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 (~88.2 ± 0.9 mV) and action potentials were observed. However, the intracellular potassium concentration ([K+]_i) based on chemical analysis and the assumption that potassium is homogeneously distributed was 41.8 ± 4.3 mM; the apparent E_K was -57.7 ± 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 23.4 ± 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+], 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, 1956; 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 concentration ([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 (McDonald 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^-5 m ouabain or cooling to 8°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+]. 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+]_i, the intracellular sodium concentration. Despite an [Na+], 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.
(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. Papillary muscles were dissected from the right ventricle, pinned in the experimental chamber, and superfused initially with G50O2 solution at 37°C.

The Krebs' media nominally had the following composition (in mM): NaCl, 113.1; KCl, 4.6; CaCl2, 2.45; MgCl2, 1.15; NaHCO3, 21.9; NaH2PO4, 3.48; and glucose, either 5 (G5) or 50 (Gw). High potassium (15 mM) medium was prepared by substituting equimolar amounts of KCl for NaCl. The solutions 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 potentials detected by the ISE and $E_m$ 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:

$$E_o = V_o + S \ln (a_k + k_{K-Na} a_{Na}).$$

$V_o$ 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).

Figure 1 illustrates typical calibration data. The slope of the ISE's response ranged from 26 to 28 mV/e-fold increase in $a_k$; this is equivalent to 60 to 64 mV/10-fold increase. The selectivity coefficient, $k_{K-Na}$, ranged from 0.039 to 0.021.

#### Calculation of $a_k$

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

$$E_i = E_m + V_o + S \ln (a_k + k_{K-Na} a_{Na}).$$

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:

$$a_k = \exp \left( \frac{\Delta E - E_m}{S} \right) \times (a_k + k_{K-Na} a_{Na}).$$

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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**Note:** The provided text is a partial transcription from a scientific paper, focusing on the methodology and equipment used in experiments involving tissue preparation and potassium activity detection using ion-selective electrodes (ISE). The text outlines the preparation of tissue samples, the solutions used, and the techniques employed for measuring membrane potentials, specifically highlighting the use of potassium liquid ion-exchange resin for calibration. The approach avoids certain issues inherent in chemical analysis by directly measuring intracellular potassium activity. The text also describes the calibration of ISEs and the calculation of intracellular potassium activity. The example provided in the text is Figure 1, illustrating typical calibration data. The slope of the ISE's response, ranging from 26 to 28 mV/e-fold increase in $a_k$, is equivalent to 60 to 64 mV/10-fold increase. The selectivity coefficient, $k_{K-Na}$, ranges from 0.039 to 0.021. The potential detected by an ISE in a cell, $E_i$, is given by Equation 2, and the calculation of $a_k$ is detailed in Equation 3. The document concludes with a note on the necessity of an independent measure of $E_m$ for the calculation of $a_k$.
a'K AND Eₘ IN HYPOXIC VENTRICLE/Baumgarten et al. 1183

Papillary muscles were rinsed (2 seconds) in isotonic choline chloride or tetramethylammonium chloride, trimmed of cut ends, and blotted by pressing between two sheets of filter paper. The wet weight was measured (Cahn, model 4700 electrobalance), and the muscle was then dried overnight in an oven at 100°C and reweighed. The wet weight minus the dry weight was taken as the total tissue H₂O. The dry tissue was placed in a polypropylene test tube encased in a heated aluminum block (about 60°C) and digested with 2 ml of 30% hydrogen peroxide over a 24-hour period. A suitable volume of diluent (lithium chloride, 15 mM) was used to dissolve the residue, and the sodium and potassium contents were determined by flame photometry (Instrumentation Laboratory, model 443).

The extracellular space of papillary muscle was estimated with ¹⁴C-inulin (New England Nuclear). The radioactivity of the medium was about 0.2 μCi/ml, corresponding to an inulin concentration of 0.3 mg/ml. Inulin was added to the Krebs' solution during the last hour of oxygenated or hypoxic incubation (see Table 1). Muscles were rinsed (2 seconds) in choline chloride, blotted with filter paper, weighed, and solubilized in 0.5 ml Soluene 100 (Packard Instrument Co.). After the addition of 10 ml Bray's solution, ¹⁴C was counted on a Packard Tri-Carb scintillation counter.

The inulin space (mean ± SE) was 27.1 ± 1.2% in control muscles (n = 6) and 27.9 ± 1.5% in hypoxic muscles (n = 5). These values are in good agreement with previous determinations using 30-minute ¹⁴C-inulin exposures (Hunter et al., 1972).

**Experimental Protocol**

Isolated papillary muscles were superfused with G₅O₂ solutions and electrically stimulated (1.5 × threshold) at 60 Hz. After a 1-hour equilibration period, G₅O₂ superfusion was continued or the muscles were made hypoxic by exposure to G₆N₂ solutions for 9.7 ± 1.7 hours. Hypoxic incubation was continued in G₅O₂ media with electrical measurements beginning at least 30 minutes later. The switch to G₅O₂ media was intended to minimize further changes during the measurement period. Incubation in a G₅O₂ solution largely prevents or quickly reverses the profound abbreviation of action potential duration but has only small effects on depressed adenosine triphosphate levels and downhill ion movements (McDonald and MacLeod, 1973).

The results are reported as mean ± standard error of the mean.

**Results**

In a well-oxygenated papillary muscle, Eₐ in Krebs' solution is nearly equal to Eₖ. This is evidenced in Figure 2, where a typical ISE recording is shown. In 4.6 mM [K⁺]₀ solution (G₅O₂), the ΔE on ISE impalement was always negative and averaged −15.3 ± 1.1 mV; Eₘ was −90.4 ± 1.3 mV. The calculated value of a'K in this muscle was 106.8 mM and Eₖ was 92.3 mV, about 2 mV negative to Eₘ.
FIGURE 2  ISE recording in oxygenated papillary muscle. Upon impalement, the potential (ΔE) recorded by the ISE became 16.4 mV more negative suggesting that \( E_m \) was 1.8 mV positive to \( E_K \) in this cell. Varying bath \([K^+]\), between 4.6 and 15 mM caused a 30.3 mV potential change. Spontaneous firing was observed during both solution changes.

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 \([K^+]_o\) 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 \([K^+]_o\) 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 G&N 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 \( E_m \) 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 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 when 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.

Figure 3 ISE and conventional microelectrode recordings in hypoxic papillary muscle. Panel A: An ISE impaled a cell, and \( \Delta E \) was -15.8 mV, suggesting \( E_m \) was 5.1 mV positive to \( E_K \) in this cell. A KCl-filled electrode was already in a second cell. The two electrodes monitored a similar response when \([K^+]_o\) was elevated to 15 mM. The ISE was then withdrawn, and the potential returned to baseline. Panel B: A stimulated action potential. The impalement was the same as that in panel A, lower trace.
surface cells, \( \Delta E \) 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 \( \Delta E \) 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 \( \Delta E \) 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 \( \Delta E \) 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** Simultaneous recordings from cells with dissimilar electrical properties. Panel A: ISE\(_1\) impaled a cell, and \( \Delta E \) 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\(_1\) 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\(_1\) did not detect the TMA while ISE\(_2\) responded. After withdrawing from cell, both ISE responded. Panel B: The response of a negative \( \Delta E \) 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. Panel B illustrates that well-polarized cells exhibiting a negative ΔE also exclude 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, E_m was −13.7 ± 1.3 mV in 4.6 mM [K+] and −15.2 ± 1.7 mV after 5 minutes in 15 mM [K']. 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'] and [Na']. 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 G5N2 for 8 hours and then G5N2 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'], and a large decrease in [K']. Even though the values of [K'], and E_m are slightly greater than those obtained by McDonald and MacLeod (1971, 1973), they lead to the same conclusion. E_m is only slightly positive to E_k in oxygenated tissue, but it is significantly negative to E_k in hypoxic tissue.

Chemical analysis techniques were also used to determine the effect of exposure to 15 mM [K']o on [K'], 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'] media, [K'] 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'] and [K']o coincides with the results of Lee and Fozzard (1975) on rabbit ventricle and Sheu et al. (1980) on sheep Purkinje fibers, who found ak to be essentially unaffected by 30 minutes of exposure to 15 mM [K']o between 2 and 50 mM.

**Discussion**

These experiments confirm several previous observations by MacLeod and coworkers (MacLeod and Daniel, 1965; McDonald and MacLeod, 1971, 1973; Hunter et al., 1972) on the consequences of prolonged (>8-hour) hypoxia in guinea pig papillary muscle. Despite a significant decrease in the average [K']o, cells with near normal E_m and action potentials can be observed. In these cells, E_m is significantly negative to the apparent E_k 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 E_k, and Resting Potential of Guinea Pig Papillary Muscle

<table>
<thead>
<tr>
<th>Condition</th>
<th>H2O (ml/kg)</th>
<th>[Na'] (mm/liter)</th>
<th>[K'] (mm/liter)</th>
<th>E_k (mV)</th>
<th>E_m (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygenated (n = 7)</td>
<td>762 ± 4</td>
<td>18.9 ± 2.0</td>
<td>148.6 ± 3.3</td>
<td>−91.3 ± 2.2</td>
<td>−90.3 ± 0.5</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>
<td>−87.1 ± 0.7</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>
<td></td>
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</tbody>
</table>

Muscle diameter was <0.7 mm and mean wet weight was 1.63 mg. All values are means ± 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 (inulin) space of oxygenated and hypoxic muscles, respectively. The apparent E_k was calculated assuming both a homogeneous potassium distribution in cell water and equal intra- and extracellular activity coefficients.

![Figure 5](image-url)
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 \([K^+]_o\). 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 negativity to \( E_K \) have an obtunded response to changes in \([K^+]_o\). These findings are distinct departures from normal. 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 \([K^+]_o\) 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:

\[
\Delta E = 26.7 \ln \frac{a_k + b a_{Na}}{a_k + b a_{Na}^\infty} + S \ln \frac{a_k + k_{K-Na} a_{Na}}{a_k + k_{K-Na} a_{Na}^\infty}
\]

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 \( k_{K-Na} \) \( a_{Na}^\infty \) is negligible (cf., Equation 4). Since the slope of the ISE is nearly Nernstian (i.e., \( S = 26.7 \)), it can be shown that:

\[
a_k^\infty = \left( \frac{k_{K-Na} T}{(a_k^\infty + b a_{Na})} - \left( \frac{b T}{(a_k^\infty + k_{K-Na} a_{Na})} \exp \left( \frac{\Delta E}{26.7} \right) \right) \right)
\]

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

Analysis of the potential changes observed upon changing both 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 \( a_k^\infty \) is the value of \( T \). We assumed that \( T \), the sum of \( a_k \) and \( a_{Na}^\infty \), 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 0.1026 ± 0.0199 for 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.

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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 $[K^+]$, 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 electrogenic 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-

![Figure 6](https://circres.ahajournals.org/content/circres/49/5/1188/F6)

**Figure 6.** The effect of $P_{Na}/P_K$ and $T$, the sum of $a_k$ and $a_\text{anc}$ on the calculated values of $a_k$ (panel A) and $E_m$ (panel B). $a_k$ for the positive $\Delta E$ cells was calculated according to Equation 7 using $+11.8$ mV for $\Delta E$ and 0.033 for $k_v$. $E_m$ was calculated from the Constant Field equation assuming that the membrane is only permeable to potassium and sodium.
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., 1974) are focal in nature. Cardiac glycosides and Wein and McDonald (1978) Influence of glucose on the transmembrane potential of anoxic papillary muscle. J Gen Physiol 235: H559-H568.

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 Strauss recently have reported $ak$ measurements in hypoxic guinea pig ventricle (Fed Proc 40: 392, 1981).

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References


Heterogeneity of intracellular potassium activity and membrane potential in hypoxic guinea pig ventricle.

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