of the dome in the epicardial cell within 1 minute (Fig 10C). The records shown in panel C were obtained after 5 minutes of exposure to ischemia plus 4-AP. As in the syncytial preparations, continued exposure to 4-AP caused a progressive decrease in the size of the notch, resulting in a progressive abbreviation of APD (time course of ≈5 to 8 minutes). In contrast, 4-AP prolonged APD in the endocardial cell.

Fig 10. Effects of ischemia or ischemia plus 4-aminopyridine (4-AP) (1 mmol/L) on action potentials recorded in endocardial and epicardial myocytes. A, Control responses elicited at a basic cycle length of 800 milliseconds. The stimulus artifacts have been deleted for clarity. B, Responses recorded after 15 minutes of superfusion with ischemic solution. "Ischemia" caused a marked abbreviation of action potential duration at 90% repolarization (APD90) in the epicardial cell and a modest shortening in the endocardial cell. C, Responses obtained 5 minutes after switching to an ischemic solution containing 4-AP (1 mmol/L). 4-AP reversed the ischemia-induced shortening of APD90 in the epicardial myocyte and also prolonged APD90 in the endocardial cell. These effects of 4-AP were similar to those obtained in syncytial tissue preparations from the corresponding ventricular layer.

Table 2 summarizes the effects of ischemia and of ischemia plus 4-AP on action potential parameters in seven epicardial and six endocardial cells. The responses obtained in dissociated myocytes were very similar to those recorded in syncytial tissue preparations from the corresponding ventricular layers.

Discussion

The present study demonstrates that tissues as well as dissociated myocytes isolated from canine ventricular epicardium are more sensitive to electrical depression by ischemic conditions than are tissues and myocytes obtained from ventricular endocardium. Simulated ischemia was found to produce electrophysiological changes in multicellular preparations nearly identical to those in single cardiac myocytes enzymatically dissociated from corresponding layers of the ventricular wall. These results suggest that the differential responsiveness of epicardium and endocardium to ischemia is in large part a result of intrinsic electrophysiological differences between the two cell types, since extracellular ionic influences and electronic interactions can be discounted in the experiments involving single myocytes.

Electrophysiological Differences Between Canine Epicardium and Endocardium

Several electrophysiological distinctions between the epicardial and endocardial layers of the canine ventricle have been described. Epicardial action potentials recorded in vitro and monophasic action potentials recorded in situ display a characteristic spike-and-dome morphology that is absent in recordings from endocardium. Evidence from microelectrode studies on isolated tissues and voltage clamp studies on disso-
TABLE 2. Effects of Simulated Ischemia and 4-Aminopyridine on Action Potential Parameters in Canine Ventricular Myocytes Isolated from the Epicardial or Endocardial Layers

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>n</th>
<th>Intervention</th>
<th>Phase 0 Amp, mV</th>
<th>Phase 1 Amp, mV</th>
<th>Notch, mV</th>
<th>Phase 2 Amp, mV</th>
<th>APD_{90}, ms</th>
<th>MDP, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epicardial</td>
<td>7</td>
<td>Control</td>
<td>114.9±6.5</td>
<td>69.2±5.0</td>
<td>45.5±7.1</td>
<td>100.4±4.1</td>
<td>225.5±32.0</td>
<td>-84.8±3.1</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>&quot;Ischemia&quot;</td>
<td>97.9±9.2*</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>59.2±13.4*</td>
<td>-69.0±2.6*</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>&quot;I&quot;+4-AP</td>
<td>109.6±5.7</td>
<td>87.0±3.2*</td>
<td>18.2±8.2*</td>
<td>87.9±2.9*</td>
<td>138.3±22.1*†</td>
<td>-70.5±1.8*</td>
</tr>
<tr>
<td>Endocardial</td>
<td>6</td>
<td>Control</td>
<td>125.1±5.9</td>
<td>109.3±4.3</td>
<td>8.4±4.5</td>
<td>107.5±5.5</td>
<td>244.0±41.0</td>
<td>-86.6±1.6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>&quot;Ischemia&quot;</td>
<td>112.3±7.8*</td>
<td>97.5±5.9*</td>
<td>5.5±4.0</td>
<td>94.9±5.8*</td>
<td>222.7±35.5</td>
<td>-72.8±2.6*</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>&quot;I&quot;+4-AP</td>
<td>111.9±7.6*</td>
<td>96.0±3.3</td>
<td>3.8±3.4</td>
<td>94.7±3.5*</td>
<td>237.4±30.3</td>
<td>-72.2±2.5*</td>
</tr>
</tbody>
</table>

Amp indicates amplitude; Notch, phase 2 amplitude minus phase 1 amplitude; APD_{90}, action potential duration at 90% repolarization; MDP, maximum diastolic potential; "I", "ischemia"; and 4-AP, 4-aminopyridine (1 to 2 mmol/L, 15 minutes of exposure). All values are mean±SD obtained at a basic cycle length of 800 milliseconds.

*P<.05 compared with same cell control; †P<.05 compared with same cell "ischemia" value.

ischemia have suggested that the spike- and-dome configuration is caused by the presence of an I_{Ca} in canine epicardium and that differences in the early phases of the action potential are in large part attributable to the prominence of this current in epicardium but not endocardium.19,22 The 4-AP sensitive transient outward current (I_{Na}) was shown to be four to five times larger in epicardial myocytes than in endocardial cells.22 In contrast, the inward rectifier, I_{K1}, was comparable in magnitude in the two cell types.22 Similar regional differences in the contribution of I_{Na} to epicardial versus endocardial action potentials have also been described in the cat23 and rabbit ventricle.24

Effects of Ischemic Conditions on Transmembrane Currents

Components of ischemia have been shown to influence one or more ionic currents when tested in vitro. The combination of hypoxia, acidosis, and zero glucose was found to decrease the inward Na+ current (I_{Na}) in canine ventricular myocardium as measured by changes in phase 0 amplitude and the maximal rate of rise of phase 0 (V_{max}).31,32 It is still unknown whether the decrease in I_{Na} during ischemia is through a direct effect on the Na+ channel or an indirect effect of the ischemia-induced membrane depolarization to decrease I_{Na} through voltage-dependent inactivation of Na+ channels. In the present study, "ischemia" caused 22±9% and 15±3% decreases in phase 0 amplitude in epicardium and endocardium, respectively (see Table 1). A decrease in I_{Na} and secondary changes in excitability may account for the slight postrepolarization refractoriness seen in both tissues during "ischemia." The mild ischemic conditions used in this and corollary studies (in which multiple impalements were used) did not produce pronounced slowing of conduction in epicardium or endocardium.

Another current known to be affected by conditions of ischemia is the inward calcium current, I_{Ca}. Acidosis directly inhibits I_{Ca} in many cardiac tissues.30-35 Kimura et al.36 reported that metabolic inhibition, a major component of ischemia, caused a greater reduction of peak I_{Ca} in epicardial versus endocardial cells from the feline ventricle (37% versus 21%). Under control conditions, however, they found no difference in I_{Ca} between the two cell types. No data are currently available for the characteristics of I_{Ca} in canine epicardial versus endocardial cells or how I_{Ca} is altered by conditions of ischemia in each cell type. However, the feline data suggest that the contribution of I_{Ca} to the action potential plateau may be quite different in epicardium versus endocardium under ischemic conditions. The rise in intracellular Ca^2+ activity ([Ca^{2+}]) that typically occurs during ischemia37-39 may reduce I_{Ca} even further.

The effects of ischemia on outward repolarizing currents in ventricular tissues are not well established. Increased K+ permeability has been reported during hypoxia in voltage-clamped feline ventricular muscle.40 Also, a Ca^2+-activated transient outward K+ current (I_{SO}) that is insensitive to 4-AP has been reported in canine myocytes.27,28 However, recent evidence suggests that I_{SO} may be a Ca^2+-activated chloride current41 rather than a Ca^2+-activated K+ current, but this point remains to be settled. Moreover, the extent to which an increase in [Ca^{2+}]) during ischemia37-39 alters these outward currents is still not fully appreciated, although some increase in the Ca^2+-activated currents would be expected.

Role of I_{Na} in the Selective Depression of Epicardium During 'Ischemia'

As discussed above, ischemic conditions, through direct or indirect interaction with ionic channels, can cause a decrease in inward currents (I_{Na}, I_{Ca}) but an increase in outward repolarizing currents, especially those that contribute to the early phases of the action potential. These changes, coupled with the marked preexisting differences in I_{Na} in epicardium versus endocardium, may provide a fundamental basis for understanding the differential sensitivities of the two tissues to the depressant effects of ischemia.

Our data suggest that the opposite effects of ischemia on inward versus outward currents (ie, inhibition versus accentuation) result in a progressively greater imbalance between the opposing inward and outward currents flowing at the end of phase 1 in epicardium. This imbalance would explain why the notch is initially accentuated and the dome subsequently abolished as ischemia progresses. In the early phases of ischemia, phase 1 of the epicardial action potential begins at less negative potentials and proceeds to more negative potentials at which the availability of I_{Ca} is diminished. As a consequence, the second upstroke giving rise to phase 2 (plateau) of the action potential and the start of phase
3 are delayed, resulting in a transient prolongation of APD (Fig 2, 15 to 25 minutes of ischemia). With longer exposure to ischemia, a further shift in the balance of currents (caused by greater inhibition of I_{Ca} and activation of I_{Ca(Ca)}) would lead to the termination of phase 1 at still more negative potentials. Eventually, the greater net outward current flow during phase 1 would overwhelm I_{Ca} and any slowly inactivating (or window) sodium current, thus causing an all-or-none repolarization. The failure of the inward currents to overtake the outward currents (mainly I_{o} and I_{C}) as they normally do at the end of phase 1 would result in abolition of the dome and thus in a marked abbreviation of APD in epicardium.

The presence of a large and early repolarizing current such as I_{o} is essential to produce all-or-none repolarization. The relatively weak I_{C} in endocardium may explain why electrical activity in this tissue is less sensitive to ischemia, since a large imbalance of currents would not be generated. In support of this hypothesis, block of I_{o} by 4-AP readily reversed the ischemia-induced depression of action potentials in epicardium (see Figs 1, 2, and 9). It should be noted that 4-AP is not a selective blocker of I_{o}, but at the concentration used (1 mM/L), its actions to inhibit the inward rectifier (I_{K}) and delayed rectifier (I_{C}) currents are minimal if present at all. Moreover, the magnitude of I_{K} would be negligible at plateau membrane potentials due to the inward rectification properties of this current.

**Rate Dependence of Action Potential Configuration During Ischemia**

Acceleration of the stimulation rate or the introduction of premature beats early in diastole also reversed the ischemia-induced depression of the plateau in epicardium (Figs 4 through 7). The epicardial action potentials elicited at faster rates and after premature stimuli show a paradoxical prolongation caused by restoration of the dome. These results can be explained on the basis of the rate dependence of I_{o} and I_{C}, which are usually opposite. The availability of I_{o} is less at fast stimulation rates and after premature stimuli because full reactivation of I_{o} requires >1500 milliseconds in canine ventricular tissue15-22,28,42 under normal conditions. The process is slowed further in depolarized tissues, since I_{o} reactivation is voltage dependent.28 In contrast to I_{o}, I_{C} generally increases in amplitude with acceleration of the stimulation rate. Thus, the repolarizing influence of I_{o} (phase 1) grows larger at slow rates, whereas the repolarizing influence of I_{C} (phase 2) increases at fast rates. Under normal conditions, this results in accentuation of the epicardial notch at slow rates or attenuation of the notch at fast rates. If ischemia inhibits I_{C} but not I_{o}, as our data suggest, the balance between I_{C} and I_{o} would shift overwhelmingly in favor of I_{o} at all but the fastest stimulation rates. This overwhelming repolarizing influence would prevent the development of a plateau in the epicardial response during "ischemia" (ie, all-or-none repolarization). Only at fast stimulation rates would I_{C} be able to overtake I_{o} and thus generate a plateau. Consequently, a decrease in the availability of I_{o}, whether through acceleration of the stimulation rate or addition of an inhibitor such as 4-AP, is effective in reversing the ischemia-induced depression of action potentials in epicardium.

Direct evidence for the effects of I_{Ca} inhibition on action potential configuration is provided by studies of Ca2+ channel blockade. Exposure to Ca2+ channel blockers or Ca2+-free solutions results in abolition of the plateau and abbreviation of the action potential in canine epicardium43-45 but only a slight abbreviation of the action potential in endocardium (A. Lukas and C. Antzelevitch, unpublished observation). The effects of Ca2+ channel blockade (under nonischemic conditions) were very similar to those of ischemia in the present study but without the membrane depolarization. These findings provide support for the hypothesis that small or moderate changes in I_{Ca} can cause marked changes in action potential configuration in canine epicardium but relatively minor changes in endocardium.

**Role of the ATP-Regulated K+ Current in the Responses to 'Ischemia'**

Another outward current that may play a role in the ischemia-induced loss of the plateau and marked abbreviation of action potentials in epicardium is the ATP-regulated potassium current, I_{KATP}.46 Activation of this time-independent outward current by reduced [ATP] levels would provide an additional repolarizing influence. We tested this hypothesis in four experiments but found that glybenclamide (20 to 50 μmol/L), an inhibitor of I_{KATP}, was unable to restore the plateau in epicardium under ischemic conditions (A. Lukas and C. Antzelevitch, unpublished observation). Possibly, the severity of "ischemia" was too mild in our study to cause significant activation of I_{KATP}.

**Differential Sensitivities of Canine Epicardium and Endocardium to Ischemia**

Our results point to the difference in I_{o} between canine ventricular epicardium and endocardium as a major factor responsible for the differential responsiveness to ischemia but do not rule out the participation of other factors. The briefier action potentials observed in epicardium after 4-AP reversal of ischemia-induced depression (Figs 1, 2, 7, and 9 and Table 2) suggest that factors other than I_{o} also sensitize the epicardium to ischemia. The persistence of postrepolarization refractoriness in the presence of 4-AP during ischemia supports this suggestion. Differences in activation of I_{KATP} or depression of I_{C} in epicardium versus endocardium as described by Furukawa et al47 and Kimura et al46 in feline ventricular cells may constitute two additional factors.

**Physiological Implications**

The greater sensitivity of ventricular epicardium to ischemia has several important consequences. Chief among these is the development of a marked dispersion of repolarization and refractoriness between epicardium and endocardium in the ischemic zone as well as between normal and ischemic myocardium. This heterogeneity provides the electrophysiological substrate for the initiation of reentrant arrhythmias, as we have demonstrated in a corollary study.48 Reentry induced under these conditions is readily abolished after introduction of inhibitors of I_{o}, consistent with the effects of these agents to diminish ischemia-induced electrical heterogeneity (see Figs 1, 2, 7, and 9). It is noteworthy that some traditional antiarrhythmics have I_{o} blocking...
actions. Imaizumi and Giles\(^{49}\) suggested that the inhibition of \(I_\text{s}\) by quinidine may be concurrent with depression of \(I_{\text{Na}}\) in the arrhythmic actions of the drug.

The biphasic restitution of action potential characteristics in epicardium suggests that supernormal conduction may be possible in epicardium under ischemic conditions. Supernormal conduction or excitability has been demonstrated in vivo with extracellular stimulating electrodes usually applied to the ventricular epicardium.\(^{50,51}\) A supernormal phase of conduction has also been reported in epicardial strips mounted in a three-chamber bath in which a central inexcitable zone was created by superfusion with an “ion-free” isotonic sucrose solution.\(^{20,21}\) Under these conditions, premature beats elicited early in diastole conducted successfully across the inexcitable zone, whereas the basic beats failed to conduct. Supernormal conduction in epicardium would be expected to be more accentuated during ischemia, since the biphasic restitution of action potential characteristics is much more pronounced once depression of the dome occurs in epicardium (Fig 6). As a result, early premature beats (in which the dome is restored) would have a higher margin of safety for conduction in ischemic epicardium than would basic beats (in which the dome is abolished) because the greater amplitude and much longer duration of premature beats would generate more local circuit current for activation of adjacent tissues.

Finally, our data suggest that loss of the action potential dome in epicardium may contribute to ischemia-induced ST segment elevation as well as T-wave alternans.\(^{52-55}\)

**Limitations of the Study**

A major criticism of in vitro models of ischemia has been that they cannot mimic many of the components of true ischemia (buildup of metabolites, \(K^+\) accumulation, low \(O_2\) tension, transmural gradients, etc.). The advantage of the in vitro models is that transmembrane electrical activity and changes in ionic currents are more easily recorded during “ischemia.” Also, the modulating effects of extracellular and electrotonic influences on the ischemic responses in tissues can be precluded in studies of single cardiac cells.

Before selecting the “ischemic” solution used in the present study, we first tested many different test solutions in which we varied [\(K^+\)], added lactate, included cyanide, etc. Also, individual components of ischemia (hypoxia, high [\(K^+\)], acidosis) were tested alone and in combination. Severe “ischemic” solutions caused abolition of the dome and eventually inexcitability in epicardium with a time course similar to that reported in vivo (several minutes). However, the onset of depression was too rapid to allow testing of stimulation protocols or drugs. Therefore, we selected an “ischemic” solution with a composition that produced depression in epicardium with a time course slow enough to provide us with a window to test drugs or interventions and thereby help elucidate the mechanism underlying the differential sensitivity to “ischemia.” Thus, the results presented in this study apply to relatively mild ischemic insults. To what extent these data apply to more severe “ischemic” conditions remains to be established.

**Acknowledgments**

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