Heterogeneity of Intracellular Potassium Activity and Membrane Potential in Hypoxic Guinea Pig Ventricle

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SUMMARY The relationship between membrane potential ($E_m$) and the potassium equilibrium potential ($E_K$) was investigated in hypoxic guinea pig papillary muscle. After more than 8 hours of hypoxia, cells with near normal $E_m$ ($-86.2 \pm 0.9$ mV) and action potentials were observed. However, the intracellular potassium concentration ($[K+]_i$) based on chemical analysis and the assumption that potassium was homogeneously distributed was $41.8 \pm 4.3$ mM; the apparent $E_K$ was $-57.7 \pm 2.9$ mV, significantly positive to $E_m$. Measurements with potassium ion-selective microelectrodes revealed that prolonged hypoxia results in at least two populations of cells with different characteristics. The first population had an intracellular potassium activity ($[K+]_i$) of 101.5 ± 1.9 mM and $E_K$ was 4.7 mV negative to $E_m$. In contrast, $E_m$ was $33.4 \pm 1.3$ mV negative to $E_m$ in the second population. These cells also exhibited a reduced sensitivity to changes in bath potassium, and calculations suggest $[K+]_i$ was about 18 mM. The existence of cell populations with a near normal and very low $[K+]_i$ can explain the intermediate value of $[K+]_i$, calculated assuming a homogenous potassium distribution. Cells with near normal $E_m$ and action potentials represent the population with near normal $[K+]_i$. Hypoxia may also cause non-uniform changes in other cellular characteristics. Circ Res 49: 1181-1189, 1981

INVESTIGATIONS of the effects of hypoxia on the potassium content of mammalian ventricle have produced seemingly paradoxical results. The resting membrane potential ($E_m$) of myocardial cells is reported to be remarkably unaffected by hypoxia, even when oxygen is withheld for a prolonged period (Trautwein and Dudel, 1956; Webb and Holland, 1966; MacLeod and Daniel, 1965). For example, incubation under hypoxic conditions for 8 hours in media containing 5 mM glucose decreases $E_m$ of guinea pig papillary muscle by only about 5 mV (MacLeod and Daniel, 1965; McDonald and MacLeod, 1971, 1973; Hunter et al., 1972). Chemical analysis of some of these same muscles revealed an intracellular potassium content ($[K+]_i$); of 27.4 mM/kg cell-water and an apparent potassium equilibrium potential, $E_K$, of $-47.4$ mV; $E_m$ was approximately 30 mV negative to the apparent $E_K$ (MacDonald and MacLeod, 1971, 1973). McDonald and MacLeod (1971, 1973) considered two possible explanations for the maintenance of a high $E_m$: (1) the intracellular potassium in hypoxic muscles is compartmentalized so that $E_K$ across the cell membrane is appropriate for $E_m$; and (2) an electrogenic pump contributes to $E_m$. Finding that $E_m$ decreased to $E_K$ in response to application of $10^{-7}$ m ouabain or cooling to $8{\degree}C$, they concluded that a component of $E_m$ was due to electrogenic Na-K pumping. This component was reported to be enhanced by exposure to high concentrations of glucose (50 mM) or potassium (15 mM).

Several features of these experimental results and implications of the conclusion remain bothersome. First, if $E_m$ is in fact negative to $E_K$, the net passive transmembrane flux of potassium would be inward directed. Thus, it seems necessary to postulate a novel transport mechanism which extrudes potassium against its electrochemical gradient in order to obtain the low $[K+]_i$. Such a transport mechanism has not been demonstrated. It cannot be the Na-K pump operating in reverse (Garrahan and Glynn, 1967) because an outward current must be produced. Second, the basis for the rapid action potential upstroke observed after prolonged hypoxia must also be explained. Hypoxia not only decreases $[K+]_i$ but also increases $[Na+]$, the intracellular sodium concentration. Despite an $[Na+]_i$ of $114.3$ mM/kg cell-water after 8 hours of hypoxia, the maximum rate of rise of phase 0 was essentially unchanged (McDonald and MacLeod, 1973). This finding cannot be explained by electrogenic Na-K pumping. It therefore seems necessary also to invoke some heterogeneity of tissue electrolytes. Perhaps cells in the muscle core are more severely damaged than those on the surface.

We have reinvestigated the effect of prolonged hypoxia on the relationship between $E_m$ and $E_K$ using potassium ion-selective microelectrodes supported by National Heart, Lung, and Blood Institute Grants HL-24847 (CMB), 23298. and by the Nova Scotia Heart Foundation (TFM).
(ISE). This approach avoids certain problems inherent in chemical analysis. Intracellular potassium activity, $a_K$, is measured in the same compartment, as probed by the conventional $E_m$-sensing microelectrode. The measurements, therefore, should relate to the basis for the maintenance of $E_m$ without first making assumptions regarding intracellular compartmentalization and activity coefficients. Further, cell-to-cell variations in $a_K$ and $E_m$ can be detected while chemical analysis techniques measure an average of the entire preparation.

The results indicate that, after more than 8 hours of hypoxia, cells with near normal $E_m$ have near normal $a_K$, and, as usual, $E_m$ is slightly positive to $E_K$. Based on ISE impalements, at least one additional population of cells has been identified. In these cells, $E_m$ is about 30 mV positive to $E_K$ and appears to be quite depolarized.

A portion of this work has been presented elsewhere in abstract form (McDonald et al., 1979).

Methods

Tissue Preparation and Solutions

Guinea pigs (female, 250–300 g) were killed by cervical dislocation. The heart was removed as rapidly as possible and immersed in modified Krebs’ solution (G50O2, see below) at room temperature. Solutions for preparation and calibration

Solutions

Tissue Preparation

and $E_m$ were equilibrated with either 95% O2-5% CO2 (O2) or 95% N2-5% CO2 (N2), and the pH was adjusted to 7.4.

Electrical Arrangements

Membrane potential was recorded with conventional 3 M KCl-filled microelectrodes (15–20 MΩ) buffered by a high input resistance unity gain amplifier (Instrumentation Laboratory, model 181), and the potential detected by the ISE was recorded through a very high input resistance electrometer (Keithley Instruments, model 618 and model 610C). A 3 M KCl-3% agar bridge was used for ground. The signals were monitored on an oscilloscope, chart recorder, and digital voltmeters with 0.1 mV resolution from which measurements were taken. Low pass filtering (down 3 dB at 12 Hz) was occasionally used to reduce the noise picked up by the ISE.

Preparation and Calibration of ISE

Ion-selective microelectrodes were fabricated (Walker, 1971) from glass blanks pulled from Pyrex 7740 capillary tubing (Corning Glass Works). Exposure to vapors of dimethyl dichlorosilane followed by baking for 1 hour at 150°C rendered the interior of the blanks hydrophobic. Potassium liquid ion-exchange resin (Corning Glass Works, 477317) was introduced into the tip of the electrode by immersion; the shaft was then filled with a salt solution containing 120 mM KCl and 30 mM NaCl. Gentle heating and manipulation with a fine glass whisker under microscopic observation was necessary to remove trapped air bubbles.

Standard techniques (Walker and Brown, 1977) were used to calibrate the ISE both before and after use. The potential, $E_o$, in a salt solution containing potassium or mixtures of potassium and sodium is given by Equation 1:

$$E_o = V_o + S \ln (a_K + k_{K-Na} a_{Na}).$$  \hspace{1cm} (1)\

$V_o$ is an empirically determined constant (mV); $S$ is the slope of the electrode’s response in potassium solutions; $a_K$ and $a_{Na}$ are the activities of potassium and sodium; and $k_{K-Na}$ is a dimensionless empirically determined selectivity coefficient. The empirical constants were defined by determining the electrode’s responses in pure solutions of KCl (3, 10, 30, 100, and 300 mM) and in mixtures of KCl and NaCl at a constant ionic strength (KCl + NaCl: 2.5 + 97.5; 5 + 95; 10 + 90 mM). The selectivity of the electrode is such that sodium is the only interfering cation of importance for the calculation of $a_K$ under the experimental conditions (Walker, 1971; Walker and Brown, 1977).

Calculation of $a_K$

The potential detected by an ISE in a cell, $E_i$, is given by Equation 2:

$$E_i = E_m + V_o + S \ln (a_K + k_{K-Na} a_{Na}).$$  \hspace{1cm} (2)\

where the intracellular ion activities of potassium and sodium are $a_K$ and $a_{Na}$, respectively. The term, $k_{K-Na} a_{Na}$, can be ignored without significant error because of the high selectivity of the ISE for potassium over sodium (see discussion). The value of $a_K$ can be calculated from the calibration data, the change in potential upon impalement with the ISE ($\Delta E = E_i - E_o$), and an independent measure of $E_m$ according to Equation 3:

$$a_K = \exp \left( \frac{\Delta E - E_m}{S} \right) \times (a_K + k_{K-Na} a_{Na}).$$  \hspace{1cm} (3)\

The average values of $\Delta E$ and $E_m$ obtained in a muscle were used to calculate a single value of $a_K$ for that muscle.

Although an independent measure of $E_m$ is necessary to calculate $a_K$, the ISE $\Delta E$ is sufficient to
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The term at the far right is a constant for each ISE (typically \( -20 \text{ mV} \)) and is defined by the composition of the bathing media and the calibration data for that electrode. Since the ISE slope is nearly that predicted by the Nernst equation, we may substitute \(-E_K\) for the middle term and write:

\[
\Delta E = E_m + S \ln \frac{a_K}{a_{K^+}} + S \ln \frac{a_N}{a_{K^+} a_{Na}} + \text{constant}. \tag{4}
\]

This allows estimation of the difference between \(E_m\) and \(E_K\) without an independent measurement of membrane potential.

**Results**

In a well-oxygenated papillary muscle, \(E_m\) in Krebs' solution is nearly equal to \(E_K\). This is evidenced in Figure 2, where a typical ISE recording is shown. In 4.6 mM \([K^+]_o\) solution (\(G_{50O_2}\)), the \(\Delta E\) on ISE impalement was always negative and averaged \(-15.3 \pm 1.1 \text{ mV}\); \(E_m\) was \(-90.4 \pm 1.3 \text{ mV}\). The calculated value of \(a_K\) in this muscle was 106.8 mM and \(E_K\) was 92.3 mV, about 2 mV negative to \(E_m\).
The ISE reversibly depolarized 30.3 mV in response to 15 mM \([K^+]_o\). This response is quite close to the prediction of 31.6 mV for a membrane which exactly obeys the Nernst equation. Our results are consistent with previous measurements of \(a_k\) in guinea-pig papillary muscle. At a \([K^+]_o\) of 4.6 mM, the same as used in the present study, Cohen and Fozzard (1979) reported that \(a_k\) was 116.1 ± 2.4 mM, and \(E_m\) was -87.9 mV, 6.6 mV positive to \(E_K\) at a \([K^+]_i\) of 3 mM, Wier (1978a, 1978b) found \(a_k\) was 99.7 mM, and \(E_m\) was -96.1 mV, 4.6 mV positive to \(E_K\).

The behavior of the ISE on impalement of hypoxic myocardium can be predicted for hypothetical relationships between \(E_m\) and \(E_K\) from Equation 5; typical calibration data give -20 mV as the value of the constant term. If the chemical measurement of \([K^+]\), accurately predicts \(E_K\), and \(E_m\) is 30 mV negative to \(E_K\), as claimed by McDonald and MacLeod (1971, 1973), a potential change of approximately -50 mV is expected on impalement of the ISE. On the other hand, a potential change of only about -15 mV would be obtained if prolonged hypoxia does not alter the relationship between \(E_m\) and \(E_K\). Figure 3 illustrates simultaneous ISE and \(E_m\) recordings from surface cells of a papillary muscle incubated in G&N 2 media for 8 hours and then maintained in G6N 2 solution. The ISE record is typical of about 65% of successful impalements in hypoxic cells. Upon impalement, the potential recorded by the ISE became more negative, and \(AE\) was -15.9 mV. This indicates that \(E_m\) is certainly not 30 mV negative to \(E_K\) in the cell probed by the ISE. Rather, the \(AE\) suggests that \(E_m\) is 5.2 mV positive to \(E_K\). The average value of \(AE\) for this group was -12.9 ± 0.6 mV.

The average \(E_m\) in five such muscles was -86.2 ± 0.9 mV. This is similar to values previously reported after 8 hours of hypoxia (McDonald and MacLeod, 1971, 1973) and not far from \(E_m\) of fresh guinea pig papillary muscle (Cohen and Fozzard, 1979). Based on averaged values for each muscle of this group of ISE impalements and \(E_m, a_k\) was 101.5 ± 1.9 mM. \(E_K\) was -90.9 ± 0.5 mV, which is 4.7 mV negative to \(E_m\). Hence, although both \(E_m\) and \(E_K\) may be slightly more positive than in oxygenated muscle, the relationship between \(E_m\) and \(E_K\) appeared to be qualitatively unchanged.

Elevation of \([K^+]_o\) to 15 mM confirmed that in these hypoxic cells is determined primarily by the potassium gradient (see Fig. 3). The potential recorded by the ISE became 27.3 mV more positive, and \(E_m\) shifted 25.2 mV in the same direction. The potentials returned to near their prior values on readmitting 4.6 mM \([K^+]_o\), medium. In nine potassium change experiments on this group of ISE impalements, the average potential change of the ISE was 27.0 ± 0.5 mV. \(E_m\) was altered an average of 25.6 ± 0.7 mV (\(n = 13\)) by the same intervention. These responses are statistically indistinguishable but are consistent with a small increase in \(a_k\) on increasing \([K^+]_o\).

Not all impalements of hypoxic muscle with the ISE yielded records similar to the one shown in Figure 3. In 35% of successful ISE impalements of
surface cells, ΔE was positive rather than negative. Figure 4, panel A, shows a typical example of these impalements. When the ISE was advanced into the muscle, the potential abruptly changed by +14.8 mV to a new stable value. The average positive ΔE observed was +11.8 ± 1.1 mV. According to Equation 5, this implies E_m was 32 mV positive to E_K.

Cells giving a positive ΔE on impalement with the ISE do not represent the same population of cells as identified by negative shifts. Not only are the relationships between E_m and E_K different, but also the responses to changes in [K^+]_o are distinguishable (Fig. 4, panel A). When [K^+]_o was increased from 4.6 to 15 mM, the positive ΔE ISE impalement depolarized by only 11 mV while E_m simultaneously recorded from a well-polarized cell decreased 27.7 mV (trace labeled E_m). Both potential changes were reversible. In eight [K^+]_o change experiments on positive shift cells, the ISE response averaged 11.6 ± 1.4 mV. This is significantly less than the response observed in either the negative ISE shift cells or in recordings of E_m from well-polarized cells (see Figs. 3 and 4, panel B).

The small changes in potential on changing [K^+]_o may imply that the ISE were in cells whose E_m was relatively insensitive to [K^+]_o. This result and the finding that E_m was approximately 32 mV positive to E_K suggests that these cells were depolarized. Depolarization of E_m can occur as an artifact of damage inflicted by the microelectrode upon impalement. The stable potential change in Figure 4, panel A, argues against this possibility. Further evidence that the ISE has impaled an undamaged cell is provided by adding 1 mM tetramethylammonium (TMA) chloride to the bath. The potassium-selective liquid ion-exchanger is exquisitely sensitive to TMA, with a selectivity over potassium of about 600:1 (Oehme and Simon, 1976). Consequently, an ISE in a damaged surface cell will record a large potential change when TMA is added. In Figure 4, panel A, a second ISE (ISE^2) has been placed just above the surface of the muscle. The

![Figure 4](https://example.com/figure4.png)

**Figure 4** Simultaneous recordings from cells with dissimilar electrical properties. Panel A: ISE, impaled a cell, and ΔE was +14.8 mV. E_m was the potential monitored during a simultaneous impalement by a KCl-filled electrode. When [K^+]_o was elevated to 15 mM, ISE^2 recorded a much smaller potential change than E_m. ISE^2 was an extracellular electrode placed just above the muscle. At bars, solution was switched to a saline containing 1 mM TMA. ISE did not detect the TMA while ISE^2 responded. After withdrawing from cell, both ISE responded. Panel B: The response of a negative ΔE impalement to a bath [K^+]_o change and exposure to 1 mM TMA. TMA was not detected by the ISE in a cell.
potential recorded from ISE2 changed nearly 45 mV when 1 mM TMA was added for 60 seconds (indicated by bar). In contrast, the potential recorded by ISE1 did not change. A 5-mV response would have been produced by only 3 μM TMA. Upon withdrawing ISE1 from the cell, the potential returned to baseline. When 1 mM TMA was reintroduced for 45 seconds, a large potential change was recorded, indicating that this ISE was also sensitive to TMA. No shift in the ISE potential was observed when 1 mM TMA-containing medium was superfused for 60 seconds.

Conventional 3 M KCl-filled microelectrode impalements in hypoxic muscle also detect cells with modified properties. As illustrated in Figure 5, a stable potential of −9 to −20 mV was sometimes obtained upon advancing. These cells did not respond to an elevation in bath [K+]o with a depolarization, but rather tended to hyperpolarize slightly. In seven cells, Em was −13.7 ± 1.3 mV in 4.6 mM [K+]o and −15.2 ± 1.7 mV after 5 minutes in 15 mM [K+]o. A paired comparison indicates that this small hyperpolarization is statistically significant (P < 0.05).

McDonald and MacLeod (1971, 1973) found that hypoxia altered tissue [K+]o and [Na+]o. To ensure that the present experiments are comparable to theirs, we determined the effect of hypoxia on tissue H2O, Na+, and K+. The results, reported in Table 1, are based on analysis done after three treatments: (1) 3- to 4-hours superfusion with G50O2 medium (oxygenated); (2) 1-hour equilibration in G50O2 followed by exposure to G50N2 for 8 hours and then G50N2 for 2-3 hours (hypoxic); and (3) after completion of the ak measurements previously described (hypoxic-ak). Hypoxia induced a slight increase in tissue H2O, a large increase in [Na+]o, and a large decrease in [K+]o. Even though the values of [K+]o and Em are slightly greater than those obtained by McDonald and MacLeod (1971, 1973), they lead to the same conclusion. Em is only slightly positive to Ek in oxygenated tissue, but it is significantly negative to Ek in hypoxic tissue.

Chemical analysis techniques were also used to determine the effect of exposure to 15 mM [K+]o on [K+]o in both oxygenated and hypoxic muscle. Tissues were treated as described above for the oxygenated and hypoxic groups except that they were switched to 15 mM [K+]o solution for 5 minutes immediately before the analysis was begun. After exposure to high [K+]o, media, [K+]o was 145.1 ± 4.6 mM (n = 4) in oxygenated control muscles and 49.2 ± 3.8 mM (n = 3) in hypoxic muscles. These values are not significantly different from the values reported in Table 1. The independence of [K+]o and Em is only slightly positive to the apparent Ek based on chemical analysis. However, our measurements with ISE show that chemical measurements of muscle potassium are misleading because potassium is not homogeneously distributed among the cells of the preparation.

**Table 1** Effect of Hypoxia on Tissue Water, Intracellular Ion Concentrations, Apparent Ek, and Resting Potential of Guinea Pig Papillary Muscle

<table>
<thead>
<tr>
<th>H2O (ml/kg)</th>
<th>[Na+]o (mM/liter)</th>
<th>[K+]o (mM/liter)</th>
<th>Em (mV)</th>
<th>EK (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygenated (n = 7)</td>
<td>762 ± 4</td>
<td>18.9 ± 2.0</td>
<td>140.6 ± 3.3</td>
<td>−91.3 ± 2.2</td>
</tr>
<tr>
<td>Hypoxic (n = 4)</td>
<td>778 ± 7</td>
<td>121.2 ± 5.1</td>
<td>47.3 ± 4.1</td>
<td>−62.2 ± 2.7</td>
</tr>
<tr>
<td>Hypoxic ak (n = 6)</td>
<td>772 ± 8</td>
<td>41.8 ± 4.3</td>
<td>−57.7 ± 2.9</td>
<td>−86.2 ± 0.9</td>
</tr>
</tbody>
</table>

Muscle diameter was <0.7 mm and mean wet weight was 1.63 mg. All values are mean ± se. Intracellular ion concentrations (mM/liter cell water) were calculated from ion and water contents of each muscle using 27.1% and 27.9% for the extracellular (muscle) space of oxygenated and hypoxic muscles, respectively. The apparent Ek was calculated assuming both a homogeneous potassium distribution in cell water and equal intra- and extracellular activity coefficients.
At least two populations of cells with different relationships between \( E_m \) and \( E_K \) are present in hypoxic muscle. The \( E_m \) of one group is almost normal, with nearly Nernstian changes in potential when [K\(^+\)]\(_o\) is altered. \( a_k \) after prolonged hypoxia is 101.5 ± 1.9 mM, only about 15 mM less than normal in this group. Wier (1978a) obtained a similar result after 2 hours of hypoxia. A second population is characterized by a positive \( \Delta E \) upon impalement with an ISE, indicating that \( E_m \) is 33.4 ± 1.3 mV positive to \( E_K \). In contrast to the first population, these cells have an obtunded response to changes in [K\(^+\)]\(_i\). These findings are distinct departures from normal and suggest that membrane properties have been altered in important ways. Ganote and co-workers (1975) have identified two populations of hypoxic cells by using morphological criteria. One group showed signs of "irreversible" injury. The cells characterized here as having a reduced response to [K\(^+\)]\(_o\) and \( E_m \) far positive to \( E_K \) may correspond to the "irreversibly" injured population. Supporting evidence for heterogeneity during hypoxia is also provided by NADH fluorescence photography (Steenbergen et al., 1977).

What is \( a_K \) in the second population of cells identified by the ISE? It is possible that the depolarized cells found with the conventional microelectrodes represent this population. In that case, \( a_K \) is 17.9 mM, and \( E_K \) is −44.2 mV, 30.5 mV negative to \( E_m \). However, the potential changes observed when bath [K\(^+\)]\(_o\), was elevated to 15 mM would then imply that \( a_K \) increased by 64\% against its concentration gradient during the brief exposure to high potassium solution. Such an increase seems unlikely, and no increase in [K\(^+\)]\(_i\) was found in hypoxic muscles exposed to high potassium for a similar time. On the other hand, consideration of Equation 3 makes it clear that \( E_m \) in cells identified by a positive \( \Delta E \) cannot be −86.2 mV, the value ascribed to the well-polarized negative \( \Delta E \) cells. An \( E_m \) of −86.2 mV and a \( \Delta E \) of +11.8 mV gives an \( a_K \) of greater than 200 mM, which is implausible. Thus, it appears that conventional microelectrodes failed to detect comparable cells, and a direct calculation of \( a_K \) cannot be made. The depolarized cells found with conventional microelectrodes may represent an entirely different population not recognized with the ISE. For example, negative charges on the myofilaments of cells with disrupted membranes can give rise to potentials of approximately −15 mV, but because potassium and sodium are in electrochemical equilibrium, the ISE will not identify these cells (Collins and Edwards, 1971). In this regard, areas of dog papillary muscle in which \( E_m \) is less than −25 mV after periods of in vivo ischemia are correlated with regions exhibiting morphological evidence of irreversible injury (McGee et al., 1978).

The approximate values of \( E_m \) and \( a_K \) in the positive \( \Delta E \) cells can also be obtained indirectly if several simplifying assumptions are permitted. Assuming that the membrane is permeable only to sodium and potassium and that \( E_m \) can be described by the Constant Field equation, the potential observed on impaling a cell with an ISE is given by:

\[
\Delta E = 26.7 \ln \frac{a_K + b a_{Na}}{a_K + b a_{Na}^*} + S \ln \frac{a_K + k_{K,Na} a_{Na}}{a_K + k_{K,Na} a_{Na}^*}
\]

where \( b \) is equal to \( P_{Na}/P_K \) and the other terms are as previously defined. Note, Equation 6 does not assume that the product of \( k_{K,Na} a_{Na} \) is negligible (cf., Equation 4). Since the slope of the ISE is nearly Nernstian (i.e., \( S = 26.7 \)), it can be shown that:

\[
a_{Na} = \left( \frac{k_{K,Na} T}{a_K + b a_{Na}^*} \right) \left( b T \left( a_K + k_{K,Na} a_{Na} \right) \exp \left( \Delta E/26.7 \right) \right) / \left( 1 - b \left( a_K + k_{K,Na} a_{Na} \right) \exp \left( \Delta E/26.7 \right) - \left( 1 - k_{K,Na} \right) \left( a_K + b a_{Na}^* \right) \right)
\]

where \( T \) equals the sum of \( a_K \) and \( a_{Na}^* \).

An estimate of \( P_{Na}/P_K \) can be made from \( \Delta V \), the change in potential when bath potassium is altered from 4.6 to 15 mM, if it is further assumed that neither the intracellular activities of sodium and potassium nor \( P_{Na}/P_K \) are modified by the change in bath potassium. The potential change observed is given by equation 8:

\[
\Delta V = E_{m,*} - E_m = 26.7 \ln \frac{a_{Na}^* + b a_{Na}}{a_K + b a_{Na}^*} - 26.7 \ln \frac{a_K + b a_{Na}}{a_K + b a_{Na}^*}
\]

The asterisk indicates values in bathing media containing 15 mM [K\(^+\)]\(_o\). Solving for \( b \) gives:

\[
b = \frac{\exp \left( \Delta V/26.7 \right) \left( a_{Na}^* - a_{Na} \right)}{a_{Na}^* - \exp \left( \Delta V/26.7 \right) \left( a_{Na}^* \right)}
\]

Analysis of the potential changes observed upon changing bath potassium results in the following estimates for \( P_{Na}/P_K \): 0.0022 ± 0.0003 in oxygenated muscle, 0.0093 ± 0.0012 in hypoxic high \( a_K \) (negative \( \Delta E \)) cells, and 0.1026 ± 0.0199 in hypoxic low \( a_K \) (positive \( \Delta E \)) cells. Estimates of \( P_{Na}/P_K \) are significantly greater in the positive \( \Delta E \) population than in either the negative \( \Delta E \) population or oxygenated cells. \( P_{Na}/P_K \) estimates based on conventional microelectrode and ISE recordings are statistically indistinguishable.

The final assumption necessary for the calculation of \( a_K \) is the value of \( T \). We assumed that \( T \), the sum of \( a_K \) and \( a_{Na}^* \), is 120 mM, which is similar to its value in fresh muscle (Cohen and Fozzard, 1979). Utilizing the average values of 11.8 mV for the positive \( \Delta E \) impalements, 0.1026 for \( P_{Na}/P_K \) and 0.033 for \( k_{K,Na} \), Equation 7 gives 18 mM for \( a_K \) in these cells. The predicted \( E_m \) is −18 mV, 26 mV positive to \( E_K \). Thus, either by this indirect analysis or if the positive \( \Delta E \) cells are of the same population as the depolarized cells found with the conventional
microelectrodes, the conclusion is the same. Some cells in hypoxic muscle have very low \( a_k \) and are quite depolarized.

The sensitivity of the indirect calculation of \( a_k \) and \( E_m \) to errors in the estimates of \( P_{Na}/P_K \) and \( T \) is illustrated in Figure 6. The calculated values are relatively insensitive to \( T \) and to \( P_{Na}/P_K \) between 0.08 and 0.16, but are quite sensitive to \( P_{Na}/P_K \) less than 0.08. \( E_m \) becomes more negative and \( a_k \) increases as \( P_{Na}/P_K \) decreases. Unfortunately, due to the nature of the simplifying assumptions and a lack of knowledge about the membrane characteristics of hypoxic cells, the magnitude of the errors in the estimates of \( P_{Na}/P_K \) and \( T \) cannot be ascertained with certainty. Consequently, the indirect estimates of \( a_k \) and \( E_m \) for the second cell population must be regarded with some caution.

Extremely low values for \( a_k \) and \( E_m \) are, however, quite reasonable and help to explain the earlier results of McDonald and MacLeod (1971, 1973). The existence of two populations of cells, one with a near normal \( a_k \) and another with a very low \( a_k \), can at least qualitatively explain the intermediate \([K^+]_i\) obtained by chemical analysis. \( E_m \) and action potential records are compatible with the \( a_k \) of the near-normal negative \( \Delta E \) population. The marked heterogeneity of \( a_k \) obviates the need to invoke electrogentic pumping or intracellular compartmentalization to explain the electrical activity of hypoxic muscle (cf., McDonald and MacLeod, 1971, 1973). It is likely that \( a_k \) also is not uniform. Further, the low \( E_m \) of the low \( a_k \)-positive \( \Delta E \) population explains why these cells were not recognized previously with conventional microelectrodes. Such a small potential change upon impalement would be regarded as artifact, and the search for a "good" impalement would continue.

McDonald and MacLeod (1971, 1973) also found that \( E_m \) depolarized by about 30 mV to the apparent \( E_K \) when \( 10^{-4} \) M ouabain was added or when the muscle was cooled to 8°C. Our results suggest that the equality of \( E_m \) and \( E_K \) was fortuitous. Large changes in \( E_m \) can occur with small changes in steady state current if the current-voltage relationship has a negative conductance region. The steady state I-V relationship of guinea pig ventricular muscle has a negative slope region, but normally this curve intersects the voltage axis only once (Traut-
wein and McDonald, 1978). Cardiac glycosides and cooling inhibit an outward current (Isenberg and Trautwein, 1974, 1975), and we suppose that this produces a large depolarization in hypoxic muscle but not in well-oxygenated muscle.

Our results suggest that some cells remain healthy while others run down dramatically. The heterogeneity of ak and Em requires that not all cells are electrically coupled. This is consistent with measurements of internal resistance in hypoxic ventricle (Wojtczak, 1979) and also occurs in diseased human atria (Ten Eick and Singer, 1979). The bases for the non-uniform consequences of hypoxia are obscure. It is interesting, however, that cell-to-cell heterogeneity of ventricular myosin has been documented (Sartore et al., 1981). Perhaps there is also heterogeneity of other enzymes. Diverse interventions result in focal damage to the myocardium. For example, alcoholic cardiomyopathy (Hibbs et al., 1965) and cardiomyopathies induced by doxorubicin (Adriamycin) and daunorubicin (Daunomycin, Rubidomycin) (Buja et al., 1973; Jaenke, 1974; Olson et al., 1974) are focal in nature.

The effect of hypoxia or other disease processes on ion, metabolite, and enzyme content is usually determined in complex multicellular preparations. It is often assumed that the distribution of the substance being measured is homogeneous. In light of the heterogeneity described here, such assumptions may not always be correct.

Note added in press: In a brief communication, Guamieri, Wallace, and Straus recently have reported ak measurements in hypoxic guinea pig ventricle (Fed Proc 40: 392, 1981).

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