

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

DENNIS S. MIURA, BRIAN F. HOFFMAN, and MICHAEL R. ROSEN

From the Departments of Pharmacology and Pediatrics, Columbia University College of Physicians and Surgeons, New York 10032

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^i) was 130.0 ± 2.3 mM (mean \pm 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^i remained the same. At $[K^+]_o = 2.0$, E_D was -97.3 ± 0.4 and $E_m = -86.0 \pm 0.7$ 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^i) and the potassium equilibrium potential (E_K). Unfortunately, there have been no reliable measurements for Purkinje fibers of a_k^i 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^i 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^i .

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

neurons (Brown et al., 1970; Kunze and Brown, 1971), *Aplysia* ganglion (Russell and Brown, 1972), and frog heart (Walker, 1971; Walker and Ladle, 1973).

We have adopted this method and used open tip microelectrodes containing a K^+ -sensitive liquid ion exchanger to determine a_K^i 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^i and transmembrane potential.

Our data show that a_K^i 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; NaH_2PO_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 $M\Omega$ 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, ~200–300 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 $10^{13} \Omega$. Only K^+ -sensitive microelectrodes with resistances from 10^9 to $10^{11} \Omega$ were used. The ion-

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^i

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

$$\Delta E = E_{tm} + \frac{RT}{F} \ln [(a_k^i + K_{KNa} a_{Na}^i)/(a_k^o + K_{KNa} a_{Na}^o)]. \quad (1)$$

Here ΔE 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}^i 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} times a_{Na}^i , is negligible compared to the a_k^i term and can be ignored.

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

$$K_{Me} = \frac{RT}{F} \ln \{a_k^o/(a_k^o + K_{KNa} a_{Na}^o)\}. \quad (2)$$

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:

$$\Delta E = E_{tm} + \frac{RT}{F} \ln \left[\frac{a_k^i}{a_k^o} \right] + \frac{RT}{F} \ln \left[\frac{a_k^o}{a_k^o + K_{KNa} a_{Na}^o} \right]. \quad (3)$$

Since the potassium equilibrium potential can be written as

$$E_K = -\frac{RT}{F} \ln (a_k^i/a_k^o), \quad (4)$$

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).

$$\Delta E = E_{tm} - E_K + K_{Me}. \quad (5)$$

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 $[K^+]_o$, the protocol was as follows: (a) control records were obtained after superfusion for 60 min with Tyrode's solution containing $[K^+] = 4$ 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 $[K^+]_o$.

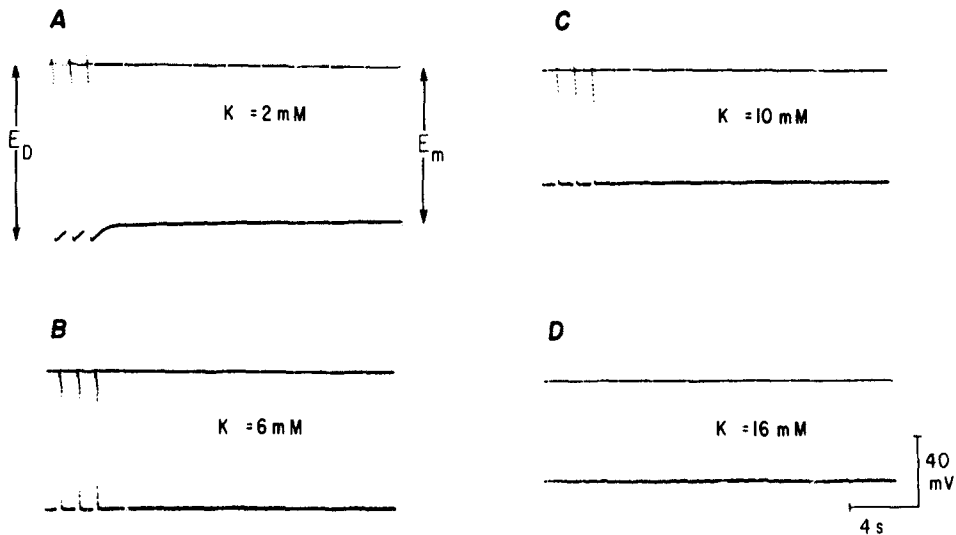


FIGURE 1. Purkinje fiber transmembrane potentials (TMP) in various concentrations of potassium in Tyrode's solution. Panel A shows the TMP in Tyrode's solution containing potassium 2 mM. The maximum diastolic potential, E_D , was determined at the greatest transmembrane potential after repolarization. The resting transmembrane 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 $[K^+]_o = 4$ 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 Cranfield, 1960), E_m , and the AP. Results at $[K^+]_o = 4$ mM are not included here. At $[K^+]_o = 2$ mM (A) E_D is high and there is a prominent phase 4 depolarization. At $[K^+]_o = 6$ mM (B), E_D and E_m are lower than in A, and there is no depolarization during phase 4. At $[K^+]_o = 10$ mM (C) E_D and E_m have decreased further and at $K^+_o = 16$ 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

TABLE I
EFFECTS OF EXTRACELLULAR POTASSIUM CONCENTRATION ON
TRANSMEMBRANE POTENTIALS

No. of preparations	5	8	5	5	4
$[K^+]_o$ (mM)	2.0	4.0	6.0	10.0	16.0
<i>A. Action potential characteristics of canine cardiac Purkinje fibers</i>					
E_D (-mV)	97.3±0.4 (50)	90.6±0.3 (50)	79.3±0.2 (50)	64.8±0.2 (50)	53.8±0.4 (30)
E_m (-mV)	86.0±0.7 (50)	84.4±0.4 (50)	76.6±0.3 (50)	63.8±0.2 (50)	53.8±0.4 (30)
AP (mV)	127.0±0.7 (50)	123.1±0.4 (50)	114.4±0.7 (50)	88.8±0.6 (50)	—
APD ₁₀₀ (msec)	373.8±4.5 (50)	349.0±4.3 (50)	308.8±2.6 (50)	211.5±2.5 (50)	—
<i>B. Values of intracellular potassium activity and E_K using the maximum diastolic potential</i>					
a_k^i (mM)	129.3±1.3 (21)	130.0±2.3 (20)	130.1±2.4 (20)	130.0±1.7 (23)	130.0±0.9 (57)
E_K (-mV)	119.0±0.3 (21)	100.6±0.5 (20)	89.9±0.4 (20)	76.2±0.3 (23)	63.6±0.2 (57)
$E_K - E_D$ (mV)	21.7	10.0	10.6	11.9	9.8
<i>C. Values of intracellular potassium activity and E_K using the resting membrane potential</i>					
a_k^i (mM)	116.6±1.4 (96)	124.2±1.3 (98)	128.8±0.6 (142)	129.9±0.7 (111)	129.7±0.7 (72)
E_K (-mV)	116.3±0.3 (96)	99.4±0.3 (98)	89.6±0.1 (142)	76.2±0.1 (111)	63.6±0.1 (72)

Where E_D is the maximum diastolic potential, E_m is the resting transmembrane potential, AP is the action potential amplitude, APD₁₀₀ is the action potential duration at 100% repolarization, a_k^i 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.

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

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^i , 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

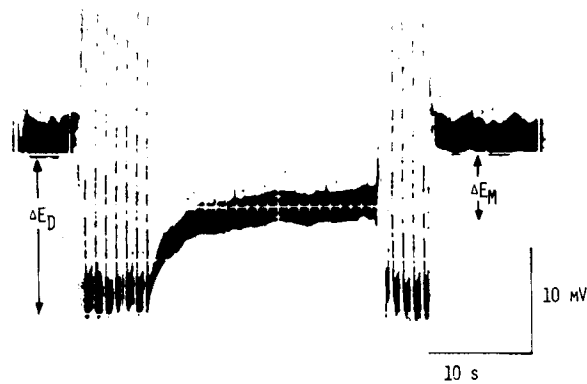


FIGURE 2. A typical response of the K^+ -sensitive microelectrode during intracellular potassium activity determination in Tyrode's solution containing potassium 4 mM. Initially, the observed microelectrode potential was due to the superfusing Tyrode's solution. After the microelectrode was advanced into the Purkinje fiber, the shift in potential, ΔE_D , was described by Eq. (5) where E_{tm} was the maximum diastolic potential, E_D . When the drive stimulus was turned off, the potential ΔE_m was measured, and E_{tm} in Eq. (5) was assumed to be the resting transmembrane potential, E_m . The drive stimulus was turned on again, and the shift in potential was again due to the transmembrane action potential. The microelectrode was withdrawn from the cell, and the observed potential again returned to the original extracellular potential.

microelectrode. The results of these calculations are summarized in Table I B and C. The value of a_k^i during superfusion with Tyrode's solution containing $[K^+]_o = 4$ mM and using E_D in Eq. (1) was 130.0 ± 2.3 mM (mean \pm SE). When E_m was used in Eq. (1) the value of a_k^i was 124.2 ± 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^i and the E_K

The effects of changing $[K^+]_o$ on the a_k^i 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^i from Eq. (1), its value remained remarkably constant (Table I B). However, when E_m was used to calculate a_k^i (Table I C) and $[K^+]_o$ was reduced to 2 mM, a_k^i was 116.6 ± 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^i 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. $\log a_K^i$ gave an estimate of a_K^i of 130.7 mM ($r = 0.99$). This estimate was based on all $[K^+]_o$ and was obtained by using the Nernst equation.

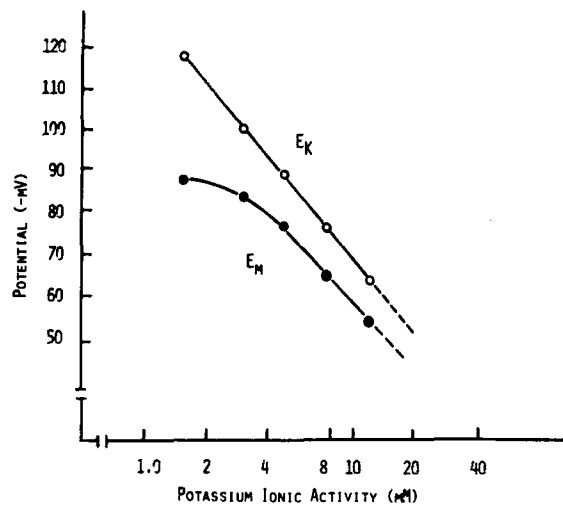


FIGURE 3. The resting transmembrane potential, E_m , and the calculated E_K are shown plotted against the potassium activity of the superfusing Tyrode's solution.

DISCUSSION

Direct measurement of the a_K^i 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^i . 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 $[K^+]_o$ between 2 and 50 mM on the resting membrane potential and a_k^i of rabbit papillary muscles. They found that the calculated value of a_k^i as determined with cation-sensitive glass microelectrodes was 82.9 mM and varied by only 1.7 mM when $[K^+]_o$ was changed from 2 to 50 mM. They also found that the calculated K^+ equilibrium potential approximated the measured E_m at values of $[K^+]_o$ greater than 5 mM, but that at low external K^+ concentrations, E_m deviated from the calculated value of E_K . This finding was expected from the results of earlier studies in which E_K had been calculated in terms of $[K^+]_o$ and estimated values of $[K^+]_i$ and which had shown that changes in $[K^+]_o$ 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 $[K^+]_o$, E_m , and E_K , but have provided evidence for a considerably higher value of a_k^i 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 a_k^i .

In the present studies we used both the E_m and the E_D to calculate a_k^i . When E_D was used as the basis for this calculation, a_k^i was approximately 130 mM for values of $[K^+]_o$ from 2 to 16 mM. When E_m was used, a_k^i was 116 mM for a $[K^+]_o$ of 2 mM, 124 mM at $[K^+]_o = 4$ mM, and approximately 130 mM at the higher values of $[K^+]_o$. Because the two methods gave different values for a_k^i at low $[K^+]_o$, it is necessary to consider whether the values of a_k^i calculated from E_m or the values calculated from E_D 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 E_D . If such an error occurred (and an error of only 2.5 mV in E_D would be required to account for the difference in a_k^i calculated for E_D and E_m when $[K^+]_o = 2$ mM), then the values calculated with E_D at low $[K^+]_o$ would be falsely high. This would not be a source of error for the value of a_k^i calculated from E_m , 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 E_D was in error.

Although Lee and Fozzard showed that a_k^i is almost constant for ventricular muscle at $[K^+]_o = 2$ –50 mM, at a $[K^+]_o$ of 2 mM, their value for a_k^i was slightly (although not significantly) less than that calculated for higher $[K^+]_o$. Page and Solomon (1960) used indirect means to measure intracellular K^+ concentrations of feline papillary muscle and showed that at $[K^+]_o = 1$ mM, $[K^+]_i$ was significantly lower than at $[K^+]_o = 5.32$ mM. Our calculation of a_k^i using E_m 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^i .

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 $[K^+]_i$ as opposed to a_k^i . 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^i that are calculated from E_D 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^i 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^i estimated from E_D would be overestimated because the value of the bulk phase $[K^+]_o$ is used in the calculation. The true value of a_k^i 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_D 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_D again to be attained in some preparations. The dissimilarity of the values for E_D 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_D and E_m results from the time and voltage-dependent decrease in i_{K_2} (Noble, 1975; Noble and Tsien, 1968). The extent to which changes in i_{K_2} 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^i 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 $[K^+]_i$ 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^i . 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^o 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^i calculated from E_D and E_m , an increase in intracellular water, could result in a decrease in a_k^i . 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^i 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^i 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^i calculations was not determined but appears to result from the time and voltage induced decrease in i_{K_2} as well as from a redistribution of K^+ across the cell membrane.

We are grateful to Dr. Normand C. Hebert of Microelectrodes, Inc., in whose laboratory Dennis S. Miura learned the technique for fabricating the potassium-sensitive microelectrode.

This work was supported in part by United States Public Health grants HL-12738 and GM-02042-07 and by a grant from the New York Heart Association.

Received for publication 9 April 1976.

REFERENCES

- ADRIAN, R. H. 1956. The effect of internal and external potassium concentration on the membrane potential of frog muscle. *J. Physiol. (Lond.)*. **133**:631-658.
- ARMSTRONG, W. M., and C. O. LEE. 1971. Sodium and potassium activities in normal and "sodium-rich" frog skeletal muscle. *Science (Wash. D. C.)*. **171**:413-415.
- ARONSON, R. S., J. M. GELLES, and B. F. HOFFMAN. 1973. A new method for producing short cardiac Purkinje fibers suitable for voltage clamp. *J. Appl. Physiol.* **34**:527-530.
- BROWN, A. M., J. L. WALKER, and R. B. SUTTON. 1970. Increased chloride conductance as the proximate cause of hydrogen ion concentration effects in *Aplysia* neurons. *J. Gen. Physiol.* **56**:559-582.
- CARMELIET, E. E. 1961. Chloride ions and the membrane potential of Purkinje fibers. *J. Physiol. (Lond.)*. **156**:375-388.
- CONWAY, E. J. 1957. Nature and significance of concentration relations of potassium and sodium ions in skeletal muscle. *Physiol. Rev.* **37**:84-132.
- COPE, F. W. 1969. Nuclear magnetic resonance evidence using D₂O for structured water in muscle and brain. *Biophys. J.* **9**:303-319.
- CORNWALL, M. C., D. F. PETERSON, D. L. KUNZE, J. L. WALKER, and A. M. BROWN. 1970. Intracellular potassium and chloride activities measured with liquid ion exchanger microelectrodes. *Brain Res.* **23**:433-436.
- DRAPER, M. H., and S. WEIDMAN. 1951. Cardiac resting and action potentials recorded with an intracellular electrode. *J. Physiol. (Lond.)*. **115**:75-94.
- HAZELWOOD, C. F., B. L. NICHOLS, and N. F. CHAMBERLAIN. 1969. Evidence for the existence of a minimum of two phases of ordered water in skeletal muscle. *Nature (Lond.)*. **222**:747-750.
- HINKE, J. A. M. 1959. Glass microelectrodes for measuring intracellular activities of sodium and potassium. *Nature (Lond.)*. **184**:1257-1258.
- HINKE, J. A. M. 1961. The measurement of sodium and potassium activities in the squid axon by means of cation-selective glass microelectrodes. *J. Physiol. (Lond.)*. **156**:314-335.
- HODGKIN, A. L., and P. HOROWICZ. 1959. Influence of potassium and chloride ions on the membrane potential of single muscle fibers. *J. Physiol. (Lond.)*. **148**:127-160.
- HOFFMAN, B. F., and P. F. CRANFIELD. 1960. *Electrophysiology of the Heart*. McGraw-Hill Book Company, New York.
- JENERICK, H. P. 1953. Muscle membrane potential, resistance and external potassium chloride. *J. Cell. Comp. Physiol.* **42**:4-48.
- KLINE, R., and M. MORAD. 1976. Potassium efflux and accumulation in heart muscle. *Biophys. J.* **16**:367-372.
- KUNZE, D. L., and A. M. BROWN. 1971. Internal potassium and chloride activities and the effects of acetylcholine on identifiable *Aplysia* neurons. *Nat. New Biol.* **229**:229-231.
- LEE, C. O., and W. M. ARMSTRONG. 1974. State and distribution of potassium and sodium ions in frog skeletal muscle. *J. Membr. Biol.* **15**:331-362.
- LEE, C. O., and H. A. FOZZARD. 1975. Activities of potassium and sodium ions in rabbit heart muscle. *J. Gen. Physiol.* **65**:695-708.
- LEV, A. A. 1964. Determination of the activity and activity coefficients of potassium and sodium ions in frog muscle fiber. *Nature (Lond.)*. **201**:1132-1134.

- LING, G. N. 1962. *A Physical Theory of the Living State: The Association-Induction Hypothesis*. Blaisdell, Inc., New York.
- LING, G. N. 1973. The physical state of solutes and water in living cells according to the association-induction hypothesis. *Ann. N. Y. Acad. Sci.* **204**:6-50.
- LING, G. N., and R. W. GERARD. 1949. The normal membrane potential of frog sartorium muscle fibers. *J. Cell Comp. Physiol.* **34**:382-396.
- NOBLE, D. 1975. *The Initiation of the Heartbeat*. Clarendon Press, Oxford.
- NOBLE, D., and R. W. TSIEN. 1968. The kinetics and rectifier properties of the slow potassium current in cardiac Purkinje fibers. *J. Physiol. (Lond.)*. **195**:185-214.
- ORME, F. W. 1969. Liquid ion-exchanger microelectrodes. In *Glass Microelectrodes*. M. Lavallee, O. F. Schanne, and N. C. Hebert, editors. John Wiley & Sons, Inc., New York.
- PAGE, E., L. P. MCALLISTER, and B. POWER. 1971. Stereological measurements of cardiac ultrastructures implicated in excitation-contraction coupling. *Proc. Natl. Acad. Sci. U. S. A.* **68**:1465-1466.
- PAGE, E., and A. K. SOLOMON. 1960. Cat heart muscle in vitro. I. Cell volumes and intracellular concentration in papillary muscle. *J. Gen. Physiol.* **44**:327-344.
- ROBINSON, R. A., and R. H. STOKES. 1965. *Electrolyte Solutions*. Butterworth & Co., (Publishers) Ltd., London.
- RUSSELL, J. M., and A. M. BROWN. 1972. Active transport of potassium by the giant neurons of the *Aplysia* abdominal ganglion. *J. Gen. Physiol.* **60**:519-533.
- SOMMER, J. R., and E. A. JOHNSON. 1968. Cardiac muscle, a comparative study of Purkinje fibers and ventricular fibers. *J. Cell Biol.* **36**:497-526.
- VASSALLE, M. 1965. Cardiac pacemaker potentials at different extracellular and intracellular K concentrations. *Am. J. Physiol.* **208**:770-775.
- WALKER, J. L. 1971. Ion specific liquid ion exchanger microelectrodes. *Anal. Chem.* **43**:89A-91A.
- WALKER, J. L., and R. O. LADLE. 1973. Frog intracellular potassium activities measured with potassium microelectrodes. *Am. J. Physiol.* **225**:263-267.