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Ca Modulates Outward Current through I_{K1} Channels

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Summary. Inward-rectifier channels in cardiac cells (I_{K1}) stabilize the resting membrane potential near the K equilibrium potential. Here we investigate the role of I_{K1} in the regulation of action potentials and link this to the influx of Ca during beating. Inward Ca current alters the open-channel probability of outward I_{K1} current. Thus Ca ions depolarize cells not only by carrying an inward current but also by blocking an outward current.

Key Words heart cells · potassium channels · inward-rectifier · calcium channels · action potential · modulation

Introduction

Although the inward current through I_{K1} channels is well characterized (Kameyama, Kiyosue & Soejima, 1983; Sakmann & Trube, 1984*a,b*), it has proven difficult to study the outward component because of the strong rectification. For this reason it is still controversial whether the small size of the outward component of I_{K1} reflects the open-channel conductance or channel gating (Kurachi, 1985; Kell & DeFelice, 1988). Rectification is important because it prevents excessive loss of K ions during action potentials, and it helps determine the beat rate and the duration of action potentials. In this paper we study the rectification of I_{K1} in spontaneously beating cells. Using different concentrations of Ca in the patch pipette, we show that the probability that I_{K1} channels open and conduct current in the outward direction is modified by the concentration of external Ca. The Ca current therefore depolarizes heart cells in two ways, directly by providing an inward current and indirectly by blocking the outward component of the I_{K1} current.

Half-saturation Mg block of the outward K current through I_{K1} channels in the absence of Ca is about 2 μM (Matsuda, Saigusa & Irisawa, 1987; Vandenberg, 1987; Matsuda, 1988). In the absence of Mg, micromolar concentrations of Ca ions also block the outward K current (Mazzanti & DiFrancesco, 1989). However, both of these results were obtained in nonphysiological conditions; the experiments were done on inside-out patches, and only Mg or Ca ions were present at the inside surface of the membrane. Here we attempt to clarify the roles of Mg and Ca ions by measuring the outward current during action potentials, conditions in which both ions are present. With normal solutions in the patch (1.3 to 4.5 mM K), I_{K1} currents as single-channel events are too small to measure (Mazzanti & DeFelice, 1988). In the present experiments the cell is bathed in a normal physiological solution, but the patch contains 60 mM K to increase the size of the current and to position its reversal potential at a midpoint in the action potential. Under these conditions a small outward current appears in virtually every patch. We identify this current as I_{K1}. Even under these favorable circumstances, patches that contain only one I_{K1} channel have extremely small outward currents on average.

Materials and Methods

Preparation

The cells are prepared by enzymatic digestion of ventricular tissue from 7-day chick hearts following the procedure of DeHaan (1967). After 12 to 24 hr in culture medium, and immediately prior to 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 were selected for this study.

Single-Channel Analysis

We screened records for patches that contained only I_{K1} channels, using characteristics of Na channels, delayed-rectifier K channels, early-outward channels, and inward-rectifier channels

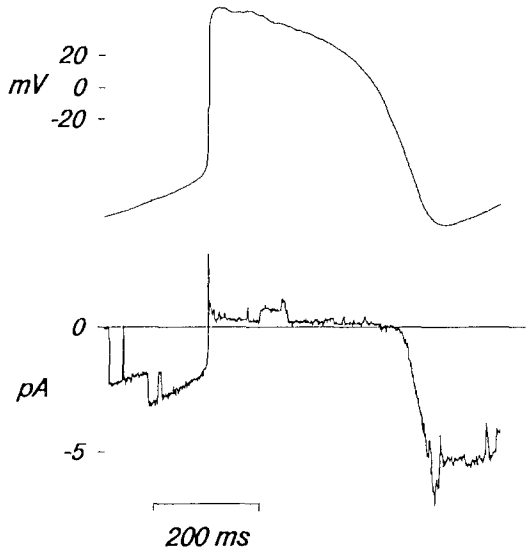

Fig. 1. Two-electrode experiment on a spontaneously beating heart cell. With 60 K solution in the pipette, the reversal potential for K ions is approximately −30 mV and the inward I_{K1} conductance is 40 pS (Mazzanti & DeFelice, 1988). The maximum diastolic potential for these cells is −80 to −90 mV and the plateau potential is 40 to 50 mV, which give driving forces for the inward and outward currents of −50 to −40 mV and 70 to 80 mV, respectively. The top trace is a sample action potential recorded with the whole-cell electrode; previous experiments indicate that spontaneously beating cells are not perfused by the whole-cell electrode (Mazzanti & DeFelice, 1987, 1988). The lower trace is the simultaneous cell-attached patch current. This patch contained at least three I_{K1} channels, as judged by multiple openings

defined in previous studies on beating cells (Mazzanti & DeFelice, 1987, 1988; Wellis, DeFelice & Mazzanti, 1990). Na currents are too small and the kinetics are too fast to be confused with the inward rectifier. Delayed-rectifier channels are larger; during the plateau phase of the action potential the smallest single-channel currents are 1-2 pA. But they have long open times and a characteristic fast flickering that is different from the outward currents observed here. To further exclude the delayed-rectifier, we recorded from patches in which both I_{K1} and I_K channels were present: E_K (the K reversal potential) is approximately the same for both, but the delayed-rectifier conductances (15, 30, and 60 pS) were nearly independent of external K, whereas inward-rectifier channel conductances are strongly dependent on external K, so that the two were easily distinguished. Early-outward channels (I_A) are approximately the size of the smallest delayed-rectifier channels, but they close during the first 50 msec after the upstroke. No driving force exists for outward Ca current during the plateau, so outward events cannot be Ca. In the absence of data on I_{Ca} during the beat, it is difficult to estimate its size but it should take away from outward currents. We eliminate background currents from each trace by subtracting blank traces, defined as those containing no obvious openings. Small unresolved Ca channel openings should be eliminated by this procedure; however, we do not exclude them entirely. Instead we rely on the observation of individual outward events (or the absence of such events) to interpret the average currents. After selecting a patch for study, we recorded up to 100 action potentials and action currents simultaneously and averaged them. Though we eliminate background currents by subtracting the average of blank traces, we use the capacitive current in the raw data to mark the action potential upstroke. The ensemble average of action currents is a miniaturization of the whole-cell current through the class of channels represented in the patch. Here the pipette solution is different from the bath solution, so we must extrapolate to normal conditions. The average action current plotted against the action potential gives instantaneous current, $i(V)$, relationships.

Recording Conditions

The cell-attached patch electrode contained 61.3 K and 70 Na (called 60 K solution); the other components were the same as the bath solution. The whole-cell electrode contained an intracellular-like solution consisting of (in mM): 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 (Corning 7052); they were coated with Sylgard (Dow Corning, Midland, MI) and fire-polished to a tip diameter of less than 1 μm just before use (electrode resistance, 5–10 MΩ). The surface area of the patch is between 5 and 7 μm^2 by geometric and capacitive (Mazzanti & DeFelice, 1987) measurements. The two-electrode recording technique is described in detail elsewhere (Mazzanti & DeFelice, 1987). We used List EPC5 and EPC7 amplifiers to measure the voltage and the current. We screened records for patches that contained only I_{K1} channels (*see* above) and recorded action potentials and action currents simultaneously. The data were stored on a VCR and analyzed using a Nicolet 4094 oscilloscope and software developed for an IBM/AT by William Goolsby. All data were filtered at 1000 Hz before analysis.

Results

Figure 1 shows the inward and outward components of I_{K1} in beating cells in a patch that contained 60 K solution and at least three I_{K1} channels. The greater the number of channels in the patch (by counting levels in the inward current), the greater the outward current. We observed this correlation in 24 cell-attached patches containing multiple I_{K1} channels. The instantaneous $i(V)$ curve for a single channel (Satin & DeHaan, 1989) is complicated by channel substrates (Sakmann & Trube, 1984*a,b*; Kell & DeFelice, 1988). In beating cells, the outward limb of the curve is not easy to determine because the current is small and the voltage during the plateau does not change much. To measure it we plotted the ensemble average of the single-channel currents, $I(V)$, rather than the instantaneous currents, $i(V)$. Figure 2*a–c* shows examples from patches containing one, two, and four I_{K1} channels. Figure 2*d* plots the ensemble average of these currents versus the action potential. The $I(V)$ curves are continuous, they display the presupposed rectification, they reverse at the presumed value of −30 mV, and they show that outward current increases in proportion to inward current, suggesting that

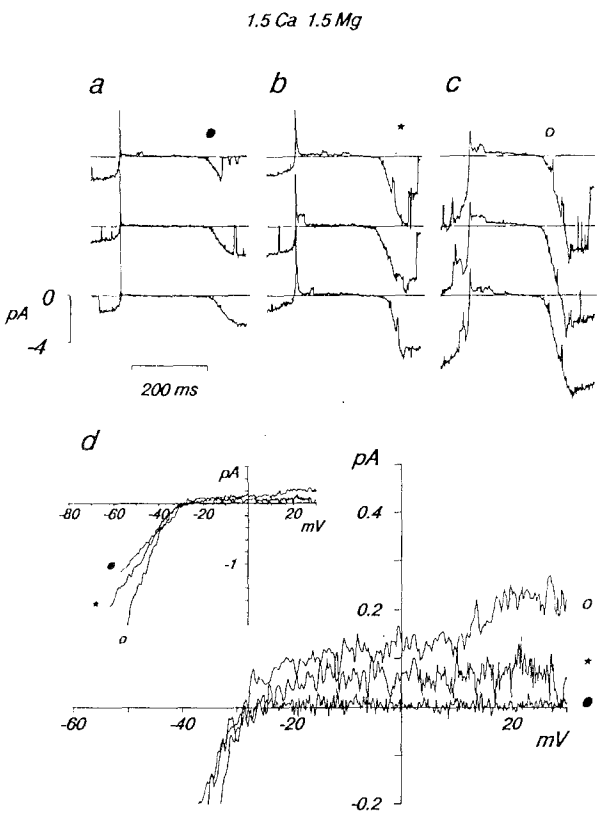

Fig. 2. (*a*) Three sample action currents (not leak-subtracted) from a patch containing one I_{K1} channel (no multiple openings in the inward direction are shown). Outward openings occur rarely. Plotting the average of 43 traces similar to those in *a* gives the trace in *d* marked with a solid dot. With one channel in the patch, the average outward current is essentially zero, in agreement with previous work (Mazzanti & DeFelice, 1988). Plotting averages of 22 traces from *b*, in which the patch contains at least two channels, and 18 traces from *c*, in which at least four channels are present, gives the $I(V)$ curves indicated by the asterisk and the open dot, respectively. The lower plot is an enlargement of the insert in the upper left-hand corner of *d*

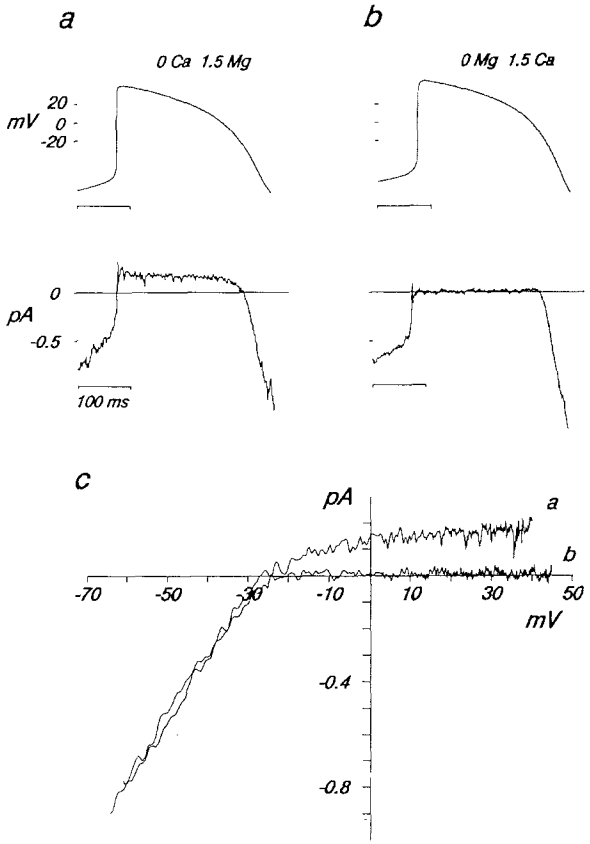

Fig. 3. (*a*) The first experiment of Fig. 2 is repeated (in which the patch contains only one I_{K1} channel; closed-dot), but without Ca in the patch. The top trace is an action potential and the bottom trace is the average of 50 action currents (leak-subtracted). (*b*) The experiment is repeated, but without Mg in the pipette. The experiments in *a* and *b* were picked for matching action potentials; 12 experiments with slightly different action potentials gave the same result. (*c*) The average action potential is plotted against the average action current for the two cases. Removing Mg or Ca does not affect the inward current, which is the same as it is when either one of the ions or both ions are present (Fig. 2*d*; solid dot). Removing Mg does not affect the outward current. Removing Ca, however, dramatically increases the average outward current through I_{K1} channels

both the inward and outward components of the $I(V)$ curves are due to the same channels.

We investigated the regulation of I_{K1} by external Ca under the following rationale: changes in bath Ca should change the amplitude of the Ca current through Ca channels and alter internal Ca concentration near the membrane. Since internal Ca controls I_{K1} rectification (Mazzanti & DiFrancesco, 1989), we asked whether conditions inside beating cells were such that the inward flow of Ca could regulate I_{K1}. To test this idea, we removed Ca from the patch only, and then repeated the experiments of Fig. 2. The result is shown in Fig. 3*a*, which demonstrates that the inward current is unaffected by removing external Ca, whereas the outward current is markedly increased by removing external Ca in the vicinity of the I_{K1} channels. Removing Ca in the patch also reduces I_{Ca}, thus increasing outward current. Although the subtraction procedure should remove such background effects (*see* Materials and Methods), we do not entirely exclude this possibility. Because the $i(V)$ curves are composed of individual action currents, we can see that the average outward current reflects the presence of individual, outwardly directed events. Thus the outward limbs of the $i(V)$ curves represent authentic outward currents, and not the absence of inward currents.

To determine whether this increase in outward current is zero Ca was due to a nonspecific effect of divalent cations on I_{K1} channels (Kell & DeFelice, 1988), we removed Mg instead of Ca. The effect in

zero Ca is not mimicked in zero Mg, which alters neither the inward nor the outward limbs of the $I(V)$ curve (Fig. 3*b*). To ask whether it is the removal of Mg that blocks the outward K current, compare Figs. 3b and 2a, which show similar results in 1.5 Mg/1.5 Ca and 0 Mg/1.5 Ca. In Fig. 3 we selected experiments with nearly identical action potentials and chose patches with only one I_{K1} channel. Thus the inward limbs of the $I(V)$ curves were identical, and the effect of Ca was clearly on the outward current alone. We obtained the same qualitative result in 12 separate experiments with slightly different action potentials and in some patches containing more than one channel.

Discussion

Our results show that under the conditions of beating, which most likely means that the internal concentrations of Mg and Ca are varying with time, a small outward current flows through I_{K1} channels. Although the experiments are done with 60 mM K in the patch pipette, the results extrapolate to normal levels of K, i.e., the reversal potential and the size of the outward current make sense in terms of the complementary whole-cell experiments. For two channels/μm^2, which is near the average density of I_{K1} channels in this tissue (Kell & DeFelice, 1988; *see also* Sakmann & Trube, 1984*a*,*b*), we estimate[1] from Fig. 2*d* that the macroscopic current in 10 K would be at least 0.75 $\mu A/cm^2$. This is near the amplitude obtained from direct measurement in these same embryonic chick ventricle cells using whole-cell recording techniques (Satin & DeHaan, 1989), and it is roughly the value found in adult mammalian ventricle cells (Beeler & Reuter, 1984).

We conclude that in beating cells in spite of strong rectification a small but finite outward I_{K1} exists. From other experiments we know that 10 μM of internal Mg would block nearly all of the outward current through I_{K1} channels (Matsuda et al., 1987; Vandenberg, 1987; Matsuda, 1988). In the absence of Mg, 10 μM Ca does the same (Mazzanti & DiFrancesco, 1989). Taking these two findings into account, our experiments would imply that during the plateau phase of the action potential the Mg and Ca concentrations inside the cell are less than 10 μM, in dramatic contrast to the generally accepted concentration of free Mg. Duchatelle-Gourdon, Hartzell and LaGrutta (1989) show that to function the delayed-rectifier in frog atrium needs internal Mg greater than 0.3 mM and probably closer to 1 mM, which is near the accepted values (White & Hartzell, 1988). Furthermore, the intracellular-like solution in our voltage recording electrode contains 2 mM concentration of Mg. We are unable to resolve this apparent discrepancy. One explanation is that the estimate of 10 μM Mg for the complete block of outward I_{K1} current is nonphysiological. It is based on experiments on cell-detached patches in rather abnormal conditions. Another possibility is that near the membrane under the normal conditions of spontaneous action potentials the concentration of Mg is much lower than the bulk concentration. We speculate that in beating cells the internal concentration of Mg near the membrane is low enough that internal Ca can bias I_{K1} rectification, and that external Ca influences the outward component of I_{K1} by flowing into the cell. Virtually every patch contains Ca channels (Mazzanti & DeFelice, 1989) that could modulate the local concentration of Ca near the inner mouth of I_{K1} channels, where the block is assumed to occur. In our experiments we arranged conditions to see the outward current through I_{K1} channels during the plateau, when Ca currents are flowing. In normal conditions the reversal potential for I_{K1} is near the maximum diastolic potential, and the environment of the channels may be quite different than during the plateau.

Internal Mg and Ca are never low enough to remove I_{K1} rectification completely. Our results suggest that Ca ions may regulate I_{K1} rectification by flowing into the cell through nearby Ca channels. By this mechanism external Ca could help regulate the stability of the resting potential, the interbeat interval, and the duration of the cardiac action potential.

[1] From Fig. 2*d*, the current for two channels, at 20 mV and in 60 K solution, has an average value of 0.045 pA. The mean density is 2 channels/μm^2, which implies 0.045 pA/μm^2 = 4.5 $\mu A/cm^2$. Assuming the outward current in 10 mM K is one-sixth that in 60 mM K gives 0.75 $\mu A/cm^2$. The conductance of inward-rectifier channels in 60 mM K is 42 pS and in 10 mM K is 14 pS (Mazzanti & DeFelice, 1988). Thus the estimate of 0.75 $\mu A/cm^2$ is a lower limit.

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