Effect of Oxygen Withdrawal on Active and Passive Electrical Properties of Arterially Perfused Rabbit Ventricular Muscle

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Oxygen withdrawal from myocardial cells leads to changes of the transmembrane action potential (mainly action potential shortening), to cellular uncoupling, and to changes of vascular permeability. This study was aimed at the simultaneous measurement of electrical activity and passive electrical properties (extracellular and intracellular longitudinal resistance) in arterially perfused rabbit papillary muscles under different conditions of changed oxygen supply. These included 1) complete anoxia (erythrocyte-free perfusate), 2) hypoxia (PO$_2$ between 23–28 mm Hg, erythrocytes present) in the presence and absence of glucose, and 3) normoxia with erythrocyte-free perfusate. Similarly to myocardial ischemia, rapid cellular uncoupling occurred only after an initial stable period of approximately 17 minutes, and it required complete anoxia. The marked shortening of the action potential developed before cellular uncoupling. In six out of eight experiments, the fibers were inexcitable when uncoupling started. In severe hypoxia, no significant change of internal longitudinal resistance was observed over 35–40 minutes. The time course of the extracellular longitudinal resistance was different from the change in intracellular resistance: A marked decrease occurred almost immediately after the onset of oxygen withdrawal. This decrease was followed by a small increase in conduction velocity, which was most likely due to a change in the interstitial compartment (edema). It was observed during anoxic as well as during hypoxic perfusion. We conclude that 1) cellular uncoupling in arterially perfused tissue requires almost complete oxygen lack and occurs with a delay of more than 10 minutes, 2) marked action potential shortening precedes uncoupling, and therefore can not simply be attributed to an increase in free, intracellular calcium, and 3) vascular endothelial function is more sensitive to oxygen withdrawal than the myocyte. (Circulation Research 1989;64:532–541)
The effect of acute myocardial ischemia on the passive electrical properties of rabbit myocardium has been determined recently. This was done with an experimental model of an arterially perfused rabbit papillary muscle that allows the measurement of passive cable properties in perfused ventricular myocardium. This method offers several advantages: 1) It enables the distinction between changes of extracellular and intracellular longitudinal resistance without interference from an extracellular shunt resistance of the superfusate. This distinction is important, because the magnitude of the extracellular resistance of densely packed myocardium is approximately the same as the intracellular resistance, and therefore, affects conduction velocity. 2) It allows the assessment of the effects of changes in blood supply or composition of perfusate on the resistive values of the extracellular and intracellular space. 3) The problem of diffusion between the superfusate and the tissue core, which exists in excised muscle, is avoided.

In hypoxic ventricular muscle, the early changes of passive cable properties have not been studied. In superfused hypoxic preparations, a 1.7-fold increase of intracellular longitudinal resistance was measured after an exposure of 30 minutes (with the assumption of a constant extracellular resistor). In isolated hypoxic sheep and calf Purkinje fibers, cellular uncoupling occurred after a relatively long delay (30 minutes). The present experiments were undertaken to assess the time course of the changes in active and passive electrical properties of arterially perfused ventricular myocardium under test conditions that included complete absence of oxygen and substrate (anoxia) and relative lack of oxygen (hypoxia). Moreover, passive properties were measured under normoxic perfusion conditions but in the absence of oxygen carriers. As will be shown, a marked decrease in oxygen content of the perfusate is necessary to produce cellular uncoupling. The resistance of the extracellular space seems more sensitive to oxygen withdrawal and decreases after partial omission of oxygen supply.

Materials and Methods

Preparation and Perfusion of Papillary Muscles

Our method for preparation and arterial perfusion of rabbit papillary muscles has been described in detail. Rabbits of either sex weighing 2–3 kg were heparinized (200 U/kg), anesthetized with an intravenous injection (pentobarbital, 50 mg/kg), and killed by cervical dislocation. The hearts were excised, and the atria, the free wall of the left ventricle, and the nonperfused parts of the right ventricle were removed. After cannulation of the septal artery, the preparation was placed in the recording chamber. The final preparation consisted of a cylindrically shaped, arterially perfused right ventricular papillary muscle. The muscle was attached to the interventricular septum at its base and ran freely through the H2O-saturated artificial atmosphere of the recording chamber (for details see Kléber and Riegger and Kléber et al). The average diameter of the preparation was 1.04±0.1 mm (±SEM, n=23).

The perfusion solution was pumped to the recording chamber by a roller pump (Ismatec, Switzerland). Perfusion pressure was measured with a transducer (Statham, Gould Instruments, Cleveland, Ohio). It was kept constant ([40–45 mm Hg) by adjustment of the perfusion flowrate (control: 80–100 ml/min×100 g), whenever the perfusate was changed from control to test solution. Therefore, the pressure–flow ratios given in Figures 5 and 8 represent a measure of vascular resistance. Equilibration between the perfusate and a mixture of O2 and CO2 was achieved with a membrane oxygenator. The relative amounts of the two gases 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 to prevent diffusion losses to the environmental atmosphere. The solution for normoxic perfusion contained washed bovine erythrocytes (hematocrit 25–30%), albumin (2 g/liter), dextran (Mw 70,000; 40 g/liter), insulin (1 U/liter), heparin (400 units/liter) and Tyrode’s solution of the following composition (mM): Na+ 149, K+ 4.5, Mg2+ 0.49, Ca2+ 1.8, Cl− 133, HCO3− 25, HPO42− 0.4, glucose 20. The temperature of the preparation was maintained at 35°C.

Measurement of Electrical Activity, Extracellular, and Intracellular Longitudinal Resistance

A detailed description and a critical evaluation of the method used to determine passive cable properties in arterially perfused ventricular myocardium is given elsewhere. In essence, excitatory and subthreshold current pulses are applied to a muscle with a predefined macroscopic shape (cylinder), which is placed in an electrical insulator (artificial atmosphere of recording chamber). In such a situation, the pathways for current flow consist of the intracellular and extracellular spaces of the muscle. Because the muscle is supplied through its arterial vasculature, methodological errors produced by electrical shunts (related to a surrounding volume conductor) are prevented.

Values for the passive cable parameters are calculated from two measurements: 1) The longitudinal tissue resistance r, (in ohms per centimeter) is measured during the application of subthreshold constant current pulses between the apical end and the base of the muscle. It equals the potential difference V0, measured between two extracellular electrodes (tungsten wire, 50 μm diameter) on the longitudinal axis of the muscle divided by the product of the current strength (I) and the interelectrode distance (Ax). For the case of an idealized linear cable, r, is equal to a resistor which is composed of
the intracellular longitudinal resistance \(r_i\) (in ohms per centimeter) and the extracellular longitudinal resistance \(r_o\) (ohms per centimeter) in parallel:\(^1^2\):

\[
\frac{1}{r} = \frac{1}{r_i} + \frac{1}{r_o}
\]

2) The ratio \(q\) of the extracellular and the intracellular longitudinal resistance \(r_i:r_o=q\) is obtained from the amplitude \(\Delta V_m\) of the transmembrane action potential (measured with floating glass microelectrodes) and the amplitude of the extracellular bipolar electrogram \(\Delta V_o\), measured between the two extracellular electrodes:\(^1^2\):

\[
q = \frac{r_o}{r_i} = \frac{\Delta V_o}{\Delta V_m - \Delta V_o}
\]

\(r_i\) and \(r_o\) are directly calculated from \(r_i\) and \(q\). Values for the intracellular \((R_i)\), the extracellular \((R_o)\), and the tissue \((R_t)\) longitudinal specific resistances (in ohms centimeter) during normal perfusion were calculated from \(r_i\), \(r_o\), and \(r_t\), respectively, the fiber diameter and an assumed extracellular to intracellular space ratio of 1:3.\(^1^8\) During hypoxia, values are expressed as relative changes of \(r_t, r_i, r_o\) and \(r_t\). Calculation of \(R_t, R_i,\) and \(R_o\) is not possible in this condition because no measurements of changes of the extracellular to intracellular space ratio during hypoxia are available at present (see Kléber et al\(^1^9\)).

In addition to the values for the active electrical properties (resting potential, action potential) and the passive resistive properties \((r_i, r_o,\) and \(r_n\)), conduction velocity \((\Theta)\) in the longitudinal direction can be obtained from the same set of measurements. It corresponds to the ratio of conduction time (time between deflection and inflection on the extracellular electrogram) and the interelectrode distance \(\Delta x\). Statistical comparison of values during control and a given test period was made by the paired t test.

**Recording Procedure**

The extracellular bipolar electrogram was recorded as the voltage difference between two tungsten wires placed on the longitudinal axis of the cylinder, the transmembrane action potential was recorded as the voltage difference between an intracellular floating glass microelectrode and the apical extracellular electrode (see Figure 1 in Kléber and Riegger\(^1^9\)). No special attempt was made to accurately determine the upstroke velocity of the transmembrane action potential in the present series of experiments. This measurement would require a very close distance (<80 \(\mu\)m) between the intracellular and the extracellular electrode to avoid interference with the large extracellular field.\(^1^3,\(^1^9\) Its realization would be technically very difficult in the present experimental setup. The extracellular and the intracellular electrodes were connected to high input impedance buffer amplifiers (Analog Device 515) and amplified by differential instrumentation amplifiers. The strength of the subthreshold constant current pulse delivered between the apex and the base of the papillary muscle was measured in the feedback loop of an operational amplifier connected between the muscle and ground. The amplified voltages and constant current traces were stored on a Signal Memory Recorder (Max Meyer Electronics, Zürich, Switzerland) at a sampling rate of 100 msec during subthreshold events and 30 msec during suprathreshold events (upstroke of the action potential, bipolar extracellular electrogram). The data were analyzed by a computer (model 9617, Hewlett-Packard, Palo Alto, California) and displayed on a graphical plotter. In the majority of the experiments, whole action potential recordings were made simultaneously on an analog strip chart recorder (Watanabe, Mark VII). The dimensions of the muscle (diameter, interelectrode distance) were measured with a micrometer in the eyepiece of the binocular microscope (magnification, \(\times 25\)). The preparations were stimulated by DC-pulses of 0.5–1.5 msec duration at double threshold strength, and at a cycle length of 479±14 msec (SEM; \(n = 23\)).

**Experimental Protocol, Production of Anoxia and Hypoxia**

Before exposure to the test solution, the fibers were perfused with normal, oxygenated perfusate for at least 30–40 minutes. Repetitive measurements of electrical activity and longitudinal resistances during this period were made in eight experiments (Table 1). During this period the \(\text{H}_2\text{O}\)-saturated atmosphere of the recording chamber contained a mixture of \(\text{N}_2\) (75%), \(\text{O}_2\) (20%), and \(\text{CO}_2\) (3%).
Anoxia and absence of substrate was produced by perfusing the preparation with erythrocyte-free and glucose-free solution (dextran and albumin present) that was equilibrated with a mixture of N₂ and CO₂ in the membrane oxygenator. The fraction of CO₂ in this gas was adjusted to produce a pH of the perfusate of 7.35–7.45. Previous experiments from our laboratory had shown that the PO₂ in erythrocyte-free solutions decreases to below 5 mm Hg if mixing between the perfusate and the environment is prevented by stainless steel tubing.

Hypoxia was produced by oxygen withdrawal in the membrane oxygenator from an erythrocyte-containing perfusate. After a single passage through the membrane oxygenator, the O₂ saturation decreased to 40–50% of normal as measured with an IL 282 Oximeter (Nanolab, Switzerland). This corresponded to an oxygen content of 5.8–7 ml O₂/100 ml perfusate and to a PO₂ of 20–25 mm Hg. For both conditions, anoxia and hypoxia, the composition of the artificial, H₂O-saturated atmosphere was changed to N₂ (95%) and CO₂ (5%). The oxygen contamination in this atmosphere was controlled in each experiment and was less than 1%.

In four experiments, the preparation was perfused with an erythrocyte-free solution under normoxic conditions (normal PO₂ in perfusate and artificial atmosphere). Each heart was subjected to only one test period lasting 30–50 minutes. The n values given in the text always refer to number of hearts.

Results

Control Perfusion

Values obtained at the beginning of the control perfusion (Table 1A) were in close agreement to those previously reported from the same preparation. The main difference between these values and those obtained from superfused excised tissue is the large value for the extracellular resistor and, consequently, for the ratio rₒ:ᵣᵣ (for discussion, see Kléber and Riegger). Table 1B shows that these values were not significantly altered by a perfusion period of 30 minutes. In particular, there was no observable decrease in extracellular longitudinal resistance or resistance to blood flow (not shown), which indicated stable perfusion conditions.

Anoxia and Glucose Withdrawal

The intracellular recordings confirmed the well-known changes in resting potential and action potential configuration. The result of a single experiment is shown in Figure 1. The main change in action potential configuration was the very rapid development of transmembrane action potential shortening in the presence of a relatively well preserved resting potential and action potential amplitude (Figure 1, top trace). In the present experiments the muscles became inexcitable to apical stimulation after 15–23 minutes. Therefore, the measurement of the ratio rₒ:ᵣᵣ became impossible after this period. The extracellular bipolar electrograms and the action potential upstrokes are shown on the middle and bottom traces of Figure 1 on a different time scale. In contrast to the amplitude of the transmembrane potential, the amplitude of the extracellular signal decreased rapidly from 63 mV (control) to 48 mV after 7 minutes and 41 mV after 14 minutes. This marked change was due to an early decrease in extracellular resistance, as shown below (Figure 4).

The mean change (n=8 different hearts) in action potential amplitude (ΔVp), extracellular voltage (ΔVₑ), and the resulting change of the ratio rₒ:ᵣᵣ=q is shown on Figure 2. There was a small but significant decrease in action potential amplitude from 99 mV (control) to 89 mV after 12.3 minutes of anoxic perfusion (Figure 2A). This was associated with a small reduction in resting membrane potential (not shown). ΔVₑ decreased from 53 mV to 29 mV during the same time interval. Figure 2B shows that the resulting decrease of the ratio q was as much as 58%, from 1.2 (control) to 0.5.

A decrease of rₒ:ᵣᵣ=q could have reflected either an increase of intracellular longitudinal resistance (rᵣ) or a decrease in extracellular longitudinal resistance (rₒ) or both. The measurement of the longitudinal tissue resistance (rᵣ) by subthreshold current pulses (which was independent of propagated activ-
Anoxic perfusion differed from control perfusion in two important respects: 1) by the absence of erythrocytes, which are likely to contribute to the lumped specific resistance of the extracellular space and 2) by increased flow rate (decreased vascular resistance), that is expected to decrease extracellular longitudinal resistance ($r_o$). In an attempt to estimate the contribution of both vascular components to the decrease of $r_o$, we compared the changes of $r_o$ with the changes in the pressure–flow ratio, as shown in Figure 5. This comparison was possible in seven out of the eight experiments in which the first measurement of $r_o$ and $r_i$ was obtained very early after the onset of hypoxic perfusion (mean, 1.6 minutes). At this time, $r_o$ had decreased by 27%, the concomitant decrease in pressure–flow ratio was 70%. Between 1.6 minutes and 9.7 minutes of anoxic perfusion the pressure–flow ratio remained almost constant, whereas $r_o$ decreased further. The comparison indicated that at least half of the decrease of $r_o$ occurred during stable perfu-
Figure 5. Comparison between the change of extracellular resistance (r_o) and the change of pressure-flow ratio (p:f) during anoxic perfusion (mean±SEM from seven of the eight experiments shown in Figures 2—4). The earliest measurements of r_o and p:f (mean 1.6 minutes) are compared with measurements obtained at a later stage (9.6 minutes). Absence of erythrocytes, withdrawal of glucose and anoxia leads to a rapid decrease of the pressure-flow ratio to a steady level of 26–30%. Extracellular resistance decreases to 73% after 1.6 minutes and to 45% after 9.6 minutes. This indicates that only 27% (or less) of the 55% decrease of r_B can be attributed to a vascular component.

The time course of the changes in conduction velocity (0) was variable among the different experiments. In all experiments, an initial increase of conduction velocity was observed, with a mean value of +25% (±11.8%, SEM, p<0.05) after 12 minutes. Afterwards, 0 continued to increase in two experiments, remained stable in one experiment, and decreased in the others. This was due to the variability in onset of uncoupling and in development of inexcitability among the individual experiments. The mean theoretical change in 0 predicted by the changes in r_o+r_j (linear cable theory, see Kléber et al 13) was +26% after 12 minutes. This indicated that changes in extracellular and intracellular longitudinal resistance were the main factors in determining the change 0 within the first 12 minutes after oxygen withdrawal.

Hypoxic Perfusion in Presence and Absence of Glucose

In 11 experiments, the erythrocyte-containing perfusate was pumped through the membrane oxygenator and exposed (during a single passage) to a mixture of N2 and CO2. This produced a marked "hypoxia" with a hemoglobin saturation of 40–50% and a PO2 of 20–25 mm Hg.

In six experiments, the effect of hypoxia on active and passive electrical properties was measured in presence of glucose. In contrast to the anoxic condition, the fibers remained excitable during test period of 30–50 minutes. There were no major changes in action potential configuration, action potential amplitude (Figure 6) remained almost constant. A major change was observed in the amplitude of the extracellular electrogram (Figure 6) and in tissue resistance (not shown); both decreased significantly during the test period. Figure 7 and Table 2 show that these changes were caused by a decrease of the extracellular resistance (to 46% after 27 minutes) whereas no cellular uncoupling occurred during the whole period of hypoxic perfusion. In this setting, the flow was monitored repeatedly in three of the six experiments. The pressure-flow ratio remained constant throughout the test period (98±3.5%, ±SEM, after 6 minutes and 101±9.5%, ±SEM, after 30 minutes) in these experiments.

The observed effects were not dependent on the presence of glucose in the hypoxic perfusate. This was shown in five experiments in which glucose was omitted. Virtually the same decrease of extracellular resistance over a test period of 30–40 minutes was measured as shown on Figure 7 and Table 2 for the presence of glucose. No uncoupling occurred during the test period.
**Table 2. Changes of Intracellular \( r_i \) and Extracellular \( r_e \) Resistance, Longitudinal Tissue Resistance \( r_L \), Amplitude of the Extracellular Electrogram \( \Delta V_e \) and Amplitude of the Transmembrane Action Potential \( \Delta V_m \) at Different Intervals During the Test Perfusion**

<table>
<thead>
<tr>
<th>Test condition</th>
<th>Parameter (mean±SEM; n)</th>
<th>Time interval after onset of perfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10–15 min</td>
</tr>
<tr>
<td>Anoxia, absence of glucose, and erythrocytes</td>
<td>( r_i )</td>
<td>+12±12; 6</td>
</tr>
<tr>
<td></td>
<td>( r_e )</td>
<td>-53±9; 6*</td>
</tr>
<tr>
<td></td>
<td>( r_L )</td>
<td>-36±7; 8*</td>
</tr>
<tr>
<td></td>
<td>( \Delta V_e )</td>
<td>-47±10; 6*</td>
</tr>
<tr>
<td></td>
<td>( \Delta V_m )</td>
<td>-12±3; 6*</td>
</tr>
<tr>
<td>Hypoxia, presence of glucose and erythrocytes</td>
<td>( r_i )</td>
<td>+1±3; 3</td>
</tr>
<tr>
<td></td>
<td>( r_e )</td>
<td>-40±9; 3*</td>
</tr>
<tr>
<td></td>
<td>( r_L )</td>
<td>-25±5; 6*</td>
</tr>
<tr>
<td></td>
<td>( \Delta V_e )</td>
<td>-27±8; 6*</td>
</tr>
<tr>
<td></td>
<td>( \Delta V_m )</td>
<td>-1±1; 6</td>
</tr>
<tr>
<td>Normoxic perfusion, absence of erythrocytes</td>
<td>( r_i )</td>
<td>-15±5; 4</td>
</tr>
<tr>
<td></td>
<td>( r_e )</td>
<td>-41±9; 4*</td>
</tr>
<tr>
<td></td>
<td>( r_L )</td>
<td>-31±3; 4*</td>
</tr>
<tr>
<td></td>
<td>( \Delta V_e )</td>
<td>-20±8; 4*</td>
</tr>
<tr>
<td></td>
<td>( \Delta V_m )</td>
<td>-3±5; 4</td>
</tr>
</tbody>
</table>

Values are given as % changes. *Statistically significant difference from control value (p<0.05).

**Normoxic Perfusion With No Oxygen Carriers**

In four experiments, the fibers were perfused with a solution containing no erythrocytes (but 40 g/l dextran and 2 g/l albumin, see "Materials and Methods"), which was equilibrated with 94% O2-6% CO2. During the test perfusion, the artificial atmosphere was composed of 94% O2-6% CO2. Within 3 minutes, the flow necessary to maintain pressure was increased by 3.5-fold (decrease of pressure–flow ratio to 29%, bottom panel of Figure 8), afterwards the pressure–flow ratio remained constant. As expected, there was no significant change in the configuration of the transmembrane action potential during the test period. The time course of the changes in intracellular and extracellular longitudinal resistances are shown on Figure 8 (top panel) and summarized on Table 2. Similarly to the hypoxic perfusion, there was a significant decrease of \( r_L \) to 50.3±13% after 28 minutes, but no significant changes of intracellular longitudinal resistance or action potential amplitude.

**Discussion**

**Changes in Intracellular Longitudinal Resistance**

The time course of cellular uncoupling in anoxic ventricular myocardium was almost identical to myocardial ischemia and qualitatively similar to uncoupling in anoxic Purkinje fibers: Uncoupling starts as a fast, precipitating process only after an initial stable phase which lasts between 12–17 minutes in ventricular myocardium and approximately 30–40 minutes in Purkinje fibers.

The present method enables the calculation of the longitudinal resistance \( r_L \) which lumps the individual resistances made up by the complex cytoplasmic pathways for current flow and the series resistors of the intercellular junctions. Our results do not allow the distinction of the changes between these two components to be calculated. However, it is likely that factors which contribute to an increased junctional resistance at a cellular level (increased free cellular calcium and/or acidosis) are responsible for the comparable effect observed in the anoxic preparation. Maintained electrical communication during the initial phase of myocardial anoxia and the close dependence of cellular coupling on intracellular Na+ and Ca2+ suggests that the anoxic myocardial cell initially is able to keep these ions at a normal or only slightly elevated level. It is in close accordance with measurements of only moderate elevation of intracellular Na+ activity in hypoxic myocardium, and with a delayed increase of intracellular calcium in early myocardial ischemia and during partial metabolic blockade. Recent experiments with isolated Purkinje fibers have shown that the contribution of a decreased intracellular pH to uncoupling is minor, and certainly less than anticipated from previous work. This might explain why the results in ischemia, in which a marked acidosis develops, are not essentially different from myocardial hypoxia, in which acidosis is small. In summary, the studies on intracellular ion activities or concentrations (by means of a variety of methods) and the present electrical data provide evidence for a close relation between cellular uncoupling, increase of cellular Na+ and Ca2+, and the onset of irreversible anoxic damage.

An explanation for maintained electrical coupling during the initial 15–17 minutes of complete anoxia is difficult to provide. It is astonishing that total inhibition of glycolysis or glycogen depletion is required to produce an immediate increase in free intracellular calcium after withdrawal of oxygen.
In the absence of complete metabolic blockade, the chemical energy synthesized by anaerobic glycolysis appears to match, for a limited initial period, with a decreased energy demand of the cell. This decreased demand probably is due to the failure of the hypoxic myocardial cell to contract\(^{30}\) and to a decreased cycling of Ca\(^{2+}\).\(^{26,31}\) A dependence of this initial phase on the amount of consumed energy would explain the longer delay before the onset of uncoupling in Purkinje fibers in which steady-state oxygen (or energy) consumption is about 25% of ventricular myocardium.\(^{32}\)

Oxygen diffusing from a superfusate into excised tissue will continuously decrease in concentration because ongoing consumption acts as a sink.\(^{33}\) This makes it difficult in superfused tissue to estimate a critical O\(_2\) level at which uncoupling develops. Thus, uncoupling was observed in ventricular tissue superfused with solutions containing up to 40 mm Hg of oxygen by Wojtizak\(^{14}\) who qualitatively demonstrated for the first time cell-to-cell uncoupling in hypoxic myocardium. In these experiments, no measurements of \(r_\lambda\) were performed in the first 30 minutes, and \(r_\lambda\) was assumed as constant. The fact that \(r_\lambda\) rose much more slowly (threefold increase after 60 minutes) may have reflected an inhomogeneity between the core and the superficial layers of the superfused fibers. In the present experiments, the oxygen transported by the microcirculation assured a more homogeneous supply. The results show that a low level of O\(_2\) is required for uncoupling to develop.

In contrast to anoxia, severe hypoxia with more than 40-50% saturation of hemoglobin and an arterial PO\(_2\) of 25 mm Hg or less did not affect electrical activity or longitudinal resistance significantly, independent of the presence of glucose.

**Electrical Activity and Uncoupling**

The well-known alterations in the cardiac action potential, which mainly consisted in a marked shortening, occurred always before cellular uncoupling, similarly to the changes observed in anoxic Purkinje fibers.\(^{15}\) Most likely, action potential shortening is due to an increased K\(^+\) conductance and to a loss of inward-going rectification of a K\(^+\) channel.\(^{34}\) Both an increased intracellular free Ca\(^{2+}\) and cytosolic ATP depletion were found to increase repolarizing K\(^+\) current.\(^{35,36}\) The dissociation between action potential shortening and uncoupling complicates the discussion of the underlying cause, however. If the anoxic action potential shortening were to be explained by an increase in intracellular Ca\(^{2+}\), the sensitivity of \(r_\lambda\) to the initial increase of intracellular Ca\(^{2+}\) would be relatively low. This could be the case in a system of series resistors in which the junctional part would be considerably smaller than the cytoplasmic part during normal function. Alternatively, the cytosolic ATP content might decrease to a very low value (<1 mM)\(^{36}\) before a significant increase of free [Ca\(^{2+}\)], developed. A low ATP content preceding the increase of free Ca\(^{2+}\) was also postulated to explain the dissociation between the increase in resting tension and the delayed increase in resting Ca\(^{2+}\) in the hypoxic ferret heart.\(^{26}\)

**Changes in Extracellular Longitudinal Resistance**

In the absence of a conducting fluid layer around the cylindrical muscle, current during propagating activity and during application of a subthreshold pulse was forced to flow through the tissues proper intracellular or extracellular spaces. Therefore, the extracellular longitudinal resistance \(r_\lambda\) represents a global parameter characterizing the resistive elements in the interstitial as well as in the intravascular spaces. It will predominantly depend on the specific resistances of the different compartments (blood, interstitial fluid) and on the size of these compartments. Values given for the volume fraction of the extracellular space range between 20–30%\(^{18,37,38}\). A value of 25% was taken to calculate the lumped specific resistance of the extracellular space, which in the present and in previous experiments\(^{12,13}\) was found to be between the resistivity of Tyrode's solution and blood. This suggests that factors such as the collagen matrix of the interstitial space\(^{39}\) and the blood cells\(^{12}\) of the intravascular space may decrease the mobility of charge carriers and increase the resistivity of the extracellular compartment relative to the Tyrode's solution. A qualitative indication of role of the intravascular space in determining the extracellular resistance was derived from the observation that arrest of flow...
(and diminution of intravascular volume) is associated with a marked and immediate increase of $r_0$.\textsuperscript{12,13} In the present experiments, we monitored the flow necessary to maintain constant perfusion pressure and compared the change in pressure–flow ratio (reflecting a change in flow at a constant perfusion pressure) to the change in $r_0$. This was to get a qualitative estimate of the effect of the vascular compartment volume and/or red blood cells on $r_0$.

During control perfusion, the pressure–flow ratio, as well as the extracellular resistance, remained stable. This reflected a constant vascular resistance and the expected steady-state between filtration and reabsorption of interstitial fluid when red blood cells,\textsuperscript{40} dextran,\textsuperscript{41} and trace amounts of albumin\textsuperscript{42} were added to the perfusate. Transition to anoxic, erythrocyte-free perfusion was characterized by a rapid increase of flow and a decrease of vascular resistance. Two types of experiments were carried out to separate the effect of erythrocyte removal from the effect of anoxia or hypoxia: 1) Normoxic perfusion with an erythrocyte-free solution, and 2) hypoxic perfusion in presence of erythrocytes. Perfusion at normal $P_O_2$ but without erythrocytes produced the same initial increase of flow rate and decrease of vascular resistance as anoxic perfusion. This indicated that the initial changes of the perfusion parameters shown on Figures 4 and 7 were mainly caused by erythrocyte removal. The observations that erythrocyte-free perfusion alone produced a smaller decrease of $r_0$ than anoxia suggested that the early change of extracellular resistance in anoxia had an extravascular component. This argument was further sustained by the finding that hypoxic perfusion (oxygen saturation of 40–50%), $P_O_2$ of 23–25 mm Hg) significantly decreased $r_0$ within the first 3 minutes without changing the pressure–flow ratio. Therefore, even partial removal of $O_2$ caused a decrease of the extravascular component of $r_0$ (i.e., an increase in microvascular permeability and formation of edema) within less than 3 minutes. Our results are consistent with macromolecular leakage reported by Sutherland and Young after repetitive, very brief (<2 minutes) periods of anoxia in the rat heart in vivo.\textsuperscript{43} The metabolic mediators of these early changes are not precisely known, however.

In all three test conditions (anoxia, hypoxia, and normoxic perfusion without erythrocytes), the extracellular resistance continued to decrease after 3–5 minutes (when the pressure–flow ratio and the flow were constant). A plateau of approximately 40% of the initial value was reached after 8 minutes in complete anoxia and after 20–25 minutes in hypoxia and normoxia. Erythrocyte removal alone (normoxia) has been reported to cause an increase in coronary microvascular permeability, which explains the decrease in $r_0$ during stable perfusion conditions. The exact reason for this red blood cell–dependent change in permeability is not clear. Most likely, it is due to direct effect of the erythrocytes and not to relative lack of $O_2$ (see McDonagh\textsuperscript{40} for discussion). The direct effect of a low $P_O_2$ to increase the permeability of the endothelial barrier, which is well documented by tracer studies,\textsuperscript{44,45} most likely explains the decrease of $r_0$ during hypoxic perfusion. In conclusion, the very rapid decrease of the extracellular resistance observed during anoxic, erythrocyte-free perfusion in the present experiments (Figure 4) was caused by both erythrocyte removal and anoxia. Whether the decrease of extracellular resistance and the associated edema formation in anoxia and hypoxia was a homogeneous process it is not clear. Metabolic inhomogeneity with anoxic zones of several hundred micrometers in diameter has been demonstrated during hypoxic perfusion of rat hearts.\textsuperscript{46} Such major extracellular inhomogeneities were unlikely in the present experiments because of the constant shape of the extracellular electrogram and the constant pressure–flow ratio during the first 10 to 15 minutes of hypoxia.

Macromolecular leakage is known to occur after reperfusion of previously ischemic myocardium.\textsuperscript{47} It seems to be associated with endothelial Ca\textsuperscript{2+} overload and is prevented by calcium entry blockade.\textsuperscript{48} The present results suggest that at least part of the leaks that become manifest on resumption of coronary flow are formed during perfusional arrest. As an electrical parameter, the extracellular resistance directly scales the amplitude of the cardiac extracellular field, and consequently, the electrocardiogram. As a parameter for the measurement of capillary leakage and associated interstitial edema, it is only indirect and qualitative. However, the simultaneous assessment of intracellular and extracellular resistances allows the comparison of the effect of the duration and the degree of hypoxia on cellular electrical activity and cell-to-cell coupling with the effect on the extracellular space. This comparison clearly showed that cellular uncoupling occurs only after a delay and requires almost complete anoxia, whereas capillary leakage is immediate and becomes manifest after partial oxygen withdrawal. This suggests that the endothelial function is more sensitive to the metabolic consequences of hypoxia than the myocytes.

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