K CHANNEL KINETICS DURING THE SPONTANEOUS HEART BEAT IN EMBRYONIC CHICK VENTRICLE CELLS

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ABSTRACT By averaging the current that passes through cell-attached patches on beating heart cells, while measuring action potentials with a whole-cell electrode, we were able to study K channels during beating. In 7-d chick ventricle in 1.3 mM K physiological solutions at room temperature, delayed-rectifier channels have three linear conductance states: 60, 30, and 15 pS. The 60 and 15 pS conductances can exist alone, but all three states may appear in the same patch as interconverting conductance levels. The delayed-rectifier conductance states have low densities (less than 10 channels per 10-μm diam cell), and all have a reversal potential near -75 mV and the same average kinetics. Outward K current through delayed-rectifier channels follows the upstroke without appreciable delay and lasts throughout the action potential. No inward current flows through delayed-rectifier channels during beating. The early outward channel has a nonlinear conductance of 18–9 pS depending on the potential. It also turns on immediately after the upstroke of the action potential and lasts on average only 50 ms. The early outward channel has an extrapolated reversal potential near -30 mV; no inward current flows during beating. The inward-rectifier has an extrapolated conductance and reversal potential of 2–3 pS and -80 mV in 1.3 mM K. Channel kinetics are independent of external K between 10 and 120 mM, and the channel conducts current only during the late repolarization and diastolic phases of the action potential. No outward current flows through inward-rectifier channels during beating. This work parallels a previous study of Na channels using similar techniques (Mazzanti, M., and L.J. DeFelice. 1987. *Biophys. J.* 52:95–100).

INTRODUCTION

To explain excitability in axons, Hodgkin and Huxley (1952) proposed that nerve membranes conduct Na and K ions through separate time-variant, voltage-dependent pathways. Most investigators now identify these macroscopic conductances with two uniform populations of membrane-spanning proteins, each forming an ion-selective channel through the bilayer. Nearly all excitable cells express Na channels with essentially the same characteristics as those inferred from Hodgkin and Huxley's model (Hille, 1984). In particular, embryonic chick ventricle cells contain pores that are virtually identical to Na channels in nerve axons (Mazzanti and DeFelice 1987*b*; Fujii et al, 1988). This resemblance between nerve and heart breaks down for the K current. Typically, a cardiac cell will incorporate five or more different kinds of K-conducting channels into its membrane, none of which compares closely to the original K current in the Hodgkin-Huxley model.

A consequence of this complexity in the K conductance is variety in the shape of cardiac action potentials. Heart cells can generate waveforms as diverse as pacemaker potentials and Purkinje fiber action potentials. The myriad patterns of cardiac excitability arise not only from K channels, however, but also from Ca channels, ligand-gated channels, electrogenic pumps, and the absolute and relative number of these pores coexisting in the membrane.

One of the earliest reconstructions of a cardiac action potential from underlying currents is the McAllister, Noble, and Tsien model of the Purkinje fiber (McAllister et al., 1975). The MNT model and all of the subsequent reconstructions contain several distinct pathways for K ions. Examples include models for adult ventricle cells (Beeler and Reuter, 1977; McDonald and Trautwein, 1978), the sinoatrial node (Yanagihara et al., 1980), pacemaker cells (DiFrancesco and Noble, 1985), and embryonic chick ventricle (Clay and Shrier, 1981; Shrier and Clay, 1987). Collectively, the outward K currents in these models balance the inward flow of Na and Ca ions. Since cardiac action potentials last hundreds of times longer than nerve action potentials, one of the striking contrasts between the two tissues is the relatively slow kinetics of the individual K currents in heart compared with those in the nerve (Hume and Giles, 1983; Kass, 1984; Giles and Shibata, 1985; Bennett et al., 1985; Simmons et al., 1986; Bennett et al., 1987).

In the traditional analysis of cardiac excitability, it is unclear whether the currents in the models correspond to individual classes of ion channels in the membranes. Noble (1984) has taken the position that the pathways found in macroscopic experiments represent individual classes of

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ion channels. Rather than providing the synthesis that many had hoped for, he argues that single-channel data from individual cells tend to support the complexity indicated by the earlier two-microelectrode, voltage-clamp experiments in multicellular preparations. In their reviews of cardiac excitability, Reuter (1984), Cohen et al. (1986), and Clapham (1988) address this issue by focusing attention on single-channel data, but the scarcity of such information and the variety of cells and conditions from which these data come frustrate any attempt to generalize, with the result that the K conductance in cardiac membranes is among the least understood of the ionic pathways.

To help clarify the relationship between macroscopic K conductances and individual K channels, we have studied a family of K channels in a single category of heart cell under uniform physiological conditions. Standard voltage-clamp studies of the K system already exist for this tissue (Clay and Shrier, 1981; Shrier and Clay, 1987; Clay et al., 1988), and the individual cells are under intensive whole-cell and patch-clamp investigation (Clapham and DeFelice, 1984; Fischmeister et al., 1984; Levi and DeFelice, 1986; Mazzanti and DeFelice, 1987*a,b*; Clapham and Logothetis, 1988). This article characterizes three kinds of K-conducting channels and investigates the average current through these channels during spontaneous beating. By comparing channel kinetics with action potentials, we have associated these classes with the macroscopic delayed-rectifier, the inward-rectifier, and the early outward current of the cardiac cell membrane. Preliminary reports of this work have appeared in Mazzanti et al. (1987) and DeFelice et al. (1988).

METHODS

Preparation

Embryonic chick ventricle cells were prepared by enzymatic digestion of 7-d chick hearts following the procedure of DeHaan (1967; Fujii et al., 1988). After 12–24 h in tissue culture medium and immediately before the experiments, the cells were washed with bath solution at room temperature. The composition of the bath (in mM) was 1.3 K, 130 Na, 1.5 Ca, 1.5 Mg, 1 SO_4, 133.5 Cl, 5 dextrose, 10 Hepes (adjusted to pH 7.35). The cells are 10 μm in diameter, and approximately half of them beat spontaneously. Only single cells or small clusters of two or three cells were selected for study.

Solutions and Electrodes

The cell-attached electrode contained one of the following proportions of K to Na, depending on the experiment: 121 mM K/10 mM Na (called 120 K), 61.3/70 (60 K), etc., to 11.3/120 (10 K). The other components in the cell-attached electrode were the same as those in the bath solution. The whole-cell electrode contained an intracellular-like solution consisting of 120 K, 0.1 Ca, 2 Mg, 122.1 Cl, 1.1 EGTA, 10 Hepes (adjusted to pH 7.4). The patch electrodes were made from a hard borosilicate glass (No. 7052; Corning Glass Works, Corning, NJ) using a programmable puller (Sachs-Flaming PC-84, Sutter Instruments, San Rafael, Ca.). The pipettes were coated with Sylgard (Dow Corning Corp., Midland, MI) as near to the tip as possible and fire-polished to an external tip diameter of ~1 μm just before use. These electrodes had resistances of 4–10 MΩ when filled with one of the standard patch electrode solutions and dipped into the bath. The estimated surface area of the patch formed with these electrodes is between 5 and 7 μm^2 by geometric and by capacitive measurements (Kell and DeFelice, 1988; Mazzanti and DeFelice, 1987*b*).

The Dual Recording Technique

The method is described in detail in Mazzanti and DeFelice (1987*a,b*). Previously, we used one or two electrodes to record current and voltage, breaking the patch at the end of the one-electrode experiments to obtain the cell potential. That method has the advantage of not perfusing the cell during the measurements, but the voltage and current are not simultaneous. The present experiments use two electrodes exclusively. The tips of the current and voltage electrodes were usually 5–20 μm apart on the surface of the cell. We used List EPC5 and EPC7 amplifiers (Medical Systems, Greenvale, NY) to measure the voltage and the current. The data were stored on a Panasonic VCR and analyzed later using a model 4094 oscilloscope (Nicolet Instrument Corp., Madison, WI) and software developed for an IBM AT by William Goolsby. All data were filtered at 1,000 Hz before analysis.

Single-Channel Analysis

We first screened the records for patches that contained only one kind of channel, i.e., no multiple openings occurred, or if they did occur each had the same conductance and reversal potential. After selecting a patch for study, we recorded up to 100 action potentials and action currents simultaneously. We eliminated the background current from each trace by subtracting the average of blank traces, i.e., action currents in which by chance no openings occurred. The ensemble average of background-corrected traces is a miniature version of the action current through the class of channels represented the member of that class in the patch. Alternatively, we plotted individual action currents against the action potential to give instantaneous, open-channel $i(V)$ curves. Fitting a straight line by eye to the open-channel current gave us an estimate of single-channel conductance and reversal potential. Lines through the center of the open-channel noise or through the extremes of the open-channel noise differed by as much as 20%, as discussed in Levi and DeFelice (1984). Here we used values from lines drawn through the center and rounded off the conductance and the reversal potential to the nearest multiple of five, e.g., 13.5 to 15 pS and -73 to -75 mV. This method works only for channels that open throughout the action potential, e.g., the channels we identify as the delayed-rectifier and the inward-rectifier. The channel specified as the early outward channel required a different method (see below under Results).

RESULTS

Three characteristically different channels conduct K during the cardiac action potential in 7 + 1 d embryonic chick ventricle. Two of these operate mainly during the plateau phase (the delayed-rectifier and early outward channels), while the third functions primarily in the diastolic phase of the action potential (the inward-rectifier). Delayed-rectifier and early outward channels appear in only one out of fifty patches and seldom last long enough to calculate an average current. When present, however, they are large and easy to observe in 1.3 mM external K. The reverse is true for the inward-rectifier; virtually every patch contains at least one channel, but in 1.3 K the background noise at 1,000 Hz exceeds the current through these channels. Since inward-rectifiers have a large conductance in high external K, steadily lowering the concentration from 120

mM to 10 mM K in separate experiments allowed us to identify these channels in nearly normal levels of external potassium.

In the Results section, we use delayed-rectifier, early outward, and inward-rectifier to indicate channels having the same approximate conductance, reversal potential, and expected action current as channels by these names already described in the literature (see Discussion for further justification).

The Delayed-Rectifier

Fig. 1 is from a two-electrode experiment in which the tips of a cell-attached and a whole-cell electrode are ~20 μm apart on the cell surface. The top panel plots simultaneously the cell action potential and the patch action current after capacitive and leak subtraction (see the Methods and Mazzanti and DeFelice, 1987*b* for details of the background subtraction). The patch current includes one channel tentatively identified as the delayed-rectifier channel. The whole-cell electrode contains an intracellular-like solution (120 mM K), and the cell-attached electrode contains bath solution (1.3 mM K). The channel conducts an outward current during the plateau phase. The bottom panel plots $i(t)$ against $V(t)$, giving an open-channel conductance of 15 pS and an extrapolated reversal potential of near −75 mV.

Besides the 15 pS delayed-rectifier channel shown in Fig. 1, two other delayed-rectifier conductances may appear during the plateau phase (Fig 2). One has a value of 60 pS (Fig. 2 *A*), and the second has a conductance of 30 pS (Fig. 2 *B*). The currents through all three of these conductances reverse at −75 mV, and all have the same average current (Fig 3). In the particular experiment shown in Fig. 2, the 60-pS conductance dropped in abrupt, nonreversing steps to 30 and then to 15 pS. The action potentials shown to the left of the sample current traces remained constant throughout the experiment.

The 60- and 30-pS conductances could be a superposition of 15-pS channels, but the following observations argue against this explanation: (*a*) the 60-, 30-, and 15-pS states often persisted as unique values of open channel conductance for several minutes, as in the experiment of Fig. 2; (*b*) the 45-pS state never appeared in any experi-

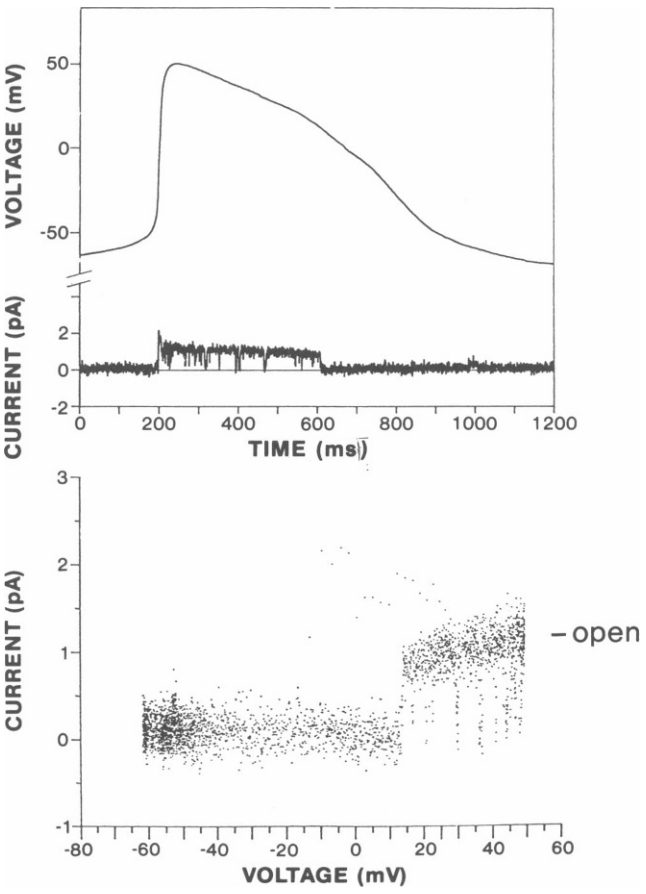

FIGURE 1 The 15-pS delayed-rectifier channel in 1.3 mM K. (*Top*) An action potential and a simultaneous action current after subtracting the background current. (*Bottom*) The instantaneous $i(V)$ curve obtained by plotting the action potential against the action current. A straight line fit by eye through the points of the open state has a slope of 15 pS and extrapolates to a reversal potential of −75 mV.

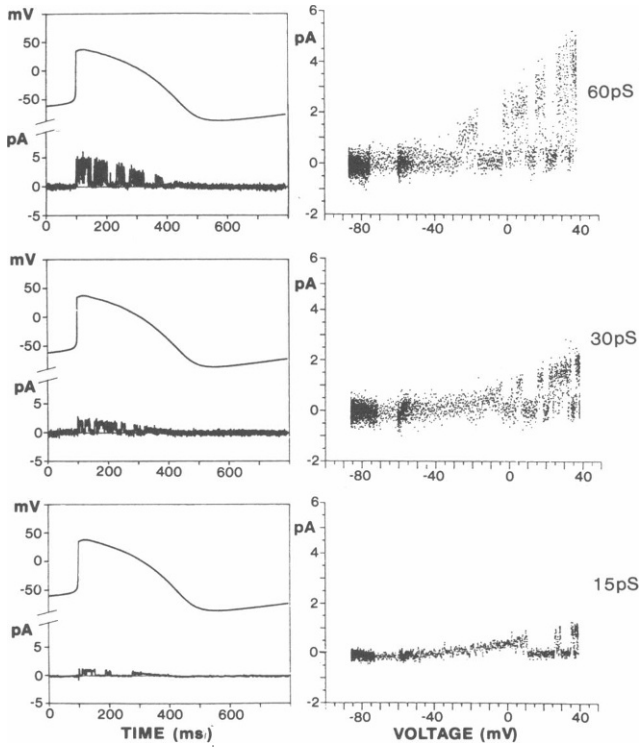

FIGURE 2 Three delayed-rectifier conductance states in 1.3 mM K. (*Left column*) Simultaneous action potentials and action currents from the three states (after subtracting the capacitive transient and leak current). (*Right column*) The corresponding $i(V)$ relations. The data are from the same patch and are in temporal order from top to bottom. During this experiment, the delayed-rectifier channel changed its conductance from 60 to 30 to 15 pS in abrupt, spontaneous steps. Each of the three states was stable for several minutes. By extrapolation, the reversal potential for each conductance is −75 mV.

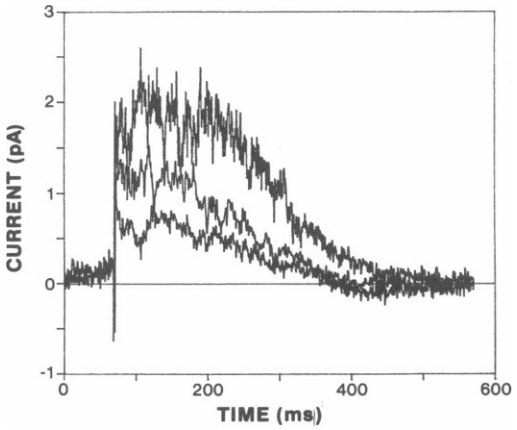

FIGURE 3 The average currents through the 60, 30, and 15 pS delayed-rectifier conductances in Fig. 2. The figure is the result of averaging 20 action currents from each of the three states. The averages are aligned at the upstroke of the respective potentials, which occur at the sharp downward deflection in the currents, an artifact of the capacitive subtraction procedure.

ment; and (*c*) in some patches, reversible transitions occurred between the 15-, 30-, and 60-pS delayed-rectifier conductances. Records containing reversible transitions of the three conductance states during action potentials are too complex to analyze; qualitatively, simultaneous openings and closings occur too often to be independent events.

In the present study, the 60-pS state occurred as a unique conductance in six patches, and 15-pS conductance occurred in four patches. The 30-pS state appeared in only two experiments, and then exclusively as a quasi-stable transition between the 15- and the 60-pS levels. During the experiment in Fig. 2, each of the three conductance states lasted for several minutes, long enough to obtain an average. Using 20 sequential traces for each conductance, Fig. 3 plots the mean delayed-rectifier currents for the 60-, 30-, and 15-pS states. The currents are virtually the same for all three conductances, and the average amplitudes are in the ratio of 4:2:1. Average currents from patches containing unique delayed-rectifier conductances of 60 and 15 pS match those in Fig. 3 except for a scaling factor.

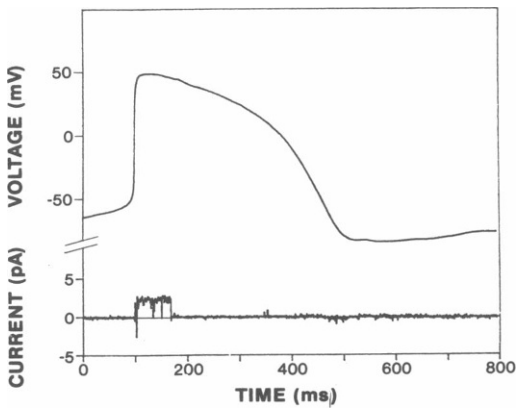

FIGURE 4 The early outward channel in 1.3 mM K. The figure shows an action potential and a simultaneous action current after subtracting the background current. This opening is atypically long for the early outward channel; the average action currents is shown in Fig. 6.

The Early Outward Channel

Fig. 4 is a plot of the action potential and a coincident opening of an early outward channel in a patch containing 1.3 mM K. For clarity, the figure shows an unusually long channel opening, but even prolonged openings conduct too briefly to estimate open-channel conductance as we did in Fig. 1. However, by either changing the pipette potential during beating or by selecting action potentials with different plateau voltages, we were able to obtain two separate estimates of channel conductance. Fig. 5 shows

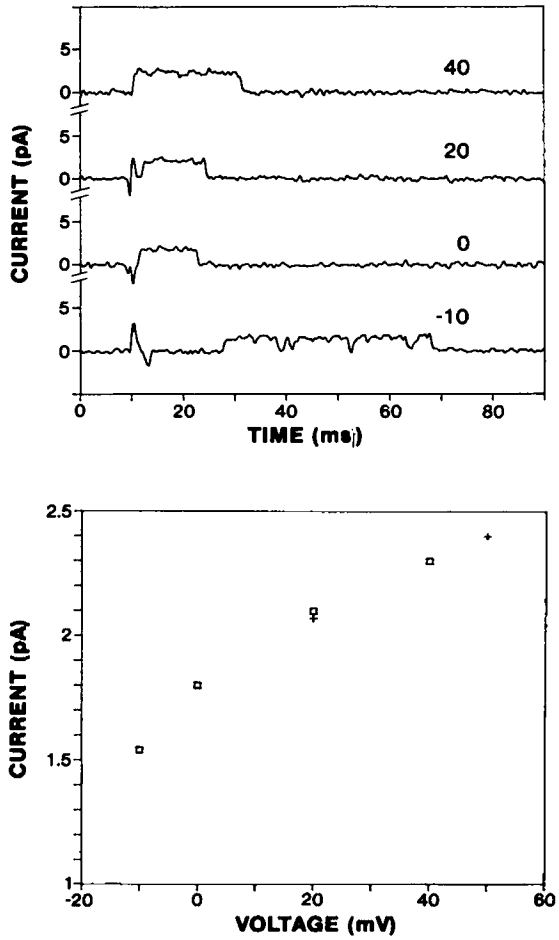

FIGURE 5 The early outward channel conductance in 1.3 mM K. (*Top*) Four action currents (after background subtraction) taken from the same patch at different pipette voltages. The absolute membrane potential (above each trace, in millivolts) takes into account the voltage of the action potential when the channels were open. The $i(V)$ plot uses the average potentials and average open channel currents. (*Bottom*) The open-channel $i(V)$ relation for the early outward channel from data in the top panel (*squares*) and from two other patches containing early outward channels (*crosses*). For the latter experiments, the channels opened during action potentials that had 20 and 50 mV overshoot potentials (the pipette potential was held at zero).

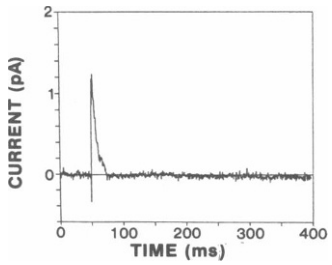

FIGURE 6 The average currents of the early outward channel during action potentials. The figure was constructed from 20 action currents by the same method used for Fig. 3.

selected openings from a cell with a constant plateau 40 mV but with varying patch potentials obtained by offsetting the pipette voltage. The bottom panel plots the open-channel current (*squares*) against the membrane potential. Fig. 5 also plots currents (*crosses*) from cells with plateau voltages of 20 and 50 mV in which we held the tip potential at 0 mV. From the $i(V)$ curve in Fig. 5, the early outward channel rectifies at positive voltages, with a slope conductance of 18 pS at 0 mV and 9 pS at 40 mV. By extrapolation, the potential at which the current would reverse is ~ -30 mV.

Fig. 6 shows the average current through the early outward channel during beating by averaging 20 beats with channel openings and subtracting the average of selected beats without openings. The early outward current comes on immediately at the upstroke and is over in 50 ms. In some experiments, evidence exists for a late component of the early outward current, whose presence may depend on the rate of beat and the action potential upstroke velocity. Late openings were rare and difficult to investigate.

The Inward-Rectifier

Fig. 7 is a recording from a patch containing one inward-rectifier channel. The cell-attached electrode contained 120 mM K. The channel conducts no current during the plateau phase, and it conducts inward current only during the repolarization and diastolic phases of the action potential. Fig. 8 illustrates how the channel behaves in various concentrations of external K. From top to bottom, the K in the patch electrode in the separate experiments represented here was 120, 60, 20, and 10 mM. To show the beat rate, these plots, unlike the previous ones, contain an unsubtracted capacitive transient. The interbeat intervals are different in each experiment, and in *D*, the lowest K concentration, the next beat is just off scale. At each concentration, the channel conducts current primarily during the diastolic phase. The lower the external K, the smaller the inward current. To study the channel in 10 mM K, it was necessary to select cells with slow beat rates since the diastolic potentials reach more negative potentials in such cells, giving rise to larger inward currents.

Fig. 9 displays the open-channel $i(V)$ curves for the

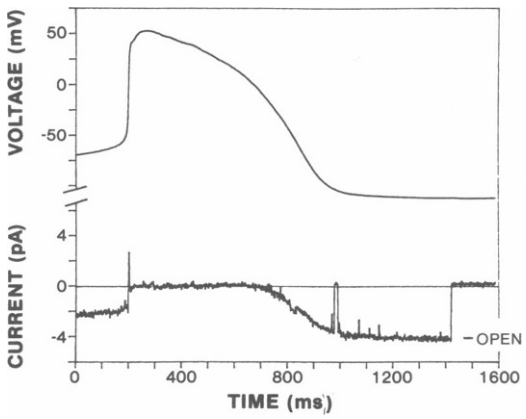

FIGURE 7 The inward-rectifier channel in 120 mM K. The top trace is an action potential and the bottom trace is a simultaneous action current after subtracting the background current. The channel carries no current during the plateau phase of the action potential. It conducts inward current during the repolarization phase and diastolic depolarization phase of the action potential.

inward-rectifier for each concentration in Fig. 8. The slope of the left limb of each graph in Fig. 8 is the inward conductance of the channel, and the voltage at which that line extrapolates to zero current is the reversal potential, though there is no conspicuous reversal of the current. Fig. 10 shows the inward conductance (*top*) and the reversal potential (*bottom*) of the inward-rectifier channel plotted against the concentration of K in the patch electrode. In

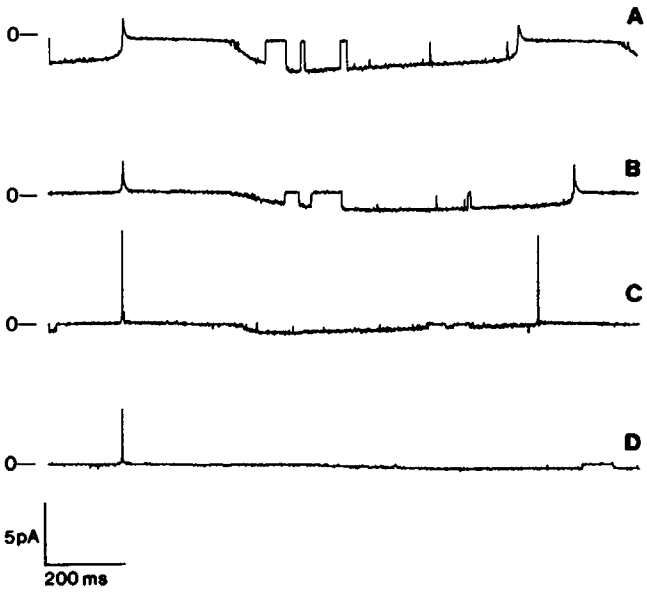

FIGURE 8 The conductance and the kinetics of the current through inward-rectifier channels in various concentrations of external K. From top to bottom, the patch electrode contained (in four separate experiments) 120, 60, 20, and 10 mM K. The sharp upward spikes in each trace are capacitive currents due to the upstroke of individual action potentials; *A–C* show a second action potential, which in *D* is just off scale. At all K concentrations, the inward-rectifier channel conducts current only during the repolarization and diastolic depolarization phases of the action potential. There is no outward current.

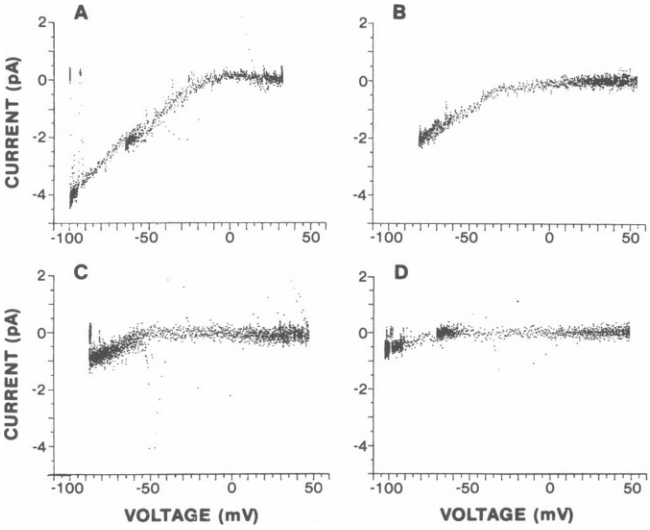

FIGURE 9 The $i(V)$ curves of the inward-rectifier in 120 (A), 60 (B), 20 (C), and 10 (D) mM K (from the data in Fig. 8).

1.3–2.5 mM K, the inward conductance extrapolates to ~2–3 pS, and the reversal potential of the channel would be close to −80 mV.

Fig. 11 shows the average current kinetics of inward-rectifier channels during action potentials. The patch electrode in these two separate experiments contained 20 and 60 mM K, but the action potentials of the cells were virtually identical, which allowed us to compare the time course of the current at the two concentrations. These cells also had approximately the same interbeat intervals (700 ± 41 ms and 820 ± 330 ms). Increasing the amplitude of the 20 K curve and shifting it to the left (to account for the lower conductance and more negative reversal potential of the channel in 20 K compared with 60 K) results in a near superposition of the two traces in Fig. 11. This correspondence indicates that the average kinetics of the inward-rectifier channel are the same in 20 mM and 60 mM external K.

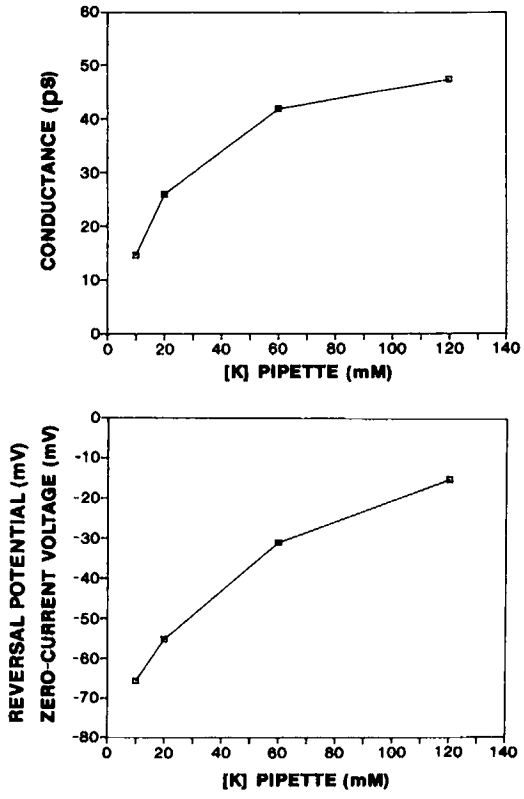

FIGURE 10 Summary of the inward conductance and the reversal potential of the inward-rectifier channel as a function of external K (from the data in Fig. 9).

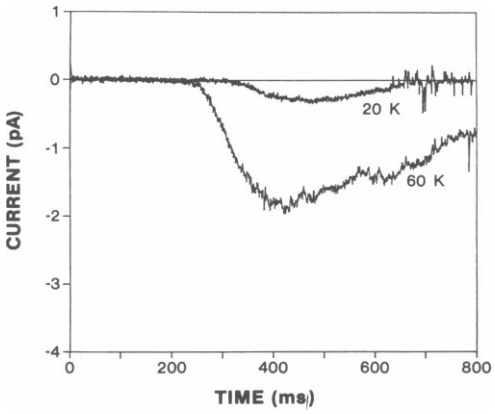

FIGURE 11 The average currents of the inward-rectifier in 20 and 60 mM external K during action potentials. The figure is constructed by the same method used in Fig. 3. The upstrokes of the two virtually identical action potentials (separate experiments) begin at $t = 0$.

DISCUSSION

The names of channels in this article are the common ones associated with macroscopic K currents; however, no strict identification of channel types with aggregate currents is possible. We base our nomenclature on the occurrence of openings during action potentials, using the traditional roles of the macroscopic cardiac currents as a guide to naming the channels. Identification and nomenclature is therefore provisional and will ultimately require quantitative modeling for exact correspondence. Other terms such as "pacemaker current" and other channels like the unitary event referred to as I_f (DiFrancesco, 1986; DiFrancesco et al., 1986) do not appear in the present work. If pacemaker channels or other K-conducting pores exist in our tissue (transient-inward channels, Na-activated channels, ATP-depletion channels, or Ca-activated channels), they are of extremely low density or, perhaps, these apparently diverse channels are redundant with a smaller set of native pores that operate when the concentration of ions and metabolites are naturally varying.

The ATP-depletion channel, originally seen in guinea-pig and rabbit heart cells superfused with cyanide (Noma, 1983), has a conductance of 65 pS in 140/140 mM K, which is comparable to our conductance of 60 pS in

1.3/140 mM K. We do not believe that our channel is the ATP-depletion channel because (*a*) the ATP-depletion channel in chick heart is 90 pS (Clapham, D. E., and Y. Kurachi, personal communication), (*b*) the kinetics of the current through the 60- and 15-pS channels are identical, and (*c*) the 15- and 60-pS channels are seen in cell-attached patches in beating cells, where internal ATP should be normal and well above the 0.2 mM Noma reports as a critical value.

None of the K-conducting pores that we have investigated in beating cells appears to reverse its current. Delayed-rectifier channels and early outward channels conduct only outward current, and the inward-rectifier channels conduct only inward current. However, we cannot exclude the possibility that small, unresolved currents flow in reverse at some time during the beat. In describing our data, we have used the term "reversal potential" to mean the voltage at which the current would change its sign if we extrapolated the $i(V)$ curve. In voltage clamp or in some other abnormal condition, all three classes of the K-conducting channels we have studied are capable of reversing their current.

The Delayed-Rectifier

Conti and Neher (1980) first measured the unitary conductance of the K channel in squid giant axons, a pore that now carries the retronym of delayed-rectifier. Extrapolating from a value of 17.5 pS measured in nonphysiological conditions, Conti and Neher concluded that the conductance of the neuronal delayed-rectifier channel is 9–11 pS, in agreement with noise measurements from the same preparation and a microscopic interpretation of the Hodgkin and Huxley model (Conti et al., 1975). Clapham and DeFelice (1984) described a 60-pS K channel in embryonic chick ventricle cells whose kinetics were suggestive of the I_x current (McDonald and Trautwein, 1978; DiFrancesco et al., 1979; Clay and Shrier, 1981; Kass, 1984), and Levi and DeFelice (1984) and Fischmeister et al. (1984) found the same channel in beating heart cells, showing that it contributed to the plateau current and to the repolarization of the action potential, the traditional role of the delayed-rectifier in heart. Thus it appeared that delayed-rectifier channels in cardiac cells had four times the conductance of the analogous channels in nerve cells. At about this same time, however, Conti et al. (1984) found that in the frog node an analysis of the current noise suggested that delayed rectification is sustained by two interconverting conductances, one with a conductance of 13 pS and the other with 60 pS.

We now realize that the lower conductance state also exists in heart. Shibasaki (1987) and Clapham and Logothetis (1988) have both characterized a delayed-rectifier channel whose conductance in the rabbit is 11 pS and in embryonic chick is 15 pS. The discrepancy in conductance may be due to the conditions of the two experiments: Shibasaki measured the conductance in 150 mM external K when the channel was carrying an inward current; Clapham and Logothetis used 1.3 to 4.5 mM K and measured the conductance under the normal condition of an outward current. The 15-pS channel studied by Clapham and Logothetis and the 60-pS channel in the previously cited studies in heart were both observed as unique conductances. In the present study we have also seen both conductances as exclusive states, but our experiments suggest in addition that these two conductance states are actually ancillary configurations of a basic 15-pS pore that can double and double again to form either a 30- or 60-pS channel, a conclusion that is supported by the observation that the reversal potential and the kinetics of all three of these delayed-rectifier conductance states are virtually identical.

The total internal K in 7 + 1 d embryonic ventricle cells is 138 mM (McDonald and DeHaan, 1973), and the free K is 83 mM (Fozzard and Sheu, 1980). Assuming a value of 80 mM K inside the cells, the Nernst potential in 1.3 mM K at room temperature is −96 mV. The reversal potential of the delayed-rectifier conductances during action potentials is −75 mV. This low value could mean that the channel is not perfectly K selective. In other tissues, however, the cardiac delayed-rectifier is highly K selective (57 mV per 10-fold change in external K in frog atrium at 20°C; Simmons et al., 1986). Clapham and Logothetis (1988) found a similarly high K discrimination for delayed-rectifier channels in a preparation close to ours. Thus, our results suggest that the reversal potential of −75 mV reflects, at least in part, a concentration of K ions in beating cells lower than the 80 mM we assumed in the above calculation.

The 15- and 60-pS conductances have extremely low densities. A delayed-rectifier channel appears in only one out of 40 patches. Assuming a lower estimate of the patch area of 1 μm^2, this frequency of observation would imply that there are less than 10 channels per cell, each cell having a surface area of ~400 μm^2. Since patches may contain more than one channel, there would appear to be not only a low, but also a highly uneven density. Clapham and DeFelice (1984) and Clapham and Logothetis (1988) compared their 60- and 15-pS conductance single-channel data with macroscopic currents, and they arrived at similarly low densities. Shibasaki found densities of a little less than one delayed-rectifier channel per μm^2, which is about 40 times higher than in 7-d, embryonic chick ventricle. If the low density for chick heart is not an artifact of the patch or whole-cell conditions, two questions remain; how the cell regulates such a small number of membrane proteins, and how the cell functions with what is likely to be not only a low, but also a variable number of K channels.

In contrast to the complications raised by the presence of three conductance states, the currents through the various delayed-rectifier channels during action potentials are simple. The average current comes on immediately after the upstroke of the action potential, lasts the duration of

the action potential, as well as its approximate shape, owing to a nearly linear open-channel $i(V)$ curve. No current appears to flow into the cell through this pathway.

Little delay exists in the current we identify as the delayed-rectifier. This apparent contradiction is not entirely unexpected in a channel with multiple closed states, for the diastolic depolarization and the finite rise-time of the action potential would shift delayed-rectifier channels toward the open state. In step-protocol experiments, the channels are typically held at negative potential for a long time, thus the channels would be more fully closed when the step occurs and the delay before opening would be more apparent (see also Yue and Marban, 1988).

The Early Outward Channel

The early outward current in heart is similar to the current described by Conner and Stevens (1971) in nerve, where it is called the transient-outward current and is usually designated by I_A. In nerve, the current regulates the frequency of action potentials. Siegelbaum and Tsien (1980) studied an analogous pathway in cardiac Purkinje fibers, and Giles and Van Ginneken (1985) ascribed a regulatory role to the same current in the rabbit heart, where it appears to be involved in the rate-dependent modulation of cells in the crista terminalis. Kukushkin et al. (1983) made a connection between the transient-outward current and the rate dependence of action potential duration in rabbit ventricle, and Nakayama and Fozzard (1987) demonstrated the adrenergic modulation of the current in canine Purkinje cells. Coraboeuf and Carmeliet (1982), Kukushkin et al. (1983), and Josephson et al. (1984) all report on a comparable current whose reversal potential (−40 mV) and kinetics (transient-outward) are similar to the properties we find in early outward channels in the chick embryo.

Though usually categorized with K channels (Cohen et al., 1986), its reversal potential implies that the early outward channel conducts other ions. Nakayama and Irisawa (1985) studied single transient-outward channels in cultured atrio-ventricular node cells of the rabbit heart. In 5.4 mM K, the channel had a linear $i(V)$ curve with a conductance of 20 pS and a reversal potential of −42 mV, near the resting potential of the cell. Both Na and K ions carried the current, and the single-channel data showed kinetics with both rapid and slow components of inactivation (approximate time constants at 40 mV of 30 and 500 ms). Nakayama and Irisawa demonstrated that at the peak of the transient, which occurred near 40 mV, only one channel per 20 μm^2 was in the open state. Taken together, the characteristics of the pore that Nakayama and Irisawa described are qualitatively similar to the early outward channel in the present study. On the other hand, Benndorf et al. (1987) found two types of early outward channels in mouse ventricle cells, one with a conductance of 12 pS and the other with 27 pS; both had a reversal potential of −70 mV, implying a unusually high K-selectivity for this category of channel. The Ca-dependent, 120-pS channel studied by Callewaert et al. (1986) carries a transient outward current, but it is probably unrelated to the early outward channel studied here.

The average current through early outward channels is larger in cells that have action potentials with fast upstroke velocities. On the other hand, cells with faster rates of beat appear to generate lower average currents. In both cases, the single-channel properties remain the same. Because of the rarity of the early outward channel, these observations on its kinetics are necessarily preliminary. The channel appears in only one out of 80 patches, implying an even lower density than the delayed-rectifier. Like the delayed-rectifier, early outward channels appear to conduct no current into the cell while beating.

The Inward-Rectifier

Sakmann and Trube (1984*a*,*b*), Trube and Hescheler (1984), and Kurachi (1985) identified single K channels in adult ventricle cells from the guinea-pig heart which showed many similarities to the macroscopic inward-rectifier (or anomalous-rectifier) current in mammalian ventricle. The current, often designated by I_{K1} or I_{bg} (for background current), exists in many kinds of cells. Beeler and Reuter (1977) and McDonald and Trautwein (1978) were among the first to describe this background current in cardiac tissue. The roles usually acribed to I_{K1} channels are that they help determine the resting potential of cells and assist in the repolarization phase of action potentials.

Sakmann and Trube found a density of 2 channels/μm^2, and in high external K the pore exhibited interconverting substates with distinct conductances. The "main conductance state," as Sakmann and Trube defined it, had a value of 3.6 pS in 5.4 K and 27 pS in 145 K. Fukushima (1981; tunicate eggs) reported a 5.2-pS channel in 50 mM external K, Bechem et al. (1983; guinea-pig atrium) found a conductance of 31 pS in 20 K, and Hume and Uehara (1985; atrial and ventricle cells) described a 32-pS inward-rectifier channel in 145 K. The only study whose unit conductance for the inward-rectifier channel was close to the values we found in 7-d embryonic chick ventricle cells is that of Kameyama et al. (1983; rabbit ventricle). This variety in the conductance of the pore may reflect actual differences in delayed-rectifiers in the diverse tissues mentioned above, but it could indicate an underlying structure that is now and then available to the inward-rectifier channel in the various conditions of these experiments.

Embryonic chick inward-rectifier channels do display substates but primarily in non-beating cells. The pore is charged (Kell and DeFelice, 1988) and the relationship between charge and conductance resembles channels in other membranes (Begenisich, 1975; Bell and Miller, 1984). The conductance of each of the substates depends linearly on external K. In beating cells we observe no

intrinsic substates; however, we do find the widely reported nonlinear relationship between channel conductance and external K, sometimes referred to as the "square root" dependence. One feature of quiescent cells compared with beating cells is that the inward-rectifier reversal potential is ~20 mV more positive when the cell is firing, suggesting again that the internal concentration of K is lower in beating cells than in non-beating cells.

Kurachi (1985) studied the kinetics of inward-rectifier channels in guinea-pig heart in 75 and 150 mM external K. He suggested that the pronounced rectification of the channel may be due to the voltage dependence of the open-channel probability rather than the open-channel conductance itself. This mechanism of rectification is unlikely to be operating in embryonic chick heart. A previous study in conditions that were similar to Kurachi's showed that channel kinetics are nearly independent of voltage in the range spanned during action potentials (Kell and DeFelice, 1988).

In some preparations, the macroscopic inward-rectifier current has an outward component (Shah et al., 1987), and Kameyama et al. (1983), Payet et al. (1985), and Kurachi (1985) have shown that individual channels conduct outward K current under certain conditions, usually high external K. During beating, we observed a pattern of flow that was essentially the same from 120 to 10 mM K and in no case observed an outward component of the current. Assuming this trend holds for normal levels of K (4.5–1.3 mM), the function of the inward-rectifier channel becomes somewhat paradoxical. Though the pore is relatively abundant, it would seem never to conduct K. No current flows out of the cell when it is beating, and current flows into the cell only below the reversal potential of the channel, which in normal levels of external K is close to −80 mV. Since the inward-rectifier has the most negative reversal potential of all the K-conducting pores we have studied, we postulate the existence of other channels or pumps whose reversal potentials are below that of the inward-rectifier.

In the absence of ATP, or under conditions of metabolic inhibition, Trube and Hescheler (1984) report that inward-rectifier channels display an 80-pS state that carries a significant outward current, in contrast to a 25-pS state that carries little or no outward current when ATP is available. Furthermore, in the absence of internal Mg ions, inward-rectifier channels do not rectify but instead have an ohmic $i(V)$ curve (Matsuda et al., 1987). The total concentration of Mg in heart cells is ~12 mM; ~5 mM of this Mg is bound to ATP, and somewhere between 0.2 and 3 mM Mg is free inside the cell (Flatman, 1984; White and Hartzell, 1988). How much of the ion and the amount of ATP that is actually available to inward-rectifier channels in the membrane is unknown, but since the active heart varies its internal concentration of these molecules through Mg-ATP metabolism, the possibility arises that the ATP or the Mg blockade of inward-rectifier channels fails during part of the beat cycle. Such a failure would, under this hypothesis, permit K ions to flow out of the cell through this pathway. Our present experiments would argue against this idea, for presumably we are measuring the currents during the natural variations that are occurring in ATP and Mg ion concentrations, or in other possible regulatory molecules. Although our data suggest that outward current never flows through inward-rectifier channels, we cannot exclude the possibility of a small unresolved outward component masked in our experiments by the capacitive and leakage currents through the patch.

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