A Time- and Voltage-dependent K⁺ Current in Single Cardiac Cells from Bullfrog Atrium

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ABSTRACT Individual myocytes were isolated from bullfrog atrium by enzymatic and mechanical dispersion, and a one-microelectrode voltage clamp was used to record the slow outward K⁺ currents. In normal [K⁺]o (2.5 mM), the slow outward current tails reverse between -95 and -100 mV. This finding, and the observed 51-mV shift of E,10-fold change in [K⁺]o, strongly suggest that the “delayed rectifier” in bullfrog atrial cells is a K⁺ current. This current, Iₖ, plays an important role in initiating repolarization, and it is distinct from the quasi-instantaneous, inwardly rectifying background current, Iₖ₉. In atrial cells, Iₖ does not exhibit inactivation, and very long depolarizing clamp steps (20 s) can be applied without producing extracellular K⁺ accumulation. The possibility of [K⁺]₉ accumulation contributing to these slow outward current changes was assessed by (a) comparing reversal potentials measured after short (2 s) and very long (15 s) activating prepulses, and (b) studying the kinetics of Iₖ at various holding potentials and after systematically altering [K⁺]o. In the absence of [K⁺]₉ accumulation, the steady state activation curve (nₑ) and fully activated current-voltage (I-V) relation can be obtained directly. The threshold of the nₑ curve is near -50 mV, and it approaches a maximum at +20 mV; the half-activation point is approximately -16 mV. The fully activated I-V curve of Iₖ is approximately linear in the range -40 to +30 mV. Semilog plots of the current tails show that each tail is a single-exponential function, which suggests that only one Hodgkin-Huxley conductance underlies this slow outward current. Quantitative analysis of the time course of onset of Iₖ and of the corresponding envelope of tails demonstrate that the activation variable, nₑ, must be raised to the second power to fit the sigmoid onset accurately. The voltage dependence of the kinetics of Iₖ was studied by recording and curve-fitting activating and deactivating (tail) currents. The resulting 1/τₑ curve is U-shaped and somewhat asymmetric; Iₖ exhibits strong voltage dependence in the diastolic.
range of potentials. Changes in the \([\text{Ca}^{2+}]_o\) in the superfusing Ringer's, and/or addition of \(\text{La}^{3+}\) to block the transmembrane \(\text{Ca}^{2+}\) current, show that the time course and magnitude of \(I_K\) are not significantly modulated by transmembrane \(\text{Ca}^{2+}\) movements, i.e., by \(I_{\text{Ca}}\). These experimentally measured voltage- and time-dependent descriptors of \(I_K\) strongly suggest an important functional role for \(I_K\) in atrial tissue: it initiates repolarization and can be an important determinant of rate-induced changes in action potential duration.

**INTRODUCTION**

The first voltage-clamp measurements of time- and voltage-dependent \(\text{K}^+\) currents, or "delayed rectification," in cardiac muscle were made approximately 20 years ago (cf. Trautwein, 1973; Vassalle, 1979). In the heart, these slow outward currents are thought to have very important physiological functions. For example, in the atrium or ventricle, their activation may trigger and control the repolarization of the action potential (D. Noble and Tsien, 1972; Carmeliet, 1977), and their subsequent decline may modulate the relative refractory period. In cardiac pacemaker tissue, the decay of the \(\text{K}^+\) currents may also underlie the pacemaker depolarization (cf. Brown, 1982; Shibata and Giles, 1985; Nakayama et al., 1984; Giles and Shibata, 1985).

Slow outward \(\text{K}^+\) currents in isolated strips, or trabeculae, of mammalian cardiac muscle have been studied extensively since the voltage-clamp technique was first applied (for review, see Carmeliet, 1977; Carmeliet and Vereecke, 1979). In the first semiquantitative study, two separate slow outward currents were identified in the range of potentials corresponding to the action potential plateau of the Purkinje fiber (D. Noble and Tsien, 1969a, b). The kinetics of each of these currents were described using the Hodgkin-Huxley formalism, but the reversal potentials (approximately \(-60\) mV) indicated that they were not carried entirely by \(\text{K}^+\) ions. Thus, they were named \(i_{n}\) and \(i_{m}\). Later studies described a slow outward current somewhat similar to \(i_n\) in ventricular trabeculae (Katzung and Morgenstern, 1977; McDonald and Trautwein, 1978a, b), in strips of sinoatrial node (DiFrancesco et al., 1979), and in small clusters of atrioventricular node cells (Kokubun et al., 1982). However, in these preparations, this current reversed at potentials much closer to \(E_K\); therefore, it was thought to be carried mainly by \(\text{K}^+\).

The slow outward currents in frog atrium have also been studied quite extensively (cf. Carmeliet and Vereecke, 1979), beginning with the work of Rougier et al. (1968) and Brown and Noble (1969a, b). Although these and later investigations (de Hemptinne, 1971a, b; Maughan, 1973; Ojeda and Rougier, 1974; Brown et al., 1976a) used a similar voltage-clamp technique (double sucrose gap) and the same preparation (amphibian atrial trabeculae), both the raw data and the interpretation of the results differed substantially. At present, it is unclear how many distinct outward currents exist in atrial tissue. Detailed data concerning the ionic selectivity of these channels are also lacking, and no quantitative results describing their time and voltage dependence are available.

Many of the disparities in the raw data in these studies may arise from technical limitations and artifacts. Thus, in multicellular trabeculae, \((a)\) it is difficult to
ensure that the preparation is "space clamped" (Johnson and Lieberman, 1971),
and (b) the K⁺ currents that are elicited may result in changes in the electrochemical
gradient (see Attwell and Cohen, 1977; Attwell et al., 1979; Brown et al., 1976b; Lammel, 1981). For example, in voltage-clamp protocols, the K⁺ currents
may produce significant accumulation and/or depletion of K⁺ in the small,
restricted extracellular spaces within the trabeculae (cf. Maughan, 1973; S. J.
Noble, 1976; Kline and Morad, 1978; Attwell et al., 1979; Brown et al., 1980;
Morad, 1980; Cohen and Kline, 1982). Since the fully activated outward currents
in atrial trabeculae are rather large (microamperes), series resistance artifacts
may also be substantial, making the transmembrane voltage variable and difficult
to measure, even under quasi-steady state conditions (cf. Johnson and Lieberman,
1971; Attwell and Cohen, 1977).

Our main objective was to carry out a quantitative analysis of the slow outward
currents in atrial tissue. The following questions were addressed: (a) Are such
currents carried mainly by K⁺? (b) How many distinct currents are there? (c)
What are the ion transfer (I-V) and the kinetic properties of these currents? (d)
What is their role in, e.g., the repolarization of the action potential? It is now
possible to obtain answers to these questions using single isolated atrial cells. We
have recently refined an enzymatic dispersion technique for isolating single cells
from the bullfrog atrium (Humen and Giles, 1981) and developed an appropriate
single-microelectrode voltage-clamp technique (Hamill et al., 1981; Hume and
Giles, 1983). In voltage-clamp measurements of the "delayed rectifier" in single
atrial cells, it is possible to assess and minimize series resistance and [K⁺]o
accumulation/depletion problems. Our results show that the slow outward current
in bullfrog atrium is carried mainly by K⁺ and that it is controlled by a single Hodgkin-Huxley gating mechanism. Some of these results have been
reported previously in abstract form (Hume and Giles, 1982; Giles et al., 1984).

M E T H O D S

M y o c y t e Dispersion and Microelectrode Recording Techniques

The method used for cell isolation was the same as that used by Hume and Giles (1983).
The microelectrode fabrication and intracellular impalement and data recording tech-
niques were identical to those outlined in Giles and Shibata (1985). In some of the
experiments, a series resistance compensation method developed by Sigworth (1980) and
previously used by Hume and Giles (1983) was employed.

S olutions

The ionic composition and the equilibration procedure for both the normal Ringer's
solution and the HEPES-buffered Ringer's solution have been described by Giles and
Shibata (1985). In some protocols, it was necessary to raise [K⁺]o; this was done by
equimolar replacement of KCl for NaCl. In separate experiments, [Ca²⁺], was increased
or decreased and/or [Mg²⁺], was altered. These solution changes were made so that the
total extracellular divalent cation concentration remained constant in order to minimize
the effects of changes in surface charge on voltage-dependent gating variables.

Tetrodotoxin (TTX) was purchased from Sigma Chemical Co., St. Louis, MO; CdCl₂
was purchased from Aldrich Chemical Co., Inc., Milwaukee, WI; LaCl₃ was purchased
from K&K Laboratories, Inc., Plainview, NY; and HEPES was obtained from Research Organics Inc., Cleveland, OH.

**Data Recording and Analysis**

In our initial experiments, membrane potentials and currents were amplified and then recorded on FM tape (model 3968, Hewlett-Packard Co., San Diego, CA). Tape speeds of 3.75 in./s (bandwidth, DC to 1.25 kHz) and 7.5 in./s (bandwidth, DC to 2.5 kHz) were used. These data were later digitized using a digital oscilloscope (model 3001, Norland Corp., Fort Atkinson, WI) and then sent to a PDP 11-70 laboratory computer (Digital Equipment Corp., Maynard, MA). Current waveforms were analyzed (cf. Lanczos, 1957) using the "Discrete" analysis program (Provencher, 1976), which determines whether the waveform is best fit by one-, two-, three-, or four-exponential functions and then calculates the time constants and initial (or final) amplitudes that best fit the data. In later experiments, voltage and current traces were digitized on-line (12 bit; ~14 kHz per channel) using a Data Translation (Marlboro, MA) 2801A board in an IBM PC microcomputer (Robinson and Giles, 1986). The subsequent analysis was done on a VAX 11-750 computer (Digital Equipment Corp.).

**RESULTS**

**General Features of the Delayed Rectifier Current**

We have shown previously (Hume and Giles, 1983) that isolated myocytes from bullfrog atrium respond to depolarizing voltage-clamp pulses with a characteristic pattern of current activation. First, a rather large (~6 nA) and transient inward Na current, \( I_{Na} \), is elicited. Next, a smaller (300 pA) and slower transient inward Ca current, \( I_{Ca} \), develops. Finally (in response to depolarizations positive to approximately ~40 mV), a slow, but maintained, time- and voltage-dependent outward current is activated. The major aim of the experiments reported here was to study in quantitative detail the kinetics and rectifier properties (ion transfer mechanism) of the time- and voltage-dependent slow outward current. As noted in the Introduction, within the past 10 years, this "delayed rectifier" current has been studied extensively by cardiac electrophysiologists. However, no agreement presently exists on many of its fundamental characteristics: (a) Which ion(s) is the major current carrier? (b) How many separate Hodgkin-Huxley conductance components underlie this current? (c) What is the size of this current in physiologically relevant voltage ranges? In the absence of these data, it has not been possible to define unambiguously the physiological role of this current during the atrial action potential.

Fig. 1 illustrates a representative pattern of the time- and voltage-dependent outward currents measured in an isolated atrial myocyte superfused in normal Ringer's solution. In this experiment, 5-s depolarizing pulses were applied from a holding potential (~80 mV) near the resting potential. The superimposed voltage records (inset, top right) and current records (digitized traces) show that when the depolarizing clamp step is positive to ~30 mV, a slowly activating outward current develops. This current becomes progressively larger and faster as the voltage steps are made more positive. In addition, these data illustrate that the current relaxes at the end of the depolarizing clamp steps and that these current tails appear to get larger as the size of current onset increases.
We have shown previously (Hume and Giles, 1983) that this current can be studied without any significant overlap or contamination from either $I_{Na}$ or $I_{Ca}$. In Fig. 1 and all subsequent records in this article, $I_{Na}$ has been selectively blocked by the addition of TTX ($3 \times 10^{-6}$ M). The Ca current, $I_{Ca}$, is present, but it is relatively small and transient. It therefore does not overlap significantly with the onset of the delayed rectifier, even at very positive potentials.

**Ion Transfer Characteristics of the Delayed Rectifier Current**

The first series of experiments was done to determine which ion carries this slow time- and voltage-dependent outward current. The data in Fig. 2 illustrate a measurement of the reversal potential. A conventional double-pulse protocol was used: the first pulse ($P_1$) depolarized the cell to +60 mV for 2 s; hence, it activated a relatively large outward current. The second pulse ($P_{II}$) clamped the transmembrane potential to various predetermined levels (in the range -80 to -105 mV) for 8 s. These data show that this outward current reverses near -95 mV. As noted previously (Hume and Giles, 1983), this reversal potential is very close to the $E_K$ calculated from K$^+$-selective microelectrode measurements (Walker and Ladle, 1973), which strongly suggests that K$^+$ is the major current carrier.
Previous studies (e.g., Brown and Noble, 1969a, b) in multicellular atrial trabeculae have indicated that the reversal potential for this current is much more positive—near −60 mV. To confirm our findings, two additional protocols were used. First, we assessed whether extracellular K⁺ accumulation or depletion was likely to be significantly influencing our reversal potential measurements. Second, we determined the selectivity of the delayed rectifier ion transfer mechanism by measuring reversal potential shifts after graded changes in [K⁺]ₒ.

The results shown in Fig. 3 provide information on the extent to which extracellular accumulation and/or depletion of K⁺ occurs and thus may influence reversal potential measurements. This protocol is similar to that shown in Fig. 2; however, there is one important difference. The duration of P₁ has been lengthened from 2 to 10 s; the amount of current it elicits therefore has increased ~10-fold. The results show that the reversal potential remains very close to −95 mV (cf. Adrian et al., 1970), which provides strong evidence against the possibility that extracellular K⁺ accumulation significantly shifts $E_K$. As a result, accurate and reproducible measurements of the reversal potential of the delayed rectifier current can be made in this isolated single cell preparation.
Further evidence that this time- and voltage-dependent current is carried mainly by K+ was obtained from the experimental results summarized in Fig. 4, which shows the shifts in the reversal potential when [K+]o was increased from 2.5 to 10 and 25 mM. At each [K+]o, the $E_{rev}$ measurement was repeated a number of times in different cells ($n = 5-12$). The data are plotted as means ± SEM ($n = 5$), and a best-fitting straight line having a slope of 52 mV/10-fold change in [K+]o has been superimposed. These results provide very strong evidence that this time- and voltage-dependent, or delayed rectifier, current is selective for K+. Therefore, we have named it $I_K$.

After establishing that K+ is the major carrier of this outward current, we determined the shape of the steady state current-voltage relation so that a mathematical description of its ion transfer mechanism could be formulated. Following the semi-empirical approach developed by Hodgkin and Huxley (1952), the ion transfer properties were obtained from measurements of the fully activated current-voltage relationship. Fig. 5 illustrates three fully activated, quasi-instantaneous $I$-$V$ curves for $I_K$, obtained in 2.5, 5.0, and 10.0 mM [K+]o Ringer's. The data points between $-125$ and $-30$ mV on each curve were obtained using a protocol very similar to the one illustrated in Fig. 3. A very long $P_1$ depolarization to $+50$ mV was used to activate $I_K$ fully; immediately thereafter, a variable $P_{II}$ pulse clamped the membrane potential to the voltages indicated. The quasi-instantaneous (after the decay of the transient capacitative current) $I$-$V$ points were obtained from the initial amplitude of the tail current. The data points positive to $-30$ mV on the $I$-$V$ curves in Fig. 5 were obtained by direct measurement of the $I_K$ elicited by long $P_1$ pulses. In these experiments, series resistance compensation was used to reduce the micropipette resistance to $\sim 2$ MΩ and hence accurately record the membrane potential. The protocol was
repeated after \([K^+]_o\) had been changed. Fig. 5 shows data from an experiment in which three solution changes were completed.

These results indicate that the ion transfer properties of \(I_K\) can be accurately described as a linear conductance, and furthermore that this current is quite \(K^+\) selective. This fully activated, instantaneous \(I-V\) can be used to provide additional information regarding the ability to space-clamp this preparation under these conditions. The cells used in these experiments had input resistances (measured at the resting potential) of \(\sim 500 \, \Omega\). The resting DC space constant \((\lambda)\) is \(\sim 900 \, \mu m\) (Hume and Giles, 1981), or three to four times the cell length. The fully activated \(I-V\) curves in Fig. 5 have slope resistances between 40 and 80 \(\Omega\). The terminated short cable space constant, \(\lambda\) (which scales according to \(\sqrt{R_m}\)) is therefore \(\sim 280 \, \mu m\), or one cell length, which indicates that spatial voltage inhomogeneity will not give rise to significant artifacts, even when \(I_K\) is fully activated.

**The Steady State Voltage Dependence of \(I_K\)**

The data in Fig. 1 provide an indication of the range of transmembrane voltages that activate \(I_K\). The voltage dependence of this gating process must be accurately determined before the physiological role of \(I_K\) can be deduced. The data in Fig.
illustrate the steady state voltage dependence of $I_K$. These results were obtained by applying longer prepulses (~30 s) than those shown in Fig. 1 to ensure that the activation of $I_K$ had reached steady state values. The initial amplitude of each decay tail was measured as an indication of the amount of activation of $I_K$. The steady state activation value, $n_\infty$, was then calculated by normalizing and accounting for the second-power relationship of the exponential function describing the tail current (see Giles and Shibata, 1985).

\[ I(t) = n_\infty \left(1 + \exp\left(\frac{V - V_n}{K}\right)\right)^{-1/2} \]

**Figure 5.** Fully activated, quasi-instantaneous current-voltage relationships for $I_K$ in 2.5 and 10 mM $[K^+]_o$. Note that all three $I-V$ curves are approximately linear in the range -110 to +20 mV and that the zero-current level (reversal potential) shifts positive as $[K^+]_o$ is increased. The $E_{rev}$ values are -95, -80, and -65 mV in 2.5, 5.0, and 10 mM $[K^+]_o$, respectively. Details of the experimental protocols and the measurements are given in the text. The linearity of these $I-V$ curves suggest that the ion transfer characteristics of $I_K$ can adequately be described as an ohmic conductance.

\[ n_\infty = \left[ \frac{I_{K,\text{tail}}}{I_{K,\text{tail,max}}} \right]^{1/2}. \]

Fig. 6 shows data from six such experiments. A Boltzmann distribution has been fitted to the averaged data points. The equation for this curve is:

\[ n_\infty = \frac{1}{1 + \exp(V_n - V_m)/K}. \]

$V_n$ (where $n_\infty = 0.5$) is approximately -15 mV, and the slope factor ($K$) is 11.36 mV.
Ik Kinetics: the Envelope of Tails

An important assumption in the use of current tails for the determination of the amount of steady state activation, or for the measurement of the kinetics of relaxation of Ik, is that this current cannot instantaneously change its amplitude. One test of the assumption (Hodgkin and Huxley, 1952) requires measurement of the "envelope of the tail currents" so that the time course of this envelope can be compared with the time course of the onset of the current.

The left-hand columns of the two panels of data in Fig. 7 show envelope-of-tails tests done in bullfrog atrial cell from a holding potential of −70 mV. The depolarizing clamp steps were to +40 (top) and 0 mV (bottom) for the various durations (see figure legend). The current onsets and tails were digitized and then superimposed photographically. Tail amplitudes were obtained using the curve-fitting procedure developed by Provencher (1976). In ~80% of all trials (some 25 experiments), the current tails were best fitted by a single exponential; therefore, a single-exponential fit was adopted in all kinetic analyses. The initial values of each of these current tails were scaled to the amplitude of the onset current; the curve drawn through the onset data points is the scaled envelope of tails.

The right-hand columns of Fig. 7 show a comparison between the time course of current onset and that of the envelope of tails. The mathematically reconstructed current waveform and experimental data (circles) are superimposed. At the two potentials shown, the agreement between the time course of the current

![Graph showing the steady state activation curve for Ik. The data for this plot were obtained as described in the text and are plotted as means ± SEM (n = 6). The data points have been fitted with a Boltzmann distribution. The threshold for activation of Ik is near −40 mV and is fully activated at approximately +30 mV.](image-url)
onset and the time course of the envelope of tails is excellent. Note that from these data and from the results shown in Fig. 1, it is apparent that at relatively negative potentials within its activation range, this current is activated with a sigmoid time course. This sigmoid time course is also illustrated in the envelopes of tails (0 mV). It is therefore necessary to use a power function ($n = 2$) for the activation variable in mathematical descriptions of the time course of $I_K$ activation (Giles and Shibata, 1985).

**Figure 7.** An envelope-of-tails test of $I_K$. If the time and voltage dependence of $I_K$ is controlled entirely by the gating mechanism, then the time course of an appropriately scaled envelope of the current tails at any fixed potential should closely match the time course of the current onset itself. The data in A show 15 superimposed current records, each made by a depolarizing voltage-clamp step from the holding potential ($-70$ mV) to $+40$ mV for various durations: $0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.8, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0$, and $10$ s. Each digitized current record was fitted to a single-exponential function. The initial, or time zero, values thus obtained were then plotted along with the time course of the $I_K$ onset, as shown on the right. The squares denote the activation phase of the current and the circles are the initial amplitudes of the current tails. The curve drawn through the onset data is the same as that used to fit the envelope of current tails, after being scaled appropriately. It is apparent that the envelope of tails matches quantitatively the time course of the onset record itself. Panel B shows a similar experiment at a less depolarized activation potential, 0 mV. Note that in these data, the sigmoid activation of $I_K$ is apparent both in the onset record and in the envelope of tails.

A second, very important (but seldom tested) assumption in Hodgkin-Huxley analysis is that the ion transfer phenomena and the voltage-dependent gating mechanism of a channel are independent phenomena. To test whether this assumption is valid for $I_K$, we (a) carried out experiments in which the steady state activation curve, $n_m$, was obtained at a holding potential of $-15$ mV, i.e., where $I_K$ is activated substantially, and (b) measured $n_m$ in two different external $K^+$ concentrations (2.5 and 25 mM).
The $n_\infty$ curve obtained from a holding potential of $-15$ mV is shown in Fig. 8A (dotted line). For comparison, the "standard" $n_\infty$ curve (Fig. 6), obtained from a holding potential of $-70$ mV, is superimposed (solid line). There appears to be no significant difference. Fig. 8B shows an additional test of the independence of the gating and ion transfer phenomena controlling $I_K$. In this experiment, the $n_\infty$ values were obtained in 25 mM $[K^+]_o$ Ringer's (dashed line). The standard $n_\infty$ curve is shown as the solid line. Once again, the difference is very small, i.e., the time- and voltage-dependent gating does not depend strongly on the magnitude or direction of $I_K$. In combination, the envelope-of-tails tests (Fig. 7) and the two additional measurements shown in Fig. 8 provide strong evidence that $I_K$ obeys conventional Hodgkin-Huxley kinetics, and that its gating and ion transfer characteristics are independent.

After establishing that current tails are valid indicators of the magnitude and
kinetics of $I_K$, we sought to obtain additional data concerning the kinetics of activation and deactivation of $I_K$ at potentials within the physiological range. Protocols similar to those for measurement of the reversal potential were used. In the range of potentials negative to the activation threshold (approximately $-30$ mV), current tails were digitized and curve-fitted. Onset records of $I_K$ positive to $-30$ mV were also analyzed. Data from six complete experiments, plotted as the rate constant ($1/\tau_n$) vs. membrane potential ($E_m$), are shown in Fig. 9A.

Knowledge of the steady state activation of $I_K (n_n)$ and of the time constant of activation or decay at a variety of potentials can be used to obtain rate constants for the opening and closing of these $K^+$ channels. The mathematical expressions for obtaining this information (Hodgkin and Huxley, 1952) are given below, and the resulting rate constant curves are shown in Fig. 9B.

\[ n_n = \frac{\alpha_n}{\alpha_n + \beta_n}, \]

\[ \tau_n = \frac{1}{\alpha_n + \beta_n}. \]

The equations for the rate constant curves are:

\[ \alpha_n (s^{-1}) = \frac{0.0087(V_m + 26.46)}{1 - \exp[-0.128(V_m + 26.46)]}, \]

\[ \beta_n (s^{-1}) = 0.285 \exp[-0.0308(V_m + 26.46)]. \]

Thus, the kinetics of $I_K$ exhibit relatively steep dependence on voltage near the resting potential and also at the plateau of the action potential (see Discussion).
**Is $I_K$ Ca$^{2+}$ Dependent?**

The data in Figs. 1 and 7 indicate that $I_K$ is activated with a significant sigmoidicity or delay. This observation, combined with the finding that a transient inward Ca$^{2+}$ current, $I_{Ca}$, precedes the activation of $I_K$, suggests that $I_K$ may be triggered, or strongly modulated, by transmembrane calcium movements (cf. Kass and Tsien, 1975; Goto et al., 1982, 1983). This possibility has been tested by comparing the size and kinetics of activation of $I_K$ after selectively inhibiting transmembrane Ca movement with La$^{3+}$ (Nathan, R. D., K. Kanai, R. B. Clark, and W. Giles, manuscript in preparation). Representative data are shown in Fig. 10. The top panel illustrates control data; the bottom panel was obtained after blockade of $I_{Ca}$ with $10^{-5}$ M LaCl$_3$. In the absence of $I_{Ca}$, there was no change in either the magnitude or the time course of $I_K$. Therefore, transmembrane Ca$^{2+}$ influx does not trigger or modulate this delayed rectifier K$^+$ current. Moreover, it is rather unlikely that any Ca$^{2+}$-dependent or voltage-dependent release of Ca$^{2+}$ from intracellular sites triggers or modulates $I_K$. The frog heart contains very little sarcoplasmic reticulum (Page and Niedergerke, 1972), and the data of Fabiato (1983) have shown that during normal contraction-relaxation cycles, there is no significant Ca$^{2+}$-induced Ca$^{2+}$ release in frog heart.
DISCUSSION

Use of Bullfrog Atrium for Studies of the Delayed Rectifier $K^+$ Current

These experiments show that this enzymatically dispersed single cardiac cell provides a very suitable preparation for the quantitative study of time- and voltage-dependent delayed rectifier $K^+$ currents. This current can be studied without any significant pharmacological manipulation and it is very consistent in size and time course from cell to cell and from preparation to preparation. Although there are quite extensive infoldings or caveolae on the surface of these cells (Hume and Giles, 1981), there does not appear to be any significant accumulation or depletion of $K^+$ even during very large and maintained current changes. Furthermore, although the delayed rectifier $K^+$ current, when fully activated, is rather large (1–3 nA), it can be shown that the cell is still adequately space-clamped in the steady state, provided that appropriate series resistance compensation is employed. Since $I_K$ is relatively slow in this preparation, the settling time of the command pulse (500 μs) is unlikely to give rise to technical or analytical problems.

Ion Transfer Characteristics of $I_K$

The data in Figs. 1–5 show clearly that the delayed rectifier current in this preparation is carried mainly by $K^+$ and that its fully activated $I$-$V$ relationship is linear in the physiological range of potentials. We have therefore described this $I$-$V$ relationship as an ohmic conductance. In this respect, the delayed rectifier current appears to be very similar to that of squid axon (Hodgkin-Huxley, 1952) but different from that of the frog node of Ranvier (cf. Armstrong, 1978; Neumke, 1982; Dubois, 1983). Interestingly, in frog cardiac pacemaker tissue (Giles and Shibata, 1985) and in single pacemaker cells from mammalian heart (Nakayama et al., 1984), the fully activated $I$-$V$ relationship shows significant inward rectification at potentials positive to $-30$ mV.

It is difficult to meaningfully compare the fully activated $I$-$V$ relationship obtained from this single cell preparation with the $I$-$V$ relations, or the rectifier ratio relations, obtained from multicellular atrial trabeculae (Brown and Noble, 1969a, b; Brown et al., 1976a; Ojeda and Rougier, 1974). In these preparations, it is very likely that extracellular $K^+$ accumulation develops during protocols designed to measure the fully activated $I$-$V$ relation. The resulting positive shift of $E_K$ and crossover of the inwardly rectifying background $K^+$ current ($i_K$) $I$-$V$ relationships (cf. Kline and Morad, 1978; Cohen and Kline, 1982; Martin and Morad, 1982) give rise to quantitative errors in the analysis of these data from multicellular tissue.

It is of interest to estimate the number of delayed rectifier channels that may be present in this cardiac cell type. The slope conductance of the fully activated $I$-$V$ relationship is $\sim 2.0 \times 10^{-3}$ S. Knowledge of the single channel conductance for the elementary events underlying $I_K$ would therefore allow a determination of the number and density of these channels in the atrium, assuming that the applied depolarizations open all of the $I_K$ channels. Our initial attempts to identify transitions corresponding to single channel currents in this preparation have been unsuccessful. However, if one takes the single channel conductance of the
putative delayed rectifier in cultured chick heart cells (62 pS, Clapham and DeFelice, 1984) or assumes that the single channel conductance is similar to that in squid axon (20 pS, Neher and Conti) or frog node of Ranvier (20-60 pS, Conti et al., 1984; for review see Latorre and Miller, 1983), then the minimum number of delayed rectifier channels in an atrial cell varies between 400 and 600. These estimates yield a density of ~0.1-0.3/μm² of cell surface area. This extremely low density, and the possibility of nonuniform distribution of these channels (cf. Beam et al., 1985), are plausible explanations for our inability to identify these channels in patch-clamp recordings. It is relatively easy to record the single channel events that underlie either \( i_k \), the inwardly rectifying background K⁺ current, or \( i_{Na} \), the transient inward Na⁺ current. We estimate (Giles, Momose, and Szabo, unpublished data; Clark and Giles, 1986) that (assuming uniform distribution) the densities of these channels in atrial cells are ~0.2-1.0/μm² and 2/μm² for \( i_k \), and \( i_{Na} \), respectively.

**Kinetics of \( i_k \) in Frog Atrium**

Our data consistently show that the decay of the macroscopic \( i_k \) records is well described by single-exponential kinetics, and that the time course of the envelope of tails very closely matches that of the onset record itself. These findings strongly suggest that only a single Hodgkin-Huxley conductance underlies the macroscopic \( i_k \) records and that its kinetics can be described mathematically by a first-order reaction. Furthermore, the data in Figs. 7-9 provide evidence that the gating process that controls the kinetics of \( i_k \) is functionally independent of the ion transfer process. It is therefore possible to obtain a quantitative description of the kinetics of \( i_k \) as a function of membrane potential (Fig. 9). A conventional U-shaped \( 1/\tau_n \) was observed.

In a variety of excitable tissues (for review see Dubois, 1983; Neumke, 1982; Conti et al., 1984), it has been reported that the kinetics of the delayed rectifier currents are better described by a sum of two exponential processes than by a single exponential. Very recently, this has also been suggested from measurements of the delayed rectifier in cardiac Purkinje fibers and in isolated Purkinje cells (Cohen et al., 1984; Ginant et al., 1985; Bennett et al., 1985). For these reasons, we attempted to study the kinetics of \( i_k \) in detail. Our analysis consisted of fitting each of the current tails to the sum of one, two, three, or four exponentials, and then using statistical criteria to determine the best fit (cf. Lanczos, 1957; Provencher, 1976). As noted earlier, this analysis consistently indicated that the kinetics of \( i_k \) tail currents were best described by a single exponential. Further insight into the details of the voltage-dependent gating of \( i_k \) may be obtained if the kinetics of the relevant gating current could be recorded and compared directly with the kinetics of the ionic current (Bezanilla, 1985; White and Bezanilla, 1985).

**The Physiological Role of \( i_k \)**

Knowledge of the reversal potential, the fully activated \( I-V \) relationship, and the kinetics of \( i_k \) makes it possible to determine the physiological role of this current in atrial cells. \( i_k \) is activated by depolarizations positive to approximately ~40
mV and hence will be turned on during the plateau of the action potential. Since the plateau of the action potential in bullfrog atrium remains positive to 0 mV for 400–500 ms, and the time constant for $I_K$ activation in this range of potentials is $\sim 1$ s, a substantial $I_K$ will be activated during each action potential. This outward current will initiate repolarization, and will then decay with a time constant of $\sim 500$ ms as the membrane potential repolarizes back to rest ($-90$ mV). $I_K$ may also be important in certain frequency-dependent changes in action potential duration. For example, in multicellular preparations and in single cells, the application of paired stimuli at various interstimulus intervals shows that there is a substantial shortening of the second action potential whenever the two stimuli occur within 400 ms (cf. D. Noble and Tsien, 1972; Carmeliet, 1977). An important contributing factor in the shortening of the second action potential is the residual activation of $I_K$.

Thus, a major determinant of the initiation of repolarization is the time- and voltage-dependent activation of $I_K$; in principle, this is similar to the repolarization process in squid axon. Our previous data (Hume and Giles, 1983), the present results, and additional computer simulations (Rasmusson, R., R. B. Clark, W. Giles, K. Robinson, D. T. Campbell, and E. F. Shibata, manuscript in preparation) indicate that the previous suggestion that the inactivation of $I_{Ca}$ initiates repolarization in frog atrium (Horackova and Vassort, 1976) is incorrect. However, it is important to note that although $I_K$ initiates repolarization, $I_{Kw}$, the inwardly rectifying background K+ current, is very important in controlling the final one-third ($-60$ to $-90$ mV) of repolarization (Hume and Giles, 1983; Giles et al., 1984). Although $I_K$ generates very little current (<10 pA) positive to 0 mV, its size is significant (~50 pA) at $-70$ mV.

These direct measurements of the K+ currents that control repolarization also provide further insights into the work of Goldman and Morad (1977a, b). Their data indicated that in ventricular trabeculae from the frog, (a) the conductance at the plateau of the action potential was approximately one-third that near the resting potential, and (b) only a very small (~10%) increase in conductance accompanied the initiation of repolarization. The first result can be explained by the strong inward rectification of $I_{Kw}$, and the second finding arises directly from the significant (but still relatively small) activation of $I_K$. The rate of repolarization ($-dV/dt$) is only $\sim 0.1$–$0.3$ V/s. Therefore, in a space-clamped atrial cell having a total capacitance of $\sim 100$ pF, a net $I_K$ of only 10–30 pA is required to generate the repolarization of the action potential. However, two additional aspects of the Goldman and Morad (1977b) work are not supported by our findings: (a) the finding that the instantaneous I-V during the plateau is linear, and (b) the suggestion that a carrier, as opposed to a conductance mechanism, may generate the repolarizing current.

The data presented in this article indicate that in this single cell preparation, no significant accumulation of K+ occurs as a consequence of the activation of $I_K$. However, this may not be the case in the intact frog heart or in isolated multicellular trabeculae. It will therefore be important to assess the extent to which K+ accumulation occurs physiologically. This can be done in mathematical simulations using the current densities measured in this study in combination
with microanatomical measurements of the sizes and distributions of the extracellular spaces in frog atrium or ventricle (Page and Niedergerke, 1972; Meyer et al., 1982; cf. Morad, 1980; Cohen and Kline, 1982; Tung and Morad, 1985; Rasmusson et al., manuscript in preparation).

The possibility that a single action potential or an action potential train may produce \( [K^+]_o \) accumulation raises the question, Does the Na\(^+\)/K\(^+\) pump contribute to the repolarization process? This is possible since the pump mechanism is electrogenic and its activity can be modulated by \( [K^+]_o \) (cf. Gadsby, 1984), and a current generated by the electrogenic Na\(^+\)/K\(^+\) pump has been identified in isolated atrial cells (Shibata et al., 1984). Our results suggest this current \( (i_p) \) arising from the pump is not importantly involved in repolarization. This \( i_p \) is (a) much smaller than \( I_K \) and (b) activated by \( [K^+]_o \) with a \( K_v \) of 1.5 mM; hence, small increases in \( [K^+]_o \) from 2.5 mM in normal Ringer's will not produce much additional current. The contribution of \( i_p \) to repolarization is therefore likely to be minimal unless this pump mechanism exhibits substantial voltage dependence (Chapman et al., 1983). Recent data from single cardiac cells indicate that in the physiological range of potentials this is not the case (Gadsby et al., 1985).

**Relationship to Previous Findings**

A review of the published data on the steady state voltage dependence, kinetics, and reversal potentials of the slow time- and voltage-dependent outward currents in frog atrial trabeculae indicates that, in general, this earlier work suggested that there were two distinct outward currents, neither of which was very selective for K\(^+\) (for review, see Carmeliet and Vereecke, 1979). Theoretical treatments of the effects of restricted K\(^+\) diffusion in small extracellular spaces, and the likelihood of spatial nonuniformity in these double sucrose gap voltage-clamp experiments, have indicated that both the differences in these earlier results and the discrepancies between many of them and those from this study may arise from these technical limitations (Attwell and Cohen, 1977; Attwell et al., 1979). However, experimental and theoretical work has been published which indicates that spatial nonuniformity is not a significant problem in voltage-clamping the K\(^+\) currents in frog atrial trabeculae (Brown et al., 1976b). Although multipexponential current changes were recorded consistently in most previous double sucrose gap experiments, one of the conductance components that was described by Brown et al. (1976a), de Hemptinne (1971a, b), and Ojeda and Rougier (1974) is quite similar to the \( I_K \) identified in this study. It is therefore likely that the double- or triple-exponential splits used in the analysis of these data provided a fairly reliable qualitative estimate of the steady state voltage dependence of the actual K\(^+\) conductance, \( I_K \). These studies on multicellular preparations may also have provided a reasonable estimate of the size and kinetics of the accumulation component. Unfortunately, however, they gave unreliable estimates of the selectivity of the channel and were unable to provide any quantitative data concerning the nature of the ion transfer mechanism.

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