Influence of Potassium Ions and Osmolality on the Resting Membrane Potential of Rabbit Ventricular Papillary Muscle with Estimation of the Activity and the Activity Coefficient of Internal Potassium

By Toshio Akiyama and Harry A. Fozzard

ABSTRACT
Resting membrane potentials of rabbit right ventricular papillary muscles were measured in modified Tyrode's solutions that were isotonic (1.0 x T), hypertonic (1.58 x T), or hypotonic (0.76 x T) at eight different concentrations of external potassium, [K]o, ranging from 0.78 to 100 mm. The amount of hyperpolarization produced by exposure to the hypertonic solutions was relatively constant with an average of 4.6 mv at all levels of [K]o except 0.78 and 1.56 mm. This potential change is much less than the 10.6 mv which would be predicted if the papillary muscles behaved as nearly perfect osmometers and the activity coefficient of intracellular potassium (K) remained constant. The amount of depolarization produced by exposure to the 0.76 x T hypotonic solutions averaged 6.8 mv at all levels of [K]o, except 0.78 mm; this value is close to the predicted value of 6.9 mv. Variations in the activity and the activity coefficient of intracellular K were introduced to explain these discrepancies. We estimated that the activity of intracellular K in 1.58 x T hypertonic solution was increased 15-23% compared with that in isotonic solution. This change is much less than the 58% that would be predicted if the papillary muscle behaved as a nearly perfect osmometer. We also estimated that the activity of intracellular K in 0.76 x T hypotonic solution fell 22-26%, which is very close to the predicted value of 24%. The activity coefficient of intracellular K appeared to fall in 1.58 x T hypertonic solution to about 0.76 of its value in isotonic solution. This decline in the estimated activity coefficient of intracellular K could not be attributed to an increase in Coulombic attractive and repulsive forces as predicted by the Debye-Hückel equation for a univalent electrolyte.

The potassium (K) gradient across the cell membrane is the major determinant of resting membrane potential, and there is an almost linear relation between the resting potential and the logarithm of the external K concentration. However, the electrochemical events producing the transmembrane potential should be responsive to the activity of the K ion rather than its concentration (1, 2). All of the K in a salt solution is free ion only as the solution approaches infinite dilution; at higher concentrations, Coulombic forces result in association of anions and cations. For univalent ions in solutions with a concentration of less than several hundred millimolar, the Debye-Hückel equation accurately predicts the reduction in activity as concentration is increased; this reduction can be described in terms of the activity coefficient (activity/concentration). A common assumption in the use of the Nernst and Goldman equations to predict the membrane potential is that the activity coefficients for extracellular and intracellular ions are the same.

Recently, activities of K ions have been measured in the squid axon (2) and the rabbit papillary muscle (3). The activity coefficients of intracellular K have been shown to be much lower than was previously thought; they are 0.605 and 0.612, respectively. We explored the importance of this lower intracellular K activity coefficient by examining the effects of altering external and internal K concentrations on the resting membrane potential in rabbit papillary muscles. Changes in the internal K concentration were produced by exposing the muscles to solutions of different osmotic pressure but the same external K concentration. Activities and activity coefficients of internal K with solutions of different osmotic pressure were estimated by the changes in resting membrane potential by assuming that the membrane was a K electrode.

Methods

All of the experiments were performed on rabbit papillary muscles. The rabbits were killed by a blow to the cervical spine, and the intact heart was rapidly...
APPARATUS

Microelectrodes of the Ling-Gerard type were made by a vertical pipette puller (David Kopf Instruments) from Kimax glass tubes with an outside diameter of approximately 0.9 mm (Kimble Products). They were filled with 3M KCl and had d-c resistances of 9 to 30 megohms and tip potentials of less than −6 mv.

Membrane voltages were recorded as the difference in voltage between an internal electrode and an external reference electrode (Ag-AgCl). After the signals had been passed through emitter followers (Picometric, Instrumentation Laboratory, Inc.), they were displayed on a Tektronix 565 oscilloscope.

The Lucite tissue chamber was 3.7 × 1.2 × 0.5 cm. The papillary muscles were placed in the chamber and held lightly in place with 5.0 silk running across the muscle. There were two channels for solution flow through the stopcock, which was located very close to the chamber. The papillary muscles were superfused at a rate of approximately 10 ml/min. The osmolalities of the solutions were determined by freezing point depression measured with a Fiske osmometer (model G-62).

SOLUTIONS

Distilled water and analytical grades of electrolytes and sucrose (Mallinckrodt Chemical Works) were used. The inorganic chemicals were made up as 0.5M or 2M stock solutions; dextrose and sucrose were prepared as 1M stock solutions. All of the solutions were cautiously mixed using calibrated micropipettes and volumetric flasks. The compositions of the solutions used are shown in Table 1. To obtain variable concentrations of K, KCl was substituted for NaCl. Hypertonic solutions were made by adding sucrose solution. Hypotonic solutions were prepared by reducing the content of NaCl. Isotonic Tyrode’s solution containing 5.4 mM K was used to store the rabbit papillary muscles until the time of the study. All of the solutions were bubbled with 95% O2-5% CO2 and warmed to a temperature between 35 and 37°C (monitored with a Tele-Thermometer, Yellow Springs Instrument Co., Inc.).

RESTING MEMBRANE POTENTIAL

Resting membrane potentials (E<sub>r</sub>) were measured to the nearest millivolt. Care was taken to impale cells of the surface layer of the preparation. The criteria for acceptance of a resting membrane potential were an abrupt deflection from the base line on impalement, a stable potential for at least 20 seconds, and an abrupt return to the same base line on withdrawal. If the initial and final base line differed by more than 2 mv, the measurement was discarded.

Results

EFFECT OF THE EXTERNAL K CONCENTRATION ON THE RESTING MEMBRANE POTENTIAL IN ISOTONIC SOLUTIONS

The rabbit right ventricular papillary muscles were kept in isotonic Tyrode’s solution containing 5.4 mM K for between 0.5 and 2 hours after dissection. All of the muscles were then held in each test solution for at least 1 hour before E<sub>r</sub> was measured. Papillary muscles that weighed around 10–15 mg were selected from the right ventricle. The experiments were done at temperatures between 35 and 37°C.

### Table 1

<table>
<thead>
<tr>
<th>Solution</th>
<th>NaCl (mEq/L)</th>
<th>KCl (mEq/L)</th>
<th>Sucrose (g/L)</th>
<th>Osmolarity (mOsm/L)</th>
<th>Volume (% of water)</th>
<th>E&lt;sub&gt;r&lt;/sub&gt; (mv)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotonic</td>
<td>141.6</td>
<td>5.4</td>
<td>140.9</td>
<td>313</td>
<td>0.78</td>
<td>5.43</td>
</tr>
<tr>
<td>Hypertonic</td>
<td>141.6</td>
<td>141.6</td>
<td>64.5</td>
<td>301</td>
<td>0.78</td>
<td>5.43</td>
</tr>
<tr>
<td>Hypotonic</td>
<td>141.6</td>
<td>5.4</td>
<td>140.9</td>
<td>313</td>
<td>0.78</td>
<td>5.43</td>
</tr>
</tbody>
</table>

In addition to the ingredients listed in the table, each solution contained NaHCO<sub>3</sub> 13.5 mM, NaHPO<sub>4</sub> 2.4 mM, CaCl<sub>2</sub> 1.8 mM, MgCl<sub>2</sub> 1.05 mM, and dextrose 11 mM.
INFLUENCE OF K AND OSMOLALITY ON $E_r$

 measured. To minimize the equilibration time when the same muscle was used in more than one test solution, the solutions were applied in order of increasing K concentration (4).

The resting membrane potentials in these solutions are shown in Table 2 and plotted against the logarithm of the external K concentration ([K]o) in Figure 1. The slope for the linear part of the curve in isotonic solution at [K]o greater than 12.5 mM was 53.6 mv per tenfold change in [K]o, which is slightly less than the 61.4 mv/decade calculated for a temperature of 36.5°C. The departure of $E_r$ from a K electrode was progressively greater at the lower [K]o, as others have previously observed (1, 4, 5).

The standard deviation for $E_r$ became progressively larger at lower [K]o (Table 2). At the lowest [K]o of 0.78 mM, the papillary muscles did not seem to be in a steady state and gave a wide variation in $E_r$ ranging from 72.0 to 102.5 mv. This finding is in agreement with the observations of Page (4), who noted that $E_r$ ranged from 60 to 117 mv at a [K]o of 1.0 mM in cat papillary muscles.

EFFECT OF THE EXTERNAL K CONCENTRATION ON THE RESTING MEMBRANE POTENTIAL IN HYPOTONIC SOLUTIONS

Following the shift from isotonic to 0.76 × T hypotonic solution (relative tonicity is expressed in terms of a multiple of isotonicity [T]) of the same [K]o, all muscles were incubated for at least 10 minutes before measurement of $E_r$. As seen in Figure 2, depolarization in 0.76 × T hypotonic solution was rapid, and repolarization to the original $E_r$ was prompt on returning to the isotonic solution. The membrane potential in hypotonic solution was unchanged for up to 1 hour at all levels of K. In one experiment using a 0.50 × T hypotonic solution with a [K]o of 6.25, however, the muscle depolarized rapidly and was irreversibly damaged in several minutes.

The amount of depolarization in the 0.76 × T hypotonic solutions was relatively constant at each [K]o, ranging from 5.7 to 7.6 mv, except for that at 0.78 mM K. At this low [K]o, the papillary muscles were damaged on exposure to the 0.76 × T hypotonic solution and failed to return to their normal resting potentials. The slope of the linear part of the curve with 0.76 × T hypotonic solutions was approximately 51.1 mv for a tenfold change in [K]o, which is very close to the 53.6 mv observed with isotonic solutions.

EFFECT OF THE EXTERNAL K CONCENTRATION ON THE RESTING MEMBRANE POTENTIAL IN HYPERTONIC SOLUTIONS

Hyperpolarization in 1.58 × T hypertonic solutions was complete within a few minutes, and it was stable for as long as 1 hour. Measurement of $E_r$ was made after 10 minutes of equilibration. After they were returned to isotonic solutions, the papillary muscles repolarized promptly to the original level. As seen in Table 2 and Figure 1, the amount of hyperpolarization was relatively constant and ranged from 3.2 to 5.5 mv at a [K]o ranging from 3.13 to 100 mM. At a [K]o of 1.56 mM, however, there was no statistically significant difference in $E_r$ between the hypertonic and isotonic states. Due to the instability of papillary muscles at a [K]o of 0.78 mM, measurements of $E_r$ in 1.58 × T hypertonic solutions were performed on only one muscle; they did not seem to be different from those for the isotonic state.

Preliminary data for rabbit atrial trabeculae are in agreement with these observations for 1.58 × T hypertonic solutions in that the degree of hyperpolarization in the rabbit atrial trabeculae was around 5 mv for a [K]o of 4.5 to 71 mM.

ESTIMATION OF ACTIVITIES OF INTRACELLULAR K IN HYPERTONIC AND HYPOTONIC STATES

The amount of hyperpolarization with 1.58 × T hypertonic solution, $\Delta E_{r1.58}$, and the amount of depolarization with 0.76 × T hypotonic solution, $\Delta E_{r0.76}$, are:

$$\Delta E_{r1.58} \approx 53.6 \log_{10}\left(\frac{a_{K1}}{a_{K1.58}}\right). \quad (1)$$

$$\Delta E_{r0.76} \approx 53.6 \log_{10}\left(\frac{a_{K0.76}}{a_{K1}}\right) - 0.54, \quad (2)$$

where $a_{K1.58}$, $a_{K1}$, and $a_{K0.76}$ are the activities of intracellular K in 1.58 × T hypertonic, isotonic, and 0.76 × T hypotonic solutions, respectively (see Appendix 1 for derivation of Eqs. 1 and 2). Since the behavior of rabbit papillary muscles was close to that of a K electrode only at the higher [K]o, estimation of activity ratios from Eqs. 1 and 2 may not be valid for the observations with lower [K]o. As shown in Table 3, at a [K]o of 12.5 mM or greater, $a_{K1.58}/a_{K1}$ ranged from 1.15 to 1.23 with an average of 1.20. This ratio is much less than 1.58, which would be expected if the papillary muscles behaved as nearly perfect osmometers and the activity coefficient of intracellular K remained constant. The ratio of $a_{K0.76}/a_{K1}$ was 0.77, close to the predicted result for ideal water movement.

ESTIMATION OF ACTIVITY COEFFICIENTS OF INTRACELLULAR K IN HYPERTONIC AND HYPOTONIC STATES

With the assumption that the papillary muscles behave as a nearly perfect osmometer for the range
Resting Membrane Potentials in Various Solutions

<table>
<thead>
<tr>
<th>[K]o (mM)</th>
<th>0.78</th>
<th>0.78</th>
<th>1.56</th>
<th>1.56</th>
<th>3.13</th>
<th>3.13</th>
<th>3.13</th>
<th>6.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative tonicity</td>
<td>0.76</td>
<td>1.0</td>
<td>1.58</td>
<td>0.76</td>
<td>1.0</td>
<td>1.58</td>
<td>0.76</td>
<td>1.0</td>
</tr>
<tr>
<td>Number of muscles studied</td>
<td>7</td>
<td>1</td>
<td>3</td>
<td>12</td>
<td>5</td>
<td>3</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Number of measurements</td>
<td>86</td>
<td>9</td>
<td>21</td>
<td>82</td>
<td>38</td>
<td>21</td>
<td>74</td>
<td>25</td>
</tr>
<tr>
<td>ER ± 1 SD</td>
<td>92.5 ± 9.4</td>
<td>95.7 ± 9.4*</td>
<td>90.7 ± 3.2</td>
<td>98.3 ± 3.4</td>
<td>97.7 ± 3.6*</td>
<td>78.5 ± 1.7</td>
<td>86.1 ± 1.7</td>
<td>91.6 ± 2.6</td>
</tr>
<tr>
<td>Range of ER</td>
<td>72-106.5</td>
<td>83-106</td>
<td>82-94</td>
<td>92-106</td>
<td>76-82</td>
<td>84-92</td>
<td>88-96</td>
<td>64-71</td>
</tr>
</tbody>
</table>

Relative tonicity is expressed in terms of a multiple of isotonicity; osmolalities were determined by freezing point depression. ER = resting membrane potential.

* P < 0.001 compared with the value in the same external K concentration at a relative tonicity of 1.0.

of relative tonicity from 0.76 to 1.58 in which the experiments were performed, the amount of hyperpolarization with 1.58 x T hypertonic solution, \( \Delta E_{R,1.58} \), and the amount of depolarization with 0.76 x T hypotonic solution, \( \Delta E_{R,0.76} \), at 36.5°C are given by the Eqs. 3 and 4, respectively:

\[
\Delta E_{R,1.58} \approx 53.6 \log_{10} \left( \frac{f_t}{f_{11.58}} \right) - 10.6, \tag{3}
\]

\[
\Delta E_{R,0.76} \approx 53.6 \log_{10} \left( \frac{f_t}{f_{10.76}} \right) - 6.9, \tag{4}
\]

where \( f_{11.58} \), \( f_t \), and \( f_{10.76} \) are activity coefficients of intracellular K in 1.58 x T hypertonic, 1.0 x T isotonic, and 0.76 x T hypotonic solutions, respectively (see Appendix 2 for derivation of Eqs. 3 and 4). The equations predict that the amount of hyperpolarization with 1.58 x T hypertonic solutions will be 10.6 mV and the amount of depolarization with 0.76 x T hypotonic solutions will be 6.9 mV, provided the activity coefficient of intracellular K remains the same in the hypotonic and hypertonic states (i.e., \( f_t = f_{10.76} \) and \( f_t = f_{11.58} \)). As shown in Table 2 and Figure 1, however, the recorded amount of hyperpolarization with 1.58 x

**Figure 1**

Influence of the potassium concentration ([K]o) and the osmolality of superfusing solutions on the resting membrane potentials (E_r) of rabbit papillary muscles. The results of E_r (mV) are plotted against [K]o (mM) on a logarithmic scale as the average value ± 1 SD obtained in 1.0 x T isotonic solutions (open circles), 1.58 x T hypertonic solutions (solid circles), and 0.76 x T hypotonic solutions (triangles). (Relative tonicity is expressed in terms of a multiple of isotonicity [T]). The lines are drawn by eye to give a good fit to the experimental points.

**Figure 2**

Time course of resting membrane potential (E_r) of rabbit papillary muscles with change in the tonicity of the superfusate at [K]o = 50 mM (top) and [K]o = 6.25 mM (bottom). The types of superfusing solutions tested are indicated in the top portion of each section: 1.0 x T = isotonic Tyrode's solution, 1.58 x T = sucrose hypertonic Tyrode's solution with a relative tonicity of 1.58, and 0.76 x T = hypotonic Tyrode's solution with a relative tonicity of 0.76. Each dot corresponds to a separate impalement.

Circulation Research, Vol. 37, November 1975
INFLUENCE OF K AND OSMOLALITY ON E_r

<table>
<thead>
<tr>
<th>[K]_o (mM)</th>
<th>12.5</th>
<th>25</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.76</td>
<td>1.0</td>
<td>1.0</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>1.58</td>
<td>1.0</td>
<td>1.0</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>0.76</td>
<td>1.0</td>
<td>1.0</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>1.58</td>
<td>1.0</td>
<td>1.0</td>
<td>1.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

T hypertonic solutions at a [K]_o of 12.5 mM or greater averaged 4.2 mv, which is much less than the predicted value of 10.6 mv. The degree of depolarization with 0.76 x T hypertonic solutions at a [K]_o of 12.5 mM or greater, however, averaged 6.7 mv, which is probably not different from the predicted value of 6.9 mv. As seen in Figure 2, papillary muscles promptly repolarized to the original level on being returned to isotonic solutions. It is unlikely, therefore, that the smaller amount of hyperpolarization with 1.58 x T hypertonic solutions relative to the value predicted by Eq. 3 was caused by movements of K ions across the cell membrane.

To test the possible role of a change in microelectrode tip potential in producing the discrepancies between the observed and the predicted amount of hyperpolarization with 1.58 x T sucrose hypertonic solutions, a total electromotive force (emf) in the circuit shown in Figure 3 was measured. The process of impalement of the papillary muscles in 1.58 x T sucrose hypertonic Tyrode's solution was simulated by switching the solution in the test compartment from 1.58 x T sucrose hypertonic solution to 1.58 x T KH2PO4 hypertonic solution, which was made hypertonic by adding KH2PO4 to isotonic Tyrode's solution. The concentration of K was 25 mM for the sucrose hypertonic solution and 118 mM for the KH2PO4 solution. Deviation of emf with 1.58 x T KH2PO4 hypertonic solution in the test compartment from the reference level was less than —1 mv for three different glass microelectrodes with d-c resistances of 9, 10 and 10 megohms. Finally, the test compartment was again filled with 1.58 x T sucrose hypertonic solution, which gave no significant deviation in the reference level. Tip potentials for each of the three microelectrodes were —4, —5, and —5 mv in 1.58 x T sucrose hypertonic solution.

In an attempt to understand the discrepancy between the observed and the predicted values of E_r with 1.58 x T hypertonic solutions, activity coefficients, f_1.58 and f_0.76, were estimated as f_1.58/xi and f_0.76/xi using Eqs. 3 and 4 (Table 3). If

<table>
<thead>
<tr>
<th>[K]_o (mM)</th>
<th>0.78</th>
<th>1.56</th>
<th>3.13</th>
<th>6.25</th>
<th>12.5</th>
<th>25</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>a_0.78</td>
<td>0.738</td>
<td>0.738</td>
<td>0.800</td>
<td>0.741</td>
<td>0.777</td>
<td>0.784</td>
<td>0.767</td>
<td></td>
</tr>
<tr>
<td>f_0.78</td>
<td>0.971</td>
<td>0.972</td>
<td>1.05</td>
<td>0.972</td>
<td>1.021</td>
<td>1.03</td>
<td>1.006</td>
<td></td>
</tr>
<tr>
<td>a_1.56</td>
<td>1.15</td>
<td>0.974</td>
<td>1.368</td>
<td>1.19</td>
<td>1.23</td>
<td>1.23</td>
<td>1.15</td>
<td></td>
</tr>
<tr>
<td>f_1.56</td>
<td>0.728</td>
<td>0.618</td>
<td>0.802</td>
<td>0.754</td>
<td>0.782</td>
<td>0.782</td>
<td>0.747</td>
<td></td>
</tr>
</tbody>
</table>

a_0 = activity of internal K ion, a_0.78 = activity in 0.76 x T hypotonic state, a_1.58 = activity in 1.58 x T hypertonic state, f_1 = activity coefficient of internal K ion, f_0.78 = activity coefficient in 0.76 x T hypotonic state, and f_1.58 = activity coefficient in 1.58 x T hypertonic state. Activities and activity coefficients of internal K were estimated using Eqs. 1 and 2 and Eqs. 3 and 4, respectively. Due to the instability of papillary muscles at a [K]_o of 0.78 mM, measurements of E_r in 1.58 x T hypertonic solutions were performed in only one papillary muscle.

Circulation Research, Vol. 37, November 1975
Oscilloscope

1.58X T sucrose hypertonic solution (Ko) 25 mM.

Test solution.

FIGURE 3

Influence of microelectrode tip potential on the measurement of resting membrane potential in 1.58 x T sucrose hypertonic Tyrode's solution. The process of impalement of papillary muscles in 1.58 x T sucrose hypertonic Tyrode's solution was simulated by switching the solution in the test compartment from 1.58 x T sucrose hypertonic Tyrode's solution to 1.58 x T (H2PO4 hypertonic Tyrode's solution. ME = microelectrode and KCl Bridge = KCl-filled agar bridge.

we assume that f1 = 0.612, as reported by Lee and Fozzard (3), then estimates of f10.76 and f11.58 can be made. These results are illustrated in Figure 4 for a comparison between KCl and KH2PO4 solutions. The average estimated activity coefficients of intracellular K in solutions with a [K]o of 3.1 to 100 mm in hypertonic and hypotonic solutions are also plotted. The change across this range of tonicity appears to be greater than that for either of the two salt solutions.

Discussion

Our observations in rabbit muscle are in keeping with those from three previous experiments in various muscles. Fozzard and Kipnis (6) found 7 mv of hyperpolarization at a [K]o of 4.7 mm with 1.9 x T sucrose hypertonic solution in the rat diaphragm, which gives a f11.58/f1 ratio of 0.685 using Eq. 3, and Gibbons and Fozzard (7) found 7.5 mv of hyperpolarization at [K]o of 18.5 to 100 mm in hypertonic and hypotonic solutions are also plotted. The change across this range of tonicity appears to be greater than that for either of the two salt solutions.

ventricular muscles of the cat, chick, guinea pig, and frog, which gives a f12.58/f1 ratio of approximately 0.65 based on their Figure 5. Our findings in rabbit papillary muscles, however, differ significantly from those in frog skeletal muscles for which the f11.58/f1 ratio is around 0.93-0.94 based on Figure 10 of Adrian's report (1). Moreover, in the frog, the degree of observed hyperpolarization with 1.75 x T sucrose hypertonic solutions agrees with the value calculated based on the assumption that frog skeletal muscles behave like nearly perfect osmometers, which suggests that f11.58/f1 is close to unity (5). Our estimated activity coefficients of intracellular K with 0.76 x T hypotonic solutions, in contrast to those with 1.58 x T hypertonic solutions, differed only slightly from the values obtained with isotonic solutions in that they ranged from 97 to 105% of the value for the isotonic state.

INTERNAL K ACTIVITY

The simplest interpretation of the dependence of E\textsubscript{r} on external K concentration is that the membrane is selectively permeable to K. A logical inference is that changes in internal K also alter E\textsubscript{r} in a predictable way. In these experiments, internal K was changed by exposing the muscle fiber to hypotonic and hypertonic solutions. Dilution of

FIGURE 4

Effect of tonicity on the estimated activity coefficients of internal K and the measured activity coefficients of KCl and KH2PO4 solutions. Dots are estimated activity coefficients of intracellular K of papillary muscles calculated using Eqs. 3 and 4. The activity coefficient of internal K in the isotonic state, f1 = 0.612 (circled dot), is from Lee and Fozzard (3). The activity coefficients of the KCl solution (open triangles) and the KH2PO4 solution (open circles) were obtained from Robinson and Stokes (13); the lines are drawn by eye to give a good fit.

Circulation Research, Vol. 37, November 1975
internal K gave the predicted change in membrane potential at all values of external K above 6.3 mM. Concentration of internal K, however, failed to produce the predicted hyperpolarization. The logarithmic relation between \( E_r \) and external K suggests that the membrane remained a good K electrode under both hypotonic and hypertonic conditions.

Recognizing the fact that the behavior of a K electrode is related to the ratio of K activities and not concentrations, we were led to consider that the failure of the fiber to hyperpolarize enough in hypertonic solution was the result of a fall in the K activity coefficient. The relation between K concentration and its activity coefficient is given by the Debye-Hückel equation for dilute solutions of univalent salts. However, the interior of the fiber is not a simple solution of KCl, and it is not safe to assume that the K activity coefficient is identical inside and outside the cell. Indeed, some measurements of K activity in ventricular muscle suggest that the apparent K activity coefficient is less than that in the bathing Tyrode’s solution. The results reported by Lee and Fozzard (3) are consistent with either compartmentalization of some of the internal K or a lower K activity coefficient. The latter might be explained by the complex nature of the interior of the cell, especially the possible multivalent nature of the cellular anions. Since we do not know the nature of the intracellular anions, we might consider that the interior resembles a \( KH_2PO_4 \), solution more closely than it does a KCl solution (Fig. 4). For this salt, the K activity is lower than it is for the equivalent KCl concentration. Furthermore, its relation to concentration is steeper in that raising the concentration causes a larger change in the activity coefficient than it does for KCl. Shown below the \( KH_2PO_4 \) curve in Figure 4 are the values for an activity coefficient estimated from these experiments, suggesting that the intracellular K salt has an even steeper relation to concentration.

A logical interpretation of the results of these experiments is that the K activity coefficient within the myocardial cell is lower than that in the bathing solution and that this difference is a consequence of the multivalent nature of the intracellular anions. To the extent that this concept is true, the use of intracellular K concentrations to estimate membrane potential under different conditions is unwise.

\( a_{K_i} \) is probably higher in these experiments than the value measured by Lee and Fozzard (3), since calculation of resting potential using \( a_{K_i} = 82.6 \) results in a predicted potential less negative than those that we observed experimentally in isotonic solutions. This conclusion is supported by extrapolation of the membrane potentials in isotonic solution (Fig. 1) to \( [K]_o = 160 \text{ mM} \) for 0 mV potential. Estimation of \( a_{K_i} \) by extrapolation may not be accurate, since it assumes that the membrane is symmetrical. In studies of glass membranes, an “asymmetry potential” has been defined, which represents the residual potential when both sides of the electrode are bathed by the same solution. The origin of this asymmetry potential in glass membranes is not clear, but it may be related to inhomogeneities in the glass (9). Some asymmetry probably also exists in the cell membrane, making the extrapolated value difficult to interpret. However, the main conclusion of this work is an excessive change in the K activity coefficient on exposure to the hypertonic solution, and this change is apparent in activity ratios, where the actual activity values are cancelled. The relations between membrane potential and \( [K]_o \) were almost parallel, so that ratios derived from extrapolated values gave the same result.

**INFLUENCE OF THE SODIUM PUMP**

There is now abundant evidence that the sodium (Na) pump can be electrogenic in nerve and muscle (10), particularly when it is stimulated by increased internal Na. Exposure of the muscle to a hypertonic solution would raise both internal K and internal Na. This change might increase active Na efflux and would tend to hyperpolarize the fiber further. Therefore, the influence of enhancement of electrogenic Na pumping would be opposite to the effects found experimentally. Since internal Na contents or activities were not measured in these experiments and no experiments were done in the presence of digitalis glycosides, we cannot be entirely sure that the Na pump had no influence on these studies.

**OTHER SOURCES OF ERROR**

Since the behavior of rabbit papillary muscles resembled that of a K electrode only at the higher \( [K]_o \), estimations of \( a_{K_{11.3}}/a_{K_1}, a_{K_{10.7}}/a_{K_1}, f_{11.3}/f_1 \) and \( f_{10.7}/f_1 \) using Eqs. 1–4 are probably not valid for the observations with lower \( [K]_o \), where the Na gradient across the cell membrane is thought to be responsible for the greater deviation of observed \( E_r \) from the Nernst equation. Possible errors intro-
duced by not counting the effects of the Cl gradient across the cell membrane in estimating the activity coefficient of internal K by measuring $E_r$ should be small, due to the small value for Cl permeability in the resting state (11).

A change in the tip potential of the microelectrode on entering the intracellular space might significantly influence the measurement of resting membrane potential. So far, we do not have any direct technique available to measure tip potential during impalement of a cell. From Table 4-2 of Agin's experiments (12), a microelectrode filled with 1M KCl should give a -1-mv difference as the K concentration of the outside solution of KCl plus 2mM CaCl$_2$ is changed from 150mM (1.0 x T isotonic state) to 237mM (1.58 x T hypertonic state). We tried to simulate the process of impalement of the papillary muscles in 1.58 x T sucrose hypertonic superfuse by switching the solution in the test compartment of Figure 3 from 1.58 x T sucrose hypertonic Tyrode's solution to 1.58 x T KH$_2$PO$_4$ hypertonic Tyrode's solution. In doing so, we observed that the shift in base line, i.e., the level of emf in the total circuit, was less than 1 mv. It seems that only a very small fraction of the large discrepancies between the observed and the predicted amount of hyperpolarization with 1.58 x T sucrose hypertonic Tyrode's solution can be explained by a systematic change in the tip potential of the microelectrodes during impalement.

**Appendix 1**

**ESTIMATION OF ACTIVITIES OF INTRACELLULAR K IN HYPERTONIC AND HYPOTONIC STATES**

The transmembrane potential for a cell that behaves like a K electrode can be predicted by the Nernst equation:

$$E_r = \frac{RT}{F} \log \left( \frac{aK_i}{aK_o} \right).$$  \hspace{1cm} (5)

where $E_r$ is resting potential, $aK_i$ and $aK_o$ are activities of intra- and extracellular K, and $R$, $T$, and $F$ have their usual meanings. The resting membrane potential in a sucrose hypertonic solution of relative tonicity 1.58 x T, $E_{1.58}$, is given by:

$$E_{1.58} = \frac{RT}{F} \log \left( \frac{aK_i}{aK_o} \right),$$  \hspace{1cm} (6)

where $aK_{i,1.58}$ is the activity of intracellular K in the 1.58 x T hypertonic state. Consequently, from Eqs. 5 and 6, the amount of hyperpolarization with hypertonic solution, $\Delta E_{1.58}$, is given by:

$$\Delta E_{1.58} = E_{1.58} - E_r = \frac{RT}{F} \log \left( \frac{aK_i}{aK_o} \right) - \frac{RT}{F} \log \left( \frac{aK_{i,1.58}}{aK_{i,1.58}} \right),$$  \hspace{1cm} (7)

where the slope of the linear part of the curve, 53.6 mv per tenfold change in external K, was used instead of the theoretical value of 61.4 mv with the base of logarithm 10 at a temperature of 36.5°C for $RT/F$. Similarly, the amount of depolarization with 0.76 x T hypotonic solutions, $\Delta E_{0.76}$, is given by:

$$\Delta E_{0.76} = E_r - E_{0.76} = \frac{RT}{F} \log \left( \frac{aK_i}{aK_o} \right) - \frac{RT}{F} \log \left( \frac{aK_{i,0.76}}{aK_{o,0.76}} \right),$$  \hspace{1cm} (8)

where $aK_{i,0.76}$ and $aK_{o,0.76}$ are activities of intra- and extracellular K, respectively.

Introducing $f_o = f_o[K_o]$ and $f_{i,0.76} = f_{i,0.76}[K_o]$, where $f_o$ and $f_{i,0.76}$ are activity coefficients of extracellular K for isotonic and hypotonic solutions, Eq. 8 is obtained.

$$\Delta E_{0.76} = \frac{RT}{F} \log \left( \frac{aK_{i,0.76}}{aK_{i}} \times \frac{f_o}{f_{i,0.76}} \right).$$  \hspace{1cm} (9)

Now, the activity coefficient of the electrolyte solution, $f_o$, up to 1M concentration is given by the empirical equation of Debye-Hückel (13):

$$\log f_o = -\frac{A[Z_iZ_j] \sqrt{T}}{1 + aZ_iZ_j} + bI,$$  \hspace{1cm} (10)

where $a$, the diameter of the hydrated K ion, was taken as 4Å, $A = 0.521$ and $b = 0.33 \times 10^4$ at a temperature of 35°C, $Z_i = 1$, and $Z_j$ are the charge on the positive and negative ions, and $I$ is the ionic strength. This equation estimates the activity coefficient of extracellular K in the isotonic and 0.76 x T hypotonic solution: $f_o \approx 0.742$ and $f_{i,0.76} \approx 0.759$. Introducing these values into Eq. 8 yields the following equation:

$$\Delta E_{0.76} \approx 53.6 \log_{10} \left( \frac{aK_{i,0.76}}{aK_{i}} \right) - 0.54.$$

**Appendix 2**

**ESTIMATION OF ACTIVITY COEFFICIENTS OF INTRACELLULAR K IN HYPERTONIC AND HYPOTONIC STATES**

To evaluate the effects of changes in osmolality on activity coefficient of intracellular K, the assumption has been made that the papillary muscles of the rabbit right ventricle behave like nearly perfect osmometers for the range of relative tonicity from 0.76 to 1.58 in which the experiment were performed. This assumption is supported in vertebr myocardial cells by the observation that cat heart muscle behave like nearly perfect osmometers (14,15). Then, $[K]$ and $[K]_{i,0.76}$, the intracellular concentration in the hypertonic and hypotonic state, respectively, can be expressed as a function of $[K]$:

$$[K]_{i,1.58} = [K] \times 1.58,$$  \hspace{1cm} (10)

$$[K]_{i,0.76} = [K] \times 0.76.$$  \hspace{1cm} (11)

Now, activities of intracellular K in isotonic, hypertonic, and hypotonic states are:

$$aK_i = f_o \times [K],$$  \hspace{1cm} (12)

_Circulation Research, Vol. 37, November 1975_
INFLUENCE OF K AND OSMOLALITY ON $E_r$

\[ a_{K(1.58)} = f_{1.58} \times [K]_{1.58}, \]  
\[ a_{K(0.76)} = f_{0.76} \times [K]_{0.76}, \]

where $f_{1.58}$, $f_{0.76}$, and $f_{0.76}$ are activity coefficients of intracellular K in isotonic, 1.58×T hypertonic, and 0.76×T hypotonic states. Introducing these values into Eqs. 1 and 2, the following equations are obtained:

\[ \Delta E_{1.58} \approx 53.6 \log_{10} \left( \frac{f_{1.58} \times [K]_{1.58}}{f_{0.76} \times [K]_{0.76}} \right) \]

\[ = 53.6 \log_{10} \left( \frac{f_{1.58}}{f_{0.76}} \right) - 53.6 \log_{10} 1.58 \]

\[ = 53.6 \log_{10} \left( \frac{f_{1.58}}{f_{0.76}} \right) - 10.6, \]  
(3)

\[ \Delta E_{0.76} \approx 53.6 \log_{10} \left( \frac{f_{0.76} \times [K]_{0.76}}{f_{1.58} \times [K]_{1.58}} \right) - 0.54 \]

\[ = 53.6 \log_{10} \left( \frac{f_{0.76}}{f_{1.58}} \right) + 53.6 \log_{10} 0.76 - 0.54 \]

\[ = 53.6 \log_{10} \left( \frac{f_{0.76}}{f_{1.58}} \right) - 6.9. \]  
(4)

References

Influence of potassium ions and osmolality on the resting membrane potential of rabbit ventricular papillary muscle with estimation of the activity and the activity coefficient of internal potassium.

T Akiyama and H A Fozzard

Circ Res. 1975;37:621-629
doi: 10.1161/01.RES.37.5.621

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/37/5/621

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/