Intra- and Extracellular Potassium Activities, Acetylcholine and Resting Potential in Guinea Pig Atria

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SUMMARY. Intracellular potassium activity in guinea pig left atria was measured using potassium ion-selective microelectrodes and conventional microelectrodes. The effects of extracellular potassium concentration and acetylcholine on both intracellular potassium activity and the relationship between the resting membrane potential and the potassium equilibrium potential were investigated. Intracellular potassium activity was 102.1 mM in bathing media with a potassium concentration of 5 mM. Neither increasing extracellular potassium concentration to 10 mM nor exposure to acetylcholine (2 × 10⁻⁶ to 10⁻³ M) significantly altered intracellular potassium activity. In contrast, intracellular potassium activity decreased to 92.9 mM in 2.5 mM potassium concentration solutions. Resting membrane potential was 18.6, 9.6, and 7.3 mV positive to the potassium equilibrium potential in 2.5, 5, and 10 mM potassium, respectively. Acetylcholine caused a significant hyperpolarization at each extracellular potassium activity, confirming that resting membrane potential was positive to the potassium equilibrium potential. Even after exposure to 10⁻³ M acetylcholine, the resting membrane potential apparently remained positive to the potassium equilibrium potential. If potassium accumulates in extracellular clefts during acetylcholine exposure, the calculated potassium equilibrium potentials are too negative, and the resting membrane potential might closely approximate the potassium equilibrium potential under these conditions. Fading of the acetylcholine-induced hyperpolarization and overshoot of the resting membrane potential on washout of acetylcholine were observed and are consistent with an accumulation of potassium during exposure to acetylcholine. In 5.0 mM potassium bathing solution, preparation-to-preparation variability of resting membrane potential can largely be explained by variability of intracellular potassium activity. The resting membrane potential is dependent on the logarithm of the intracellular potassium activity with a slope of —54.2 mV/10-fold increase in the intracellular potassium activity (r = —0.869). For fibers suprafused with 2.5 mM extracellular potassium, this relationship has a slope of —28.5 mV/10-fold increase in intracellular potassium activity (r = —0.454), and other factors must be invoked to explain the variability of the resting membrane potential. (Circ Res 54: 65–73, 1984)

THE introduction of ion-selective microelectrodes (ISE) for potassium with tip diameters of less than 0.5 µm (Walker, 1971) has made direct measurement of cytoplasmic potassium ion activity feasible in cardiac muscle while avoiding the problems associated with chemical analysis of tissue ion content. The intracellular activity of potassium (ak) has been studied in mammalian ventricle (Lee and Fozzard, 1975; Cohen et al., 1982; Guarnieri and Strauss, 1982; McCullough and Singer, personal communication), Purkinje fibers (Miura et al., 1977; Lee and Fozzard, 1979; Sheu et al., 1980), sinoatrial node (Grant and Strauss, 1982), and atrioventricular node (Baumgarten, 1982), as well as in amphibian atria, ventricle, and sinus venosus (Walker and Ladle, 1973). In these myocardial tissues, ak is reported to range from 64 to 130 mM.

Except for a brief mention in a report on rabbit sinoatrial node (Grant and Strauss, 1982) and a preliminary report on diseased human atrium (Singer et al., 1981), measurements of ak in mammalian atria have not been described. In view of the critical importance of potassium to electrophysiological events in mammalian atria and the wide range of ak obtained in various other myocardial tissues, it is desirable to fill this gap. Therefore, the effects of extracellular potassium concentration and acetylcholine (ACH) on both ak and the relationship between resting membrane potential and the potassium equilibrium potential were examined in guinea pig atria. Further, the basis for variability of Eₘ was considered.

A portion of this work previously has been presented in abstract (Singer et al., 1980).

Methods

Tissue Preparation and Solutions

Guinea pigs (Hartley strain, 250–300 g, either sex) were killed by cervical dislocation. The heart was removed as
rapidly as possible and rinsed in room temperature oxygenated modified Tyrode’s solution. Strips, approximately 4 x 6 mm, were dissected from the left atrium, pinned in the experimental chamber with the endocardial surface up, and superfused with oxygenated modified Tyrode’s solution at 37°C. Stimuli were delivered at 1–1.5 Hz via a pair of fine silver wires insulated except at the ends. Conventional 3 or KCl-filled microelectrodes (15–25 MΩ) were used to record membrane potential, and K⁺-selective ISE (5–20 Gill) were used to determine aK. Low pass filtering (down 3 dB at 12 Hz) was needed occasionally to reduce the noise picked up by the ISE.

The Tyrode’s media nominally had the following composition (in mm): NaCl, 150; KC1, 5.0; CaCl2, 1.8; MgCl2, 1.1; glucose, 10.9; HEPES, 5.0. Solutions were equilibrated with 100% O2, and the pH was adjusted to 7.4 with NaOH.

Measurements of Em and aK were made at potassium concentrations ([K+]o) of 2.5, 5.0 and 10 mm, substituting equimolar amounts of KCl and NaCl. For studies on the effect of ACh, the chloride salt (Sigma Chemicals) was added from a concentrated stock solution freshly prepared before each experiment.

Preparation and Calibration of ISE

Ion-selective microelectrodes were fabricated by the method of Walker (1971) as modified (Baumgarten et al., 1981). Glass blanks were coated with dimethyldichlorosilane, then K⁺ liquid ion exchanger (Corning Glass Works, #477317) was introduced into the tip of the electrode, and the shaft was filled with a solution of 400 mm KCl and 100 mm NaCl.

Standard techniques (Walker and Brown, 1977) were used to calibrate the ISE both before and after use. The potential, Ew, in a salt solution containing potassium or mixtures of potassium and sodium is given by:

\[ E_w = V_0 + S \ln(a^+_K + k_{K-Na} \cdot a_{Na}) \]  

\( V_0 \) is an empirically determined constant (mV), S is the slope of the electrode’s response in potassium solutions, \( a^+_K \) and \( a_{Na} \) are the activities of potassium and sodium, and \( k_{K-Na} \) is a dimensionless empirically determined selectivity coefficient. The empirical constants were defined before and after experimental measurements by determining the electrode’s responses in pure solutions of KCl (1, 3, 10, 30, 100, and 300 mm) and in constant ionic strength mixtures of KCl and NaCl (KCl + NaCl: 2 + 168; 4 + 166; 6 + 164 mm) at 37°C. The ISE potential in each superfuse could be predicted from the calibration data.

Figure 1 illustrates typical calibration data. The slope of the ISE’s response ranged from 26 to 28 mV/e-fold increase in \( a^+_K \); this is equivalent to 60 to 64 mV/10-fold increase. The selectivity coefficient, \( k_{K-Na} \), ranged from 0.02 to 0.05.

Calculation of \( a^+_K \)

The potential detected by an ISE in a cell, \( E_w \), is given by:

\[ E_w = E_m + V_0 + S \ln(a^+_K + k_{K-Na} \cdot a_{Na}) \]  

where the intracellular ion activities of potassium and sodium are \( a^+_K \) and \( a_{Na} \), respectively. The term \( k_{K-Na} \cdot a_{Na} \) can be ignored without significant error because of the high selectivity of the ISE for potassium over sodium and the high \( a^+_K/a_{Na} \) ratio. With an independent measurement of \( E_m \) and the calibration data, \( a^+_K \) can be calculated from a rearrangement of Equation 2:

\[ a^+_K = \exp \left( \frac{E_m - E_w - V_0}{S} \right) \]  

or from the change in potential upon impalement with the ISE (\( \Delta E = E_w - E_m \)) according to:

\[ a^+_K = \exp \left( \frac{\Delta E - E_w}{S} \right) \times \left( a^+_K + k_{K-Na} \cdot a_{Na} \right) \]  

Because Equation 3 utilizes S and \( V_0 \) while Equation 4 utilizes \( S \) and \( k_{K-Na} \), the two methods for calculating \( a^+_K \) have a different sensitivity to occasionally occurring drift in the calibration parameters. In each case, \( a^+_K \) was calculated by the method that minimized the effect of any drift. To define the characteristics of a preparation under a particular set of conditions, 6 to 12 impalements at different sites were made with both the conventional microelectrode and the ISE. The averages of the potentials obtained were used to calculate a single value of \( a^+_K \). In some experiments it was desirable to follow the response to an intervention over time. In these, several brief (≥1 minute) impalements were made with each electrode, and then a pair of representative impalements were maintained for the necessary interval. The \( a^+_K \) during this period was calculated from the pair of continuous recordings. Although an independent measurement of \( E_m \) is required to calculate \( a^+_K \), the ISE \( \Delta E \) alone is sufficient to estimate the difference between \( E_m \) and \( E_w \) in the impaled cell (Walker and Brown, 1977; Baumgarten et al., 1981):

\[ \Delta E \approx E_m - E_w + S \ln \left( \frac{a^+_K}{a^+_K + k_{K-Na} \cdot a_{Na}} \right) \]  

The term at the far right is a constant for an ISE and is defined by the composition of the bathing media and the calibration data for that electrode.
Baumgarten et al. \(a_k\) and \(E_m\) in Guinea Pig Atria

**Results**

**Effects of \([K^+]_o\) on \(a_k\), \(E_m\), and \(E_K\)**

To characterize the potassium distribution in atria and to determine the effect of \([K^+]_o\) and \(a_k\), multiple impalements were made with both ISE and conventional 3 M KCl-filled microelectrodes in preparations exposed to superfusates containing 2.5, 5.0, or 10 \(\text{mM} [K^+]_o\) for at least 30 minutes. Figure 2 illustrates typical impalements in quiescent atrial muscle. Upon advancing the ISE into a cell, the potential recorded became 5–15 mV more negative.

The calculated values of \(a_k\) are given in Table 1. In 5.0 \(\text{mM} [K^+]_o\) media, \(a_k\) was 102.1 ± 3.8 \(\text{mM}\). Recent estimates of \([K^+]_o\) in guinea pig atria based on chemical analysis give 128 \(\text{mM}\) (Glitsch et al., 1976) and 135 \(\text{mM}\) (McDonald and MacLeod, 1973) under conditions similar to those used here. If these estimates of \([K^+]_o\) apply to the preparations we studied, the apparent intracellular activity coefficient for \(K^+\) would be 0.75 or 0.79. These values are reasonably close to the \(K^+\) activity coefficient of the bathing media, 0.73, and suggest that most intracellular \(K^+\) is free in the cytoplasm of atrial muscle. Since \(a_k\) and \([K^+]_o\) were not measured in the same preparations, it is difficult to exclude totally the possibility that a small fraction of \(K^+\) is bound or compartmentalized (Lee and Fozzard, 1975).

Increasing \([K^+]_o\) to 10 \(\text{mM}\) did not significantly modify \(a_k\) (see Table 1). On the other hand, \(a_k\) was significantly reduced in preparations superfused with low \([K^+]_o\). In 2.5 \(\text{mM} [K^+]_o\) solutions, \(a_k\) was 92.9 ± 2.6 \(\text{mM}\), nearly 10% less than its value in 5.0 \(\text{mM} [K^+]_o\) media. A fall in \(a_k\) upon exposure to 2.5 \(\text{mM} [K^+]_o\) media can also be demonstrated with continuous impalements (see Fig. 3). In this experiment, \(a_k\) fell from 110 to 97 \(\text{mM}\) on lowering \([K^+]_o\) from 5.0 to 2.5 \(\text{mM}\) and returned to 106 \(\text{mM}\), nearly its previous value, within 12 minutes of readmitting 5.0 \(\text{mM} [K^+]_o\) media. Multiple impalements made beginning 30 minutes after readmitting 5.0 \(\text{mM} [K^+]_o\) media indicated complete restoration of \(a_k\), although recovery of \(a_k\) was consistently slower than its fall. In contrast to these results, continuous impalement experiments switching between 5.0 and 10 \(\text{mM} [K^+]_o\) media failed to reveal changes in \(a_k\). Thus, the data from these continuous impalement experiments are consistent with those from the multiple impalement experiments presented in Table 1.

Also presented in Table 1 are values of \(E_m\) and \(E_K\). These findings indicate that \(E_m\) of atrial muscle remains significantly positive to \(E_K\), even when \([K^+]_o\) is raised to more than twice its physiological level. The difference between \(E_m\) and \(E_K\) was 18.6 ±

### Table 1

<table>
<thead>
<tr>
<th>([K^+]_o) ((\text{mM}))</th>
<th>(a_k) ((\text{mM} ± \text{SE}))</th>
<th>(E_m) ((\text{mV} ± \text{SE}))</th>
<th>(E_K) ((\text{mV} ± \text{SE}))</th>
<th>(E_m - E_K) ((\text{mV} ± \text{SE}))</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>1.81</td>
<td>92.9 ± 2.6</td>
<td>-86.5 ± 0.8</td>
<td>-105.1 ± 0.8</td>
<td>18</td>
</tr>
<tr>
<td>5.0</td>
<td>3.62</td>
<td>102.1 ± 3.8</td>
<td>-79.5 ± 0.5</td>
<td>-89.1 ± 1.1</td>
<td>9</td>
</tr>
<tr>
<td>10.0</td>
<td>7.24</td>
<td>101.6 ± 3.1</td>
<td>-63.1 ± 0.5</td>
<td>-70.5 ± 1.0</td>
<td>6</td>
</tr>
</tbody>
</table>

\(E_K\) calculated using \(a_k\) and \(a_{K^+}\). \(n\), number of preparations.
0.8, 9.6 ± 0.9, and 7.3 ± 1.5 mV in 2.5, 5.0, and 10 mM [K+]o media, respectively. This analysis is based on the average of multiple impalements in each preparation. In addition, the conclusion that $E_m$ does not equal $E_\text{K}$ can be confirmed for individual cells of the preparation by considering the potential change detected upon impalement of the ISE (ΔE). The ΔE reflects the difference between $E_m$ and $E_\text{K}$ in the impaled cell plus a constant that is dependent on ISE properties and the composition of the bathing solution (see Eq. 5). The observed ΔE were −13.5 ± 0.7, −10.0 ± 0.5, and −6.7 ± 0.8 in 2.5, 5.0, and 10 mM [K+]o media, respectively (see Fig. 1). These values are significantly different than −34, −22, and −13 mV, the predictions of ΔE if $E_m$ equals $E_\text{K}$. Thus, the observed ΔE confirmed the conclusion that $E_m$ was 6–20 mV positive to $E_\text{K}$ in the cells impaled by the ISE.

The Relationship between $a'_\text{K}$ and $E_m$

It is pertinent to consider whether the observed dispersion of $E_m$ among the preparations studied can be attributed to variability of $a'_\text{K}$ as has previously been suggested in Purkinje fibers (Sheu et al., 1980) and frog ventricle (Walker and Ladle, 1973). Figure 4 shows the relationships between $E_m$ and the logarithm of $a'_\text{K}$ in both 5.0 and 2.5 mM [K+]o media. Each point represents the average of all the impalements in a single preparation. With the exclusion of one outlying experiment, the best linear fit to the data in 5.0 mM [K+]o media (panel A) has a slope of −54.2 mV/10-fold increase in $a'_\text{K}$. Although the slope of the relationship is significantly less than the Nernst equation prediction of −61.5 mV ($P < 0.001$), the atrial cell membrane nevertheless behaves approximately as a potassium electrode under this condition, and most of the dispersion of $E_m$ can be attributed to variability of $a'_\text{K}$. The limited range of observed $a'_\text{K}$ is insufficient, however, to determine whether the experimental data are better fit by the Goldman-Hodgkin-Katz equation or by the Nernst equation.

In contrast to the results in 5.0 mM [K+]o media, the variability of $a'_\text{K}$ was insufficient to explain the distribution of $E_m$ in preparations bathed in 2.5 mM [K+]o media (see Fig. 4, panel B). The slope of the best linear fit to the data was −28.5 mV/10-fold increase in $a'_\text{K}$. Despite the fact that the response of $E_m$ to changes in extracellular potassium is markedly less than that expected from the Nernst equation at 2.5 mM [K+]o, the Goldman-Hodgkin-Katz equation still predicts that $E_m$ should change by about 61 mV/10-fold change in $a'_\text{K}$ over the range of $a'_\text{K}$ observed. This prediction is obtained because $a'_\text{K}$ is much greater than $P_{\text{Na}}/P_{\text{K}} \times a'_\text{Na}$ and $P_{\text{Cl}}/P_{\text{K}} \times a'_\text{Cl}$. Thus, factors other than $a'_\text{K}$ must determine the variability of $E_m$ at low [K+]o. Perhaps the sensitivity of $P_{\text{K}}$ or electrogenic Na-K pumping to low [K+]o or the voltage dependence of membrane permeabilities differ.

Meaningful analysis of the relationship between $E_m$ and $a'_\text{K}$ in 10 mM [K+]o media was not feasible because the ranges of $a'_\text{K}$ and $E_m$ were both quite small.

Effects of Acetylcholine

It is well-known that acetylcholine (ACh) hyperpolarizes atrial muscle by increasing the potassium permeability of the membrane (e.g., Trautwein and...
Düdel, 1958; Rayner and Weatherall, 1959). Consequently, \( E_m \) approaches \( E_k \). The maximum hyperpolarization achieved with "saturating" doses of ACh are presented in Table 2. At all \([K^+]_o\) explored, ACh induced a significant hyperpolarization confirming that \( E_m \) was positive to \( E_k \) in the absence of ACh. Although the average hyperpolarizations seen with \( 10^{-3} \) m ACh were slightly larger than those with \( 10^{-4} \) m ACh, the differences were not statistically significant.

Exposure to ACh for 10-20 minutes, even at these pharmacological concentrations, does not detectably alter \( a_k \). In 20 experiments, simultaneous impalements with both ISE and conventional microelectrodes were maintained throughout exposure to ACh and its washout in 2.5, 5.0, or 10 mM \([K^+]_o\) media. At each \([K^+]_o\), the average potential changes recorded by each electrodes were within 1 mV and statistically indistinguishable. Since \( a_k \) is proportional to the difference in these potentials (see Eq. 3), the results indicate that \( a_k \) was unchanged by ACh.

The relationship between \( E_k \) and \( E_m \) in the presence of ACh is also given in Table 2. Despite the ACh-induced hyperpolarization, \( E_m \) appears to remain significantly positive to \( E_k \) at each \([K^+]_o\) studied. This calculation assumes that the potassium activity in the extracellular clefts of the preparation remains the same as in the bulk phase of the superfusate. However, augmentation of \( K^+ \) efflux with ACh exposure (Trautwein and Düdel, 1958; Rayner and Weatherall, 1959) may result in an accumulation of \( K^+ \) in extracellular clefts, and in a shift of \( E_k \) to more positive potentials (Maughan, 1973; Baumgarten and Isenberg, 1977; Kunze, 1977). This would have the effect of reducing the difference between \( E_m \) and \( E_k \).

Although these experiments were not designed to test whether ACh-induced hyperpolarization results in the accumulation of \( K^+ \) in extracellular clefts, some data that may be consistent with this view were obtained. If accumulation occurs during ACh exposure, after the initial hyperpolarization, a gradual decrease in \( E_m \) is expected as \( E_k \) shifts toward more positive potentials. Conversely, the membrane should transiently depolarize beyond the control level on washout of ACh and return to control \( E_m \) as cleft \([K^+]_o\) is restored to normal.

Records consistent with changes in cleft \([K^+]_o\) are shown in Figure 5. In panel A, the preparation was exposed to \( 10^{-4} \) m ACh in 2.5, 5.0, and 10 mM \([K^+]_o\) media. The ACh-induced hyperpolarization was not well maintained. Instead, \( E_m \) reached a maximum and then declined by 3.8, 1.8, and 0.8 mV, respectively. Thus, the steady state ACh-induced hyperpolarizations were only about one-half their initial amplitudes. Such time dependence was observed in almost all preparations exposed to \( 10^{-4} \) or \( 10^{-3} \) m ACh, although the magnitude of the decline was variable. On the other hand, the predicted transient depolarization upon washout of ACh was almost never detected with these doses of ACh. The failure may result from the prolonged washout necessary to decrease the ACh concentration in the bath and in extracellular clefts to a low level. Transient depolarizations on washout were a more common finding in fibers exposed to a lower and more physiological concentration of ACh, \( 2 \times 10^{-6} \) m. A prominent example is illustrated in Figure 5, panel B. Simultaneous impalements with a conventional microelectrode and an ISE both show transient 2-mV depolarizations beyond the baseline on washout that reach a peak in 1.5 minutes and then return to baseline within a similar time. Transient depolarizations were noted during washout of \( 2 \times 10^{-6} \) m ACh in about 40% of the experiments conducted in 2.5 mM \([K^+]_o\) media. However, a significant decline of the ACh-induced hyperpolarization was observed only infrequently in these experiments. In four experiments, following washout of ACh, preparations were reexposed to the same ACh concentration about 3 minutes after \( E_m \) returned to its resting level. Both the amplitude and the time dependence of the response were reproducible. Alternative interpretations of the time dependence of the effects of ACh will be considered later.

### Table 2

<table>
<thead>
<tr>
<th>([K^+]_o) (mM)</th>
<th>(a_k) (mM)</th>
<th>ACh (m)</th>
<th>Control ( E_m ) (mV ± SE)</th>
<th>( \Delta E_m ) (mV ± SE)</th>
<th>( E_m - E_k ) (mV ± SE)</th>
<th>( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>1.81</td>
<td>(10^{-4})</td>
<td>-87.6 ± 0.9</td>
<td>-7.0 ± 0.6</td>
<td>10.5 ± 0.6</td>
<td>15</td>
</tr>
<tr>
<td>2.5</td>
<td>1.81</td>
<td>(10^{-3})</td>
<td>-85.4 ± 1.0</td>
<td>-8.0 ± 0.6</td>
<td>11.7 ± 0.8</td>
<td>25</td>
</tr>
<tr>
<td>5.0</td>
<td>3.62</td>
<td>(10^{-4})</td>
<td>-79.8 ± 0.6</td>
<td>-3.7 ± 0.3</td>
<td>5.6 ± 0.6</td>
<td>13</td>
</tr>
<tr>
<td>5.0</td>
<td>3.62</td>
<td>(10^{-3})</td>
<td>-78.7 ± 0.4</td>
<td>-4.2 ± 0.3</td>
<td>6.1 ± 0.5</td>
<td>20</td>
</tr>
<tr>
<td>10.0</td>
<td>7.24</td>
<td>(10^{-4})</td>
<td>-62.8 ± 0.8</td>
<td>-2.2 ± 0.2</td>
<td>4.8 ± 0.8</td>
<td>12</td>
</tr>
<tr>
<td>10.0</td>
<td>7.24</td>
<td>(10^{-3})</td>
<td>-63.9 ± 0.8</td>
<td>-2.3 ± 0.3</td>
<td>3.6 ± 0.8</td>
<td>17</td>
</tr>
</tbody>
</table>

Control \( E_m \) observed before addition of acetylcholine (ACh); \( \Delta E_m \) maximum hyperpolarization of \( E_m \) during exposure to ACh; \( E_m - E_k \) difference between maximum \( E_m \) during exposure to ACh and \( E_k \); \( n \), number of experiments.
atria is either 1.5 mM, based on measurements of hyperpolarization on rewarming and membrane resistance (Glitsch et al., 1978), or 0.2 mM, based on measurements of K⁺-activated Na⁺ efflux (Glitsch et al., 1976). Values of 1–2 mM have also been obtained in other cardiac tissues by several different techniques (e.g., Portius and Repke, 1967; Erdmann et al., 1971; Gadsby, 1980; cf., Deitmer and Ellis, 1978; Eisner and Lederer, 1980). If the estimates of 1–2 mM for $K_m$ are correct, reduction of $[K^+]_o$ to 2.5 mM is expected to inhibit active K⁺ uptake significantly, while increasing $[K^+]_o$ to 10 mM should have a relatively small stimulating effect. Nevertheless, factors other than the $[K^+]_o$-dependence of the Na-K pump are also important in determining the steady state $a_K$ attained at low $[K^+]_o$. The present results (see Table 1) indicate that the driving force for K⁺ efflux is greater at low $[K^+]_o$. On the other hand, the fall in $a_K$ is resisted by a decrease in $P_K$ at low $[K^+]_o$ (Carmeliet, 1961; Haas et al., 1966) and by a stimulation of the Na-K pump due to an increase in $a_K$ (Page and Storm, 1965; Glitsch et al., 1976; Glitsch and Pusch, 1980). The ability of some cardiac preparations to maintain a constant $a_K$ in the face of low $[K^+]_o$, while others cannot, may stem from quantitative differences in the operation of these homeostatic mechanisms.

Even at normal mammalian $[K^+]_o$ (i.e., 4–5.4 mM), $a_K$ appears to vary considerably in different cardiac preparations. The value of $a_K$ obtained for guinea pig left atria in the present study is substantially the $a_K$ of 80.2 mM in rabbit right atria at 4.0 mM $[K^+]_o$ (Grant and Strauss, 1982), but is similar to the $a_K$ of 100 mM in diseased human right atrial appendage (Singer et al., 1981). In Purkinje fibers, $a_K$ is 124.2 mM in dog (Miura et al., 1977) and is 121.4 mM (Lee and Fozzard, 1979) or 99.5 mM (Sheu et al., 1980) in sheep. In rabbit ventricle, $a_K$ is 82.6 mM (Lee and Fozzard, 1975), whereas in guinea pig ventricle, it is 116.1 mM (Cohen et al., 1982) or 104.6 mM (Guarnieri and Strauss, 1982), and, in diseased human ventricle, 100 mM (McCullough and Singer, personal communication). Further, in 2.5 mM $[K^+]_o$ solution, $a_K$ in guinea pig atria is greater than in frog atria (Walker and Ladle, 1973).

It is not possible to determine rigorously whether the reported differences in $a_K$ result from species differences, variation in the state of the preparations, or errors in the determination of $a_K$. However, it is interesting to note that under some circumstances preparation-to-preparation variability of $E_m$ can be explained by variability of $a_K$. $E_m$ is dependent on the logarithm of $a_K$ with a slope that approaches −61 mV/10-fold increase in $a_K$ in guinea pig atria superfused with 5.0 mM $[K^+]_o$ [slope = −54 mV (Fig. 4, panel A)] in sheep Purkinje fibers with 5.4 mM $[K^+]_o$ [slope = −73 mV (Sheu et al., 1980)] and in frog ventricle with 2.5 mM $[K^+]_o$ [slope = −57 mV (Walker and Ladle, 1973)]. Differences between the observed $E_m$ in dog (Miura et al., 1977) and sheep Purkinje fibers can also be explained on this basis.

Discussion

Intracellular Potassium Activity

Potassium ion-selective microelectrode measurements indicate that $a_K$ in guinea pig atrium is 102 mM in 5.0 mM $[K^+]_o$. Neither increasing $[K^+]_o$ to 10 mM nor exposure to ACh ($2 \times 10^{-4}$ to $10^{-3}$ M) has a significant effect on $a_K$. In contrast, reduction of the superfusate $[K^+]_o$ from 5.0 to 2.5 mM causes $a_K$ to decrease by 9%. A decrease in $a_K$ is also observed at low $[K^+]_o$ in Purkinje fibers (Miura et al., 1977; Sheu et al., 1980) but not in rabbit ventricle (Lee and Fozzard, 1975) and cat ventricle, however, $[K^+]_o$ decreases at low $[K^+]_o$ (Page and Solomon, 1960; Page et al., 1964).

It seems reasonable to attribute the fall in $a_K$ primarily to the $[K^+]_o$ dependence of the Na-K pump. Although not all of the details of its function are known, estimates of the Michaelis-Menten constant, $K_m$, with respect to $[K^+]_o$ have been made. Glitsch and co-workers reported that the $K_m$ in guinea pig

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*Lee and Fozzard (1975) used NAS 27-04 glass ISE which have a substantially lower selectivity for K⁺ over Na⁺ ($K_{Na} = 0.2-0.4$) than the liquid ion-exchanger ISE used here. Consequently, a small decrease in $a_K$ upon exposure to low $[K^+]_o$ media would have been difficult to detect if it were associated with an equimolar increase in $a_K$. 

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$E_m$ and $E_{IS}$ electrodes are the same. This demonstrates that $a_K$ is not altered by ACh.

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(Sheu et al., 1980), whereas differences between \( E_m \) in rabbit (Grant and Strauss, 1982) and guinea pig atria (present results) cannot. On the other hand, in guinea pig atria superfused with 2.5 mM \([K^+]_o\), the relationship between \( E_m \) and \( a_K \) is distinctly different from that predicted by the Nernst or Goldman-Hodgkin-Katz equations [slope = -28.5 mV (Fig. 4, panel B)]. A similar relationship between \( E_m \) and \( a_K \) has also been noted in frog atria and sinus venosus at 2.5 mM \([K^+]_o\), [slope ≈ -30 mV (Walker and Ladle, 1973)]. The most likely explanation of this discrepancy with Nernst or Goldman-Hodgkin-Katz theory is a significant preparation-to-preparation variability of \( P_{Na}/P_K \). \( P_K \) decreases as \([K^+]_o\) is lowered (e.g., Carmeliet, 1961; Haas, 1966), and, consequently, \( P_{Na}/P_K \) increases. Perhaps the sensitivity of \( P_K \) to reduced \([K^+]_o\) differs between preparations. This is certainly the case for Purkinje fibers. Some preparations depolarize to about -40 mV on exposure to 2.7 mM \([K^+]_o\) media while others remain well-polarized with \( E_m \) near -80 mV. Sheu et al. (1980) estimated \( P_{Na}/P_K \) as 0.163 in the former group and as 0.029 in the latter. Differing sensitivities of the Na-K pump to \([K^+]_o\) might also contribute to the inability to predict \( E_m \) from \( a_K \).

Relationship between \( E_m \) and \( E_K \)

Atrial \( E_m \) is significantly positive to \( E_K \) by 18.6, 9.6, and 7.3 mV at 2.5, 5.0, and 10 mM \([K^+]_o\) respectively. It is not surprising that \( E_m \) is positive to \( E_K \) over the entire physiological range of \([K^+]_o\). Such an arrangement is mandatory if ACh is to hyperpolarize to \( E_K \) (Trautwein and Dodel, 1958; Rayner and Weatherall, 1959). Indeed, the occurrence of hyperpolarizations on exposure to ACh strongly supports the conclusion that \( E_m \) is positive to \( E_K \) at each \([K^+]_o\) examined.

The relationship between \( E_m \) and \( E_K \) is not the same in all cardiac tissues. These differences are illustrated in Figure 6. Atrial muscle more closely resembles Purkinje fiber than ventricular muscle in this regard. Lee and Fozzard (1979) found that \( E_m \) was within 2 mV of \( E_K \) in rabbit ventricle at \([K^+]_o\) of 5.0 mM or greater. In contrast, results in Purkinje fibers indicate that \( E_m \) is 10–15 mV positive to \( E_K \) at \([K^+]_o\) and remains 5–12 mV positive to \( E_K \) at elevated \([K^+]_o\) (Miura et al., 1977; Sheu et al., 1980). Thus, although it is possible to estimate \( E_K \) from \( E_m \) in ventricle (at \([K^+]_o\) of 5 mM or greater), this is not the case in atria or Purkinje fibers.

One possible source of error in the calculation of \( E_K \) is the assumption that \( a_K \) in the clefts of the preparation equals that in the bulk phase of the bathing media. Whereas alterations in the cleft \([K^+]_o\) have been documented during transient changes in \( E_m \) (e.g., Maughan, 1973; Baumgarten and Isenberg, 1977; Kunze, 1977), no convincing evidence has been presented for such changes in the steady state. Thus, we believe that the calculated values of \( E_K \) presented in Table 1 and Figure 6 are accurate. If cleft and bulk phase \([K^+]_o\) are not equal, the discrepancy should be smallest at the highest \([K^+]_o\).

The situation during exposure to ACh is more complex in that ACh increases \( P_K \) and \( K^+ \) efflux (Ten Eick et al., 1976; Belardinelli and Isenberg, 1983). Although this does not cause a measurable change in the \( a_K \) of the large volume of the cell, it may lead to an accumulation of \( K^+ \) in the small volume of the extracellular clefts (e.g., Maughan, 1973; Baumgarten and Isenberg, 1977; Kunze, 1977) and overestimation of the driving force on \( K^+ \) (see Table 2 and Figure 5). In fact, relatively small increases in cleft \( a_K \) (1.00 mM in 2.5 mM \([K^+]_o\), 0.96 mM in 5.0 mM \([K^+]_o\), and 1.10 mM in 10 mM \([K^+]_o\)) would be sufficient to make \( E_m \) equal to \( E_K \) during 10⁻³ M ACh.

FIGURE 6. The relationships between \( a_K \) and both \( E_m \) and \( E_K \) in guinea pig atria (panel A), rabbit ventricle (panel B), and dog Purkinje fiber (panel C). \( E_m \) is indicated by filled circles, \( E_K \) by open circles, and maximum \( E_m \) after exposure to ACh by triangles. When \([K^+]_o\) is 5 mM or greater, \( E_m \) closely approximates \( E_K \) in ventricle but not in atria or Purkinje fiber. Although ACh hyperpolarizes atria, \( E_m \) remains to remain positive to \( E_K \). However, \( E_K \) was calculated from the measured \( a_K \) and the superfusate \( a_K \). Although exposure to ACh does not alter \( a_K \), ACh probably does result in an accumulation of \( K^+ \) in extracellular clefts. This would make the calculated value of \( E_K \) negative to its true value during exposure to ACh. Data in panel B were plotted from Table 3 of Lee and Fozzard (1979) and those of panel C from Table 1A and 1C of Miura et al. (1977).
exposure. Although it is uncertain whether changes of this magnitude occur, it seems highly probable that at least some accumulation is induced by ACh, shifting $E_K$ toward more positive potentials. Direct evidence for this view is provided by voltage clamp experiments on frog atria that revealed an ACh-dependent positive shift of the reversal potential for a $K^+$ current (Gamier et al., 1978) and by extracellular ISE recordings in rabbit sinoatrial node that identified an ACh-dependent increase of $a_{\text{Cl}}$ in the subendocardial space monitored by the ISE (Spear et al., 1979). Moreover, our observations that ACh-induced hyperpolarizations often decrease with time and that $E_m$ transiently depolarizes beyond its steady state value on washout of ACh are also consistent with accumulation.

**Time-Dependence of ACh Response**

Although it is likely that an accumulation of cleft K$^+$ during ACh exposure and its subsequent fall during washout modify the response to ACh (Garnier et al., 1978; Spear et al., 1979), additional factors may contribute to the time course of $E_m$. A fading of the ACh-induced hyperpolarization and an overshoot of $E_m$ on washout have been noted previously in both atrial muscle and sinoatrial node (Sato, 1968; Burke and Calaresu, 1972; Jalife et al., 1980; Tokimasa et al., 1981). Moreover, our observations that ACh-induced hyperpolarizations are not restored in bullfrog atria for as much as 15 minutes after removal of drug, whereas $E_m$ and conductance changes return to control values within a few minutes (Tokimasa et al., 1981). Such a dissociation of recovery of $E_m$ and responsiveness to ACh was not seen in the present experiments on guinea pig atria. Furthermore, the ACh-induced current in single isolated atrial cells, a preparation not subject to K$^+$ accumulation, does not exhibit fading or desensitization (Momose et al., in press). In any case, it is not clear how desensitization would explain the transient depolarization on ACh washout. Several other possible explanations for the transient depolarization on washout of ACh also seem unlikely. The transient depolarization is unaffected by inhibition of the Na-K pump, tetrodotoxin, 3-4-600 (a slow channel blocker), curare, or pentolinium, and is only somewhat attenuated by 1 $\mu$M propranolol (Nishiye et al., 1982). Nevertheless, it would be premature to conclude from the evidence at hand that transient changes in $E_K$ are the full explanation for the time-dependent effects of ACh on $E_m$.

The biphasic character of the in vivo $E_m$ responses to both ACh and its washout are strikingly reminiscent of the biphasic chronotropic responses to prolonged vagal stimulation or application of muscarinic agonists in vivo. Both a fading of the negative chronotropic effect (vagal escape) and a transient positive chronotropic effect on cessation of muscarinic receptor activation (postvagal tachycardia) are well documented (e.g., Burke and Calaresu, 1972; Loeb and Vassalle, 1978, 1979; Martin et al., 1982). Biphasic alterations in sinus node automaticity of this type could be explained, at least in part, in terms of biphasic changes in sinoatrial node maximum diastolic potential similar to the biphasic $E_m$ response to ACh observed here. Consideration of other membrane effects (e.g., on phase 4 depolarization and threshold potential) and of the systemic effects of vagal stimulation is also required, however, for a full explanation of these phenomena. Irregularities in the first few returning sinus cycles following termination of supraventricular tachycardias may also be explicable on this basis.

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