The Combined Effects of Hypoxia, High K\(^+\), and Acidosis on the Intracellular Sodium Activity and Resting Potential in Guinea Pig Papillary Muscle

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SUMMARY. Several reports have shown that electrical and ionic changes occurring in acute myocardial ischemia can be closely mimicked by exposure of tissue to hypoxic, acid-, and glucose-free solutions at elevated [K\(^+\)]\(_o\). In the present work, this approach was chosen to distinguish between the combined effects of hypoxia, substrate withdrawal, and acidosis, and the effects of two different levels of [K\(^+\)]\(_o\) (4.7 mM and 11.5 mM) on intracellular sodium activity and resting membrane potential. Measurements were made with microelectrodes in isolated guinea pig papillary muscles. In normoxia at 4.7 mM [K\(^+\)]\(_o\), intracellular sodium activity was 7.5 mM (±1.9 mM, SD) during stimulation at 1 Hz. Combined hypoxia, substrate withdrawal, and acidosis increased intracellular sodium activity significantly, by 3—4 mM in 4.7 mM [K\(^+\)]\(_o\) and by approximately 2 mM in 11.5 mM [K\(^+\)]\(_o\), after 9—10 minutes. Increasing [K\(^+\)]\(_o\) in normoxic solution decreased intracellular sodium activity by 1.9 mM (±1.3 mM, SD). The transition from normal (4.7 mM [K\(^+\)]\(_o\)) Tyrode’s solution to “ischemic solution” (hypoxia, acidosis, substrate withdrawal, 11.5 mM [K\(^+\)]\(_o\)) was associated with a small initial increase and a subsequent decrease of intracellular sodium activity. The steady state level after 12 minutes was not significantly different from the level in normal Tyrode’s solution. The secondary decrease of intracellular sodium activity coincided with the gradual development of inexcitability and was absent in quiescent preparations. Combined hypoxia, acidosis, and glucose-withdrawal produced a depolarization by 7—10 mV at 4.7 mM and at 11.5 mM [K\(^+\)]\(_o\), probably reflecting cellular potassium loss and extracellular potassium accumulation in the restricted extracellular space. Our results suggest that alteration of intracellular sodium in myocardial ischemia results from two opposing changes: (1) a partial inhibition of the sodium-potassium pump by hypoxia, glucose-withdrawal, and acidosis, and (2) a decrease of passive sodium influx following extracellular potassium accumulation. Moreover, the present results support previous findings that net cellular potassium loss from ischemic cells is not (fully) compensated by an equivalent gain of intracellular sodium. (Circ Res 58: 249–256, 1986)
papillary muscle. It has been shown that the combination of elevated $[K^+]_o$, hypoxia, and acidosis closely imitates the changes of membrane potential (Morena et al., 1980) and of impulse conduction (Kagiya et al., 1982) observed in myocardial ischemia. Under these experimental conditions, it proved possible (for the first time) to maintain two simultaneous intracellular electrode impalements over a relatively long period of time in a beating papillary muscle and, thus, to assess relatively small changes of intracellular Na$^+$ activity under different experimental conditions.

Methods

Preparations, Recording Chamber, and Solutions

Guinea pigs (body weight, 300–400 g) were killed by a blow to the head. The heart was rapidly removed and placed in a preparation chamber, where papillary muscles less than 4–5 mm long and less than 1 mm in diameter were excised from the right ventricle. Occasionally, muscles also were taken from the left ventricle and split longitudinally, forming a strip of the same diameter as the intact preparations from the right ventricle.

The Perspex recording chamber consisted of three compartments separated by two rubber membranes. The mid-compartment (width, 1 mm) was perfused with isotonic sucrose solution containing $10^{-5} M$ Ca$^{++}$ (Kleber, 1973). The left compartment contained isotonic KCl solution, and the right compartment was perfused with the test solution. The papillary muscle was introduced through holes in the two rubber membranes, with the apex of the preparation in the test compartment. Normally, the length protruding into the test compartment was 1.5–2 mm. For stimulation, rectangular 1.0-msec current pulses were applied between the left compartment (cathode) and the test compartment (anode, electrical ground) at double threshold strength. The sucrose gap arrangement was chosen in order to obtain close to simultaneous excitation of the cells in the test compartment. If the current threshold increased during an experimental intervention (at elevated $[K^+]_o$, with or without hypoxia), stimulus current strength was increased up to a maximum value of four times the initial value. The experimental temperature was kept between 36°C and 37°C. During superfusion at normal $P_o$, two different test solutions were used: (1) Tyrode’s solution at normal pH (7.4) and with normal $[K^+]_o$ (4.7 mm), and (2) Tyrode’s solution at normal pH and with elevated $[K^+]_o$ (11.5 mm). During exposure to hypoxia, three different test solutions were used, all with a low pH (mean 6.77, see below): (1) Tyrode’s solution with normal $[K^+]_o$, (2) glucose-free Tyrode’s solution with normal $[K^+]_o$, and (3) glucose-free Tyrode’s solution with elevated $[K^+]_o$ (11.5 mm). The composition of normal Tyrode’s solution was as follows (in mM): Na$^+$, 153.5; K$^+$, 4.7; Ca$^{++}$, 1.3; Mg$^{++}$, 0.6; Cl$^-$, 136.5; HCO$_3^-$, 25; H$_2$PO$_4^-$, HPO$_4^{--}$, 0.4; glucose, 20. In the solution containing 11.5 mM K$^+$, the Na$^+$ was reduced by 6.8 mM. This small reduction will cause a decrease of $a'_N$ by approximately 5% (Chapman et al., 1983), and effect which was neglected in our experiments. To obtain normoxic conditions and normal pH, gases the solutions, with a constant mixture of 95% O$_2$ and 5% CO$_2$ (Carba Gas, Switzerland) at 37°C. Hypoxia and acidosis were achieved by equilibrating the solutions with a mixture of N$_2$ and CO$_2$. The volume fraction of CO$_2$ in this mixture was adjusted to yield a pH of 6.77 (±0.05, so, $n = 31$). All connections between the glassware used for storage of the solutions and the recording chamber were made of stainless steel to prevent gas exchange between the virtually O$_2$-free test solutions and the surrounding atmosphere. In this way, a $P_o < 5$ mm Hg was obtained in the recording chamber, as measured by an O$_2$ microelectrode (J. Streit, unpublished data).

Measurement of Intracellular Na$^+$ Activity and Membrane Potential

Intracellular sodium activity ($a_{Na}$) was measured by microelectrodes (borosilicate glass) filled with the neutral Na$^+$ carrier ETH 227 (Steiner et al., 1979). Several reports have described the fabrication and calibration procedure of such Na$^+$-sensitive microelectrodes (e.g., Chapman et al., 1983). The details of the preamplifiers and amplifiers used to process the microelectrode signals were given in an earlier report (Kleber, 1983).

In the test compartment, each papillary muscle was impaled by a conventional microelectrode and a Na$^+$-sensitive microelectrode (interelectrode distance, <0.8 mm). The distance from the sucrose compartment was chosen to be greater than 1 mm to avoid the effects of sucrose diffusion into the test compartment (Kleber, 1973). Both potentials recorded from each electrode (conventional microelectrode, $V_{Na}$; Na$^+$-sensitive electrode, $V_{Na^+}$) were referred to the potential of a third microelectrode placed in the superfusing solution close to the impaling sites. Calibration curves relating $V_{Na^+}$ to $a_{Na}$ were obtained before and after an experiment (at 34–36°C) using the calibration chamber described by Weingart and Hess (1984). For calculation of $a_{Na}$, an activity coefficient of 0.764 was used (Bates et al., 1970). In the calibration solutions, the sum of Na$^+$ and K$^+$ was always 151.1 mm. The solutions contained in addition 3 mM Mg$^{++}$, 5 mM HEPES at a pH of 7.0 and Cl$^-$. The selectivity of the neutral Na$^+$ carrier, ETH 227, and interference from K$^+$ and Ca$^{++}$ have been discussed previously, e.g., by Cohen et al. (1982) and by Vasalle and Lee (1984). In the present experiments, increased K$^+$ efflux during hypoxia (Vleugels and Carmeli, 1976; Rau et al., 1977) and the consequent decrease of $a_K$ (Wier, 1978) might have changed the potential recorded by the Na$^+$ electrode. At a selectivity coefficient $K^+/Na^+$ of 0.01 to 0.02 (e.g., Dagostino and Lee, 1982), an assumed decrease of $a_K$ from a normal value of 100 mm (Wier, 1978; Baumgarten et al., 1981) by one-third would mimic a decrease of $a_{Na}$ by 0.3–0.6 mm. Therefore, this small source of interference was ignored in the analysis of the data.

A pen recorder (Watanabe mark VII) served for recording of $V_{Na}$, $V_{Na^+}$, and $V_{Na^+}-V_{Na}$. To obtain $V_{Na^+}-V_{Na}$ and $V_{Na^+}$ were filtered by two low-pass filters (corner frequency, 0.1 Hz) before subtraction (Lee and Dagostino, 1982; Vasalle and Lee, 1984). A single papillary muscle was subjected to a maximum of three hypoxic periods lasting no longer than 15 minutes. After these hypoxic periods, changes of $a_{Na}$ and $V_{Na}$ were always reversible. When longer periods were studied, the preparations were not used for further measurements. Only those experiments during which both impalements remained stable before and during a certain intervention were taken for analysis. In most cases, both electrodes were withdrawn into the bulk solution after a measurement, and were reimpaled before reexposure to a test solution. In the original recordings of $V_{Na^+}-V_{Na}$ shown in Results, the voltage scale (in mV) has been converted to $a_{Na}$ (in mm). The summarized values of $V_{Na}$, $V_{Na^+}$, $a_{Na}$, $a_{Na}$, and pH are
expressed as mean ± s.d. Statistical comparison of \( V_M \) and \( a_{Na} \) values before and during an experimental intervention was made by the paired samples t-test.

**Homogeneity of Resting Potential**

Theoretically, the potential error introduced by the measurement of \( V_M \) and \( a_{Na} \) with separate electrodes in different cells might become important during hypoxia: this is because the electronic interaction between neighboring cells will probably decrease as a consequence of an increase in internal longitudinal resistance (Wojtczak, 1979) and an increase in membrane conductance (Vleugels et al., 1980). Therefore, the problem of homogeneity of \( V_M \) was addressed in two papillary muscles (three measurements) by the simultaneous impalement with two electrodes (\( V_{M1}, V_{M2} \)), as illustrated in Figure 1. This figure shows the membrane potentials during continuous impalements (top and middle tracings) of two intracellular electrodes situated 0.8 mm apart. The bottom trace depicts the differential voltage \( V_{M2}-V_{M1} \). During the 11-minute period indicated by the bar from 1 to 2, the fiber was exposed to hypoxic, glucose-free solution at low pH; during the period 2 to 3, it was superfused with normoxic Tyrode’s solution at a \( [K^+]_o \) of 11.5 mM. After a period of exposure to normal Tyrode’s solution, the two electrodes were withdrawn into the extracellular space (arrow).

During both interventions, \( V_{M1} \) and \( V_{M2} \) decreased by the same amount and produced no change in the differential tracing \( V_{M2}-V_{M1} \). Upon withdrawal, the difference between \( V_{M1} \) (—86 mV) and \( V_{M2} \) (—84.5 mV) caused a shift of \( V_{M2}-V_{M1} \) by —1.5 mV. In all three control measurements, no change of \( V_{M2}-V_{M1} \) was observed during exposure to hypoxic solutions, whereas the shift upon withdrawal amounted to —1.5 mV, 0 mV, and 1 mV, respectively. This indicates the order of magnitude of differences in tip potential or resting potential which can affect the measurement of \( a_{Na} \) in a random fashion. However, interventions such as exposure to hypoxic or “high [K+]” solutions will not introduce additional inhomogeneity of resting potential or variability in the measurement of \( a_{Na} \).

**Results**

**Effects of Hypoxia and Acidosis on Membrane Potential and Intracellular Sodium**

Mean \( a_{Na} \) in 15 different muscles amounted to 7.5 mM (±1.9 mM, 33 impalements) during normoxic perfusion at \( [K^+]_o \) of 4.7 mM and at a stimulation frequency of 1 Hz. Under the same conditions, a mean resting potential of —83.4 mV (±3.6 mV; \( n = 33 \)) was obtained.

The effect of hypoxic, glucose-free and acidic solutions (\( [K^+]_o \) of 4.7 mM) on membrane potential and intracellular sodium activity in a single experiment is shown in Figure 2. Intracellular Na\(^+\) activity increased from an initial level of 5.1 mM to a plateau level within 10 minutes, which, on average, was 5 mM higher than the control level. Resting potential decreased with a similar time course from —86 mV to a new level of —80 mV. The changes of resting potential (\( \Delta V_M \)) and intracellular sodium activity (\( \Delta a_{Na} \)) expressed as mean values with standard deviations from six experiments (in six different preparations) are depicted in Figure 3 and listed in Table 1. All preparations were paced at 1 Hz. Resting membrane potential depolarized to a steady level after 11 minutes, which was 8.3 mV (±4.5 mV) less negative than the resting potential in normal Tyrode’s solution. In none of the experiments was spontaneous diastolic depolarization observed. The loss of resting potential was statistically significant (\( P < 0.05 \)) between 2 and 15 minutes of exposure to hypoxic, acidic, and glucose-free medium. Intracellular Na\(^+\) activity started to increase within the first 2 minutes after the change of the superfusate. The increase of \( a_{Na} \) reached a peak between 10 and 12 minutes (3.5 ± 2.7 mM at 10 minutes). Subsequently, a secondary minor decline occurred to values 2.5–3 mM above the control level (statistical significance \( P < 0.05 \)) was obtained for values between 6 and 14 minutes and between 22 and 30 minutes. It is not clear whether this secondary decrease was of biological relevance or whether it reflected random variation. It was not related to a change in amplitude or maximum upstroke velocity of the action potential.

In six additional experiments (five different preparations), the changes of \( V_M \) and \( a_{Na} \) were recorded after exposure to hypoxic, acidic Tyrode’s solution which contained 20 mM glucose (Table 1). The...
Elevation of extracellular K⁺ is known to decrease resting potential and to reduce intracellular sodium activity (Eisner et al., 1981; January and Fozzard, 1984). During early myocardial ischemia, an accumulation of extracellular potassium by approximately 12-15 mM and a concomitant decrease of resting potential by about 30 mV takes place within the first 10 minutes after cessation of perfusion (Kleber, 1983).

To assess the combined effects of hypoxia, acidosis and glucose with daration on VM and aNa at elevated [K⁺]o, we exposed papillary muscles first to normal Tyrode’s solution containing 11.5 mM [K⁺]o. Subsequently, we changed the superfusion to the hypoxic, acidic, and glucose-free solution at the same [K⁺]o. This caused an additional depolarization of VM to -56 mV. In contrast, intracellular sodium activity increased from 2.8 to 4.8 mM, i.e., to slightly below the control level in normal Tyrode’s solution. Subsequent exposure to normoxic Tyrode’s solution containing 4.7 mM [K⁺]o was followed by the return of VM and aNa to their initial values. The mean changes of aNa and VM obtained in five experiments, caused by hypoxia, glucose withdrawal, and acidosis at a [K⁺]o of 11.5 mM, are shown in Figure 5 and in Table 1. Resting membrane potential became more positive, and aNa increased significantly during the 9-

### Table 1

<table>
<thead>
<tr>
<th>Control solution</th>
<th>Test solution</th>
<th>Rate (Hz)</th>
<th>n</th>
<th>VM (mV)</th>
<th>aNa (mM)</th>
<th>ΔVM (mV)</th>
<th>ΔaNa (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Tyrode</td>
<td>Hypoxia, acidosis, no glucose, 4.7 mM [K⁺]</td>
<td>1</td>
<td>6</td>
<td>82.8 ± 3.8</td>
<td>6.2 ± 1.8</td>
<td>8.3 ± 4.5*</td>
<td>3.5 ± 2.7*</td>
</tr>
<tr>
<td>Normal Tyrode</td>
<td>Hypoxia, acidosis, 20 mM glucose, 4.7 mM [K⁺]</td>
<td>1</td>
<td>5</td>
<td>78.8 ± 1.9</td>
<td>7.6 ± 0.7</td>
<td>9.0 ± 3.3*</td>
<td>1.6 ± 1.0*</td>
</tr>
<tr>
<td>Normal Tyrode</td>
<td>Hypoxia, acidosis, no glucose, 11.5 mM [K⁺]</td>
<td>1</td>
<td>5</td>
<td>59.2 ± 2.6</td>
<td>3.8 ± 1.4</td>
<td>7.3 ± 2.3*</td>
<td>2.2 ± 1.3*</td>
</tr>
<tr>
<td>Normal Tyrode</td>
<td>Hypoxia, acidosis, no glucose, 11.5 mM [K⁺]</td>
<td>1</td>
<td>8</td>
<td>85.1 ± 3.2</td>
<td>7.9 ± 1.6</td>
<td>26.8 ± 1.7*</td>
<td>-0.9 ± 1.6</td>
</tr>
<tr>
<td>Normal Tyrode</td>
<td>Normal Tyrode, 11.5 mM [K⁺]</td>
<td>1</td>
<td>8</td>
<td>81.4 ± 3.0</td>
<td>6.9 ± 1.8</td>
<td>19.5 ± 3.3*</td>
<td>-1.9 ± 1.3*</td>
</tr>
</tbody>
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Results are expressed as mean ± SD. Tyrode = Tyrode’s solution. * Denotes statistical significance of changes in aNa and VM (P < 0.05).
minute period. After 9 minutes, ΔV_M averaged 7.3 ± 2.3 mV and Δa_{Na} was 2.2 ± 1.3 mM. The results shown in Figures 3 and 5 indicate that induction of hypoxia and acidosis is followed by a reduction of resting membrane potential and an increase of intracellular sodium activity, both at extracellular [K+]_o of 4.7 mM and 11.5 mM.

In ischemia, potassium accumulation develops within the first minutes after interruption of coronary flow. In order to mimic the transition from normoxic to ischemic conditions, we changed superfusion from normal Tyrode's solution ([K+]_o 4.7 mM) to hypoxic, acidic, and glucose-free solution containing 11.5 mM K* "(ischemic Tyrode)" for 11 minutes. Between times 1 and 2, resting potential decreased from −87 mV to −60 mV. Intracellular Na+ activity increased initially from 6.5 mM to 7.5 mM after 6 minutes. The occurrence of small amplitude potentials was associated with a secondary decrease of a_{Na} to 6.2 mM. Between time marks 2 and 3, the preparation was exposed to normoxic Tyrode's solution at a K* of 11.5 mM. This resulted in a slight hyperpolarization. At time 3, the papillary muscle was reexposed to normal Tyrode's solution.

The results of eight experiments (four preparations) in which Δa_{Na} and ΔV_M were recorded after a change from normal Tyrode's solution (4.7 mM [K+]_o) to hypoxic, acidic, and glucose-free medium at a [K*] of 11.5 mM are presented in Figure 7 (square symbols) and in Table 1. For comparison, they are superimposed on the changes of resting membrane potential and intracellular Na+ activity caused by elevation of extracellular [K*] alone, and the subsequent return to normal Tyrode's solution (circles, eight experiments in 10 preparations; see also Table 1). In both types of experiments, the preparations were stimulated at 1 Hz. Resting potential in the hypoxic condition declined to a level which was 7.0–7.5 mV more positive than in normoxic Tyrode's solution at a K* of 11.5 mM. Intracellular Na+ activity decreased, as expected, as a consequence of the elevation of extracellular K* alone. The decrease amounted to 1.9 ± 1.3 mM (Table 1). Intracellular Na+ activity during exposure to "ischemic" solution showed a biphasic time course. After an initial small increase of about 1 mM...
within 4-5 minutes, a secondary decrease to a steady level occurred which was 0.9 mM (±1.6 mM) below the initial level in normal Tyrode’s solution. This final aNa level (reached after 12 minutes in “ischemic” solution) was not significantly different from the level in normal Tyrode’s solution. The small biphasic change was probably real, although it was not statistically significant. This was suggested from the coincidence of occurrence of small amplitude potentials (average time 3.8 minutes, Figure 7) with the onset of the secondary decrease of aNa. After the transition from activity (1 Hz) to quiescence, a decrease of Na+ influx and, hence, of aNa is to be expected (Deitmer and Ellis, 1980; Cohen et al., 1982; Lee and Dagostino, 1982). Such an effect was regarded as important, because the gradual development of inexcitability, as shown, for example, in Figure 6, also occurs in the first minutes of acute myocardial ischemia (Downar et al., 1977). Therefore, we verified the dependence of aNa on rate by measuring aNa in quiescent muscles. In the absence of stimulation, aNa in normal myocardium is lower than during activity (Deitmer and Ellis, 1980; Cohen et al., 1982; Lee and Dagostino, 1982). In our experiments, this difference of aNa (steady state value after 5 minutes) between the quiescent state and stimulation at 1 Hz amounted to 1.5 mM (±1.4 mM, n = 9; P < 0.05). In addition, no biphasic response was observed in three additional fibers exposed to the solution mimicking "ischemia" but kept unstimulated. Intracellular Na+ activity remained practically stable, the change amounting to -0.3 mM (±1.4 mM) after 9 minutes. The absence of the secondary decrease in the quiescent state supported the interpretation that, during superfusion with "ischemic" solution, the secondary decrease of aNa was related to the development of inexcitability and, thus, inactivity of the muscle.

**Discussion**

**Effect of Hypoxia, Acidosis, and Extracellular K+ on Intracellular Sodium Activity**

In papillary muscles stimulated at 1 Hz and exposed to normal Tyrode’s solution, intracellular Na+ activity was found to be 7.5 mM (±1.9 mM). This value compares closely to previous measurements in cardiac Purkinje fibers (Glitsch and Pusch, 1980; Cohen et al., 1982; Sheu and Fozzard, 1982; Vassalle and Lee, 1984) and in isolated cardiac muscle (Cohen et al., 1982; Sheu and Fozzard, 1982; Chapman et al., 1983).

The main purpose of this study was to investigate the effects of hypoxia, acidosis, and glucose withdrawal on aNa at normal and elevated [K+]o. These conditions were chosen because it has been shown that the changes of resting potential and action potential measured in ischemic hearts (Downar et al., 1977) can be mimicked closely by adding K+ to a hypoxic, acidic, and glucose-free perfusate (Morena et al., 1980). This analogy is not surprising, because both accumulation of extracellular K+ (Hill and ettes, 1980) and tissue acidification (Garlick et al., 1979) take place in early ischemia, in addition to oxygen and substrate withdrawal.

The level of intracellular Na+ is determined primarily by the balance between passive Na+ influx and active Na+ efflux, the latter mediated predominantly by the Na+/K+ pump. At a constant [K+]o of 4.7 mM, the effect of hypoxia and acidosis (in the absence of glucose) was to increase aNa by 3-4 mM; this plateau level was reached within 10 minutes. The most probable explanation for this shift is a reduction in the activity of the sodium pump. Several reports are in favor of this hypothesis. (1) A reduction of Na+/K+-ATPase activity by 25% after 30 minutes of ischemia has been demonstrated by Bersohn et al. (1982) in sarcolemmal vesicles from rabbit myocardium. (2) The free energy change for hydrolysis of ATP, which provides the chemical driving force for phosphorylation of the Na+/K+-ATPase, decreases in the first 10 minutes (Kammermeier et al., 1982; Fiolet et al., 1984) with a time course similar to the change in aNa in our experiments. In part, this decrease is due to a change of inorganic phosphate (P) which accumulates 3-fold and reaches a plateau after 4 minutes in hypoxic ferret ventricle (Allen et al., 1985). In erythrocytes, an increase of P reduces the rate of sodium pumping without affecting the affinity relation between internal Na+ and the Na+/K+ pump (Garay and Garrahan, 1975).

Complete inhibition of Na+/K+ pumping by withdrawal of extracellular K+ or application of ouabain (Ellis, 1977; Eisner and Lederer, 1979) is followed
by an increase of $a_{Na}^*$ up to levels ranging from 15–20 mM. In addition to the present results, which show a significantly smaller increase of $a_{Na}^*$, studies on extracellular $K^+$ accumulation during early ischemia (Weiss and Shine, 1982; Kléber, 1983) have provided evidence that inhibition of the $Na^+/K^+$ pump cannot be complete. Qualitatively, the persistence of $Na^*/K^+$ pumping was demonstrated in those studies by a transient extracellular $K^+$ depletion upon a sudden decrease in stimulation rate. This effect was prevented by the application of a cardiac steroid (Klüber, 1983).

The present results indicate that, during exposure to hypoxia and acidosis, $a_{Na}^*$ is also modified by changes in $Na^+$ influx. Theoretically, this may involve several mechanisms: (1) a change in $Na^+$ permeability ($P_{Na}$) of the sarcolemmal membrane, (2) $Na^*/Ca^{++}$ or $Na^*/H^+$ exchange, (3) changes in membrane potential with consequent changes of the electrochemical gradient for $Na^+$, and (4) rate-dependent changes of $Na^+$ influx. At present, no experimental evidence for changes of $P_{Na}$ after O$_2$ withdrawal is available. Acidosis per se was reported to decrease $Na^+$ influx in nerve (Bass and Moore, 1983). An increase of $Na^+$ entry in exchange for $Ca^{++}$ or $H^+$ extrusion may occur. However, no detectable rise in intracellular free [Ca$^{++}$] was found in early hypoxia (Allen and Orchard, 1984), and the increase in intracellular free [H$^+$] is too small (Allen et al., 1985) to explain a detectable increase in [Na$^+$]. However, $a_{Na}^*$ in the present report was modified by changes of membrane potential. The decrease of $a_{Na}^*$ upon increasing $[K^+]_o$ probably was caused by reduction of $Na^+$ influx consequent to a decrease in the electrochemical driving force for $Na^+$ in the resting cell, and by a partial inactivation of $Na^+$ channels, thereby decreasing $Na^+$ influx during action potentials. Within the range of $[K^+]_o$ tested, modulation of $Na^*/K^+$ pumping (i.e., $Na^+$ efflux) by changes in $[K^+]_o$ is expected to play a minor role (for discussion, see e.g., January and Fozzard, 1984). In addition to the above effect of elevated $[K^+]_o$ per se (Fig. 7), the combination of high $[K^+]_o$, hypoxia, acidosis, and the absence of glucose leads to a gradual development of inexcitability (e.g., Fig. 6) which is associated with a decrease of $a_{Na}^*$. The same phenomenon is regularly observed between 4 and 7 minutes of acute myocardial ischemia (e.g., Downar et al., 1977). This may be regarded as equivalent to the transition from a stimulated to a quiescent fiber. The levels of internal sodium which accompany the transition from normal to "ischemic" Tyrode's solution was associated with a reduction of $V_M$ which was significantly greater than the reduction caused by elevation of $[K^+]_o$ from 4.7 to 11.5 mM in normal Tyrode's solution (Fig. 7). Net cellular $K^+$ loss, which most likely was the cause of this depolarization, occurred without a concomitant increase of $a_{Na}^*$. The mechanism of net $K^+$ efflux and the identity of the ions which account for electroneutrality remain to be elucidated (see Kléber, 1984).

**Effect of Hypoxia and Acidosis on Resting Membrane Potential**

In both myocardial ischemia and hypoxia, changes of resting membrane potential ($V_M$) closely follow the changes of potassium equilibrium potential [$E_K$ (Baumgarten et al., 1981; Guarnieri and Strauss, 1982; Kléber, 1983)]. In hypoxic muscle, a decrease of intracellular $K^+$ activity from 99–86 mM has been reported after 2 hours of hypoxic superfusion (Wier, 1978). At later stages, a more pronounced decrease of intracellular $K^+$ activity occurs (Baumgarten et al., 1981; Guarnieri and Strauss, 1982). In the early stage of hypoxia, intracellular $K^+$ depletion results from an increase of $K^+$ efflux (Vleugels and Carmeliet, 1976; Rau et al., 1977). In the case of myocardial ischemia, where no washout of $K^+$ from the extracellular space can take place, a marked increase in $[K^+]_o$ has been reported (e.g., Hill and Gettes, 1980). Our present results suggest that the depolarization which develops after exposure to hypoxia and acidosis at either $[K^+]_o = 4.7$ mM or 11.5 mM in the bulk solution was related to extracellular $K^+$ accumulation in the restricted extracellular fiber space, in addition to loss of intracellular $K^+$. Estimation of intracellular potassium activity from the observed membrane potential and from $[K^+]_o$ in the superfusate (by means of the Nernst equation) yields values that are considerably lower than the 86 mM found by Wier (1978) after 2 hours of exposure to hypoxia. This indicates that the extracellular $[K^+]_o$ which effectively contributes to the level of resting potential was higher than in the bulk solution.

Our results confirm indirectly that cellular $K^+$ loss in myocardial ischemia is not fully compensated by an equivalent gain of intracellular $Na^+$ (Kléber, 1983). This is suggested from the observation that the transition from normal to "ischemic" Tyrode's solution was associated with a reduction of $V_M$ which was significantly greater than the reduction caused by elevation of $[K^+]_o$ from 4.7 to 11.5 mM in normal Tyrode's solution (Fig. 7). Net cellular $K^+$ loss, which most likely was the cause of this depolarization, occurred without a concomitant increase of $a_{Na}^*$. The mechanism of net $K^+$ efflux and the identity of the ions which account for electroneutrality remain to be elucidated (see Kléber, 1984).
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