Effects of Pinacidil on Electrophysiological Properties of Epicardial Border Zone of Healing Canine Infarcts
Possible Effects of $K_{ATP}$ Channel Activation

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Background—$K_{ATP}$ channels, activated by ischemia, participate in the arrhythmogenic response to acute coronary occlusion. The function of these channels in border zones of healing infarcts, where arrhythmias also arise, has not been investigated. Do these channels remain maximally activated during infarct healing, or do they downregulate after a period of time? Both might preclude further activation.

Methods and Results—Myocardial infarction was produced in dogs by ligation of the left anterior descending coronary artery. Impulse propagation in the epicardial border zone (EBZ) of 4-day-old healing infarcts was mapped during administration of pinacidil, a $K_{ATP}$ channel activator, directly into the EBZ coronary blood supply. Pinacidil restored conduction and excitability when the EBZ was initially inexcitable and had large regions of block (6 of 8 experiments). This allowed reentrant circuits to form in the EBZ, causing tachycardia (4 of 8 experiments). In hearts with an initially excitable EBZ, pinacidil shortened the effective refractory period and abolished conduction block at short cycle lengths (7 experiments). This effect prevented initiation of reentry (1 of 2 experiments).

Conclusions—The response to pinacidil indicates that $K_{ATP}$ channels in the EBZ remain functional and can be activated to influence electrophysiological properties and arrhythmogenesis. (Circulation. 2002;105:2309-2317.)

Key Words: arrhythmia • ischemia • infarction

The $K_{ATP}$ channel is activated by a reduction in ATP that occurs during acute ischemia and has been implicated in early ischemic changes in electrophysiology, contractility, and rhythm and result from an acute coronary artery occlusion. Occlusion of a coronary artery eventually results in infarction, but some ischemic cells may survive to form infarct border zones. The epicardial border zone (EBZ) is often the site of reentry that causes tachycardia in the healing phase of infarction (4 to 5 days after coronary occlusion). Time-dependent remodeling of membrane channels involved in depolarization ($I_{Na}$) and repolarization ($I_{to}$, $I_{CaL}$) resulting from the prolonged ischemic period causes alterations in EBZ myocyte action potentials. There is no information, however, on $K_{ATP}$ channel function. Because metabolic abnormalities may persist in border zone myocytes long after the onset of acute ischemia, do these channels remain maximally activated or do they lose activity (downregulation) after a period of time? If they downregulate, are they capable of being reactivated? Our hypothesis is that activation of $K_{ATP}$ channels in border zones of healing infarcts can still have significant influences on electrophysiological properties and arrhythmogenesis. To test this hypothesis, we investigated the effects of pinacidil, a specific $K_{ATP}$ channel opener, on electrophysiological properties of the EBZ.

Methods

Myocardial infarction was produced in 18 mongrel dogs by occlusion of the proximal left anterior descending coronary artery (LAD). Four days after occlusion, the dogs were anesthetized with pentobarbital sodium (20 to 25 mg/kg IV). Two ECG leads and arterial blood pressure were monitored. A 292-bipolar-electrode array was used to map the EBZ. Bipolar stimulating electrodes were positioned around the center recording electrodes in the mapping array (center stimulation) and at the basal and lateral margins of the array (see Figure 1 in Reference 11). Stimulating electrodes were also located on the right ventricle adjacent to the LAD. For induction of ventricular tachycardia, programmed stimulation with single, double, or triple premature stimuli was used from each of the 4 stimulation sites.

The effective refractory period (ERP) of noninfarcted epicardial muscle was measured at the LAD stimulating electrodes and in the infarcted region at the center of the EBZ. Conduction velocities and anisotropic ratio in the EBZ were calculated from activation maps generated during center stimulation.

We administered drugs directly into the EBZ through a catheter placed into the LAD just distal to the occlusion to eliminate systemic vascular effects that provoke autonomic reflexes and to minimize drug effects on normal myocardium. Retrograde flow through
Effects of Pinacidil on the EBZ
Experiments were divided into 2 groups. In 8 dogs (group 1), the ventricles could not be stimulated through the electrodes in the center of the EBZ at the maximum stimulus strengths (10 ma and 10 ms duration) (inexcitable EBZ). In 10 dogs (group 2), the EBZ was excitable and was activated during stimulation from the central electrodes.

Effects of Pinacidil on Group 1 (Inexcitable EBZ)

Conduction
Figures 2A, 3A, and 4 show controls. Figure 2A shows recordings from the EBZ around the central stimulus site (bottom 4 traces) and between the central stimulus site and the LAD margin (next 4 traces) (diagram of electrode array in Reference 11) during stimulation from the center of the electrode array (cycle length 350 ms) in one experiment. Propagated wavefronts could not be initiated by central stimulation, but stimulus artifacts occur in the bottom 4 traces. Electrogroms reflecting activation of the outer margins of the EBZ by the sinus rhythm occur in the next 4 traces. In these hearts, activation of the EBZ during sinus rhythm and stimulation from electrodes outside the EBZ often showed a high degree of block. In Figure 3A (from a different experiment), the first 2 impulses at the left (bottom trace) are the last in a train stimulated at the LAD electrodes (cycle length 300 ms) before a premature impulse (coupling interval 180 ms). The stimulated impulse at the left activates sites at the LAD margin of the electrode array (bottom 5 traces, arrow) but does not propagate to the center of the EBZ (top 3 traces, block at horizontal line). The next (last) basic stimulated impulse before the premature impulse does activate the EBZ. The map in Figure 4 (S1) shows that activation of this stimulated impulse propagates from the LAD margin (asterisk) toward the center of the EBZ, where the wavefront blocks (isochrone 80; thick black line; also horizontal line after second impulse in Figure 3A). Propagation around the line of block (dashed line) activates its distal side in an antidromic direction at 130 and 170 ms (see reversal of activation sequence [arrow] in Figure 3A). The premature impulse also activates only the margin of the EBZ and not the center (Figure 3A and Figure 4 [S2]).

Intracoronary pinacidil improved propagation. Figure 2B shows activation at all recording sites after pinacidil (arrows) during central stimulation at the 350-ms cycle length (compare with Figure 2A). Propagation occurred from the center, near the stimulation electrodes (electrode 189), to the margins of the electrode array. Figure 3B shows that pinacidil restored propagation of basic and premature impulses to sites that were not activated before drug administration (electrogroms at all sites; compare with Figure 3A) during LAD stimulation. In the map in Figure 5 (S1), activation was initiated at the LAD (asterisk) after pinacidil and propagated through regions in which it blocked before pinacidil (compare with Figure 4 [S1]). The entire EBZ is activated within 80 ms (Figure 5 [S1]), compared with 180 ms in control (Figure 4 [S1]). Conduction of premature impulses was also restored (Figure 5 [S2]; detailed description below). Similarly, conduction was restored in the other experiments in this group.

Results

Local Effects of Intracoronary Pinacidil
At doses of pinacidil that exerted electrophysiological effects in the EBZ (see below), no significant effects on systemic blood pressure (control 124±32/76±25 mm Hg versus pinacidil 123±35/75±26 mm Hg, P=0.72), sinus cycle length (control 329±28 ms versus pinacidil 330±28 ms, P=0.72), and ERP of the noninfarcted right ventricle (control 136±18 ms versus pinacidil 133±12 ms, n=6, P=0.54) were observed.

Effects of Pinacidil on Group 1 (Inexcitable EBZ)
The effects of the acidified alcohol diluent alone were investigated as a control in the same volumes injected with pinacidil in 3 dogs in which there was no center pacing. The diluent did not restore activity in any experiment.

**Induction of Ventricular Tachycardia**

Ventricular tachycardia often arises from reentrant circuits in the EBZ after premature activation. In group 1, there were extensive regions of inexcitability that prevented the formation of circuits (Figures 3A and 4 [S2]). Neither sustained nor unsustained tachycardia was induced during control in any experiment. After pinacidil, excitability and conduction were restored to the EBZ in 6 of 8 experiments (Figures 2B, 3B, and 5). Sustained tachycardia was then induced in 4 experiments (Figure 6, A and B). Figures 3 and 5 show the mechanism for the induction of tachycardia. During basic drive, the EBZ was activated without block (Figure 5 [S1]). The premature impulse (Figure 5 [S2]) blocked along a horseshoe shaped line (black line) after 70 to 90 ms; there is no longer a large area of unexcited myocardium, as in Figure 4 [S2]. Block of the premature impulse is also shown in Figure 3B (short arrow and horizontal line). Activation occurred around both ends of this line of block (dashed line), with the distal side activated between 170 and 190 ms. The retrogradely conducting wavefront propagated back across this region of block (Figure 5 [T1], isochrones 10 to 60) to cause the first impulse of the tachycardia (also see retrograde activation in Figure 3B, long black arrow from top to bottom). Activation continued with this wavefront dividing into 2, 1 moving toward the basal margin at the right and 1 moving toward the apical margin at the left. This figure-8 pattern of activation continued during the tachycardia (Figure 5 [T2]).

In 2 dogs in which tachycardia was not induced, excitability and conduction in the EBZ were not restored by pinacidil. In 2 other dogs, despite restoration of excitability and conduction, tachycardia still could not be induced. In 3 experiments, the diluent alone did not result in arrhythmia induction because excitability was not restored to the EBZ.

**Effects of Pinacidil on Group 2 (Excitable EBZ)**

**Refractoriness and Conduction**

In 10 dogs, the ventricles could be paced from the stimulating electrodes in the center of the recording array at the lowest stimulus strength (2 ma, 2 ms duration). At longer stimulus cycle lengths, there were no areas of conduction block. Isochrones were elliptical, with the long axis of the ellipse parallel to the long axis of the myocardial fiber bundles (identical to the activation pattern in Figure 7, D and F). Conduction velocities in the longitudinal and transverse directions were 60±20 and 23±8 cm/sec, respectively.
effects of pinacidil on conduction velocity in either direction (63±15 and 22±10 cm/sec; \(P>0.5\)) were observed. As the stimulus cycle length was decreased during central pacing, failure of 1:1 conduction resulted from failure to capture the central region. Figure 7, A through C, shows electrograms and maps during stimulation of the EBZ in one experiment at the longest cycle length that failed to capture the ventricles 1:1 (170 ms). Every other stimulus initiated a propagated

**Figure 3.** A, Selected recordings from EBZ during programmed stimulation in control. Bottom 5 traces (62, 64, 65, 66, 67) are near margin with noninfarcted myocardium; top 3 (68, 69, 70) are from near center of EBZ. For location see Figure 4. Arrows indicate direction of activation; horizontal black lines indicate block. B, Electrograms recorded from same sites after administration of pinacidil. Time marks, 100 ms.

**Figure 4.** Maps during last basic drive (left, S1) and premature stimulus (right, S2) in control in same experiment as Figure 3. Electrode array is represented with margin adjacent to LAD above and apex at left. Small numbers show activation times at each recording site. Isochrones, drawn at 10-ms intervals, are labeled with larger number. Thick black lines indicate conduction block; arrows point out direction of wave-front propagation. Bar at lower right is 14 mm. Circled activation times indicate recording sites from Figure 3. This same format is used for all mapping figures. Large asterisk indicates LAD margin.
wavefront (Figure 7B, arrows). The activation patterns during this period of stimulation are shown in panels A and C. In A, the wavefront originates at the center (asterisk). Activation spreads toward the left side of the map (arrows) and reaches the apical margin (below) after 50 to 60 ms. The stimulated wavefront does not spread from the site of stimulation toward the right margin. Activation of the right half of the mapped region begins from the margins (above and at right), and these wavefronts move toward the center. The next stimulated impulse did not excite the center of the EBZ, and the EBZ was not activated during this time period. The third stimulated impulse (Figure 7C) excited the center (asterisk). These wavefronts collided with other wavefronts, possibly of sinus origin, moving from the margins toward the center (arrows).

Administration of pinacidil decreased the cycle length at which the EBZ could be captured by stimuli from the central electrodes from $185 \pm 47$ to $158 \pm 50$ ms ($P<0.01, n=5$). In the experiment illustrated in Figure 7, the electrograms in panel E show that after pinacidil, the EBZ responded 1:1 at a stimulus cycle length of 170 ms. Activation maps are shown for 2 of the stimulated impulses in D and F. For each stimulated impulse, activity originates at the central stimulation site (asterisk) and spreads toward the margins.

Improvement in the response of the EBZ to rapid stimulation is related to shortening of the ERP by pinacidil. For the entire series, ERP at the center of the EBZ decreased from $172\pm49$ to $141\pm21$ ms ($P=0.12$) at a stimulus strength of 2 times diastolic threshold.

In 4 experiments, no effects on conduction velocity or refractoriness in the EBZ were observed after administration into the LAD of the diluent alone in the maximum amount. In the longitudinal direction, conduction velocity was $70\pm4$ cm/sec in control and $75\pm9$ cm/sec after the diluent ($P=0.47$). In the transverse direction, conduction velocity was $39\pm9$ cm/sec in control and $38\pm14$ cm/sec after the diluent ($P=0.89$). ERP at the center of the EBZ was $191\pm44$ ms in control and $192\pm54$ ms after the diluent ($P=0.89$).

**Induction of Ventricular Tachycardia**

Sustained ventricular tachycardia was induced in 2 dogs and nonsustained tachycardia or ventricular fibrillation in another 4. Pinacidil prevented the induction of sustained tachycardia in 1 of the 2 experiments (Figure 6, C and D). Nonsustained ventricular tachycardia or ventricular fibrillation could still be induced after pinacidil in the other experiments. Figure 8 shows maps of tachycardia induction before pinacidil. During the basic drive from the LAD electrodes (top left), activation spread across the EBZ. The premature impulse that initiated tachycardia (top right) blocked along the 80-ms isochrone (thick black line, small arrows). Activation around the line of block reached the distal side after 170 ms. The wavefront then propagated retrogradely through the blocked region, back

![Figure 5](http://circ.ahajournals.org/)

**Figure 5.** Top left, Activation pattern of basic drive (S1); top right, activation pattern of stimulated premature impulse (S2); bottom left, first reentrant impulse (T1); bottom right, second reentrant impulse (T2) during tachycardia in Figure 6B after pinacidil. Circled activation times indicate recording sites from Figure 3B. Bar at lower right is 10 mm. Asterisk indicates LAD.
toward the LAD margin (lower left; isochrones 0 to 70), as the first reentrant impulse. This reentrant wavefront then divided into 2, 1 moving to the left toward the apical margin and 1 to the right toward the basal margin (isochrones 80 to 120). Two parallel and functional lines of block formed that resulted in a figure-8 reentrant circuit during the sustained tachycardia (lower right). After pinacidil, the premature impulses at all coupling intervals no longer blocked but rather propagated directly to the lateral margin (Figure 9 [S2]). In the experiment in which tachycardias could still be initiated after pinacidil, conduction block of premature impulses was not prevented by pinacidil, although the refractory period of the EBZ was decreased.

Discussion

Role of $K_{ATP}$ Channels in Ischemic Arrhythmias

$K_{ATP}$ channels are activated when ATP levels fall during acute ischemia. The increase in conductance that results causes efflux of potassium into the extracellular space and shortening of repolarization. It was originally hypothesized that the shortening of repolarization acts as a protective mechanism by limiting calcium entry, although more recent studies ascribe the protective role to mitochondrial ATP channels. Activation of the $K_{ATP}$ channels during acute ischemia is arrhythmogenic; the extracellular potassium accumulation and shortening of the refractory period favor the occurrence of reentrant excitation. Pharmacological agents that increase $K_{ATP}$ channel conductance have effects...
similar to those of ischemia on action potentials and are also arrhythmogenic.14,16,20,21

The focus on the arrhythmogenic role of K<sub>ATP</sub> channel activation has been during acute ischemia.4,14,17 During this period, epicardial muscle that eventually forms the EBZ undergoes more severe electrophysiological alterations than endocardial muscle,22,23 partly related to greater activation of the K<sub>ATP</sub> channels.24 During subsequent days, muscle cells in the EBZ undergo changes in ion channels that control repolarization, resting membrane potential, and depolarization and are related to changes in ion channel protein synthesis.8 The function of K<sub>ATP</sub> channels in such longer-term ischemia has not been explored. These channels might be persistently activated if ATP levels remain reduced or might be downregulated and lose function. Therefore, we determined whether we could activate K<sub>ATP</sub> channels pharmacologically and, if so, what the effects of activation on electrophysiological and arrhythmogenic properties of the EBZ would be.

Administration of Drugs into the EBZ
As a prelude to this study, we developed a new method to investigate the effects of pharmacological agents on EBZ: administration of drugs directly into the border zone through its local circulation to avoid systemic effects and effects on other regions that might confound the interpretation of the experiments. When a catheter is inserted into the LAD distal to the coronary occlusion, there is significant retrograde blood flow through it. This retrograde blood flow may be involved in maintaining survival of the EBZ. Blood flow may originate from collaterals from the proximal LAD or from the circumflex coronary artery. Blood flow varies significantly among hearts, and therefore, dilution of an injected drug also varies.

Effects of K<sub>ATP</sub> Channel Activation on EBZ
The results of our experiments answer the question we posed: K<sub>ATP</sub> channels are still functional in the healing infarct border zone, and activation can alter the electrophysiology of this region. The channels are not maximally activated or downregulated, which might prevent further activation.

As in our previous studies, the EBZ could be classified into 2 groups with different electrophysiological properties.25 In group 1, the EBZ was inexcitable to pacing stimuli applied directly to it and had large regions of block during pacing from outside the EBZ, even at long cycle lengths. Ventricular tachycardia cannot be induced because excitable border zone myocardium is necessary for the occurrence of reentrant
circuits. After pinacidil, pacing stimuli excited the border zone myocardium, and block of propagating wavefronts was reduced. Increased outward current caused by activation of KATP channels may have caused hyperpolarization to restore excitability and conduction. Once activity was restored, the induction of reentry causing ventricular tachycardia was possible, further affirming the role of this region in arrhythmogenesis. A similar mechanism of arrhythmogenesis may be involved when an acute ischemic event is superimposed on a healing infarct, a situation that is particularly prone to the occurrence of ventricular tachycardia and fibrillation. Administration of pinacidil was not arrhythmogenic in hearts in which the EBZ was excitable before administration of pinacidil. The muscle in the EBZ of group 2 did not appear to have the depressed membrane potentials characteristic of group 1 and, therefore, may have been less affected by the acute ischemic insult caused by the LAD occlusion. Pinacidil had no effect on conduction at long cycle lengths, whereas it shortened the ERP, effects similar to those described for potassium channel openers on normal myocardium. In 1 of 2 experiments, the initiation of reentry was prevented by pinacidil. Initiation is dependent on conduction block of premature impulses in regions of long refractoriness; therefore, shortening of refractoriness by pinacidil prevented the occurrence of the block in this experiment. In the other experiments, premature impulses still blocked after pinacidil, although the lines of block were shortened.

One limitation of the interpretation of the results of this study is that pinacidil also has vasodilator effects. Administration of this drug into the LAD may have increased coronary flow to the EBZ. Saltman et al demonstrated a 15% increase in coronary blood flow by pinacidil in the isolated rabbit heart. It is possible that the local administration of pinacidil caused some increase in collateral blood flow into the EBZ and that this may have influenced the electrophysiological response. It is unlikely, however, that a small increase in collateral blood flow would have such a marked effect on electrophysiological properties as occurred in our experiments.

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