Passive Electrical Properties, Mechanical Activity, and Extracellular Potassium in Arterially Perfused and Ischemic Rabbit Ventricular Muscle

Effects of Calcium Entry Blockade or Hypocalcemia

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The relation among passive electrical resistive properties, longitudinal conduction velocity, extracellular potassium concentration, [K+]o, and mechanical activity was investigated in the isolated rabbit papillary muscle during normal arterial perfusion and no-flow ischemia in the presence and absence of verapamil, or a reduced extracellular Ca²⁺ concentration [Ca²⁺]o. During normal arterial perfusion, verapamil (0.5 μM, free [Ca²⁺]o=1.0 mM) and hypocalcemic blood perfusate (free [Ca²⁺]o=0.4 mM) reduced the maximal isometric twitch tension by 48% and 78%, depolarized the resting membrane by +3 and +7 mV, decreased the extracellular longitudinal resistance (rL) by 15% and 26%, and increased conduction velocity by 4% and 6%, respectively. The changes in conduction velocity during these interventions were consistent with those predicted by linear cable theory (+3% and +9%) for the observed changes in rL. In contrast, verapamil shortened whereas a reduced [Ca²⁺]o, lengthened action potential duration. Comparison of simultaneously measured longitudinal whole tissue resistance (r), intracellular longitudinal resistance (rL), [K⁺]o, and resting tension during ischemia showed a close association between abrupt cell-to-cell electrical uncoupling, development of ischemic contracture, and the secondary rise of [K⁺]o, which all started to develop after approximately 15 minutes of ischemia. Electrical cell-to-cell uncoupling was completed within 15 minutes. In the presence of verapamil, the relation among the onset of electrical cell-to-cell uncoupling, secondary rise of [K⁺]o, and onset of ischemic contracture in ischemia was qualitatively the same as in its absence; however, these events were postponed by approximately 10 minutes, and the rates of contracture development and uncoupling were diminished. Conduction velocity decreased after 12 minutes of ischemia from 54 to 36 cm/sec in the presence of and from 61 to 46 cm/sec in the presence of verapamil. This slowing effect on impulse conduction could not be attributed to changes of electrical cell-to-cell coupling because at this time an increase in rL had not yet taken place. In the presence of a reduced [Ca²⁺]o, the resting tension and rL increased almost immediately after the onset of ischemia. Although the resting tension rose progressively throughout the course of ischemia, the rL showed a biphasic increase characterized by an early transient increase that reached a peak at 8 minutes (+87%) and a second, irreversible increase beginning at approximately 12 minutes. This final onset of electrical cell-to-cell uncoupling and the secondary rise of [K⁺]o were not different from the findings with a normal [Ca²⁺]o. The decrease of conduction velocity was greater with reduced than with normal [Ca²⁺]o, and ischemic conduction block occurred earlier. In conclusion, our results demonstrate that the abrupt onset of electrical cell-to-cell uncoupling, ischemic contracture, and the second phase of extracellular K⁺ accumulation are closely coupled, which suggests that they are triggered by a common event that probably involves an increase in cytosolic free [Ca²⁺]. Verapamil postpones the onset and slows the rate of development of the irreversible changes of electrical and mechanical function in ischemia. In contrast, reduced [Ca²⁺]o results in a rapid increase of intracellular resistance in association with a rise in resting tension during reversible ischemia. The differences between the effect of Ca²⁺ entry blockade and reduced [Ca²⁺]o are likely to be explained by their different effects on membrane potential during normoxia and ischemia, and consequently on Na⁺/Ca²⁺ exchange. (Circulation Research 1990;66:1461–1473)
Acute myocardial ischemia is characterized by a rapid transition from normal electrical function to a state of metabolic, ionic, and electrical instability, which is associated with a high incidence of malignant ventricular arrhythmias.\(^1\)

The metabolic as well as the ionic and electrical changes appear to occur in distinct phases. During the first few minutes after arrest of myocardial perfusion, the predominant occurrences are production of lactic acid, which is self-inhibited afterwards;\(^2,3\) cellular loss and extracellular accumulation of K\(^+\);\(^4,5\) rapid electrical depolarization with development of inexcitability;\(^6\) and loss of active tension development.\(^7\) With a latency of several minutes, a second phase of cellular K\(^+\) loss takes place,\(^8\) mean free cytosolic Ca\(^{2+}\) concentration increases,\(^8,9\) electrical cell-to-cell uncoupling occurs,\(^10\) and ischemic contracture develops.\(^7\) Both the second phase of extracellular K\(^+\) accumulation\(^4\) and cell-to-cell electrical uncoupling with development of conduction block\(^10\) have been shown to be only partially reversible, indicating that these latent changes reflect the onset of irreversible cell damage. Although the metabolic, ionic, electrical, and mechanical changes have been relatively well characterized individually, their exact interrelations and, especially, the separation between causal and resultant events have not been fully established. This lack of precise correlation of electrical, ionic, and mechanical changes may be due to the difficulty of applying different kinds of methods simultaneously to the same experimental preparation and of comparing results obtained among different models of myocardial ischemia and among different animal species.

Recently, we developed a method to measure passive electrical properties (resistances of the extracellular and intracellular space) in arterially perfused ventricular rabbit myocardium.\(^11\) The preparation consists of a cylindrically shaped papillary muscle in which electrical cable analysis (including the measurement of cell-to-cell electrical coupling) can be performed during conditions of normal perfusion and no-flow ischemia.\(^10\) An advantage of this preparation is that drugs acting on the myocytes can be brought to their sites of action through the intact microvasculature. Thus, the relatively long diffusion distances present in superfused preparations are avoided.

In the present study, this experimental preparation was used for comparison of the changes in the amplitude and duration of the action potential, resting membrane potential, longitudinal conduction velocity, and passive cablelike electrical properties, especially the process of cell-to-cell electrical uncoupling, with the changes of mechanical activity and the extracellular accumulation of K\(^+\) by the simultaneous measurement of these parameters. Moreover, the effect of reduction of extracellular Ca\(^{2+}\) concentration or pharmacological block of Ca\(^{2+}\) entry before ischemia on the time course and the interrelation among these parameters were investigated.

As will be shown, irreversible cell-to-cell electrical uncoupling, the secondary extracellular K\(^+\) accumulation, and ischemic contracture appear to be closely associated, which suggests that these changes in electrical properties, ionic homeostasis, and mechanical properties are triggered by a common event.

**Materials and Methods**

**Preparation and Perfusion of Papillary Muscles**

The method for the preparation of the isolated arterially perfused rabbit papillary muscle has been previously described in detail.\(^10,11\) Rabbits of either sex weighing 2–3 kg were anticoagulated with heparin (200 units/kg i.v.) and anesthetized with sodium pentobarbital (50 mg/kg i.v.). Whereas the animals were deeply anesthetized, the hearts were rapidly excised, placed in Tyrode’s solution (4\(^\circ\) C), and transported to a dissection tray. The atria, left ventricular free wall, and nonperfused portion of the right ventricle were removed. The left ventricular septal surface of the tissue was secured to a wax platform that contained a ground electrode. The septal artery was cannulated and perfused with the following solution: insulin 1 unit/l, heparin 400 units/l, albumin 2 g/l, dextran (M, 70,000) 40 g/l, and Tyrode’s solution composed of (mM) Na\(^+\) 149, K\(^+\) 4.5, Mg\(^{2+}\) 0.49, Ca\(^{2+}\) 1.8, Cl\(^−\) 133, HCO\(_3\)\(^−\) 25, HPO\(_4\)\(_2−\) 0.4, glucose 20. The total ischemic time before perfusion was less than 6 minutes in each experiment.

Once the preparation was placed in the recording chamber, it was maintained at 36\(^°\)–37\(^°\) C and perfused with a solution composed of the previous perfusate plus washed bovine erythrocytes (hematocrit 25–30\%). The perfusion solution was pumped to the recording chamber by a roller pump (Ismatec, Zurich, Switzerland). Perfusion pressure in the septal artery was measured with a Statham transducer (Gould, Cleveland, Ohio) and maintained between 40 and 45 mm Hg by adjustment of the perfusion flow rate (80–100 ml/min 100 g). This perfusion pressure is normal for an artery with a diameter of 120–160 \(\mu\)m because in rabbit about 40–50% of the peripheral coronary resistance is located in vessels with a diameter >150 \(\mu\)m.\(^12\) Equilibration between the perfusate and a mixture of \(O_2\), \(N_2\), and \(CO_2\) was achieved with a membrane gas exchanger. The pH was continuously monitored during the experiment, and the relative amounts of \(O_2\), \(N_2\), and \(CO_2\) were adjusted to yield a pH of the perfusate of 7.35–7.45. Stainless steel tubing was introduced between the membrane...
gas exchanger and the recording chamber for prevention of diffusional losses.

Tension Measurement

Figure 1 shows the experimental preparation, which consisted of a cylindrically shaped, arterially perfused right ventricular papillary muscle suspended in a H2O-saturated atmosphere. The tendinous end was fixed to a force transducer and stimulated through a platinum wire. The base of the muscle protruded through a small ring. Both the transducer and the ring were fixed to two independent micromanipulators. Their respective positions defined the resting length of the muscle, which was usually slightly stretched to about 30% of slack length. This ring at the base of the muscle prevented transmission of the septal contraction to the force transducer. The force transducer consisted of a piezoresistive element (SensoNor, Horton, Norway). After amplification the sensitivity of the transducer was 50.7 mV/mN.

Measurement of Active and Passive Cablelike Electrical Properties

The amplitude of the transmembrane action potential (ΔV_m) was measured with an intracellular floating glass microelectrode (borosilicate glass, DC tip resistance 15–20 mΩ when filled with 3 M KCl). The two extracellular electrodes (E1 and E3 in Figure 1) consisted of thin polyethylene tubes that contained a fine silk thread (diameter, 45 µm) and were backfilled with 150 mM NaCl. Electrical contact between the muscle and the electrode was made only via the electrolyte-silk bridge. In such a way, mechanical damage of the preparation by the extracellular electrode was prevented, and a high degree of DC stability was obtained (shift, <1 mV/hr).

The method for the determination of the passive cablelike electrical properties in the isolated arterially perfused ventricular muscle has been described in detail elsewhere. In brief, excitatory (0.5–2.0 msec duration at double-threshold strength) and subthreshold (20 msec duration) current pulses were applied to the apical end of the papillary muscle. During excitation, the ΔV_m and the amplitude of the extracellular bipolar electrogram (ΔV_o) were taken to determine the ratio (q) of the extracellular longitudinal resistance (r_o) and the intracellular longitudinal resistance (r_i) as

\[
q = r_o/r_i = |\Delta V_o|/(|\Delta V_m| - |\Delta V_o|)
\]

The longitudinal whole tissue resistance, r_i (in kΩ/cm), was obtained from the subthreshold voltage (V_o), the interelectrode distance (Δx), and the strength of the subthreshold current pulse (I). According to linear cable theory, r_i is equal to r_o and r_i, in parallel:

\[
r_i = V_o/(I \times \Delta x) = r_o \times r_i/(r_o + r_i)
\]

The intracellular longitudinal resistance, r_i (kΩ/cm), and the extracellular longitudinal resistance, r_o (kΩ/cm), are calculated from the equations that use q and r_i.

Changes of r_i, r_o, and r_i produced by verapamil or a reduced extracellular Ca^{2+} concentration in the presence and absence of ischemia are expressed as relative changes compared with the values before the intervention. Calculation of the specific resistances for whole tissue, R_i (Ω cm); the intracellular space R_i (Ω cm); and the extracellular space, R_o (Ω cm), is not possible under these conditions because measurements of relative changes of the extracellular and intracellular space in the presence of verapamil or a reduced extracellular Ca^{2+} concentration or during ischemia are not available.

Longitudinal conduction velocity (θ, in cm/sec) was calculated from the conduction time (time between deflection and inflection on the extracellular electrogram) and the interelectrode distance.

Measurement of Extracellular Potassium Concentration

The voltage sensitive to the extracellular K^+ activity (ΔE_K) was measured as the potential difference between an extracellular reference electrode and a miniature extracellular potassium ion-selective electrode (constructed, tested, and calibrated as previously described). The mean response per 10-fold
change in K⁺ activity for all experiments was 56.8±0.7 mV (±SEM, n=38). An activity coefficient of 0.74615 was taken for conversion of activity measurements to K⁺ concentration [K⁺].

**Recording Procedure**

The extracellular bipolar electrogram was recorded between electrodes E₁ and E₂ (Figure 1), placed on the longitudinal axis of the papillary muscle. The diameter of the muscle and the interelectrode distance were measured with a graduated reticle in the eyepiece of the binocular microscope (magnification, ×25). The mean diameter of the cylindrical preparations was 1.05±0.04 mm (±SEM, n=38). The mean interelectrode distance was 1.66±0.08 mm (±SEM, n=38). The transmembrane action potential was recorded as the voltage difference between an intracellular floating glass microelectrode and the apical extracellular (see Figure 1). The extracellular and intracellular electrodes were connected to high-input impedance amplifiers (model 515, Analog Devices, Norwood, Massachusetts). The buffered signals were fed to conventional differential amplifiers. Measurement of the strength of the subthreshold constant current pulse (delivered between apex and the base of the muscle) used the feedback loop of an operational amplifier connected between the preparation and ground. Analog signals were converted to digital information and stored on a Signal Memory Recorder (Max Meyer Electronics, Zurich, Switzerland) at a sampling rate of 100 μsec during subthreshold events and 30 μsec during suprathreshold events (i.e., upstroke of the action potential, bipolar extracellular electrogram). The data were transferred to a computer (model 9617, Hewlett-Packard, Palo Alto, California) analyzed, and then displayed on a graphical plotter. In addition, whole recordings of action potential, perfusion pressure, contractile parameters, and extracellular K⁺ concentration were made on an analog strip chart recorder (Mark VII, Graphtec, Zurich, Switzerland) in the majority of the experiments.

**Experimental Protocol**

In most experiments the extracellular K⁺ concentration, electrical parameters, and contractile parameters could be measured simultaneously. In each experiment the interstimulus interval was held constant during the period of ischemia (mean for all experiments 535±13 msec, n=38; mean for control experiments 541±37 msec, n=15; mean for verapamil experiments 550±28 msec, n=14; mean for reduced calcium experiments 501±17 msec, n=9). In each experiment control measurements were obtained after the preparation had stabilized in the chamber for 30–45 minutes. For determination of the effect of verapamil or a reduced extracellular Ca²⁺ concentration during perfusion, measurements were made after 30 minutes of perfusion with control perfusate, a perfusate containing verapamil hydrochloride (0.5 μM; Knoll AG, Liestal, Switzerland), or a reduced extracellular free Ca²⁺ (0.40±0.18 mM).

The concentration of verapamil was chosen for investigation of effects that were predominantly related to calcium entry blockade rather than a mixture of calcium and sodium entry blockade. Hiramatsu et al. found that rate of rise of the action potential was not affected by 1 μM verapamil. Thus, it is likely that verapamil at a concentration of 0.5 μM would have only a minimal effect on the inward sodium current. A verapamil solution was made on the day of each experiment and added to a portion of the control perfusate. Ischemia was produced by the abrupt and complete interruption of arterial perfusion. During the preischemic period the H₂O-saturated atmosphere of the recording chamber contained a mixture of N₂ (75%), O₂ (20%), and CO₂ (5%). One minute before the interruption of perfusion, the H₂O-saturated atmosphere of the recording chamber was adjusted to contain a mixture of N₂ (95%) and CO₂ (5%). The Po₂ of the H₂O-saturated atmosphere of the recording chamber during ischemia was shown to be less than 5 mm Hg. Each preparation was subjected to only one ischemic period. During ischemia, measurements of the subthreshold events, mechanical activity, and extracellular K⁺ were obtained at 1-minute intervals for up to 50 minutes. Measurements of action potentials were performed approximately every 1–2 minutes (separate impalements in the majority of the experiments).

**Statistical Analysis**

Statistical comparisons of values during control and each test period were made by analysis of variance or Student’s paired t test, as appropriate. All results are given as mean±SEM.

**Results**

**Effect of Calcium Entry Blockade or Reduced Extracellular Calcium Concentration on Active and Passive Cablelike Electrical Properties During Normal Perfusion**

Table 1 shows the normal values of the electrical parameters, mechanical activity, and extracellular K⁺ concentration for the arterially perfused rabbit papillary muscle. The electrical parameters, which include the resting membrane potential (RMP), \( \Delta V_m \), \( \Delta V_o \), action potential duration (APD), \( R_n \), \( R_m \), and \( \Theta \), are consistent with values previously reported from this laboratory. The ratio of the extracellular to intracellular resistance was 0.95. The magnitude of the isometric twitch tension (T₁) was in the range of values measured in excised superfused cardiac muscle.

The electrical parameters were not significantly affected by a 30-minute control perfusion (Table 2); the isometric T₁ decreased slightly (11%, \( p=0.012 \)). However, verapamil at a concentration of 0.5 μM reduced the measured isometric T₁ by 48% (Table 2). It also reduced APD but did not alter the amplitude of the transmembrane action potential. Its main effect on the electrical properties was to decrease the
TABLE 1. Control Values During Normal Perfusion

<table>
<thead>
<tr>
<th></th>
<th>RMP (mV)</th>
<th>ΔV_m (mV)</th>
<th>ΔV_o (mV)</th>
<th>APD_{90} (msec)</th>
<th>APD_{80} (msec)</th>
<th>R_i (1cm)</th>
<th>R_r (1cm)</th>
<th>R_o (1cm)</th>
<th>Θ (cm/s)</th>
<th>[K^+] (mM)</th>
<th>T_i (mM/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SEM</td>
<td>−78±1</td>
<td>103±2</td>
<td>49±2</td>
<td>153±5</td>
<td>184±6</td>
<td>209±7</td>
<td>66±3</td>
<td>59±2</td>
<td>4.4±0.2</td>
<td>6.4±0.7</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>30</td>
<td>35</td>
<td>35</td>
<td>31</td>
<td>31</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>34</td>
<td>31</td>
<td>32</td>
</tr>
</tbody>
</table>

Calculation of longitudinal specific resistances for whole tissue, intracellular space, and extracellular space were based on assumed extracellular volume fraction of 0.25. ^11^ RMP, resting membrane potential; ΔV_m and ΔV_o, amplitudes of transmembrane action potential and extracellular electrogram, respectively; APD_{90} and APD_{80}, action potential duration at 50% and 80% repolarization, respectively; r_i, longitudinal resistance of whole tissue; r_r, intracellular longitudinal resistance; r_o, extracellular longitudinal resistance; Θ, conduction velocity; [K^+], extracellular potassium concentration; T_i, twitch tension.

TABLE 2. Changes of Electrical Properties, Mechanical Activity, and Extracellular K^+ After 30 Minutes of Perfusion

<table>
<thead>
<tr>
<th></th>
<th>RMP (mV)</th>
<th>ΔV_m (mV)</th>
<th>ΔV_o (mV)</th>
<th>APD_{90} (msec)</th>
<th>APD_{80} (msec)</th>
<th>r_i (%)</th>
<th>r_r (%)</th>
<th>r_o (%)</th>
<th>Θ (%)</th>
<th>[K^+] (mM)</th>
<th>T_i (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>−1±1</td>
<td>−2±2</td>
<td>−1±1</td>
<td>−1±5</td>
<td>−1±7</td>
<td>−2±2</td>
<td>−1±3</td>
<td>−1±4</td>
<td>−5±3</td>
<td>0.2±0.3</td>
<td>−11±4</td>
</tr>
<tr>
<td>Mean±SEM</td>
<td>11</td>
<td>12</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>10</td>
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<tr>
<td>n</td>
<td></td>
<td>11</td>
<td>14</td>
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<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verapamil</td>
<td>7±2</td>
<td>−7±3</td>
<td>−10±2</td>
<td>37±5</td>
<td>34±3</td>
<td>−18±5</td>
<td>−5±3</td>
<td>−26±7</td>
<td>6±2</td>
<td>0.2±0.4</td>
<td>−78±3</td>
</tr>
<tr>
<td>Mean±SEM</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Low [Ca^{2+}]_o</td>
<td>0.062</td>
<td>0.172</td>
<td>0.022</td>
<td>0.015</td>
<td>NS</td>
<td>0.030</td>
<td>0.006</td>
<td>0.050</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control vs. verapamil</td>
<td>0.002</td>
<td>NS</td>
<td>&lt;0.0001</td>
<td>0.003</td>
<td>NS</td>
<td>0.001</td>
<td>0.006</td>
<td>NS</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control vs. low [Ca^{2+}]_o</td>
<td>0.083</td>
<td>0.042</td>
<td>0.015</td>
<td>&lt;0.0001</td>
<td>0.040</td>
<td>NS</td>
<td>0.059</td>
<td>NS</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Relation Among Electrical Properties, Mechanical Activity, and Rise of Extracellular Potassium in Ischemia

Simultaneous measurements of electrical activity, passive electrical properties, mechanical activity, and extracellular K^+ concentration from an individual experiment are shown in Figure 2. The expected early changes in action potential configuration (decrease of amplitude, shortening) were associated with an increase in extracellular K^+ As described by others, the first increase of extracellular K^+ took place almost immediately after arrest of perfusion. ^4^ ^5^ Subsequently, extracellular K^+ showed a plateau and rose again rapidly after 14 minutes. This secondary increase of extracellular K^+ occurred simultaneously with an almost abrupt increase of the r_i, which rose from 118% to 184% between 15 minutes and 16 minutes after arrest of perfusion. Mechanical activity showed an immediate decrease and developed tension vanished between 5 and 10 minutes of ischemia despite the presence of high-amplitude action potentials. Resting tension remained unchanged in the first phase of ischemia and increased when electrical cellular uncoupling developed after 14–15 minutes.

The mean values of changes of r_i and r_o from 14 experiments are shown on the left in Figure 3. On the
right, the values are replotted versus a relative time scale for precise visualization of the time course of cell-to-cell electrical uncoupling. Time zero in each individual experiment corresponds to a 150% level of $r_t$. The results are similar to those reported previously. The initial increase of $r_t$ occurring during the first 3 minutes of ischemia was due to an abrupt increase of $r_s$ (diminution of the intravascular space after arrest of perfusion). A small (17±7%) and transient increase of $r_t$ ($p=0.014$) was measured at 8 minutes; $r_t$ was completely recovered by 12 minutes. The rapid secondary increase of $r_t$ was due to an increase of $r_s$ (i.e., rapid cellular electrical uncoupling). Determination of $r_t$ beyond values of 250–300% was not possible because of discontinuities in the action potential upstrokes or in the extracellular electrograms.

In this study, we extended the measurement of $r_t$ (which was possible in the inexcitable muscle) up to 40 minutes after arrest of perfusion. The increase in $r_t$ slowed rapidly after 30 minutes of uncoupling and reached an almost steady level. Taking account of the ratio $r_s/r_t$ during control (Table 1) and the increase of $r_s$ during the initial 15 minutes of ischemia, complete uncoupling (setting $r_s=\infty$) in a theoretical cable was expected to increase $r_t$ to a value of 230%. This was close to the experimental level of approximately 250% reached after 30 minutes (Figure 3, top panels).

One of the major goals of this study was comparison of the time course of gross cell-to-cell electrical uncoupling with the increase in extracellular $K^+$ and the development of rigor and/or contracture. In Figure 4, data from nine experiments obtained simul-
Effect of verapamil. The simultaneously measured changes in longitudinal tissue resistance, $r_t$, extracellular potassium concentration, $K^+$, and resting tension, $r$, were compared. The three parameters are plotted versus time after onset of ischemia in the left panels and versus relative time (time 0 at $r_t=150\%$) in the right panels. Comparison of the three parameters indicates that there is a close association between cell-to-cell electrical uncoupling, the secondary rise of extracellular $K^+$, and the increase of resting tension, which all were initiated after approximately 15 minutes of ischemia. Comparison of these three parameters in individual experiments showed that the times of onset of the changes were separated by no more than 2–5 minutes. In eight of the nine experiments the sequence of changes in these parameters was consistent; that is, an increase in resting tension preceded the increase in $r_t$. The increase in $r_t$ was either coincident with or closely followed by the secondary rise in extracellular $K^+$. In contrast, the early phase of extracellular $K^+$ accumulation was neither related to a change of $r_t$ nor to an increase in resting tension.

Effect of Calcium Entry Blockade in Ischemia

The relation between $r_o$, change of extracellular $K^+$, and resting tension in the presence of verapamil is shown in Figure 5. Qualitatively, the time course of all three parameters was the same as in the absence of verapamil. The early, small change of $r_t$ was due to an increase in $r_o$ (not shown), whereas the later steep increase at 25 minutes was caused by cell-to-cell electrical uncoupling. Similar to the results shown in Figure 4, cellular uncoupling was closely associated with the secondary increase of extracellular $K^+$ and to the development of ischemic contracture (or rigor).

Figure 4. Left Panel: Relation among changes in longitudinal tissue resistance, $r_t$ (top); extracellular potassium concentration (middle); and resting tension (bottom) during acute myocardial ischemia. Changes in extracellular $K^+$ and resting tension are relative to their values obtained immediately before ischemia. Right panel: Changes in $r_t$ (top), extracellular $K^+$ (middle), and resting tension (bottom) plotted on relative time scale. For each experiment, value corresponding to a change of $+50\%$ of $r_t$ (top) was assigned relative time 0 (mean±SEM, n=9). See text for explanation.
the magnitude and a delay in the onset of ischemic contracture (or rigor).

**Effect of Reduced Extracellular Calcium in Ischemia**

The relation among $r_r$, the change of extracellular $K^+$, and resting tension during ischemia in the presence of a reduced extracellular Ca$^{2+}$ is shown in Figure 7. Surprisingly, despite the large reduction of the isometric twitch tension produced by the reduced extracellular Ca$^{2+}$ during normoxic perfusion, the time to the onset of cell-to-cell electrical uncoupling, as measured by $r_r$, and the secondary rise of extracellular K$^+$ were not significantly different from control. Furthermore, in contrast to the findings with normal extracellular Ca$^{2+}$, the resting tension began to increase immediately after arrest of perfusion and, consequently, the secondary rise of extracellular K$^+$ appeared to be dissociated from the development of ischemic contracture (or rigor). Figure 8 shows that the small constant increase of the resting tension measured consistently during early ischemia in the presence of a reduced extracellular Ca$^{2+}$ concentration was associated with a significant transient increase in $r_r$. Hence, this finding was in contrast to the experiments carried out in the presence of verapamil, where no increase in $r_r$ or resting tension was observed during the initial phase of ischemia.

**Discordant Effects of Calcium Entry Blockade and Reduced Extracellular Calcium on Resting Membrane Potential, Action Potential Duration, and Conduction Velocity During Myocardial Ischemia**

Perfusion with blood containing either verapamil or a reduced extracellular Ca$^{2+}$ produced discordant
effects on RMP and APD during myocardial ischemia. As shown in Figure 9 (top), verapamil produced a positive shift of the RMP during control perfusion and attenuated ischemia-induced depolarization of the resting membrane. In the presence of a reduced extracellular Ca\(^{2+}\), the relatively large depolarization of the resting membrane during normal perfusion persisted during ischemia relative to control and verapamil groups. The bottom panel of Figure 9 shows that the amount of the shortening of the APD\(_{90}\) produced by verapamil and the lengthening of APD\(_{90}\) by reduced extracellular Ca\(^{2+}\) during normal perfusion persisted during the first 12 minutes of ischemia. These relations were similar for APD\(_{50}\).

Ca\(^{2+}\) entry blockade delayed the pronounced decrease of conduction velocity, \(\Theta\), during myocardial ischemia significantly. After 12 minutes \(\Theta\) decreased from 54±2 to 36±2 cm/sec \((n=8)\) in the absence of and from 61±4 to 46±3 cm/sec \((n=7)\) in the presence of verapamil. These values relate to the propagation velocity present at a time when the \(r_i\) had not yet increased and, therefore, indicate that this difference was due to an effect of verapamil on the ischemic action potential, in addition to delay of onset of electrical cell-to-cell uncoupling. In the presence and absence of verapamil, a small transient increase of \(\Theta\) was consistently observed. Complete conduction block occurred 1–2 minutes after the abrupt onset of electrical cell-to-cell uncoupling.

**Figure 7.** Comparison of changes in longitudinal tissue resistance, \(r_i\) (top panel); extracellular \(K^+\) (middle panel); and resting tension (bottom panel) during acute myocardial ischemia in presence of reduced extracellular Ca\(^{2+}\).

**Figure 8.** Comparison of changes in intracellular longitudinal resistance, \(r_i\) (top panel), and resting tension (bottom panel) during acute myocardial ischemia in presence of verapamil (○) with effect of reduced extracellular Ca\(^{2+}\) (■).

**Figure 9.** Changes of resting membrane potential (RMP, top panel) and action potential duration (APD\(_{90}\), bottom panel) during acute myocardial ischemia in presence (○) and absence (■) of verapamil, or reduced extracellular Ca\(^{2+}\) (■). Values at time of interruption of coronary flow (0 minutes) were measured after 30 minutes of normoxic test perfusion (C).
absence and presence of verapamil. In the presence of the reduced extracellular Ca\(^{2+}\), conduction velocity diminished very rapidly from 66±3 to 34±8 cm/sec after 12 minutes, and propagation failed in five of eight experiments before 15 minutes, that is, at a time before a marked increase of \(r_h\) had taken place.

**Discussion**

**Relation Among Electrical Properties, Mechanical Activity, and Rise of Extracellular Potassium in Ischemia**

The early, reversible phase of myocardial ischemia has been well described by its rapid electrical changes (depolarization, depression of action potential, development of inexcitability,6,14 mechanical activity (decrease of active tension development7), and changes of extracellular K\(^+\).4,5 The changes of electrical activity are mainly associated with the accumulation of extracellular K\(^+\) and acidosis, which are the dominant ionic changes during this period.23 Intracellular [Na\(^+\)] in guinea pig heart14 and mean free [Ca\(^{2+}\)] in rat heart8 and ferret heart9 seems to be maintained for an initial period varying between 6 and 15 minutes among animal species. Our finding of a normal \(r_h\) is in accordance with these measurements of intracellular Na\(^+\) and Ca\(^{2+}\), because calcium affects cell-to-cell coupling24 and the regulation of the intracellular levels of both ions is closely interrelated.25 The present results provide further confirmation that, under normal conditions, early extracellular K\(^+\) accumulation is dissociated from the cytosolic increase of mean [Ca\(^{2+}\)] and [Na\(^+\)], cell-to-cell uncoupling, and the development of contracture. This dissociation implies that the very early disturbances of impulse conduction (associated with 1a-type reentrant arrhythmias30) that occur with a high incidence in regional ischemia do not involve cellular uncoupling. The exact mechanism of early cellular K\(^+\) loss still remains to be elucidated (for hypothesis see Kléber27) and is not further clarified by the present investigation.

Both the ionic changes (extracellular K\(^+\)) and the electrical changes10 are known to be partially irreversible after the beginning of cell-to-cell electrical uncoupling and of the secondary increase of extracellular K\(^+\). The present observation that the onsets of contracture, of cell-to-cell uncoupling, and of the secondary increase of extracellular K\(^+\) are closely coupled suggests that these changes are triggered by a common event, which is of critical importance for explanation of the onset of ischemic damage. In addition, high-energy phosphates and mean free intracellular Ca\(^{2+}\) demonstrate a phasic time course after coronary occlusion and have turning points very similar to the parameters measured in this study: 1) The ATP potential (−dG/d\(\zeta\)-ATP) decreases from approximately 57 kJ/mol (control) to a plateau level of approximately 45 kJ/mol. The rate of decrease of −dG/d\(\zeta\)-ATP is small during this intermediate plateau phase, and a fast secondary decrease of −dG/d\(\zeta\)-ATP occurs after approximately 10 minutes (ischemic rat heart). Interestingly, the time course of −dG/d\(\zeta\)-ATP is similar in myocardial ischemia and myocardial hypoxia.2,28 and, for the case of myocardial hypoxia, the secondary decrease of cellular ATP levels coincides with the onset of contracture (rat29).

2) Mean intracellular free [Ca\(^{2+}\)] increases rapidly and simultaneously with resting tension after an initial period of stability, which lasts for approximately 6–10 minutes in rat8 and for approximately 15 minutes in ferret.9 These comparisons make it most probable that all the above-mentioned changes (rapid ATP consumption, ionic imbalance due to failure of energy-dependent pumps, development of contracture, cell-to-cell uncoupling) are caused by a common event that involves a sudden increase of cytosolic free calcium. Intracellular acidosis probably does not function as the main trigger of cellular electrical uncoupling, because the time course and magnitude of cellular electrical uncoupling are similar in hypoxia30 and ischemia,10 yet the intracellular pH changes are significantly different (ischemia31; hypoxia32). The apparent lack of a major effect of protons as the mediator of cellular uncoupling during ischemia is not surprising given that gap junctional conductance in ventricular myocytes decreased only at a pH <6.5,33 although modification of the effect of calcium by hydrogen ion cannot be excluded.34

Once a critical increase of free intracellular [Ca\(^{2+}\)] is reached, the observed rapid and simultaneous development of ischemic contracture, cell-to-cell electrical uncoupling, and secondary extracellular K\(^+\) accumulation is readily explained by a self-perpetuating process: energy consumption will be increased by activation of the contractile proteins and ion pumps. The resulting energy imbalance and decrease of the ATP potential will close a circuit of positive feedback through a decrease in pump rates and a resulting further increase of intracellular Na\(^+\) and Ca\(^{2+}\). This action will further increase resting tension and cause the observed second phase of extracellular K\(^+\) accumulation (decrease of active K\(^+\) influx) and cell-to-cell uncoupling. Such a self-perpetuating process would also explain our observation that electrical cell-to-cell uncoupling is completed rapidly (within approximately 15 minutes) after the onset of ionic imbalance.

A detailed appreciation of all the complex (known or hypothetical) factors that may cause an abrupt increase of cytosolic free [Ca\(^{2+}\)] in ischemic tissue is not possible on the basis of the present results. In essence, the level of free intracellular [Ca\(^{2+}\)] is modulated by cytosolic buffering and by exchange processes between the cytosol and separated compartments (extracellular space, sarcoplasmic reticulum, and the mitochondria), and dysfunction of any of these mechanisms may cause an increase in free intracellular [Ca\(^{2+}\)]. However, the fact that the initial, reversible phase of ischemia is associated with extracellular K\(^+\) accumulation and concomitant membrane depolarization may have an impact on the
source of the cytosolic [Ca2+] increase in myocardial ischemia. The critical energy level necessary to maintain the electrochemical gradient for Na+ at the surface membrane decreases during this early phase, partially because of membrane depolarization, partially because of the decrease of Na+ influx (acidosis; inhibition of Na+/Ca2+ exchange, probably by ATP depletion). This explains the initial maintenance of a low intracellular Na+ activity in ischemia, despite partial inhibition of Na+/K+ pumping. Consistent with this view is the observation that cytosolic [Na+] remains low in ischemic rat skeletal muscle until the free energy of hydrolysis of ATP falls below 49 kJ/mol. In contrast, the critical level of chemical energy for maintenance of the Ca2+ gradient at the sarcoplasmic reticulum will not be affected by surface membrane depolarization. For the case of skeletal muscle, the failure of the sarcoplasmic reticulum to maintain a gradient for Ca2+ has been evoked as a major mechanism for the development of contracture during fatigue. The hypothesis of a primary Ca2+ release from an intracellular store is further sustained by the observation that (in myocardial hypoxia) the increase in resting tension occurs before the cellular uptake of Ca2+ from the extracellular space.

Even in a preparation as small as a rabbit papillary muscle with 300–1,500 cells per cross-sectional area and 5,000–20,000 cells in the tissue segment between the recording electrodes, metabolic inhomogeneities are likely to complicate the exact comparison of contracture, cell-to-cell uncoupling, and cellular K+ loss. The fact that the first increase of resting tension was observed 2–5 minutes before the increase of the lumped $r_e$ might have several possible explanations: 1) Ischemic contracture might, at a cellular level, precede the increase of cytosolic [Ca2+] by a small time period, 2) the difference between the onset of resting tension and cell-to-cell uncoupling might reflect a difference in sensitivity to Ca2+ between the contractile system and the intercellular connections, and/or 3) inhomogeneous development of contracture and cell-to-cell uncoupling might be detected earlier with the tension measurement than with the measurement of lumped $r_e$. Although electrical cable analysis allows exclusion of major electrical discontinuities, microscopic inhomogeneities have been suggested as the only possible explanation for the transient increase of conduction velocity (Figure 10), which is regularly observed immediately before macroscopic uncoupling. Thus, the characteristic change of conduction velocity observed in our experiments favors the third possibility.

Discordant Effects of Calcium Entry Blockade or a Reduced Extracellular Ca2+ on Cable Properties, Electrical Activity, and Mechanical Activity During Normal Perfusion and Ischemia

Verapamil (0.5 μM) reduced the maximal isometric twitch tension by 48% and the arterial pressure-flow ratio (i.e., peripheral vascular resistance) by 14%. No effect was found on the $\Delta V_m$ as previously reported by others. As in Purkinje fibers, cell-to-cell electrical coupling in normoxia was not altered by verapamil. In contrast to previous studies, a consistent small change (+4%) in conduction velocity, $\Theta$, was observed. Most of this change is accounted for by the significant decrease of the $r_e$, which occurred concomitantly with the decrease of peripheral vascular resistance. In hearts perfused with erythrocyte-free solution such an effect of verapamil is not found, and is not to be expected, because the hearts are maximally vasodilated and have an increased interstitial space with a lower $r_e$ than normal. The small increase of extracellular K+ (0.5 mM) consistently observed after administration of verapamil may have added to the increase of conduction velocity because of the expected increase of excitability, although the effect of verapamil on the increase of extracellular K+ was minor and probably not relevant for the changes in the electrical and mechanical behavior of the papillary muscle.

Preservation of conduction in early ischemia by calcium entry blockade has been reported in a number of studies. The present results demonstrate that this effect occurs in the absence of any difference between the control cell-to-cell electrical uncoupling and, therefore, must be attributed solely to preservation of the excitability of the membrane. In part, the preservation of conduction is likely to be explained by the action of verapamil to decrease the early, immediate extracellular K+ accumulation and the associated change of resting membrane potential (see Figures 6 and 9). The reduction of extracellular K+ accumulation during the first 35 minutes of ischemia caused by verapamil approached statistical significance ($p=0.057$). However, due to the large variation in extracellular K+ at a given time from one experiment to another, we could not show a statistically significant difference at each minute during ischemia. One of the factors contributing to this variation was the relatively large range of stimulation rates. Both the time of onset of cell-to-cell electrical uncoupling and the level of extracellular [K+]...
reached during the plateau are directly related to stimulation rate\textsuperscript{50} (Cascio and Kléber, unpublished observations). Independent of its effect on ischemic depolarization, verapamil prevents the depression of the action potential upstroke for a given level of resting membrane potential.\textsuperscript{44} Several products of ischemic metabolism (such as H\textsuperscript{+} and lactate\textsuperscript{31}), which occurs at a slower rate in the presence of verapamil, may decrease the amount of depolarizing ionic current during the action potential upstroke.

Qualitatively, the phasic time course of the change in electrical cell-to-cell coupling and the characteristic relation between longitudinal resistance, resting tension, and extracellular K\textsuperscript{+} accumulation were the same in the presence and absence of calcium entry blockade. However, verapamil postponed the onset and slowed the rate of electrical cell-to-cell uncoupling, of the secondary rise of extracellular K\textsuperscript{+}, and of the rise in resting tension. These effects may be taken to indicate that both the rise of cytosolic free [Ca\textsuperscript{2+}]\textsuperscript{2} above the level at which uncoupling and contracture develop and the rate of Ca\textsuperscript{2+} rise during the process of uncoupling are affected. A variety of factors may mediate these effects: 1) a decreased energy requirement with preservation of energy-rich phosphate compounds in the presence of verapamil,\textsuperscript{52} 2) the maintained ability of the mitochondria to buffer calcium, and 3) a decreased calcium concentration in the sarcoplasmic reticulum. In the latter case, a decrease of the sarcoplasmic reticulum calcium by verapamil would reduce the energy required to maintain a given level of cytosolic Ca\textsuperscript{2+}, and, consequently, the cells would tolerate a lower ATP potential. Thus, calcium entry blockade might evidence both a slowing of the rate at which chemical energy is consumed (with a longer preservation of the ATP potential) and greater tolerance toward a fall of the cytosolic ATP potential.

It should be emphasized that in the present experiments and in all situations that implicate interruption of myocardial perfusion (ischemia), calcium entry blockade is supplied before the test period. Therefore, at the time of onset of ischemia, the energy demand of the tissue is reduced. Consequently, the rate of anaerobic metabolism and the accumulation of ischemic metabolites in general will be affected by Ca\textsuperscript{2+} entry blockade. To what extent inhibition of Ca\textsuperscript{2+} influx through Ca\textsuperscript{2+} channels during ischemia contributes specifically to the observed changes is not evident from such experiments.

For reduction of the energy demand of the muscle without pharmacological calcium entry blockade, experiments were performed in the presence of a reduced extracellular Ca\textsuperscript{2+} concentration. Although the reduction of extracellular Ca\textsuperscript{2+} significantly reduced the isometric twitch tension to an even greater extent than did calcium entry blockade, the onset of markers of irreversible injury during ischemia, such as the secondary K\textsuperscript{+} rise and the final abrupt increase in \( r \), did not differ from control. In contrast to control and verapamil, a reduction of the extracellular Ca\textsuperscript{2+} was associated with an early rapid and transient rise in \( r \), and an early continuous increase in resting tension. This early cellular electrical uncoupling and increase in resting tension are probably due to an increase in cytosolic free [Ca\textsuperscript{2+}] mediated by electrogenic Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange. The prolonged action potential duration and the positive shift of the resting membrane potential produced by the reduced extracellular Ca\textsuperscript{2+} (which persisted through the early phase of ischemia) predicts to favor the retention of intracellular Ca\textsuperscript{2+} and to overweight the effect of a reduction of the transmembrane chemical gradient.\textsuperscript{53–55} The apparently paradoxical effect of a reduction of extracellular [Ca\textsuperscript{2+}] to increase intracellular [Ca\textsuperscript{2+}] also leads to maintenance or accumulation of Ca\textsuperscript{2+} in the sarcoplasmic reticulum.\textsuperscript{56,57} This condition most likely explains the observation that verapamil but not the reduction of extracellular Ca\textsuperscript{2+} delayed the onset of irreversible ischemic damage.

Acknowledgments

We wish to express our thanks to Ms. Doris Baumgartner and Dr. Celestino Cigada for their technical assistance.

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Cascio et al. Ca²⁺ Blockade and Cable Properties in Myocardial Ischemia


KEY WORDS: verapamil, ischemia, contraction, myocardial cable properties, extracellular potassium, extracellular calcium.
Passive electrical properties, mechanical activity, and extracellular potassium in arterially perfused and ischemic rabbit ventricular muscle. Effects of calcium entry blockade or hypocalcemia.

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doi: 10.1161/01.RES.66.6.1461

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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