The Effect of Extracellular Potassium on the Intracellular Potassium Ion Activity and Transmembrane Potentials of Beating Canine Cardiac Purkinje Fibers

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ABSTRACT We used open tip microelectrodes containing a K+-sensitive liquid ion exchanger to determine directly the intracellular K+ activity in beating canine cardiac Purkinje fibers. For preparations superfused with Tyrode’s solution in which the K+ concentration was 4.0 mM, intracellular K+ activity (a_k) was 130.0 ± 2.3 mM (mean ± SE) at 37°C. The calculated K+ equilibrium potential (E_K) was −100.6 ± 0.5 mV. Maximum diastolic potential (E_D) and resting transmembrane potential (E_m) were measured with conventional microelectrodes filled with 3 M KCl and were −90.6 ± 0.3 and −84.4 ± 0.4 mV, respectively. When [K+]o was decreased to 2.0 mM or increased to 6.0, 10.0, and 16.0 mM, a_k remained the same. At [K+]o = 2.0, E_D was −97.3 ± 0.4 and E_m −86.0 ± 0.7 mV; at [K+]o = 16.0, E_D fell to −53.8 ± 0.4 mV and E_m to the same value. Over this range of values for [K+]o, E_K changed from −119.0 ± 0.3 to −63.6 ± 0.2 mV. These values for E_K are consistent with those previously estimated indirectly by other techniques.

INTRODUCTION

Potassium has many important effects on the electrophysiological properties and electrical activity of cardiac fibers. Many of the data describing how these effects are brought about have been obtained from studies on mammalian Purkinje fibers (Carmeliet, 1961; Draper and Weidmann, 1951; Hoffman and Cranefield, 1960; Noble, 1975; Vassalle, 1965). Interpretation of these data often requires a precise estimate of the intracellular potassium activity (a_k) and the potassium equilibrium potential (E_K). Unfortunately, there have been no reliable measurements for Purkinje fibers of a_k and E_K and how these values change as a function of changes in extracellular potassium concentration ([K+]o). It has been difficult to estimate a_k for Purkinje fibers because of the structural complexity of the tissues, the limitations of the techniques used for chemical determination of intracellular potassium content of disrupted tissues, and the uncertain relationship between intracellular potassium concentration ([K+]i) and a_k.

Recently, Walker (1971) introduced a method for the direct determination of a_k that uses open tip microelectrodes containing a K-sensitive liquid ion exchanger. These open tip microelectrodes were used to measure a_k in Aplysia

We have adopted this method and used open tip microelectrodes containing a K+-sensitive liquid ion exchanger to determine $a_k$ in beating canine cardiac Purkinje fibers. We have used this method to measure the effects of changing $[K^+]_o$, and thus $E_K$, on $a_k$ and transmembrane potential.

Our data show that $a_k$ is constant at 130 mM over a range of values for $[K^+]_o$ from 2.0 to 16.0 mM and that the values of $E_K$ determined from these data are in good agreement with estimates made by use of voltage-clamp and other methods.

**Materials and Methods**

**Tissue Preparation**

Mongrel dogs weighing 20-30 kg were anesthetized with intravenous sodium pentobarbital, 30 mg/kg. The heart was removed rapidly through a right lateral thoracotomy and placed in chilled, oxygenated Tyrode's solution (containing, in millimoles per liter: NaCl, 137; NaHCO$_3$, 12; Na$_2$HPO$_4$, 1.8; MgCl$_2$, 0.5; dextrose, 5.5; CaCl$_2$, 2.7; KCl, 4, equilibrated with 95% O$_2$-5% CO$_2$). Free-running Purkinje fiber bundles and attached segments of myocardium were excised from both ventricles and placed in a beaker containing Tyrode's solution maintained at room temperature and bubbled with 95% O$_2$-5% CO$_2$.

Purkinje fiber bundles, trimmed of most of their attached ventricular muscle, were placed in a Lucite tissue bath (Aronson et al., 1973) and superfused with Tyrode's solution maintained at 37°C. The volume of the fluid bathing the preparation in the tissue bath was 2.0 ml and the Tyrode's solution was pumped into the chamber at a rate of 7-8 ml/min.

The preparation was driven at a cycle length of 1,000 ms by stimuli isolated from ground (Bioelectric Instruments Isolator ISB 2.5) and delivered through Teflon-coated bipolar silver wire electrodes.

Transmembrane potentials were recorded through 3 M KCl-filled microelectrodes. These were coupled by an Ag-AgCl interface to an amplifier (Bioelectric Instruments PAD-1) with high input impedance and input capacity neutralization. The output was displayed on one channel of a cathode ray oscilloscope (Tektronix model 564, Tektronix, Inc., Beaverton, Ore.). Records of transmembrane potentials were calibrated with a known 100-mV signal delivered between the tissue chamber and ground.

**Preparation of the Potassium-Sensitive Microelectrode**

Open tip microelectrodes were pulled to a tip diameter of less than 1 μm by use of a vertical electrode puller (Model 700 C, David Kopf Instruments, Tujunga, Calif.). The microelectrodes were fabricated from chromic acid-cleaned borosilicate glass (Pyrex 7740) 2.0 mm OD by 1.0 mm ID. When the microelectrodes were filled with 3 M KCl their tip resistances were 10-20 MΩ and their tip potentials were less than 5 mV. Microelectrodes were siliconized by being dipped into a 2% solution of dimethylpolysiloxane (Dow Corning Corp., Midland, Mich.) in xylene, and then air dried for 15 min. Then, 300-300 μm of each microelectrode tip was filled with a K$^+$-sensitive liquid ion exchange resin (Corning, 477317). The space above the ion exchange resin was filled with 1.0 M KCl which served as an internal reference solution. A chlorided silver wire was inserted into the KCl as the internal reference element.

The ion-sensitive microelectrode was connected to a Teledyne Philbrick (Dedham, Mass.) 1029 Operational Amplifier which provided an input impedance of $10^{12}$ Ω. Only K$^+$-sensitive microelectrodes with resistances from $10^8$ to $10^9$ Ω were used. The ion-
Intracellular Potassium Activity

Sensitive microelectrodes were calibrated in solutions of pure KCl; their response was proportional to the logarithm of the K+ activity and linear in concentration from 1 to $10^{-4}$ M KCl. The selectivity coefficient was determined by the method of Walker (1971).

Measurement of $a_k$

Potentials measured by the K+-sensitive microelectrode resulted from the following: (a) the difference between the intracellular and extracellular K activities, $a_k$ and $a_k^e$; (b) the effect of intracellular and extracellular sodium activity, $a_{Na}$ and $a_{Na}^e$; and (c) the transmembrane potential, $E_{tm}$. The potential recorded through the K+-sensitive microelectrode ($\Delta E$) is described by the following equation:

$$\Delta E = E_{tm} + \frac{RT}{F} \ln \left[ \frac{(a_k + K_{KNa}a_{Na})}{(a_k^e + K_{KNa}a_{Na}^e)} \right].$$

Here $\Delta E$ is the measured potential; $R$ is the universal gas constant; $T$ is absolute temperature; $F$ is the Faraday; and $K_{KNa}$ is the selectivity coefficient for a given K+-sensitive microelectrode. The $a_{Na}$ has been shown by others (Orme, 1969; Lee and Fozzard, 1975) to be low in cardiac muscle. Since the selectivity coefficient for each microelectrode is chosen to be small (less than 0.03), the product, $K_{KNa}a_{Na}$, is negligible compared to the $a_k$ term and can be ignored.

It is convenient to define a K+-sensitive microelectrode characteristic constant as:

$$K_{Me} = \frac{RT}{F} \ln \left[ \frac{a_k}{a_k^e (a_k + K_{KNa}a_{Na})} \right].$$

This constant, $K_{Me}$, is determined by the initial calibration procedure and is unique to each microelectrode. Eq. (1) can be rearranged in such a manner that:

$$\Delta E = E_{tm} + \frac{RT}{F} \ln \left[ \frac{a_k}{a_k^e} + \frac{RT}{F} \ln \left( \frac{a_k}{a_k + K_{KNa}a_{Na}} \right) \right].$$

Since the potassium equilibrium potential can be written as

$$E_k = -\frac{RT}{F} \ln (a_k/a_k^e),$$

Eq. (3) now can be simplified by using Eq. (2) for the K-sensitive microelectrode characteristic constant and Eq. (4) for the K equilibrium potential to give Eq. (5).

$$\Delta E = E_{tm} - E_k + K_{Me}.$$

A maximum of six impalements was made with each K+-sensitive microelectrode. This limit was imposed because when the electrode was used for a greater number of impalements its characteristics tended to change. At the end of each experiment, each microelectrode was recalibrated. If its properties were not the same before and after the experiment, the results were discarded.

After the K+-sensitive microelectrode was advanced into the Purkinje fiber, two types of records were obtained: (a) the potential during the stimulated action potential, and (b) the potential 20 s after discontinuation of the drive stimulus. The maximum diastolic potential ($E_D$) was determined at the greatest negative potential after action potential repolarization. The resting transmembrane potential ($E_m$) was determined 20 s after discontinuation of the drive stimulus (Fig. 1).

Experimental Protocol

A Purkinje fiber bundle was superfused with Tyrode’s solution for 60 min before initial control records of the transmembrane potentials were obtained. Records of $E_m$ were
obtained 20 s after discontinuation of the drive stimulus. To study the effects of changing 
\([K^+]_0\) the protocol was as follows: (a) control records were obtained after superfusion for 
60 min with Tyrode's solution containing \([K^+] = 4 \text{ mM}\); (b) the \([K^+]\) in Tyrode's solution 
was changed (KCl for NaCl) to the desired value and after superfusion for 60 min with 
this solution, records of the transmembrane potentials were obtained; (c) additional 
measurements of the transmembrane potential were made every 20 min during the next 
hour and at the end of this period multiple impalements were made with both the 3 M 
KCl-filled electrode and the K+-sensitive microelectrode; and (d) \(A_k\) then was calculated 
for each value of \([K^+]_0\).

\[ A \]

\[ B \]

\[ C \]

\[ D \]

**FIGURE 1.** Purkinje fiber transmembrane potentials (TMP) in various concentrations 
of potassium in Tyrode's solution. Panel A shows the TMP in Tyrode's solution 
containing potassium 2 mM. The maximum diastolic potential, \(E_D\), was determined 
at the greatest transmembrane potential after repolarization. The resting trans-
membrane potential, \(E_m\), was determined 20 s after discontinuation of the drive 
stimulus. Panel B shows the TMP in potassium 6 mM; note the decreased phase 4 
depolarization and loss of transmembrane potential compared to Panel A. Panel C 
shows the TMP in potassium 10 mM. In potassium of 16 mM, the fiber was 
inexcitable (panel D).

**RESULTS**

**Effects of Extracellular Potassium on the Transmembrane Potential**

The control data at \([K^+]_0 = 4 \text{ mM}\) and the effects of changing the extracellular 
K+ concentrations to 2, 6, 10, and 16 mM are summarized in Table I A. Fig. 1 is a 
representative experiment showing the effect of varying extracellular K+ concentration on \(E_D\), the slope of phase 4 depolarization (Hoffman and Cranefield, 
1960), \(E_m\), and the AP. Results at \([K^+]_0 = 4 \text{ mM}\) are not included here. At \([K^+]_0 = 
2 \text{ mM}\) (A) \(E_D\) is high and there is a prominent phase 4 depolarization. At \([K^+]_0 = 
6 \text{ mM}\) (B), \(E_D\) and \(E_m\) are lower than in A, and there is no depolarization during 
phase 4. At \([K^+]_0 = 10 \text{ mM}\) (C) \(E_D\) and \(E_m\) have decreased further and at \(K^+_0 = 10 
\text{ mM}\) (D) \(E_m\) is still lower and the fiber is inexcitable.
Intracellular Potassium Activity in Purkinje Fibers

After control measurements of the Purkinje fiber transmembrane potentials had been obtained, records were made by use of the K⁺-sensitive microelectrode as shown in Fig. 2. The shift in the recorded potential as the Purkinje fiber was impaled with the K⁺-sensitive microelectrode is described by Eq. (5). After several cycles, during which the Purkinje fiber was driven, the stimulus was turned off, the potential of the K⁺-sensitive microelectrode was recorded for 20 s, and then the driving stimulus was reinitiated. The K⁺-sensitive microelectrode then was withdrawn from the cell and the potential in the superfusate was recorded.

In many experiments, reinstitution of the drive stimulus resulted in partial extrusion of the K⁺-sensitive microelectrode from the cell. In this situation it was impossible to determine the value for $E_D$ after the drive was reinitiated (i.e., the values measured for $E_D$ before and after stimulus cessation were not consistent). For this reason these experiments were not used in the calculation of $E_D$, and

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**Table I**

**EFFECTS OF EXTRACELLULAR POTASSIUM CONCENTRATION ON TRANSMEMBRANE POTENTIALS**

<table>
<thead>
<tr>
<th>No. of preparations</th>
<th>5</th>
<th>8</th>
<th>5</th>
<th>5</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>[$K^+_{le}$ (mM)]</td>
<td>2.0</td>
<td>4.0</td>
<td>6.0</td>
<td>10.0</td>
<td>16.0</td>
</tr>
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**A. Action potential characteristics of canine cardiac Purkinje fibers**

<table>
<thead>
<tr>
<th>$E_o$ (-mV)</th>
<th>97.3±0.4</th>
<th>90.6±0.3</th>
<th>79.3±0.2</th>
<th>64.8±0.2</th>
<th>53.8±0.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td></td>
</tr>
<tr>
<td>$E_m$ (-mV)</td>
<td>86.1±0.7</td>
<td>84.4±0.4</td>
<td>76.6±0.3</td>
<td>63.8±0.2</td>
<td>53.8±0.4</td>
</tr>
<tr>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td></td>
</tr>
<tr>
<td>$AP$ (mV)</td>
<td>127.8±0.7</td>
<td>128.1±0.4</td>
<td>114.4±0.7</td>
<td>88.8±0.6</td>
<td>--</td>
</tr>
<tr>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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</tr>
<tr>
<td>$APD_{100}$ (msc)</td>
<td>573.8±4.5</td>
<td>349.0±4.3</td>
<td>308.8±2.6</td>
<td>211.5±2.5</td>
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</tr>
<tr>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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</table>

**B. Values of intracellular potassium activity and $E_m$ using the maximum diastolic potential**

<table>
<thead>
<tr>
<th>$a_k$ (mM)</th>
<th>129.3±1.3</th>
<th>130.0±2.3</th>
<th>150.1±2.4</th>
<th>130.0±1.7</th>
<th>130.0±0.9</th>
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</thead>
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<tr>
<td>(21)</td>
<td>(20)</td>
<td>(20)</td>
<td>(20)</td>
<td>(20)</td>
<td>(20)</td>
</tr>
<tr>
<td>$E_k$ (-mV)</td>
<td>119.0±0.3</td>
<td>100.6±0.5</td>
<td>89.9±0.4</td>
<td>76.2±0.3</td>
<td>63.6±0.2</td>
</tr>
<tr>
<td>(21)</td>
<td>(20)</td>
<td>(20)</td>
<td>(20)</td>
<td>(20)</td>
<td>(20)</td>
</tr>
<tr>
<td>$E_k - E_o$ (mV)</td>
<td>21.7</td>
<td>10.0</td>
<td>10.6</td>
<td>11.9</td>
<td>9.8</td>
</tr>
<tr>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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</table>

**C. Values of intracellular potassium activity and $E_K$ using the resting membrane potential**

<table>
<thead>
<tr>
<th>$a_k$ (mM)</th>
<th>116.6±1.4</th>
<th>124.2±1.3</th>
<th>128.8±0.6</th>
<th>129.9±0.7</th>
<th>129.7±0.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>(96)</td>
<td>(98)</td>
<td>(142)</td>
<td>(111)</td>
<td>(72)</td>
<td></td>
</tr>
<tr>
<td>$E_k$ (-mV)</td>
<td>116.3±0.3</td>
<td>99.4±0.3</td>
<td>89.6±0.1</td>
<td>76.2±0.1</td>
<td>63.6±0.1</td>
</tr>
<tr>
<td>(96)</td>
<td>(98)</td>
<td>(142)</td>
<td>(111)</td>
<td>(72)</td>
<td></td>
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</table>

Where $E_o$ is the maximum diastolic potential, $E_m$ is the resting transmembrane potential, $AP$ is the action potential amplitude, $APD_{100}$ is the action potential duration at 100% repolarization, $a_k$ is the intracellular potassium activity, $E_k$ is the calculated potassium equilibrium potential. Numbers are expressed as the mean plus or minus the standard error. The number of impalements is in parenthesis.

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there is a relatively low \( n \) for determinations of \( E_D \) in Table I B. In many of these experiments we nonetheless were able to measure \( E_m \). These values were retained if the "0" level recorded before impalement and after extrusion of the microelectrode was unchanged and if the calibration curve obtained for the microelectrode after its removal from the cell was likewise unchanged.

In order to determine \( a_K \), Eq. (1) was solved for this term. The activity coefficient of K\(^+\) in solution, according to the extended Debye-Hückel equation (Robinson and Stokes, 1965), is 0.75. The values of the transmembrane potentials \( (E_{tm}) \) used in Eq. (1) were, first, the maximum diastolic potential, \( E_D \), and then the resting membrane potential, \( E_m \), measured with the 3 M KCl-filled microelectrode. The results of these calculations are summarized in Table I B and C. The value of \( a_K \) during superfusion with Tyrode's solution containing \([K^+]_o = 4\) mM and using \( E_D \) in Eq. (1) was \( 150.0 \pm 2.3 \) mM (mean \( \pm \) SE). When \( E_m \) was used in Eq. (1) the value of \( a_K \) was \( 124.2 \pm 1.3 \) mM (Table I C). This value is 5.8 mM less than that determined when \( E_D \) was used.

**Effects of [K\(^+\)]\(_o\) on \( a_K \) and the \( E_K \)**

The effects of changing [K\(^+\)]\(_o\) on the \( a_K \) and \( E_K \), are summarized in Table I B and C. When [K\(^+\)]\(_o\) was increased to 6, 10, and 16 mM or decreased to 2 mM and \( E_D \) was used to calculate \( a_K \) from Eq. (1), its value remained remarkably constant (Table I B). However, when \( E_m \) was used to calculate \( a_K \) (Table I C) and [K\(^+\)]\(_o\) was reduced to 2 mM, \( a_K \) was \( 116.6 \pm 1.4 \) mM. This value is 12.7 mM lower than that predicted with \( E_D \). When the extracellular K\(^+\) concentration in Tyrode solution
was raised to 10 mM, $E_m$ approached $E_D$, and $a_k$ was approximately 130 mM for both methods of calculation (Table I C). $E_K$ was calculated by using Eq. (4). The difference between $E_K$ and $E_D$, shown in Table I B, remained constant at approximately 10 mV when the extracellular $K^+$ concentration was equal to or greater than 4 mM. The value of $E_m$, as expected, was less than $E_K$ as shown in Fig. 3. Moreover, the value of $E_D$, while less than $E_K$, still more closely approximated $E_K$ at low $[K^+]_o$ than did $E_m$ (Table I). A least mean square analysis of the $E_m$ vs. log $a_k$ gave an estimate of $a_k$ of 130.7 mM ($r = 0.99$). This estimate was based on all $[K^+]_o$ and was obtained by using the Nernst equation.

![Graph showing $E_K$, $E_m$ vs. log $a_k$](image)

**Figure 3.** The resting transmembrane potential, $E_m$, and the calculated $E_K$ are shown plotted against the potassium activity of the superfusing Tyrode's solution.

**DISCUSSION**

Direct measurement of the $a_k$ in cardiac Purkinje fibers is of primary importance in evaluating the relationships between $[K^+]_i$ and electrical activity. However, a variety of factors may influence the recorded values of $a_k$. The activity coefficient for intracellular $K^+$ at physiologic concentrations may not equal that in the extracellular solutions because of unknown effects of the following factors which cannot be predicted accurately: (a) the degree of hydration of ions and effects on their activity coefficients at physiologic concentrations; (b) Bjerrum ion pair or other complex ion formations; (c) the degree of hydration of large polyvalent protein molecules.

Other investigators have used cation-sensitive glass microelectrodes to determine intracellular sodium and potassium activities for a variety of tissues (Hinke, 1959, 1961; Lev, 1964; Lee and Fozzard, 1975). The calculated activity coefficients for intracellular sodium ion were much lower than those predicted by ionic theories. The evidence thus indicated that intracellular sodium is partly sequestered rather than in a totally free state. The intracellular $K^+$ activity coefficient, however, has been shown to be similar to the extracellular $K^+$ activity coefficient (Hinke, 1961; Cornwall et al., 1970; Armstrong and Lee, 1971; Lee
and Armstrong, 1974; Lee and Fozzard, 1975). This implies that at most only a small fraction of the intracellular K⁺ is sequestered. It has been proposed, therefore, that the sequestered portion of the K⁺ ions, if any, can be ignored in the determination of the transmembrane K⁺ equilibrium potential. The assumption that the state of K⁺ within cardiac Purkinje fibers is the same as the ionic activity in a bulk solution of similar ionic strength is supported by the present investigation.

Lee and Fozzard (1975) determined the effect of changes in [K⁺]₀ between 2 and 50 mM on the resting membrane potential and aₖ of rabbit papillary muscles. They found that the calculated value of aₖ as determined with cation-sensitive glass microelectrodes was 82.9 mM and varied by only 1.7 mM when [K⁺]₀ was changed from 2 to 50 mM. They also found that the calculated K⁺ equilibrium potential approximated the measured Eₘ at values of [K⁺]₀ greater than 5 mM, but that at low external K⁺ concentrations, Eₘ deviated from the calculated value of Eₖ. This finding was expected from the results of earlier studies in which Eₖ had been calculated in terms of [K⁺]₀ and estimated values of [K⁺]ᵢ, and which had shown that changes in [K⁺]₀ modify membrane potassium permeability and conductance (Ling and Gerard, 1949; Jenerick, 1953; Adrian, 1956; Conway, 1957; Hodgkin and Horowicz, 1959). Our studies have shown the same type of relationship between [K⁺]₀, Eₘ, and Eₖ, but have provided evidence for a considerably higher value of aₖ in Purkinje fibers than Lee and Fozzard found for ventricular muscle. This result is not surprising, because Purkinje fibers maintain a significantly higher level of resting membrane potential than do myocardial fibers and from the Nernst equation might be expected to have a higher aₖ.

In the present studies we used both the Eₘ and the E₉ to calculate aₖ. When E₉ was used as the basis for this calculation, aₖ was approximately 130 mM for values of [K⁺]₀ from 2 to 16 mM. When Eₘ was used, aₖ was 116 mM for a [K⁺]₀ of 2 mM, 124 mM at [K⁺]₀ = 4 mM, and approximately 130 mM at the higher values of [K⁺]₀. Because the two methods gave different values for aₖ at low [K⁺]₀, it is necessary to consider whether the values of aₖ calculated from Eₘ or the values calculated from E₉ are in error. It is possible that, because of its high resistance/capacitance, the voltage recorded by the K⁺-sensitive microelectrode may have lagged behind transmembrane potential at E₉. If such an error occurred (and an error of only 2.5 mV in E₉ would be required to account for the difference in aₖ calculated for E₉ and Eₘ when [K⁺]₀ = 2 mM), then the values calculated with E₉ at low [K⁺]₀ would be falsely high. This would not be a source of error for the value of aₖ calculated from Eₘ, because this voltage was recorded during maintenance of a steady transmembrane potential. The time constant of our electrodes was short enough (10-40 ms) that we do not believe that our measurement of E₉ was in error.

Although Lee and Fozzard showed that aₖ is almost constant for ventricular muscle at [K⁺]₀ = 2-50 mM, at a [K⁺]₀ of 2 mM, their value for aₖ was slightly (although not significantly) less than that calculated for higher [K⁺]₀. Page and Solomon (1960) used indirect means to measure intracellular K⁺ concentrations of feline papillary muscle and showed that at [K⁺]₀ = 1 mM, [K⁺] was significantly lower than at [K⁺]₀ = 5.32 mM. Our calculation of aₖ using Eₘ is in
agreement with the findings of Page and Solomon in that, as \([K^+]_o\) decreases below 6 mM, there is an associated decrease in \(a_K\).

The quantitative differences between the data of Page and Solomon and our study probably reflect both differences between Purkinje fibers and ventricular muscle and differences caused by the methods used to measure \([K^+]_o\) as opposed to \(a_K\). Nevertheless, we believe that there is reason to assume that superfusion with solutions containing quite low concentrations of \(K^+\) very probably causes some loss of fiber \(K^+\).

There are other possible causes of the differences in \(a_K\) that are calculated from \(E_o\) and \(E_m\) at normal and low values of \([K^+]_o\). These include: (a) an increase in \([K^+]\) at the surface of the cell membrane; (b) an increase in intracellular water; (c) a change in \(a_K\) between the action potential and resting potential; or (d) the effects of other ions on the measurement. For the first of these possibilities, an increase in extracellular \([K^+]\) at the cell surface, there are two areas to explore. It is possible that when the Purkinje fibers are stimulated repetitively and as a result generate action potentials at a regular rate, there may be a net loss of \(K^+\) from the fibers during the time when transmembrane potential is strongly positive to \(E_K\) and, further, that because of physical limitation to diffusion, this potassium does not equilibrate rapidly with the superfusate. If active reuptake of \(K^+\) in exchange for intracellular \(Na^+\) were strongly dependent on \([K^+]_o\), during repetitive activity there might be a small increase in \(K^+\) just outside the surface membrane. An alternative is that during phases 2 and 3 of the transmembrane action potential there is a net efflux of \(K^+\) sufficient to increase \([K^+]_o\) just outside the surface membrane but that during the initial part of phase 4 the \(K^+\) concentration in this region is brought back into equilibrium with the \(K^+\) concentration in the bulk phase because of active reuptake and diffusion.

To consider first the possibility that during repetitive stimulation the steady-state \(K^+\) concentration just outside the surface membrane is higher than in the bulk phase we must evaluate several factors. Clearly, if \([K^+]_o\) just outside the surface membrane were higher than that in the superfusate, the value of \(a_K\) estimated from \(E_o\) would be overestimated because the value of the bulk phase \([K^+]_o\) is used in the calculation. The true value of \(a_K\) would be closer to the value calculated in terms of \(E_m\). However, if during repetitive activity \(K^+\) just outside the membrane surface increased, one would expect that during the period of electrical activity the value of \(E_o\) would decrease progressively, as reported for rapid stimulus rates by Kline and Morad (1976). In our studies at a relatively slow stimulus rate that decrease did not occur. At \([K^+]_o = 2\) mM when the stimulus was discontinued and the membrane depolarized to \(E_m\), however, several cycles were required for \(E_o\) again to be attained in some preparations. The dissimilarity of the values for \(E_o\) for the last action potential in a series and the first in the subsequent series after determination of \(E_m\) is consistent with a change in the steady-state value of \([K^+]_o\) just outside the surface membrane during repetitive activity as compared to the resting state at low \([K^+]_o\), but this did not happen in all preparations. Further, convincing evidence has been presented in voltage-clamp studies on Purkinje fibers that the change in transmembrane potential between \(E_o\) and \(E_m\) results from the time and voltage-dependent decrease in \(i_{KS}\) (Noble, 1975; Noble and Tsien, 1968). The extent to which changes in \(i_{KS}\) are
responsible for the disparity between $E_D$ and $E_m$ at low $[K^+]_o$ and the extent to which this might further be modified by significant $K^+$ loss to the extracellular space remains an important question.

The other aspect of this consideration is very likely to contribute to the different values of $a_K$ estimated from $E_D$ and $E_m$. It seems almost certain that during phases 2 and 3 of the action potential the next efflux of $K^+$ results in a transient increase in $[K^+]$ immediately adjacent to at least some parts of the surface membrane. After transmembrane potential has attained its maximum value, active reuptake of $K^+$ would restore the $K^+$ concentration in this small volume to its steady-state value and this probably is equal to or almost equal to the concentration in the bulk phase. The transient accumulation of $K^+$ just outside the surface membrane would give rise to a shift in measured $E_D$ and result in an error in the estimate of $a_K$. To determine whether or not there is such a phasic change in $[K^+]_o$ just outside the surface membrane it is necessary to measure $a_K$ immediately adjacent to the membrane during phases 2, 3, and 4. In studies on frog ventricle Kline and Morad (1976) have provided evidence for this type of phasic change in $[K^+]_o$; data for Purkinje fibers are not available but the same mechanism probably operates for this latter tissue because of its geometry and the frequent occurrence of small-diameter clefts (Page et al., 1971; Sommer and Johnson, 1968).

The second possibility mentioned as a cause of differences in $a_K$ calculated from $E_D$ and $E_m$, an increase in intracellular water, could result in a decrease in $a_K$. If at low external $K^+$ concentrations the membrane permeability to chloride and sodium ions increased relative to $K^+$ permeability (Carmeliet, 1961), there could be a net uptake of water. However, there is no direct evidence to support this possibility. The third consideration is that the $K^+$ actually may undergo a change in state between conditions obtaining during the action potential and those obtaining in a Purkinje fiber at rest. According to the association-induction hypothesis (Ling, 1962, 1973; Cope, 1969; Hazelwood et al., 1969), cellular water exists in an ordered state and intracellular ions are adsorbed onto cellular sites. This theory suggests the possibility of a change in state of intracellular cations. Finally, the effect of other ions interfering with the determination of $a_K$ can be considered. However, the selectivity coefficient of the ion-sensitive microelectrode for $K^+$ over the major interfering ions, sodium and calcium (Walker, 1971), and the intracellular concentrations of the interfering cations (Orme, 1969) indicate that this would not contribute significantly to the microelectrode potential.

In summary, we conclude that over a wide range of $[K^+]_o$ the $a_K$ values calculated from $E_D$ and $E_m$ are identical. At low $[K^+]_o$, $E_D$ and $E_m$ both deviate from the theoretical $E_K$. The basis for the difference in $E_m$ and $E_D$ and the resultant $a_K$ calculations was not determined but appears to result from the time and voltage induced decrease in $i_K$, as well as from a redistribution of $K^+$ across the cell membrane.

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