Rate-Dependent Effects of Hypoxia on Internal Longitudinal Resistance in Guinea Pig Papillary Muscles

Yoshihiro Hiramatsu, Jack W. Buchanan, Stephen B. Knisley, and Leonard S. Gettes

We have studied the independent and combined effects of 30 minutes' exposure to hypoxia and an increase in stimulation frequency from 0.5 Hz to 3.0 Hz on internal longitudinal resistance \( (r_l) \) and conduction in guinea pig papillary muscles through the use of the voltage ratio method with air as the external insulator. Increasing stimulation frequency from 0.5 to 3.0 Hz in the presence of \( \text{O}_2 \) caused no significant change in \( r_l \). Hypoxia to a level of \( \text{PO}_2 = 30 \text{ mm Hg} \) caused an increase in \( r_l \) that averaged 13.7\% at a stimulation frequency of 0.5 Hz and 46\% at 3.0 Hz. In all experiments, the increase in \( r_l \) during hypoxia at 3.0 Hz was greater than the increase at 0.5 Hz, but conduction velocity did not change at either rate. These results indicate that hypoxia causes rate-dependent cellular uncoupling but, under the conditions of our experiments, does not cause significant changes in conduction. (Circulation Research 1988;63:923–929)

The velocity of impulse propagation in the heart depends on a variety of electrophysiological and geometric parameters.\(^1\)–\(^3\) One dimensional cable theory suggests a predictable relation between the maximal rate of rise of the action potential upstroke \( (V_{max}) \), internal longitudinal resistance \( (r_l) \), and conduction velocity.\(^4\) Previous studies in our laboratory\(^3\) have confirmed this predictability when interventions designed to primarily affect \( V_{max} \) were employed. Hypoxia, one of the several factors that constitute ischemia, has been reported to cause an increase in \( r_l \)\(^5\) and, after 30 minutes of exposure, to slow conduction.\(^6\) Thus, hypoxia appears to contribute independently to the increase in \( r_l \) associated with simulated and actual ischemia in papillary muscle preparations\(^5\),\(^6\) and may contribute to the associated conduction slowing.\(^3\)

In the intact heart, the conduction slowing induced by ischemia is rate-dependent.\(^1\)\(^1\),\(^\text{\textsuperscript{12}}\) However, the independent contribution of hypoxia to this rate-dependent change is not known. An increase in stimulation frequency by itself causes only slight changes in \( V_{max} \) and conduction velocity,\(^5\) while its effects on \( r_l \) are controversial. Some have reported an increase,\(^5\),\(^1\)\(^3\),\(^1\)\(^4\) while others have reported no change.\(^1\)\(^5\),\(^1\)\(^6\)

There are no studies that address the combined effects of an increase in rate and hypoxia on \( r_l \) and conduction. The purpose of our study was to determine the independent and combined effects of hypoxia and an increase in stimulation frequency on \( r_l \) in guinea pig papillary muscles and to correlate any changes in \( r_l \) with associated changes in conduction velocity. It was our hope that these results would ultimately contribute to our understanding of the role of stimulation frequency on the conduction slowing that occurs in the setting of myocardial ischemia.

Materials and Methods

Guinea pigs weighing 350–400 g were anesthetized with methoxyflurane. The hearts were rapidly removed, and right ventricular papillary muscles were excised. The papillary muscle preparations used in these experiments were characterized by a lack of visible branching, a maximal diameter equal to or less than 1.6 mm, and a length equal to or greater than 6 mm. Preparations were placed on a small rubber platform, were covered by a thin layer of nylon mesh, and were superfused from the tendinous end with a roller pump (Figure 1; Cole-Parmer, Chicago, Illinois) to maintain a constant rate of 2.8–3.2 ml/min. The composition of the Tyrode's solution superfusing the preparation was (mM) NaCl 125, KCl 5.4, CaCl\(_2\) 1.8, MgCl\(_2\) 1.05, NaHCO\(_3\) 24, NaH\(_2\)PO\(_4\) 0.42, and glucose 5. The pH ranged from 7.3 to 7.5, and temperature was maintained at 36\(^\circ\)–37\(^\circ\) C. The control solution was gassed with 95\% \text{O}_2-5\% \text{CO}_2. Hypoxia was produced by gassing with 95\% \text{N}_2-5\% \text{CO}_2 and by filling the...
Perspex-lined Faraday cage in which the experimental chamber was located with nitrogen under slight positive pressure. This enabled us to maintain a Po2 between 20 and 30 mm Hg. Suction applied to the cut end of the preparation was adjusted to provide a thin uniform layer of fluid coursing over the surface of the fiber.

The preparations were stimulated at the cut end by bipolar electrodes spaced 0.5 mm apart with pulses of 1 msec duration and two times diastolic threshold strength.

**Electrical Measurements**

We used our modification\(^1\) of the voltage ratio method originally reported by Weidmann\(^2\) for determination of \(r_e\). In all experiments, three glass micro-electrodes filled with 3 M KCl and having a resistance of 15–25 MΩ were used simultaneously (Figure 1). Electrodes 1 (intracellular) and 2 (extracellular) were placed 1.5–2.5 mm from the stimulating electrodes. Electrode 3 (extracellular) was placed at 1–2 mm from the tendinous end. The local transmembrane action potential (\(V_m\)) was recorded between electrodes 1 and 2. The intracellular potential (\(V_i\)) was recorded between electrodes 1 and 3, while the extracellular potential (\(V_o\)) was recorded between electrodes 2 and 3. The ratio of the internal longitudinal resistance and the external longitudinal resistance, \(r_i/r_o\), was determined by the ratio of \(V_i\) to \(V_o\) as originally described by Weidmann\(^2\):

\[
\frac{V_i}{V_o} = \frac{r_i}{r_o} \quad (1)
\]

We measured total resistance (\(r_{tot}\)) in each experiment by recording the potential drop between the extracellular electrodes when subthreshold constant current pulses were introduced into the extracellular space through separate electrodes at opposite ends of the preparation.\(^1\) Weidmann\(^2\) showed that the steady-state intracellular and extracellular potential changes caused by the current pulse were the same beyond two or more length constants from the end of the muscle. We intentionally positioned the extracellular recording electrodes away from the ends so that current in the region between the recording electrodes flowed only in the longitudinal direction. Therefore, the region between recording electrodes was electrically equivalent to the parallel combination of intracellular and extracellular longitudinal resistance. \(r_o\) was calculated using the following equations:

\[
r_{tot} = r_i + r_o \quad (2)
\]

\[
r_o = \frac{r_{tot} V_i}{V_o + 1} \quad (3)
\]

In our preparation, the fluid outside the muscle caused a lower \(r_o\) than that reported for the original silicon oil preparation\(^2\) or in the recently reported perfused rabbit papillary muscle preparation.\(^1\) However, \(r_o\) in our preparations was sufficiently high to permit an accurate measurement of \(V_o\). Intracellular resistivity (\(R_i\)) was calculated from the values of \(r_i\), the distance (l) between the extracellular electrodes used to record \(V_o\), and intracellular area (A) determined as previously discussed.\(^1\)

\[
R_i = r_i \cdot \frac{A}{l} \quad (4)
\]

The voltage signals and the electronically differentiated upstrokes of \(V_m\) were passed through high

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**FIGURE 1.** Diagram of experimental preparation (left) and signals recorded by the three electrodes (right). These signals are shown at a slow sweep speed (top) and a rapid sweep speed (bottom). Also shown in the bottom right panel is the second time derivative of the \(V_o\) signal (\(d^2V_o/dt^2\)) from which conduction time (C.T.) was determined.
input impedance differential amplifiers having a frequency response of direct current to 8,000 Hz, displayed on a Tetronix 565 dual beam oscilloscope and photographed on Polaroid film. In addition, the waveforms were stored automatically every 2 minutes on a Norland 3001 DMX programmable digital oscilloscope. The sampling rate was 20 kHz, and the voltage resolution was 0.05 mV (12 bits). After each experiment, the digitized waveforms were retrieved and automatically measured to provide action potential amplitude, action potential duration at 90% repolarization (APD90), and the maximum rate of rise of the action potential upstroke (Vmax) as well as magnitude of Vr and Vo. Conduction velocity was determined using time derivatives of the Vo signal (Figure 1) as previously described.17

For acceptance into the study, we required that the Vo signal have a clearly defined plateau and that the control values for the amplitudes of Vm, Vr, and Vo, determined at the same point in time, satisfy the following criteria:

\[ V_m \geq 105 \text{ mV}; \quad V_r \geq 100 \text{ mV}; \quad V_o \geq 5 \text{ mV}. \]

We also required that the resting potential be more negative than -80 mV, Vmax \( \geq 150 \text{ V/sec, and APD}_{90} \geq 150 \text{ msec.} \)

Values are expressed as mean ± SD. Statistical significance was determined with Student’s t test for paired and unpaired data, as appropriate.

**Results**

The mean values of the various parameters, obtained during superfusion with the control Tyrode’s solution in the 11 experiments included in this report, are shown in Table 1. These control values are similar to those we reported previously17 in the validation of our modification of the Weidmann method.18

Our initial three experiments were designed to determine the effect of an increase in stimulation frequency from 0.5 Hz with 50 minutes under normoxic conditions. These results are shown in Table 2. An increase in stimulation frequency caused a decrease in Vm and Vo in each experiment, while their ratio (V/Vo) increased by 10% and 9% in two experiments. An increase in stimulation frequency caused a decrease in Vr and Vo in each experiment, while their ratio (V/Vo) increased by 10% and 9% in two experiments. The effects of hypoxia at 0.5 Hz were determined before the stimulation frequency was increased to 3.0 Hz. Table 3 shows the mean values for the various parameters measured before and after each hypoxic period in the six experiments. Figure 2 shows the time course of the changes in r0, V0, and conduction velocity in these experiments. The control values are not significant.

**Table 1. Control Values (mean ± SD) (n = 11)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vm (mv)</td>
<td>110.4 ± 4.1</td>
</tr>
<tr>
<td>Vr (mv)</td>
<td>10.9 ± 2.4</td>
</tr>
<tr>
<td>V/Vo (kΩ)</td>
<td>10.7 ± 3.3</td>
</tr>
<tr>
<td>rm (kΩ)</td>
<td>0.79 ± 0.18</td>
</tr>
<tr>
<td>r0 (kΩ)</td>
<td>9.6 ± 4.7</td>
</tr>
<tr>
<td>A (cm²)</td>
<td>1.0 ± 0.4 x 10⁻²</td>
</tr>
<tr>
<td>I (cm)</td>
<td>0.35 ± 0.03</td>
</tr>
<tr>
<td>R0 (Ω cm)</td>
<td>248.4 ± 94.5</td>
</tr>
<tr>
<td>Θ (cm/sec)</td>
<td>67.3 ± 6.3</td>
</tr>
</tbody>
</table>

V0, intracellular potential; Vr, extracellular potential; rm, total resistance; r0, internal longitudinal resistance; A, intracellular area; I, distance between extracellular electrodes used to record V0; R0, intracellular resistivity; Θ, conduction velocity.

**Table 2. Effect of Increasing Stimulation Frequency in Presence of O2 (n = 3)**

<table>
<thead>
<tr>
<th>Frequency</th>
<th>0.5 Hz</th>
<th>3.0 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vm (mv)</td>
<td>114.4 ± 3.0</td>
<td>109.3 ± 2.9</td>
</tr>
<tr>
<td>Vr (mv)</td>
<td>8.5 ± 1.8</td>
<td>7.9 ± 1.1</td>
</tr>
<tr>
<td>V/Vo (kΩ)</td>
<td>14.0 ± 3.7</td>
<td>14.2 ± 2.6</td>
</tr>
<tr>
<td>rm (kΩ)</td>
<td>0.85 ± 0.27</td>
<td>0.83 ± 0.26</td>
</tr>
<tr>
<td>r0 (kΩ)</td>
<td>13.2 ± 6.7</td>
<td>13.0 ± 5.5</td>
</tr>
<tr>
<td>R0 (Ω cm)</td>
<td>262.0 ± 120.8</td>
<td>271.3 ± 140.6</td>
</tr>
<tr>
<td>Θ (cm/sec)</td>
<td>71.6 ± 0.8</td>
<td>71.9 ± 4.9</td>
</tr>
</tbody>
</table>

(V0 = [0.90 ± 0.5] x 10⁻² cm², I = 0.35 ± 0.4 cm)

V0, intracellular potential; Vr, extracellular potential; rm, total resistance; r0, internal longitudinal resistance; A, intracellular area; I, distance between extracellular electrodes used to record V0; R0, intracellular resistivity; Θ, conduction velocity.

Table 3. Effect of 30 Minutes of Hypoxia at Stimulation Frequencies of 0.5 and 3.0 Hz (n = 6)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (normoxia)</th>
<th>Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>0.5 Hz</td>
<td>3.0 Hz</td>
</tr>
<tr>
<td>Vm (mv)</td>
<td>108.3 ± 3.6</td>
<td>105.9 ± 4.1*</td>
</tr>
<tr>
<td>Vr (mv)</td>
<td>11.7 ± 1.9</td>
<td>10.2 ± 1.9*</td>
</tr>
<tr>
<td>V/Vo (kΩ)</td>
<td>9.5 ± 2.2</td>
<td>10.7 ± 2.5*</td>
</tr>
<tr>
<td>rm (kΩ)</td>
<td>0.74 ± 0.16</td>
<td>0.75 ± 0.17</td>
</tr>
<tr>
<td>r0 (kΩ)</td>
<td>8.0 ± 3.1</td>
<td>9.1 ± 3.6*</td>
</tr>
<tr>
<td>R0 (Ω cm)</td>
<td>251.5 ± 91.3</td>
<td>285.5 ± 108.9*</td>
</tr>
<tr>
<td>Θ (cm/sec)</td>
<td>64.9 ± 7.3</td>
<td>65.2 ± 7.8</td>
</tr>
</tbody>
</table>

(V0 = [1.13 ± 0.5] x 10⁻² cm², I = 0.35 ± 0.03 cm)

V0, intracellular potential; Vr, extracellular potential; rm, total resistance; r0, internal longitudinal resistance; A, intracellular area; I, distance between extracellular electrodes used to record V0; R0, intracellular resistivity; Θ, conduction velocity; *p<0.05 compared with control; †p<0.05 compared with hypoxia at 0.5 Hz.
FIGURE 2. Mean±SD of changes in $r$, and conduction velocity ($\Theta$) from the six experiments in which the effects of hypoxia were determined first at a stimulation frequency of 0.5 Hz and then at a stimulation frequency of 3.0 Hz. Each hypoxic period is shaded.

significantly different from the control values of the entire group of 11 experiments shown in Table 1. During hypoxia at 0.5 Hz, $V_i$ and $V_o$ both decreased significantly, but the decrease in $V_o$ exceeded that in $V_i$, resulting in a significant increase in $V_i/V_o$. There was no significant change in $r_0$. Thus, the increase in $r_i$ was similar to the increase in $V_i/V_o$ and averaged 13.7%. During this period, conduction velocity did not change significantly. The preparation was then superfused with the control (normoxic) solution for 50 minutes. $r_i$ increased by an additional 4% during the first 5 to 10 minutes following reoxygenation. All values then returned to within 5% of their control levels. Stimulation frequency was next increased to 3.0 Hz for 20 minutes, while normoxic conditions were maintained. Oxygen was then replaced with nitrogen, and stimulation at 3.0 Hz was continued for 30 additional minutes. During this time, $V_i/V_o$ increased progressively to 54% greater than control, $r_o$ decreased by 5%, and $r_i$ increased by 46%. Conduction velocity did not change. On restoration of $O_2$ while stimulation frequency was maintained at 3.0 Hz, $r_i$ increased by an additional 8%. All values then returned toward the control levels.

In each of the six experiments, the increase in $r_i$ at 3.0 Hz was greater than that observed at 0.5 Hz (Figure 3).

Next, we performed two experiments to determine if exposure to hypoxia at 0.5 Hz influenced the changes in $r_i$ recorded during hypoxia at 3.0 Hz. In these experiments, the effects of hypoxia at 3.0 Hz were studied immediately after the initial control. The results are illustrated in Figure 4. The changes in $r_i$ and conduction are similar to those recorded in the six experiments in which the effects of hypoxia at 3.0 Hz were determined after an initial hypoxic exposure at 0.5 Hz. These results indicate that the changes associated with hypoxia at 3.0 Hz were not influenced by the prior hypoxic period.

The changes in action potential duration, action potential amplitude, and $V_{max}$ induced by hypoxia at the two stimulation frequencies are shown in Figure 5. Action potential duration and action potential amplitude decreased significantly after 30 minutes of hypoxia at each stimulation frequency. $V_{max}$ was less at 3.0 Hz than at 0.5 Hz but did not change significantly following the induction of hypoxia at either frequency. These results are similar to those reported by others.8,19

Discussion

The purpose of our study was to determine if increasing stimulation frequency changed $r_i$ in the absence and presence of hypoxia.

Our modification17 of the voltage ratio method, originally described by Weidmann18 and subsequently used by several groups of investigators7,20-22 was used to determine the changes in $r_i$ induced by hypoxia alone, by an increase in stimulation frequency alone, and by the combination of the two. The method uses the concept of local circuit theory to equate changes in the ratio of intracellular and extracellular potentials to the ratio of the intracellular and extracellular resistances, that is, $V_i/V_o=r_i/r_o$. The validity of this approach depends on $r_o$ not changing or that any changes in $r_o$ be included in the calculation.

In our modification of this method, air rather than silicone oil is used as the external insulator. We have previously published the results of studies establishing the validity of this modification17 and have shown that $r_{max}$ does not change by more than 5% throughout 4 hours of superfusion, provided that
flow rate and total ionic content of the superfusate are constant. In the present experiments, flow rate was held constant in each experiment, and the total ionic content of the superfusate was not changed. Moreover, \( r_0 \) and \( r_{\text{tot}} \) did not change by more than 5% throughout the course of the studies and thereby provided an indirect check on the constancy of the extracellular fluid layer. Under control conditions the values of all parameters measured in this study were similar to those which we have previously reported, and the values of \( R_t \) are similar to those previously reported by others. Calculation of \( R_t \) depends on the intracellular cross-sectional area (\( A \) in Equation 4). Changes in intracellular and extracellular volume occur during ischemia. Possible membrane concentration gradients of osmotically active particles due to Na-K pump failure or anaerobic metabolism may cause an increase in cell volume during hypoxia. If intracellular cross-sectional area increased during hypoxia, the calculated \( R_t \) shown in the tables underestimated the true changes. However, \( r \), rather than \( R_t \), is the resistance encountered by the propagating action potential, and changes in cell volume are incorporated into the measurements of \( V_t \), \( V_o \), and \( r_0 \), from which \( r_t \) is determined.

Our study indicates that 30 minutes of hypoxia to a level of \( P_{O_2} = 30 \) mm Hg increases \( r_t \) by 13.7% when the stimulation frequency is 0.5 Hz and by 46% when the stimulation frequency is 3.0 Hz. However, the increase in stimulation frequency does not increase \( r_t \) under normoxic conditions. An increase in \( r_t \) is thought to be caused primarily by an increase in gap junctional resistance and thus is indicative of cellular uncoupling. Increases in intracellular sodium, intracellular calcium, and intracellular hydrogen are each capable of increasing \( r_t \) and of uncoupling cells. It has been shown that an increase in stimulation frequency is associated with an increase in intracellular sodium, which is most likely responsible for the decrease in \( V_{\text{max}} \). This might lead to an increase in intracellular calcium via the Na-Ca exchange mechanism. Bredikis et al reported that in normoxic atrial trabeculi, an increase in stimulation frequency to 10-15 Hz for 15-30 minutes caused a 60% increase in input resistance, which was shown mathematically to reflect a similar increase in \( r \). The conclusion that an increase in driving frequency increases \( r_t \) was supported by the studies of Spach et al. In contrast, neither Pressler nor Davidenko and Antzelevitch were able to document a stimulation frequency dependent increase in \( r_t \) in normoxic Purkinje fibers. We did not observe a significant increase in \( r_t \) in response to the sixfold increase in driving rate from 0.5 to 3.0 Hz under normoxic conditions. It is possible that had our fibers been stimulated at rates similar to those employed by Bredikis et al, the increase in \( r_t \) would have been more impressive. On the other hand, it is also possible that such rapid stimulation might have rendered the fibers hypoxic and thus could have complicated interpretation of the results.

In our study, 30 minutes of exposure to hypoxic glucose-containing solutions increased \( r_t \) by nearly 15% at a stimulation frequency of 0.5 Hz. This is greater than our previously reported results but is less than the results of Ikeda and Hiraoka, who reported a 22% increase in \( r_t \) after 30 minutes of hypoxia in canine ventricular muscles. These differences may reflect different levels of \( P_{O_2} \) in the hypoxic superfusate.

The change in \( r_t \) induced by hypoxia alone is less than the change that occurs when fibers are superfused with solutions altered to more closely mimic ischemia.
in stimulation frequency was performed 60 minutes after superfusion with the hypoxic glucose-free solutions was instituted. By that time, \( r_i \) had already increased by 85–300%. Although the individual effects of hypoxia and the absence of glucose on this rate-dependent effect were not determined, increasing glucose to 50 mM attenuated the increase in \( r_i \) that occurred at the stimulation frequency of 1 Hz.

The combination of an increase in stimulation frequency and hypoxia could result in acceleration of anaerobic glycolysis, a greater accumulation of lactate, and a greater degree of intracellular acidosis than that which occurs with hypoxia at 0.5 Hz. This may be one mechanism underlying the rate-dependent increase in \( r_i \) that we have observed. A greater increase in intracellular sodium than occurs purely as a function of either intervention alone may also occur and increase intracellular calcium via the Na-Ca exchange mechanism.

The restoration of \( O_2 \) produced a transient further increase in \( r_i \) at both 0.5 and 3.0 Hz. This observation is consistent with the observation that the restoration of \( O_2 \) following hypoxia leads to a transient further fall in intracellular pH in both ferret papillary muscles and sheep Purkinje fibers.33

We were unable to document a significant degree of conduction slowing in association with the changes in \( r_i \) induced by 30 minutes of hypoxia at either 0.5 or 3.0 Hz. This result is consistent with the observations of Furuta et al.9 In that study of rabbit papillary muscles, 40 minutes of hypoxia caused a decrease in conduction velocity from 57 to 48 cm/sec. However, the decrease did not appear until the fibers had been exposed to hypoxia for 25 to 30 minutes. One dimensional cable theory predicts that the increase in \( r_i \), induced by hypoxia that we have observed should have caused a 6% slowing of conduction at 0.5 Hz and an 18% slowing of conduction at 3.0 Hz if no changes other than those in \( r_i \) occurred. Our inability to document such slowing suggests that other factors such as changes in membrane capacitance or changes in the relation between resting and threshold potentials may have occurred. Our requirements that the electrode remain in the same cell throughout the entire protocol was associated with slight changes in tip potential which precluded an accurate determination of resting potential throughout the course of our experiments. Others have reported that hypoxia alone causes a 1–2 mV depolarization of the resting potential in the presence of glucose19 and a 4–5 mV depolarization when glucose is absent.37 Such changes could have balanced the anticipated slowing of conduction by bringing resting potential closer to threshold potential. A similar mechanism has been proposed to explain the supernormal conduction that occurs in association with moderate elevations of extracellular potassium or spontaneous depolarization of the diastolic potential.38–41

The ultimate goal of our study was to acquire insight into the causes of the rate-dependent con-
duction slowing reported to occur in the setting of myocardial ischemia. Clearly, ischemia is a multicomponent phenomenon of which hypoxia is but a single factor. Our results suggest that hypoxia does contribute to the increase in r, associated with ischemia and that the increase is rate-dependent but, under the conditions of our study, does not of itself cause rate-dependent conduction slowing.

References

Key words: conduction • passive membrane properties • cellular uncoupling • hypoxia
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