Effects of Ischemia on Discontinuous Action Potential Conduction in Hybrid Pairs of Ventricular Cells

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Background—Acute ischemia often occurs in cardiac tissue that has prior injury, resulting in spatially inhomogeneous distributions of membrane properties and intercellular coupling. Changes in action potential conduction with ischemia, which can be associated with release of catecholamines, may be particularly important in tissue that has discontinuous conduction resulting from prior infarction, hypertrophy, or myopathy.

Methods and Results—Isolated guinea pig ventricular myocytes were electrically coupled by a coupling-clamp circuit to a comprehensive computer model of a guinea pig ventricular myocyte to assess alterations in the critical value of coupling conductance required for action potential conduction from the real cell to the model cell when the real cell was exposed to a solution that included hypoxia, acidosis, and an elevated extracellular potassium concentration to simulate acute ischemia. The “ischemic” solution increased critical coupling conductance from 6.2±0.1 to 7.4±0.2 nS and decreased the associated maximum conduction delay from 31±1 to 23±1 ms (mean±SEM, n=11). The ischemic solution plus 1 µmol/L norepinephrine decreased critical coupling conductance from 5.9±0.2 to 5.0±0.1 nS and increased maximum conduction delay from 31±2 to 54±4 ms (mean±SEM, n=8).

Conclusions—The release of catecholamines with ischemia, in a setting of partially uncoupled cells, may play a major role in producing long conduction delays, which may allow reentrant pathways. (Circulation. 1999;99:1623-1629.)

Key Words: arrhythmia ■ catecholamines ■ conduction ■ ischemia

Many episodes of acute myocardial ischemia occur in tissue that is already abnormal, ie, that has spatially inhomogeneous distributions of membrane properties and intercellular coupling resulting from prior injury. Sudden cardiac death is commonly associated with a preexisting structural abnormality1 from prior infarction, hypertrophy, or myopathy that may serve as a substrate from which arrhythmias can arise. Conduction becomes slow and discontinuous, with an irregular (zigzag) spatial pattern.2 Surviving muscle fiber bundles are separated by connective tissue, and extracellular recordings show fractionated electrograms with discrete deflections.3 Action potentials in these regions have prepotentials and notches due to electrotonic interactions with nearby cells.4 During acute ischemia, excessive concentrations of catecholamines are observed within the unperfused myocardium.5 Depletion of catecholamines or treatment with β-blockers decreases the incidence of ischemia-induced arrhythmias.6

In normal myocardium, fast sodium current (I_{Na}) is responsible for excitability and conduction. Under conditions of discontinuous conduction, however, L-type calcium current (I_{Ca,L}) may play a major role in sustaining conduction.7-10 For a pair of electrically coupled ventricular myocytes, we have previously shown that block of I_{Ca,L} significantly increased conduction delay when cells were relatively uncoupled but had no effect on conduction when cells were well coupled.7 Our further study using pharmacological modulation of I_{Ca,L}8 suggested that the facilitating effects on conduction produced by β-agonist activity might counteract the inhibitory effects on conduction of acute ischemia.

Techniques have been developed11 to study isolated cardiac cells under conditions incorporating many of the known phenomena of myocardial ischemia, including hypoxia, elevated potassium, lowered pH, elevated lactate, and lack of glucose. Combining these techniques with our model-clamp technique,12 we have studied the effects of varying coupling conductance for a pair of electrically coupled cells in which the leader (stimulated) cell is a real isolated guinea pig ventricular cell (either in a normal Tyrode’s solution, an “ischemic” solution, or an ischemic solution with a β-agonist drug) and the follower cell is a model guinea pig ventricular cell represented by the Luo-Rudy model.13

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Cell Isolation

Single ventricular myocytes were prepared from adult guinea pigs weighing 200 to 800 g. After decapitation, the heart was excised and consecutively perfused with normal Tyrode’s solution (5 mL/min, 3 to 5 minutes), nominally Ca²⁺-free Tyrode’s solution (10 minutes), and nominally Ca²⁺-free enzyme solution (12 to 14 minutes). Both ventricles were cut into pieces, gently triturated in nominally Ca²⁺-free Tyrode’s solution to which 180 μmol/L Ca²⁺ was added, and stored at room temperature. Samples of the cell suspension were placed in the recording chamber, and cells were allowed to settle for 5 to 10 minutes, after which superfusion with normal Tyrode’s solution was started (1 mL/min, 35.0±0.5°C). Only single cells that were quiescent with preservation of their rod-shaped appearance were studied. Membrane capacitance, input resistance, and (2-Hz, 2-ms) stimulus current threshold were 163±15 pF, 15.2±1.4 MΩ, and 3.14±0.16 nA (mean±SEM, n=19), respectively.

Methods

Cell Chamber

Figure 1A and 1B illustrates the design of the cell chamber to produce the ischemic conditions for isolated cells. During ischemia, the cell chamber was layered with argon, preventing diffusion of atmospheric oxygen into the bath solution. Partial oxygen pressure was continuously monitored.

Electrophysiological Recording

Whole-cell patch-clamp recordings were made with custom-built dual amplifiers using relatively high resistance patch pipettes (4 to 6 MΩ when filled with pipette solution) to minimize intracellular dialysis. Series resistance was compensated up to 90%. Apart from zeroing the potential before touching the cell surface, no attempts were made to correct for liquid junction potential. In control experiments, action potential configuration and transmembrane currents remained stable for 40 to 60 minutes.

Solutions

Normal Tyrode’s solution contained (in mmol/L) NaCl 140, KCl 1.8, MgCl₂ 1.0, HEPES 10.0, glucose 5.5 (pH 7.4 with NaOH) and was saturated with 100% O₂. Nominally Ca²⁺-free Tyrode’s solution was the same, without the CaCl₂. Enzyme solution contained 0.06 U/mL collagenase-B, 0.19 U/mL collagenase-P, and 40 mg/mL trypsin inhibitor (Boehringer-Mannheim) in nominally Ca²⁺-free Tyrode’s solution. During the last 5 minutes of the enzymatic isolation, 0.28 U/mL protease-XIV (Sigma Chemical Co) was added. Pipette solution contained (in mmol/L) KCl 140 and HEPES 10 (pH 7.2 with KOH). The ischemia solution contained (in mmol/L) NaCl 120, KCl 8.0, CaCl₂ 1.8, MgCl₂ 1.0, PIPES 10.0, and sodium lactate 20 (pH 6.8 with NaOH) and was saturated with 100% N₂.

Coupling an Isolated Ventricular Cell to a Model Cell

Figure 1C diagrams our method of dynamically coupling a real cell to a real-time solution of a mathematical cell model with an ohmic coupling conductance. The computer-controlled system, for each 80-μs time step Δt, samples the membrane potential , injects this current into the real cell, and integrates the Luo-Rudy model with as an additional ionic membrane current. To minimize effects of cell size, we scaled such that the real cell and the model cell were of equal size, both exhibiting an effective current threshold of 2.6 nA.

Statistics

For each cell, we compared action potential parameters, critical coupling conductance, and maximum conduction delay for control versus ischemia using a paired t test with a significance level of P<0.05. Comparison of action potential parameters between groups was performed with a Student-Newman-Keuls test after ANOVA. Data are presented as mean±SEM.

Results

Experimental Procedure

Figure 2 illustrates our experimental procedure, with the Tyrode’s solution bathing the cell switched to the ischemic solution for 17 minutes (horizontal bar). During the initial control period, PO₂ was 215 Pa (158 mm Hg), action potential duration at 90% repolarization (APD₉₀) was 138 ms, and resting membrane potential (RMP) was −85 mV. During

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ischemia, $P_0$ rapidly falls to $<13.6$ Pa ($<10$ mm Hg) within 5 minutes. The $APD_{90}$ shows some fluctuations, with a small decrease to $\approx 125$ ms until late in the ischemic period, when a large, rapid drop to $\approx 60$ ms occurs, at which time we switched back to control conditions. The RMP changes very rapidly with the ischemia (because of the elevated potassium) and remains stable at $-76$ mV. On reestablishment of control conditions, $P_0$ and RMP rapidly return to their initial control levels, whereas $APD_{90}$ remains slightly shorter after recovering from the large drop at the end of the ischemic period.

We applied this procedure of an $\approx 20$-minute exposure to ischemic conditions to 11 cells. For times at 8 to 10 minutes into the ischemic period, these cells showed, in addition to the changes in RMP and $APD_{90}$, a decrease in $APD_{50}$, a decrease in maximum rate of rise of the action potential upstroke ($V_\text{max}$) (not significant, $P>0.05$), and a 4-mV reduction in overshoot potential (Table, left columns). The large, rapid drop in $APD_{90}$ at the end of the ischemic period was observed for 5 of the 11 cells and is most likely due to hypoxia-induced activation of ATP-sensitive potassium current.\cite{14}

**Conduction Under Ischemic Conditions**

Figure 3 shows results from coupling the real guinea pig ventricular cell of Figure 2 to the Luo-Rudy model cell during the initial control period. Figure 3A shows membrane potential and coupling current for the 6-second period of coupling with a $G_c$ of 6.6 nS as well as the preceding and following 2-second periods of uncoupling. The real cell is stimulated at 2 Hz, and no stimuli are applied to the model cell. After coupling has been established, some but not all action potentials of the real cell propagate to the model cell: action potentials 3, 6, and 11 fail to propagate, as indicated by their short duration and monophasic coupling current transient. At $G_c=6.7$ nS, however, every real cell action potential propagates to the model cell (Figure 3B), thus establishing 6.7 nS as the critical value of $G_c$ for successful, uniform propagation under control conditions. At $G_c<6.6$ nS, action potentials propagate less frequently or not at all; at $G_c>6.7$ nS, conduction was uniformly successful (not shown).

For the same cell, we also determined critical $G_c$ after 8 minutes of ischemia. At $G_c=7.8$ nS, action potentials 3 and 8 during coupling fail to propagate (Figure 4A), but at $G_c=7.9$ nS, there is uniform propagation (Figure 4B). Because of electrotonic interactions, the amount of depolarization of the real cell is slightly reduced during coupling. From Figures 3 and 4, we see that critical $G_c$ has been increased by the ischemic conditions from 6.7 to 7.9 nS.

Figure 5 contrasts propagation at critical $G_c$ under control versus ischemic conditions by expanding Figures 3B and 4B, respectively, to illustrate only the last action potential propagated (asterisks). Under control conditions (Figure 5A), there is a rapid upstroke, followed by a partial repolarization of the real cell as current is transferred from real cell to model cell. When the model cell activates after a 28-ms delay, it rapidly depolarizes, producing a second phase of depolarization of the real cell. Under ischemic conditions (Figure 5B), the real cell shows a depolarized RMP and a slightly decreased peak amplitude, and the conduction time is decreased to 24 ms. Because for both Figure 5A and 5B, the value of $G_c$ was the minimum value that allowed uniform conduction, these values of 28 and 24 ms represent the maximum conduction delay under control versus ischemic conditions, showing that the ischemia decreased the maximum conduction delay. Similar results were obtained for each of the 10 other cells studied under ischemic conditions.

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**Action Potential Parameters of Single Guinea Pig Ventricular Cells After 8 to 10 Minutes of Exposure to Simulated Ischemic Conditions**

<table>
<thead>
<tr>
<th></th>
<th>Control for Ischemia</th>
<th>Ischemia</th>
<th>Control for Ischemia + NA</th>
<th>Ischemia + NA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$APD_{50}$, ms</td>
<td>167±10</td>
<td>134±9*</td>
<td>166±11</td>
<td>174±11†</td>
</tr>
<tr>
<td>$APD_{90}$, ms</td>
<td>191±11</td>
<td>154±8*</td>
<td>188±10</td>
<td>190±10†</td>
</tr>
<tr>
<td>RMP, mV</td>
<td>-83.4±0.4</td>
<td>-74.2±0.7*</td>
<td>-84.7±0.7</td>
<td>-75.2±0.9*</td>
</tr>
<tr>
<td>$V_{\text{max}}$, V/s</td>
<td>300±32</td>
<td>251±35</td>
<td>347±31</td>
<td>299±33</td>
</tr>
<tr>
<td>OS, mV</td>
<td>40.3±0.6</td>
<td>36.5±1.0*</td>
<td>40.3±0.8</td>
<td>36.7±0.8*</td>
</tr>
</tbody>
</table>

OS indicates overshoot potential. Values are mean±SEM for control vs ischemic conditions (Ischemia, $n=11$) and control vs ischemic conditions including 1 μmol/L norepinephrine (Ischemia+NA, $n=8$).

$^*P<0.05$ vs control.

$^†P<0.05$ vs Ischemia.
Conduction Under Ischemic Conditions With Norepinephrine

For another 8 cells, we performed the same procedure of exposure to ischemic conditions but also including 1 μmol/L norepinephrine (ischemia + NA) in the ischemic solution. Like the 11 cells that were exposed to ischemic conditions without the norepinephrine, the cells showed a 9-mV depolarization of RMP, a decrease in $V_{\text{max}}$ (not significant, $P = 0.09$), and a 4-mV reduction in overshoot potential (Table, right columns). However, no changes in APD$_{50}$ or APD$_{90}$ were observed. Also, the large, rapid drop in APD$_{90}$ at the end of the ischemic period was observed for only 1 of these 8 cells.

Figure 6 illustrates, in the same format as Figure 5, results from 1 of the 8 cells exposed in the ischemia + NA solution. We determined that critical G$_c$ was 6.2 nS in the control solution and 5.2 nS in the ischemia + NA solution (not shown). The results under control conditions (Figure 6A) are very similar to those shown for a different cell in Figure 5A. In the ischemia + NA solution (Figure 6B), the partial repolarization during the conduction process is much slower and is maintained at a higher voltage level, allowing for a much longer maximum conduction delay (60 ms). Similar results were obtained for each of the 7 other cells studied in the ischemia + NA solution.

Statistical Comparison of Conduction in the Two Test Solutions

Effects on conduction of the application of ischemia or ischemia + NA are summarized in Figure 7. Note that each experimental intervention has its own control data. For the ischemia (n = 11), the critical coupling conductance increases from 6.2 ± 0.1 to 7.4 ± 0.2 nS and the associated maximum conduction delay decreases from 31 ± 1 to 23 ± 1 ms. For the ischemia + NA (n = 8), there is a decrease in critical coupling conductance from 5.2 nS to 6.2 nS.
conductance from 5.9 ± 0.2 to 5.0 ± 0.1 nS and an increase in maximum conduction delay from 31 ± 2 to 54 ± 4 ms.

Conduction at a Constant Coupling Conductance in the Two Test Solutions

Figure 8 emphasizes the differences in the discontinuous conduction process between control conditions, ischemia, and ischemia+NA at a constant coupling conductance of 8.0 nS. For the same real cell as used for Figures 2 through 5, we have plotted the results under control conditions (Figure 8A) versus ischemia (Figure 8C). Similarly, for the same real cell as used for Figure 6, we contrast propagation under control conditions (Figure 8B) and ischemia+NA (Figure 8D). Under control conditions (Figure 8A and 8B), coupling current flowing from each of the real cells to the model cell induces an early partial repolarization to −10 to −15 mV, and the conduction delay is 12 ms.

During ischemia (Figure 8C), both the peak amplitude of the real cell action potential and the potential after this peak amplitude are reduced. Because the driving force for coupling current is the difference in membrane potential, less coupling current is flowing from real cell to model cell during the process of propagation. The real cell continues to repolarize to −18 mV until, with a 22-ms delay, the model cell reaches its activation threshold and fires its action potential.

During ischemia+NA (Figure 8D), the potential after the peak of the real cell action potential is maintained at a higher level than in control solution, with a small repolarization during conduction to only +25 mV. As a consequence, coupling current is larger than under control conditions, and it takes less time (11 ms) to activate the follower cell. Thus, Figure 8 demonstrates that, for discontinuous conduction at constant Gc, impulse propagation is largely impaired in the ischemia solution but enhanced in the ischemia+NA solution.

Changes in Ionic Currents

To unmask changes in ionic currents underlying the changes in conduction properties shown in Figures 3 through 8, we tested for changes in I_{Na}, I_{Ca,L}, and the inward rectifier and delayed rectifier potassium currents (I_{K1} and I_{K}, respectively) as major contributors to the activation and plateau phase of the action potential.13,15 In accordance with data from the literature,15 the transient outward current (I_{to}) appeared to be absent in our cells (not shown). Under both ischemic conditions and ischemic conditions plus norepinephrine, only a slight decrease in V_{max}, reflecting activation of I_{Na}, was observed (Table).

For 4 of the 19 cells studied—2 cells exposed to the ischemia solution and 2 cells exposed to the ischemia+NA solution—we applied voltage clamp protocols under control conditions and after 10 to 15 minutes of ischemia, ie, directly after the determination of action potential parameters, critical coupling conductance, and maximum conduction delay at 8 to 10 minutes of ischemia. Resulting current-voltage relations are shown in Figure 9, which contrasts data from a cell exposed to the ischemia solution (Figure 9A and 9B) and a separate cell exposed to the ischemia+NA solution (Figure 9C and 9D).

The amplitude of the peak current, reflecting I_{Ca,L}, decreased in the ischemic solution (Figure 9A) but showed an ∼4-fold increase in the ischemia+NA solution (Figure 9C). Under ischemic conditions, the quasi–steady-state current,
I effects of increased extracellular potassium concentration on crossover in the negative voltage range, as expected from the model of 2 cells can be scaled to represent the conduction group conductance is limiting the conduction. Our use of a successive activation of groups of cells for which the inter-

istic of conduction in ischemic tissue are indications of

Figure 9. Current-voltage relations of single guinea pig ventricu-

ular cells after 10 to 15 minutes of exposure to ischemic condi-
tions: peak current (left) and quasi–steady-state current (right) elicited on 500-ms depolarizing voltage clamp steps from holding potentials of −40 and −80 mV, respectively. Step interval was 1 second. A and B, Control versus ischemic conditions. C and D, Control conditions versus ischemic conditions including 1 μmol/L norepinephrine (NA).

reflecting $I_{K1}$ and $I_{K2}$ shows a shift in reversal potential and a
crossover in the negative voltage range, as expected from the effects of increased extracellular potassium concentration on $I_{K1}$, whereas no major changes are observed in the voltage range positive to −10 mV (Figure 9B). Under ischemic conditions including norepinephrine, however, quasi–steady-

state current shows a large increase in the latter voltage range (Figure 9D).

Discussion

We have used isolated guinea pig ventricular myocytes in an experimental system in which an isolated cell exposed to either normal Tyrode’s solution or an ischemic solution can be dynamically coupled to a real-time solution of a mathematical model of a guinea pig ventricular cell. Our use of the ischemic solution attempts to recreate the major modulators of acute ischemia to be tested in terms of critical coupling conductance and maximum conduction delay for action potential propagation across a pair of cells in which coupling conductance is much lower than the normal value. We are basically asking: If action potential conduction is already discontinuous, perhaps from some prior injury, what is the effect of the acute imposition of a simulated ischemic condition? Our use of “normal” cells and a normal ventricular model for this experimental model is based on previous work showing that in healed myocardial infarctions, the resting membrane potential has returned to nearly normal values, although the membrane properties of surviving cells may be somewhat abnormal, with decreased calcium currents, sodium current, and $I_{Ca,L}$.

Geometrical Considerations

The slow conduction and fractionated waveforms characteristic of conduction in ischemic tissue are indications of successive activation of groups of cells for which the inter-group conductance is limiting the conduction. Our use of a model of 2 cells can be scaled to represent the conduction between 2 groups of cells under the conditions that within each group the cells are well coupled and essentially isopotential. That this condition is met under certain ischemic conditions is indicated by the brief duration of each component of the fractionated signals and also from direct micro-electrode recordings of cells within the groups. Thus, 2 groups of 1000 cells connected by a conductance of 8000 nS would have the same characteristics of conduction as 2 single cells connected by a conductance of 8 nS. In the case of connections between large groups of cells, the interconnections might be a short bridge of cells, rather than a direct electrical connection between 1 cell of each group. The major geometrical limitation of our model system is that we are currently restricted to a pair of cells without connections to other cells or groups of cells. Thus, the effects of tissue loading are not taken into account.

Conduction Under Ischemic Conditions

Our results show that the application of ischemia increases the critical value of coupling conductance required for action potential propagation and decreases the associated maximum conduction delay. The analysis is somewhat complex, because it involves 3 factors: the ability of a proximal cell (leader cell) to supply current, the coupling conductance, and the requirements for charge to activate the distal cell (follower cell). For this experimental model system, we used the Luo-Rudy model cell as the distal cell, and we did not modify the model description during the ischemia so that the requirements for activation of the distal cell would be unchanged. During the experiment, we controlled the coupling conductance, and this became a dependent variable to characterize the amount of coupling conductance required for successful propagation. The actual mechanism by which the ischemia decreases the ability of the real cell to supply current to the distal cell (as shown by the increased value of critical coupling conductance) cannot be uniquely determined by these experiments but is probably explained by an approximately 50% decrease in $I_{Ca,L}$ during the metabolic inhibition, as observed in our voltage-clamp experiments (Figure 9A). A similar decrease in $I_{Ca,L}$ was observed by Cordeiro et al. As a result, the amount of partial repolarization of the proximal cell during the conduction process is increased and the driving force for coupling current is lowered, as we have previously shown with pharmacological modulation of $I_{Ca,L}$.

Conduction Under Ischemic Conditions With Norepinephrine

Our results with the application of ischemic conditions including 1 μmol/L norepinephrine show that the presence of the norepinephrine completely reverses the effects of the simulated ischemic condition with respect to discontinuous propagation. With the norepinephrine, the critical coupling conductance is decreased, and the maximum conduction delay is increased. The actual mechanism by which the ability of the real cell to supply current to the distal cell is increased (as shown by the decreased value of critical coupling conductance) is probably explained by an increase in $I_{Ca,L}$ (Figure 9C), which, despite the concomitant increase in outward current (Figure 9D), results in a slower rate of partial
repolarization of the proximal cell during the conduction process and an increased driving force for coupling current.

Limitations of This Study
Our results suggest that the release of catecholamines with ischemia, in a setting of partially uncoupled cells, may play a major role in producing the long conduction delays that may allow the establishment of a reentrant pathway. In our experimental model system, the application of simulated ischemia, without the catecholamine, actually decreased the maximum conduction delay that could occur. Nevertheless, it is important not to extrapolate these results for cell pairs too literally for the much more complex situation of the spatially inhomogeneous application of ischemia as well as the spatial inhomogeneity of coupling conductance and intrinsic action potential properties.

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