brief communication

Internal and external K^+ help gate the inward rectifier

Ira S. Cohen,* Dario DiFrancesco,‡ Nancy K. Mulrine,§ and Peter Pennefather‖
*Department of Physiology and Biophysics, State University of New York at Stony Brook, Stony Brook, New York 11794; ‡Dipartmento di Fisiologia e Biochimica Generali, University di Milano, Milan, Italy; §Department of Pharmacology, University College London, London, United Kingdom; and ‖Faculty of Pharmacy, University of Toronto, Toronto, Canada

ABSTRACT Recent investigations have demonstrated substantial reductions in internal $[K^+]$ in cardiac Purkinje fibers during myocardial ischemia (Dresdner, K. P., R. P. Kline, and A. L. Wit. 1987. *Circ. Res.* 60:122–132). We investigated the possible role these changes in internal K^+ might play in abnormal electrical activity by studying the effects of both internal and external $[K^+]$ on the gating of the inward rectifier iK_1 in isolated Purkinje myocytes with the whole-cell patch-clamp technique. Increasing external $[K^+]$ had similar effects on the inward rectifier in the Purkinje myocyte as it does in other preparations: increasing peak conductance and shifting the activation curve in parallel with the potassium reversal potential. A reduction in pipette $[K^+]$ from 145 to 25 mM, however, had several dramatic previously unreported effects. It decreased the rate of activation of iK_1 at a given voltage by severalfold, reversed the voltage dependence of recovery from deactivation, so that the deactivation rate decreased with depolarization, and caused a positive shift in the midpoint of the activation curve of iK_1 that was severalfold smaller than the associated shift of reversal potential. These changes suggest an important role of internal K^+ in gating iK_1 and may contribute to changes in the electrical properties of the myocardium that occur during ischemia.

INTRODUCTION

Inwardly rectifying K^+ currents are present in a wide variety of muscle (2–4) and non-muscle cells (5–7). In Purkinje fibers a current of this type, called iK_1, sets the resting potential near the Nernst equilibrium potential for K^+, and modulates the fiber's natural automatic rate (8). Inward rectification refers to the positive correlation between activation and inward current through these channels. The channels activate upon changes of membrane potential in the negative direction. The potential required for half-maximal activation and the rate of activation shift in parallel with E_K when the extracellular $[K^+]$, K_o, is changed. Thus the kinetics and steady-state open probability of these channels depend on K_o (9, 10). Much smaller effects of changes in internal $[K^+]$, K_i, on these properties of the inward rectifier have been reported (9, 11). We have examined the actions of both K_o and K_i on the inward rectifier in Purkinje myocytes. At a given voltage, increases in K_o increased the rate of activation, slowed the rate of deactivation, and increased steady-state open probability, as previously reported in other cells (9, 11). Decreasing pipette $[K^+]$ from 145 to 25 mM, hence reducing K_i, however, had several dramatic and surprising effects. The rate of activation decreased severalfold at a given voltage. The voltage-dependence of deactivation changed sign so that the deactivation rate decreased as membrane potential increased in the positive direction. A positive shift of between 5 and 12 mV in the midpoint of the steady-state activation curve was also observed but this shift was considerably less than the shift in E_K of ~25 mV. These results suggest that K_i has an important role in controlling the kinetics of gating of iK_1. This finding is relevant to the understanding of cardiac pathology since recent reports have demonstrated substantial reductions in K_i during ischemia (1) or hypoxia (12).

Address all correspondence to Dr. Ira S. Cohen, Department of Physiology and Biophysics, H.S.C., SUNY at Stony Brook, Stony Brook, NY 11794-8661.

METHODS

The experiments were performed on isolated canine Purkinje myocytes (see reference 13 for more details) cooled to temperatures 8°–10°C and constant to 0.5°C within a given experiment. The whole-cell voltage-clamp technique with series resistance compensation and leak subtraction was employed. Rectangular voltage clamp steps were used to activate iK_1. The resultant current developed exponentially. Currents were digitized and characterized in terms of time constant and initial amplitude using a log-linear least-squares fitting procedure.

In the external solution, Cd^{2+} and Mn^{2+} were added to block calcium currents. NaCl was replaced by choline chloride (ChCl) to minimize Na-dependent inactivation of iK_1 (14). A Tyrode solution with 12 mM K_o contained (in mM) 130 ChCl, 10 KCl, 0.5 $CaCl_2$, 0.5 $MgCl_2$, 0.1 $CdCl_2$, 1 $MnCl_2$, 5 Hepes, neutralized with ~2 mM of KOH to pH 7.4. In 42 and 82 mM K^+ solutions 30 or 70 mM KCl was substituted for an equal amount of choline chloride.

The high K^+ pipette contained (in mM) 120 KCl, 1 $MgCl_2$, 5 Hepes, 5 EGTA, and 5 Na_2ATP neutralized with ~25 mM KOH to pH 7.2. The filling solution for reduced K pipettes was identical except that the KCl

was replaced by 100 mM ChCl. The total K^+ in this solution was thus 25 mM.

Pipette resistance ranged initially from 1 to 3 MΩ rising to 2–8 MΩ after the whole-cell recording mode was established. The extent of dialysis of myocytes was followed by monitoring the reversed potential of iK_1. Typically this shifted relatively slowly requiring 30–60 min to reach a steady state. The time constant (τ) of dialysis, when membrane K transport is neglected, is predicted by the following formula, $\tau = VR/D\rho$, where V is cell volume, R is pipette resistance, D is the diffusion constant of potassium, and ρ is the resistivity of the pipette filling solution (15, 16). Under our conditions the predicted time constant is roughtly 5–20 min. Significant membrane K^+ transport might speed this equilibration somewhat, but a slow dialysis is not unexpected (16).

We have measured the liquid junction potentials between the external solutions of highest and lowest $[K^+]$ and each pipette solution. For the high K^+ pipette solution this potential was −0.5 mV with the high K^+ external solution and −4.3 mV with the low K^+ external solution. For the low K^+ pipette solution this potential was +7.0 mV with the high K^+ external solution and +2.6 mV with the low K^+ external solution. We have not corrected our results for this offset.

Even at steady state, K_i estimated from E_K did not appear to equal pipette $[K^+]$ (see Fig. 2). This difference may reflect the entry of K^+ into the cell from the external medium due to the action of ion transport systems and the electrochemical gradient. Because of this incomplete exchange between the pipette contents and the cell interior, it is not possible to correct for this liquid junction potential offset.

RESULTS

Fig. 1 illustrates our basic finding. Lowering the internal $[K^+]$ alters the kinetics of the inward rectifier. We examined activation and deactivation with a three-pulse protocol. A voltage clamp step in the negative direction activates most of the inwardly rectifying current. This is followed by a step to more positive potentials to allow deactivation. A third pulse to the same level as the first step then allows estimation of the amount of recovery during the preceding positive going step. This protocol is necessary to allow estimation of the deactivation since switch off of the current at potentials more positive than E_K is very rapid. Fig. 1 *A* shows sample records obtained with the normal, 145 mM, intracellular $[K^+]$, and 42 mM K_o. Upon stepping to −50 mV from a membrane potential of −10 mV there is a rapid activation of inward current. Full activation occurs within 10–20 ms. Similarly deactivation at +10 mV is largely complete within 10 ms. This is illustrated by the increase in current size during the second step to −50 mV after a variable interval at +10 mV. The time course of the deactivation is graphically depicted in Fig. 1 *B*. It is apparent that the rate of deactivation increases at more positive potentials. At potentials above 0 mV, the halftime for deactivation is of the order of 1–10 ms.

Fig. 1 *C* illustrates a similar experiment performed on another myocyte also in 42 mM K_o but with a reduced intracellular $[K^+]$. The activation time constant at −40 mV in this case is almost 100 ms, and the halftime of deactivation at potentials above 0 mV is of the order of

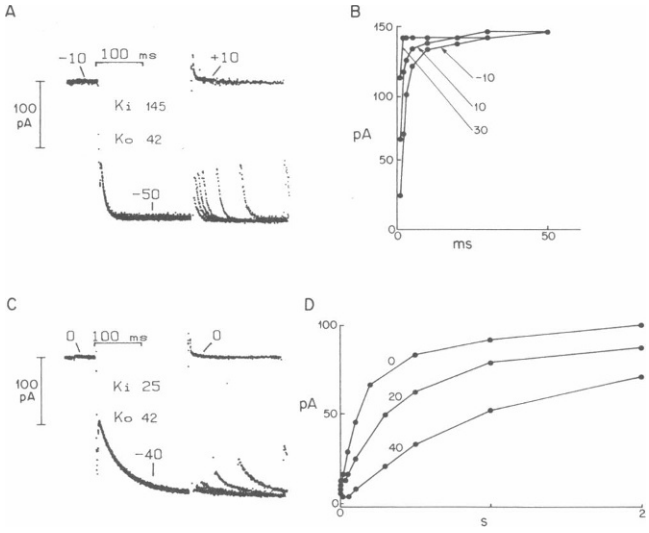

FIGURE 1 Sample membrane currents and responses to a three-pulse protocol in isolated Purkinje myocytes. (*A*) Current records obtained with 145 mM K^+ in the pipette solution. In the voltage clamp protocol the myocyte was held at −10 mV and the potential was then stepped in the negative direction for 200 ms to −50 mV. After the 200-ms step, the potential was stepped to +10 mV for a variable time period and then returned to −50 mV a second time for 200 ms. (*B*) The amplitude of the membrane current in response to the second pulse to −50 mV is plotted against the time spent at −10, +10, or +30 mV. (*C*) The same protocol as that illustrated in *A*, executed on a different Purkinje myocyte with pipette $[K^+]$ at 25 mM. The holding potential was 0 mV. The first pulse was a 200 ms command to −40 mV followed by a variable period at 0 mV and then a second command to −40 mV for 200 ms. (*D*) The amplitude of the membrane current in response to the second pulse to −40 mV is plotted against the time spent at 0, +20, or +40 mV. The contribution of a linear leakage current has been subtracted from all records.

100–1,000 ms. Thus lowering internal $[K^+]$ can slow the kinetics of activation and deactivation by up to 1–2 orders of magnitude. In the plot at the right a second important difference is illustrated. The rate of deactivation now decreases as membrane potential becomes more positive.

A common property of inward rectifiers thus far studied is that the activation curve shifts with external $[K^+]$ in accordance with the Nernst equilibrium potential for K^+, but the midpoint of this curve has little or no dependence on K_i. We examined the activation curve for the inward rectifier in Purkinje myocytes in light of these previous findings. A two-pulse protocol was employed. A conditioning pulse to various potentials was followed by a pulse to a more negative test potential where the fraction of current not already activated could be measured. In this manner the activation curve was constructed. In order to draw smooth lines through data points in Fig. 2, *A* and *B*, the sigmoidal activation curves were linearized by plotting ln [(maximal conductance/conductance) − 1] versus voltage. A slope factor was then determined by linear

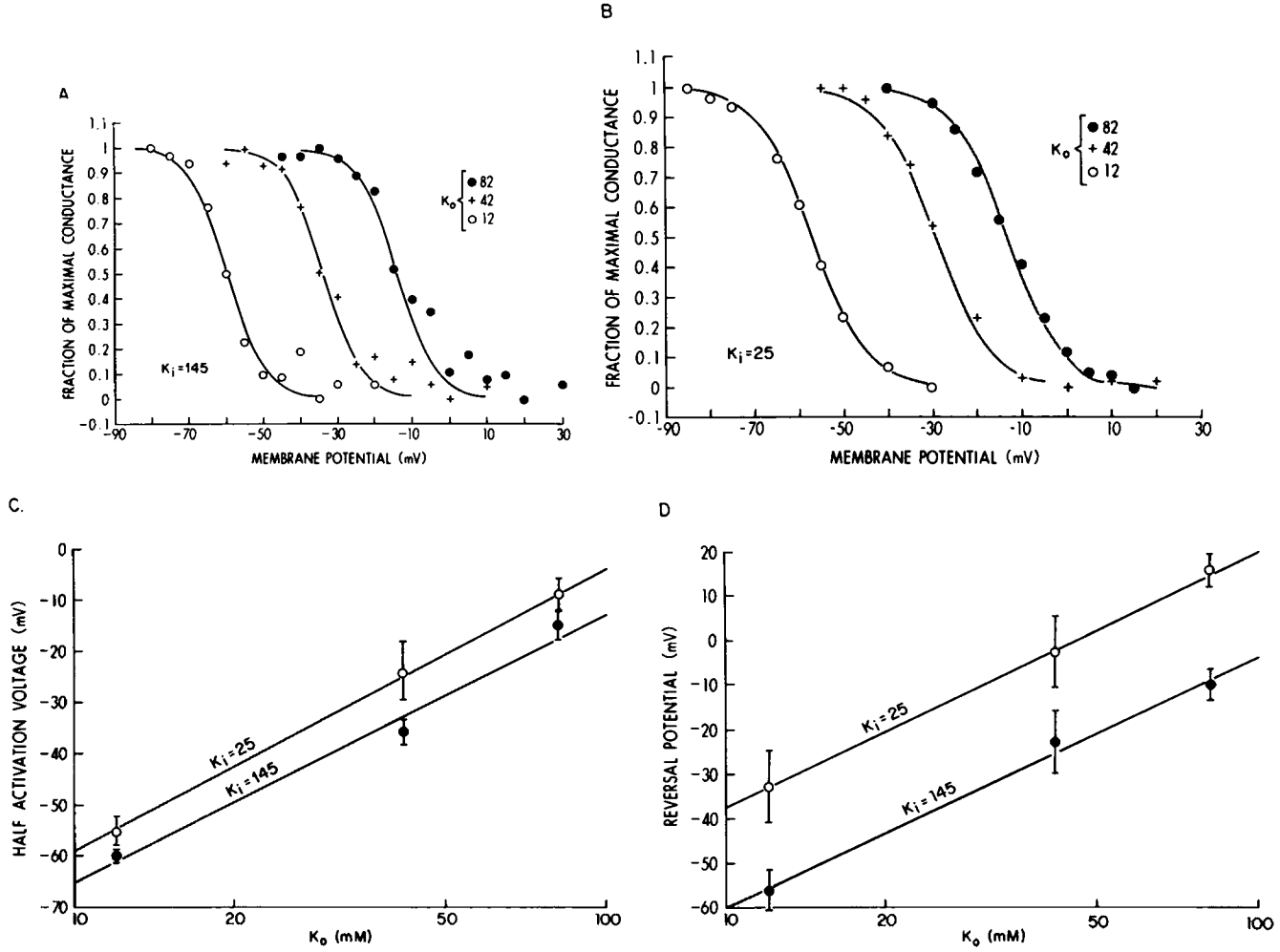

FIGURE 2 The effects of external and internal [K^+] on the activation and reversal of the inward rectifier. Activation curves were constructed from a standard two-pulse protocol. (*A*) Normal K^+ pipette: The smooth curves are generated by a Boltzmann two-state model $P_{open} = 1/[1 + \exp(V - V_{mid})/S]$ with a slope factor S of e-fold/5 mV, where V_{mid} is the half activation voltage, 9/3/86 cell 3. (*B*) Reduced K^+ pipette: The smooth curves are generated by a Boltzmann two-state model with a slope factor S of e-fold/6 mV. 8/28/86 cell 1. (*C*) A semilog plot of the external [K^+] against the half activation voltage. Reducing the pipette [K^+] shifts the activation midpoint 5–12 mV in the positive direction. The bars indicate the standard deviation of the deviation of the measurements. For the normal pipette [K^+] the least squares regression line has a slope of 53 mV and at reduced pipette [K^+] the slope is 55 mV per 10-fold change in external [K^+]. (*D*) A semilog plot of the external [K^+] against the reversal potential of the inward rectifier. Reducing pipette [K^+], shifts the reversal ~25 mV. The bars indicate the standard deviation of the measurements. For normal pipette [K^+] the least squares regression line has a slope of 56 mV and had an intercept of 0 mV of 118 mM. With reduced pipette [K^+] the slope is 57 mV per 10-fold change in external [K^+] and had an intercept at 0 mV of 46 mM. These intercepts provide estimates of the internal [K^+] under the two recording conditions. These numbers are clearly different from pipettes [K^+] (see text). K_i labeled on each figure is the pipette [K^+].

regression. For all our data obtained with a pipette containing 145 mM [K^+], the mean (±SD) slope factor was e-fold per 5.6 ± 1.7 mV (n = 15). For data obtained using a pipette containing 25 mM [K^+] the slope factor was e-fold per 6.7 ± 1.3 mV (n = 9). This difference was not statistically significant, but it should be noted that a similar effect of K_i on the steepness of the activation curve for other inward rectifiers has been previously reported (9, 11).

Fig. 2 *A* illustrates activation curves constructed in solutions containing 12, 42, and 82 mM K_o and at 145 mM K_i. The activation curve shifts along the potential axis in accordance with the Nernst potential for K^+. This is illustrated in Fig. 2 *C* where the mean (±SD) half activation voltages are plotted against K_o. The best fit to a log-linear regression gives a slope of 53 mV per 10-fold change in K_o; close to that expected for a perfect K^+ electrode. The kinetics of activation and deactivation also shifted when K_o was altered in accordance with the changes in E_K. This is in agreement with previous findings in skeletal muscle, oocytes, and guinea pig ventricular muscle.

Activation curves constructed by the same protocol but with a reduced K_i are shown in Fig. 2 *B*. As with normal K_i there is a shift of the midpoint of the activation curve with reductions in external K^+ from 82 to 42 and 12 mM. The means for half activation voltages are illustrated by the open circles in Fig. 2 *C*. Again there is a Nernstian dependence of the midpoint of the activation curve on the external $[K^+]$ with a slope of 55 mV per 10-fold change in K_o. However, at the 10 mM intercept, the relation is shifted ~6 mV in the positive direction with low K_i. This effect of internal $[K^+]$ on the position of the activation curve is in the same direction as the effect on the reversal potential of the current, but it is not as large. The reversal potentials measured by a two-pulse protocol are plotted in Fig. 2 *D* versus K_o for normal and reduced K_i as a function of K_o. In both normal and low K_i the reversal potential is Nernstian with changes in K_o. The least squares regression lines give slopes of 56 and 57 mV per 10-fold change in K_o for the normal and low K_i solutions, respectively. There is, however, about a 25-mV shift in the reversal potential of the inward rectifier in the depolarizing direction when the internal $[K^+]$ is reduced.

The effect of lowering internal $[K^+]$ on the activation of the inward rectifier is further illustrated in Fig. 3. At both normal and reduced intracellular $[K^+]$'s the activation follows a single exponential time course with a 10-fold increase in time constant for roughly a 50-mV increase in membrane potential (see legend, Fig. 3). The major difference between normal and reduced K_i is the activation rate. At a reduced K_i the kinetics of activation are shifted between 25 and 30 mV in a negative direction (in the opposite direction to the change in the K^+ equilibrium potential), implying much slower kinetics at low than normal K_i.

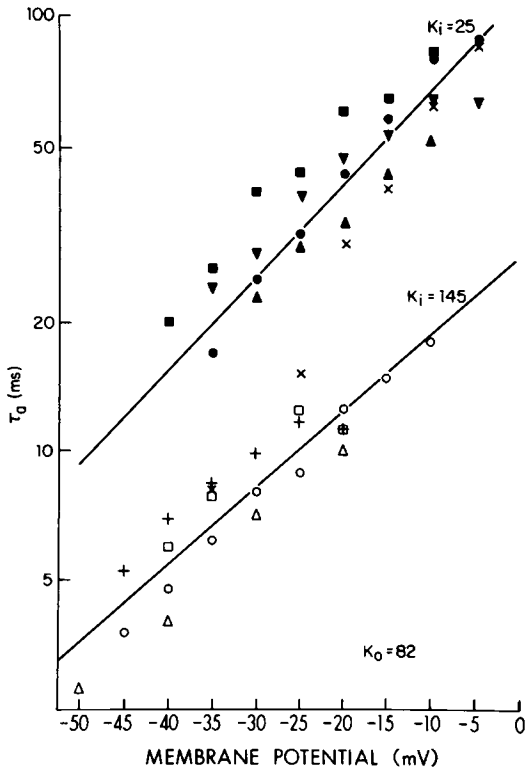

FIGURE 3 A semilog plot of the time constant of activation against membrane potential for both reduced and normal pipette $[K^+]$, at an external $[K^+]$ of 82 mM. Similar results were obtained in 42 and 12 mM K_o. The least squares regression lines for normal and reduced pipette $[K^+]$ are nearly parallel predicting 10-fold increases in activation time constant for 56- and 47-mV depolarizations, respectively. However, when recorded with pipettes containing 25 mM $[K^+]$, potentials between 25 and 30 mV more negative were required to attain activation time constants comparable to those recorded with pipettes containing 145 mM $[K^+]$.

DISCUSSION

Our results have demonstrated that in canine Purkinje myocytes K_i as well as K_o affects the kinetics of activation, the voltage dependence of steady-state activation, and the kinetics and voltage dependence of deactivation of the inward rectifier. Moreover, we have shown that shifts in the activation curve produced by K_o can be dissociated from shifts in the reversal potential. Activation thus is not a function simply of the movement of K^+ through iK_1 channels. Rather our results are more consistent with models where activation of inwardly rectifying K^+ channels is proportional to the voltage-dependent occupancy of binding sites for external K^+ on the channel protein (see reference 17). In other words the channel acts like a K^+-activated K^+ channel. Indeed, the action of K_o on the iK_1 current is somewhat analogous to that of internal calcium on calcium-activated K^+ channels (see reference 18).

The effects of lowering internal K^+ are more difficult to explain. One possibility is that K_i allosterically modifies the interaction of K_o with binding sites that regulate gating of the channel. The reduced rate of activation seen with low K_i then would reflect a reduced rate of interaction of K_o with the channel. The apparent increase in potency of K_o, with respect to steady-state activation, produced by lowering K_i would imply that the rate of dissociation of K_o from the channel is reduced to an even greater extent than the rate of association. Whatever the origin of the effects of K_i on iK_1, it is clear that both internal and external K^+ regulate the steady-state and kinetic properties of this current. The changes observed are due to internal K^+ rather than to the K^+-substitute used (choline), because in preliminary experiments similar results have been obtained using tetramethylammonium or *N*-methyl-D-glucamine to replace internal K^+ (unpublished observations). Hagiwara and Yoshii (11)

have also noted a decrease in activation rate when K_i was reduced. Thus, K_i may influence other inward rectifiers in a manner similar to its effects on iK_1.

Separation of gating and rectification is consistent with blockade of the channel by an internal ion (either Mg^{2+} [19–21] or Ca^{2+} [22]). At normal K_i we are unable to discriminate between rectification and gating because of the rapid time course of both processes (19). However, it is clear that instantaneous rectification and gating can be dissociated (see Fig. 1). Both voltage-dependent isomerization of the channel and blockade by internal Mg^{2+} may contribute to rectification of iK_1 in the physiologic range of potentials.

Recent evidence suggests that the internal $[K^+]$ can decrease to half its normal value in ischemia (1). Our results suggest this reduction in K_i should slow both activation and deactivation of the inward rectifier with little shift in the position of the activation curve on the voltage axis. When K_i declines E_K and thus the resting potential become more positive. At these more positive potentials the conductance due to iK_1, and hence, the membrane conductance should be much reduced, increasing excitability. Further, the reduced deactivation rate with its reversed voltage dependence (see Fig. 1) might allow the fraction of iK_1 activated between action potentials to persist during action potentials and contribute along with the ATP-sensitive K^+ channel (23) to the well known shortening of the action potential in hypoxic conditions (24).

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