Origin of the potassium and voltage dependence of the cardiac inwardly rectifying K-current (I_{K1})

Peter Pennefather,* Carlos Oliva,‡ and Nancy Mulrine§

*Faculty of Pharmacy, University of Toronto, Toronto, Ontario, M5S 2S2 Canada; ‡Department of Physiology and Biophysics, State University of New York at Stony Brook, Stony Brook, New York 11794 USA; and §Department of Pharmacology, University College London, London, United Kingdom

ABSTRACT Using various voltage clamp protocols, we have examined the activation and deactivation kinetics of I_{K1} recorded in dissociated myocytes obtained from canine purkinje fibers. Exponential current relaxations following step changes of the membrane potential were characterized at several different K levels (5, 12, 42, and 82 mM) and several voltages (K reversal potential ± 40 mV). We have interpreted our data according to a K-activated, K-channel model of I_{K1} gating. Our data suggests that at least two binding sites for extracellular K must be occupied before the channel opens and occupancy of about three more higher affinity sites for K on the open channel will slow the closing of that channel. In our model, the voltage dependency of gating arises from a combination of three voltage dependent steps: (*a*) isomerization between open and closed states, (*b*) binding of K, and (*c*) occupancy of the channel by internal Mg. Lowering internal K to 40 mM causes major changes in the voltage and K dependence of I_{K1} gating. However, these changes could be accounted for in our model by relatively small ($\approx$20 to 30 mV) shifts in the voltage dependence of several of the steps that govern gating. Our data further suggest that there is an interaction between both extracellular and intracellular K levels and the ability of intracellular Mg to block the I_{K1} channel.

INTRODUCTION

The inwardly rectifying potassium (K) current of cardiac myocytes (I_{K1}) derives its name from its ability to pass current more easily in the inward direction. Hyperpolarization to potentials negative to the potassium reversal potential (V_K) leads to an increase in the contribution of this channel to the membrane conductance of myocytes. The underlying channel is not simply activated by hyperpolarization because when external K is increased, the region of rectification shifts in parallel with the potassium reversal potential (Noble, 1965). Similar currents have been observed in many other cell types (Katz, 1949; Hagiwara and Takahashi, 1974; Constanti and Galvan, 1983; Stanfield et al., 1985).

Two major classes of theories have been formalized to account for this type of inward rectification. The first class postulates that a positively charged intracellular blocking particle is driven into the channel by depolarization and is flushed out of the channel by inward current (Armstrong, 1969; Hille and Schwartz, 1978). The second class proposes that gating of the channel is regulated by binding of external K to a number of sites on the channel (Horowicz et al., 1968; Ciani et al., 1978; Carmeliet, 1982; Cohen et al., 1989). We will refer to these two models as the blocking particle model and the K-activated K-channel model respectively.

Recently, the blocking particle model has been supported by the observation that physiological levels of intracellular Mg (see Matsuda et al., 1987; Vandenberg, 1987) or Ca (Mazzanti and DiFrancesco, 1989) are effective at blocking outward currents through single I_{K1} channels. However, whole cell recording of I_{K1} clearly exhibit time and voltage-dependent gating that is distinct from the almost instantaneous blockade by Mg (see Matsuda et al., 1987). More recently, Oliva et al. (1990) and Ishihara et al. (1989) have presented evidence that voltage dependent gating, independent of Mg, accounts for the majority of inward rectification around V_K. In this paper, we describe the influence of K and voltage on the kinetic and steady-state properties of the time dependent gating of I_{K1} that is predominant around V_K. We show how a K-activated K-channel model can account for that gating.

In principle, electrophysiological characterization of the steady-state and time-dependent properties of I_{K1} currents and channels, and the modification of those properties by changing K levels and transmembrane potential can provide sufficient constraints so that a unique set of parameters defining a kinetic model of the modulation by voltage and K will be consistent with the electrophysiological data. In practice, the range of potentials and K levels that can be examined is limited. We discuss the extent to which the parameters of our model can be defined from our data.

The core of our model is suggested by two basic observations. First, voltage jump current relaxations, that reflect the time dependent gating of I_{K1}, exhibit a

Address correspondence to Dr. Pennefather.

Dr. Oliva's present address is Dupont-Merck Pharmaceuticals Co., Experimental Station, Wilmington, Delaware 19880-0400.

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single exponential component (see Saigusa and Matsuda, 1988; Harvey and TenEich, 1988; Cohen et al., 1989; Ishihara et al., 1989). The time constants of that component exhibit a bell shaped dependence on voltage, with the maximal time constant occurring at the voltage where the channels are open 50% of the time (V_{mid}). These observations suggest that a single rate limiting step governs the interconversion between open and closed channels. In our model we have assumed that an isomerization step is rate limiting and that K is always in equilibrium with the binding sites that regulate isomerization. Second, increasing $[K]_o$ shifts the relation between voltage and the time constant of I_{K1} relaxations in parallel with V_K without having a major effect on the shape of that relation or on the magnitude of the maximal time constant (see Saigusa and Matsuda, 1988). Therefore, at a given voltage, increasing $[K]_o$ must increase the rate of activation of I_{K1} and decrease the rate of deactivation. Our model proposes that this behavior arises because binding of K to closed channels is required before the channel can open, and further that binding of K to the open channel increases the open state probability. The slowing of the closing rate as the result of binding K ions reflects an increase in affinity of the binding site for K, upon channel opening.

As a result of the reciprocal interactions between channel opening and K binding our model predicts that internal Mg can indirectly influence the binding of external K to the sites that regulate gating. This hypothesis is based on the evidence that Mg can trap the I_{K1} channel in an open but partially blocked state (see Matsuda, 1988; Ishihara et al., 1989; Oliva et al., 1990). By preventing channel closing at positive potentials, Mg will also maintain the high affinity state of the channel for K at those potentials. Cohen et al. (1989) have shown that lowering K_i leads to a reversal of the voltage dependence of I_{K1} deactivation; the rate of closing of channels exposed to low K_i is decreased by depolarization. Here we show that a relatively small increase in the potency of Mg_i to plug the channel and trap the channel in a plugged open state combined with a slowing of the opening and closing rates, can account for many of the dramatic changes in I_{K1} kinetics that are associated with lowering K_i. A preliminary report of this work has appeared previously in abstract form (Pennefather et al., 1987).

THEORY

In this section we outline the kinetic model used to describe our data and show how the data places constraints on the various parameters that determine the predictions of the model. Our hypothesis is that the I_{K1} channel is a K-activated K-channel. In this regard, our model is similar to that used by Moczydlowski and Latorre (1983) to analyze the influence of voltage and calcium on the large conductance, calcium-activated K-channel.

As mentioned in the Introduction, gating of I_{K1} appears to be governed by a single rate limiting step where both opening and closing rates are voltage and K dependent. This situation is depicted by Scheme 1:

$$C_T \underset{b'(V,K)}{\overset{a'(V,K)}{\rightleftharpoons}} O_T$$

SCHEME 1

where $a'(V, K)$ and $b'(V, K)$ are voltage and K dependent rate constants. Throughout the text, we will use the convention that when one variable is a function of another variable, the symbol representing the dependent variable will be followed by a bracket containing an italicized symbol representing the independent variable. C_T is the sum of all closed states and O_T is the sum of all open states.

With simple kinetic systems such as Scheme 1, the steady-state open and closed probability due to gating, P_g and Q_g respectively, are such that

$$P_g = (1 + b'/a')^{-1} \tag{1a}$$

$$Q_g = (1 + a'/b')^{-1} \quad \text{and} \tag{1b}$$

$$P_g = 1 - Q_g. \tag{1c}$$

Also, the time constant, τ, of current relaxations associated with voltage jumps is such that $\tau = (a' + b')^{-1}$. Therefore, by determining τ and P_g, one can determine the macroscopic opening, (a') and closing (b'), rate constants since,

$$a' = P_g/\tau \tag{2a}$$

$$b' = Q_g/\tau. \tag{2b}$$

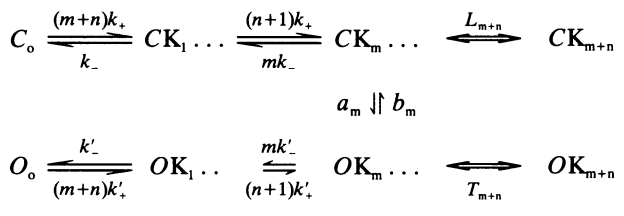
Our experimental approach was to determine how τ and P_g varied with voltage and K. A model was then formulated that allowed the rate constants, a' and b', to be factored into components that depended on either voltage, $[K]_o$, or the level of an intracellular blocking particle such as Mg. Details of the model and derivations of the relations that define the model are given in the appendices. What follows is a summary of the model and an outline of how the relations predicted by the model can be used to deduce a unique set of model parameters that are consistent with our data.

K^+ Ion dependence

In our model, the relaxation rates are dependent on external K because isomerization only occurs when a

certain fraction of the K binding sites on the channel are occupied. For simplicity we have assumed that all of those sites are structurally equivalent. Although the affinity of the binding sites for K increases upon opening of the channel we have assumed that binding is rapid an always in equilibrium. The binding can then be defined simply by an equilibrium dissociation constant.

Our model postulates that the channel contains a number of binding sites for K and that opening and closing of the channels is restricted to or is predominant at a single level of occupancy. If, at that level of occupancy, m binding sites are occupied by K, and n sites are free, this situation is depicted by Scheme 2:



SCHEME 2

Here, k_+ and k'_+ are the forward rate constants for K binding to a given binding site on the closed and open states of the channel, respectively. The backward rate constants for this binding are k_- and k'_-, respectively. For transitions between each level of occupancy, these rate constants are multiplied by a statistical factor that reflects the number of ways a given transition can occur. Thus, for the binding of the first K ion there are $m + n$ equivalent sites for the ion to chose from. On the other hand, there is only one way in which the transition between the monoliganded stated and the unliganded state can occur. The rate constants a_m and b_m describe the isomerization step between the open and closed state of the channel that is associated with m molecules of K. O_0 and C_0 are open and closed channels that have not bound K. OK_i and CK_i are open and closed channels associated with i molecules of bound K. In Scheme 2 we have only indicated those channels associated with 0, 1, m, and $m + n$, K ions. However, all states of occupancy between 0 and $m + n$ are possible. Because binding rates for K are assumed to be fast relative to the isomerization rates, binding can be defined by equilibrium dissociation constants, $L = k_-/k_+$ and $T = k'_-/k'_+$ for closed and open states respectively.

Such a model allows increase in the level of K ions to increase the rate of opening of the channel because opening requires binding of K. Further binding of K ions to the open channel reduced the probability of occurrence of that state which can close and in this way allows increases in K concentration to slow the rate of closing. That a single isomerization dominates the transition between open and closed states is supported by the observation that voltage jump relaxations exhibit a simple exponential time course during both activation and deactivation. This observation also supports the assumption that binding of K is fast relative to isomerization and that voltage jump relaxations reflect transitions primarily between two predominant channel states, open and closed.

In the Appendix we show that Scheme 2 predicts the following relations between $[K]_o$ and the opening and closing rate constants a' and b':

$$a' = \frac{a}{(1 + K/L)^n(1 + L/K)^m} \quad \text{and}$$

$$b' = \frac{b}{(1 + K/T)^n(1 + T/K)^m} \qquad (3a)$$

where

$$a = a_m[(m + n)!]/m!n! \text{ and } b = b_m[(m + n)!]/m!n!$$

$$\text{also, } b'/a' = (b/a)(T/L)^n[(L + K)/(T + K)]^{n+m}. \qquad (3b)$$

For K to increase activation in Scheme 2, L (the dissociation constant for K binding to the closed channel) must be greater than T (the dissociation constant for K binding to the open channel). In other words, the open state must have a higher affinity for K than the closed state. If L is much greater than T, then at the voltage where activation is half maximal, V_{mid}, and for a range of voltages around V_{mid}, most of the closed channels will not be associated with K whereas most of the open channels will be associated with K. Our data suggests that this situation seems to be the case because for many millivolts positive and negative to V_{mid}, a' and b' are very sensitive to external K; a' increases as external K is increased and b' decreases. This implies that, for voltages around V_{mid}, $a' \ll a$ and $b' \ll b$. It implies further that, $(L/K) \gg 1$ and $(T/K) \ll 1$. Therefore, Eqs. 3a and b can be approximated by,

$$a'(V) = a(V)[K/L(V)]^m \text{ and } b'(V) = b(V)[T(V)/K]^n \qquad (3c)$$

$$\text{also, } b'(V)/a'(V) = [b(V)/a(V)][T(V)]^n[L(V)]^m/K^{n+m}. \qquad (3d)$$

We show below that equations 3c and d can be used to deduce the magnitude of the parameters m and n from the way in which $a'(V)$ changes with K.

Voltage dependence of channel gating

The relaxation rates are voltage dependent because of gating charges associated with the channel that move within the membrane field either prior to or during the isomerization step. Our model considers three types of gating charges, those associated with (a) the isomeriza-

tion step itself, (*b*) the binding of external K, and (*c*) the binding of internal Mg.

The voltage dependence of the dissociation constants that define the binding of K, can be expressed as follows:

$$L(V) = L(0) \exp(zVF/RT) \quad \text{and} \tag{4a}$$

$$T(V) = T(0) \exp(zVF/RT), \tag{4b}$$

where z is the effective gating charge governing binding of K. For simplicity, in these derivations we only consider effective gating charges; it is assumed that the charged particle involved in gating experiences the entire membrane field (see Almers, 1978). F/RT has its standard meaning (see glossary) and, in our experiments, is equal to 1/24.4 mV (T = 10°C). In all subsequent equations, F/RT will be represented by β. Both L and T get bigger (e.g., K becomes less potent) as voltage becomes more positive. The voltage dependence of binding may arise from K moving to a binding site within the membrane field or from access of K to the binding site being dependent on the movement through the membrane field of a charged gating particle intrinsic to the channel.

If the isomerization between open and closed states also is voltage dependent, independently of the binding of K, with $a(V)$ decreasing and $b(V)$ increasing with depolarization then,

$$a(V) = a(0) \exp(-x\beta V) \quad \text{and} \tag{5a}$$

$$b(V) = b(0) \exp(y\beta V), \tag{5b}$$

and

$$\frac{b(V)}{a(V)} = \frac{b(0)}{a(0)} \exp(z'\beta V), \tag{6}$$

where x and y define the influence of membrane field on the gating particles governing the opening and closing isomerization steps respectively and the effective gating charge governing isomerization, z', is equal to $(y + x)$. The parameters x and y are a product of the gating charges involved and the fraction of the membrane field through which they move during gating. Again, for simplicity that fraction is assumed to be 1. Eqs. 4 and 5 combined with Eq. 3 will allow us to deduce the magnitude of the effective gating charges z and z'. The voltage dependence of Mg binding to and blockade of I_{K1} channels is discussed below.

Estimation of model parameters *m* and *n*

Using the equations described above we have derived relations that allow us to estimate the number of K binding sites, m and n, and to place constraints on other parameters of the model. In this section, we outline those relations and how they are used. The derivations of the relations are found in the Appendix.

The slope of the relation between ln (a') and voltage gives information about the number of ion involved in activating the channel. We show in the Appendix (see Eq. A8, *b*) that, assuming Eq. 3*c* is a good approximation,

$$d \ln(a')/dV = -\beta(x + mz). \tag{7}$$

This relation indicates that the voltage dependence of a' is dependent on the summation of two types of gating charges, that governing isomerization (x) and that governing binding of K (mz). The relation between ln (a') and K at a given voltage should have a slope equal to m. Similarly, the relation between steady-state activation and K should have a slope equal to $m + n$. Because we have examined I_{K1} gating in only a few concentrations of K those relations at a given voltage are ill defined. However, it is possible to use all of our data and factor out the voltage dependence of binding of K and of channel isomerization by considering ratios of slopes of observed relations and in this way obtain a best estimate of m, $m + n$, and hence of n. To estimate m, we considered the voltage at which a' has a given value, u, (e.g., $V_{a'=u}$) and how that voltage changes when $[K]_o$ is changed. In the Appendix we show (Eq. A9) that,

$$m = [d \ln(a')/dV]/[\Delta \ln(K)/\Delta V_{a'=u}]. \tag{8}$$

The parameter $(n + m)$ was derived by considering the ratios of two other slopes: (*a*) the slope of the relation V_K vs V_{mid} (e.g., dV_K/dV_{mid}) and (*b*) the slope factor (S) defining the steady-state voltage activation curve. In the Appendix, we show (Eqs. A11 and A14) that,

$$dV_K/dV_{mid} = z + z'/(n + m), \tag{9}$$

and

$$1/S = \beta(z' + z[m + n]). \tag{10}$$

Therefore,

$$1/(n + m) = \beta S(dV_K/dV_{mid}). \tag{11}$$

Influence of an intracellular blocking particle on channel gating

Here we describe how an intracellular charged blocking particle will influence the gating of I_{K1} predicted by the model depicted by Scheme 2. In subsequent discussions, we will equate this blocking particle with Mg but, it should be noted that physiological levels of Ca may also act in a manner analogous to Mg (Mazzanti and DiFrancesco, 1989; Mazzanti and DeFelice, 1990). Oliva et al. (1990) have proposed a model describing the effect of

internal Mg on gating of I_{K1} channels. This model, which is essentially the same as that proposed by Matsuda (1988), was used to account for an observation of excess outward current due to I_{K1} over that predicted by a simple Boltzman two-state model such as Scheme 1. Matsuda (1988) and Matsuda et al. (1989) have presented evidence suggesting that the I_{K1} channel is composed of three equivalent pores in parallel which gate in a concerted fashion but, which can be independently plugged by Mg from the inside and Rb and Cs from the outside. Their evidence suggested further that if any of the bores of a channel complex are occupied by a blocking ion, the other bores cannot close. Oliva et al., 1990 pointed out that because depolarization positive to V_K can favor both the closing associated with voltage dependent gating of I_{K1} channels as well as favoring channel plugging by Mg paradoxically, there can be more outward current in the presence of Mg than otherwise would be expected by gating alone. This result arises because of the presence of partially plugged channels that cannot gate closed.

The physical existence of parallel pores remains to be verified. For example, an alternative explanation for Matsuda's results is that a common channel arises from the association of three or more equivalent subunits each of which possesses a Mg binding site. Because of symmetry, the binding of Mg to any of those sites could reduce single channel conductance to an equivalent extent. Nevertheless, a kinetic scheme describing the effect of Mg on either parallel assemblies of channels or a single channel made from equivalent subunits would be the same.

If the binding of Mg is assumed to be fast relative to isomerization and equivalent for each bore or subunit, it can be defined simply by an equilibrium dissociation constant, J. If r is the number of equivalent binding site for Mg. The relative single channel conductance of each of the open channel forms will be $(r - i)/r$, where i is the number of Mg ions actually associated with the channel.

For simplicity we assume that Mg can only reach its binding site when the channel is in an open state and has no direct effect on the binding of the external K ions that control gating of the channel (e.g., no effect on L and T). Because the channel will only close if none of the r binding sites are associated with Mg, the rate of closing of I_{K1} channels (b') will be reduced in the presence of Mg. If p is the probability that a given bore of the channel is not blocked by Mg then, in the presence of Mg, p^r will be the probability that none of the channel bores are blocked by Mg. As a result, Eq. 3*d* should be modified as follows:

$$b'(V) = p(V)^r b(V)[T(V)/\mathrm{K}]^n \qquad (12)$$

Therefore, $b'(v)$ can decrease with voltage rather than increase, provided p is appreciable in the range of voltage studied, and has a dependence on voltage that is both greater than and in the opposite direction from that of the deactivation rate unmodified by magnesium blockade (see Appendix B).

Cohen et al. (1989) showed that lowering internal K leads to a reversal of the voltage dependence of b', a reduction of the steepness of the steady-state activation curve, and to a decrease in the value of a' and b' at V_{mid}. We show below that the first two of these effects can be explained if lowering K_i enhances the ability of Mg to block the I_{K1} channel. The third action can be explained partially by this action.

Oliva et al. (1990) pointed out that the relation between p and membrane potential could be derived from the degree to which outward current exceeds that expected from the rectification of inward current. They found that the derived value of p was not a simple function of voltage but was in fact related to membrane potential minus the potassium equilibrium potential $(V–V_K)$. This result is predicted if the I_{K1} channel is a multi-ion pore where K can knock out a blocking particle from the channel (Armstrong, 1969; Hille and Schwartz, 1978). Our present results also suggest that the value of p at a given voltage must be increased when external K is increased. We show in Appendix B how, when the dependence of p on $[K]_o$ is considered, Eq. 12 becomes

$$b'(V, \mathrm{K}) = [J(V, \mathrm{K})/\mathrm{Mg}]^r b(V) T(V)^n (\mathrm{K_o})^{rz''-n}/(\mathrm{K_i})^{rz''}, \qquad (13)$$

where z'' is the effective gating charge governing the binding of Mg to the channel. This equation predicts that the relation between ln (b') and K_o will have a slope equal to $rz'' - n$. So that if Mg blockade is appreciable and if rz'' is greater than n then, b' will increase as K_o increases.

GLOSSARY

I_{K1} = current through inwardly rectifying potassium channel
K = potassium
K_o, K_i = external and internal K
O, C = open and closed states of I_{K1} channels
O_T, C_T = sum total of all O and C states
OK_i, CK_i = O and C states associated with i molecules of bound K
L, T = dissociation constants of equivalent K binding sites defining affinity for closed and open channels

J = dissociation constant for binding of internal Mg to open channel

J' = the portion of J that is independent of external K

r = number of binding sites for Mg on the channel

m = minimum number of K ions that must bind to the channel before opening can occur

$m + n$ = total number of equivalent K binding sites on the channel that are involved in gating

a, b = microscopic opening and closing isomerization rate constants

a', b' = macroscopic opening and closing rates

P_g, Q_g = open and closed state probabilities dependent on gating alone

V = membrane voltage

$V_{a'=u}, V_{b'=u}$ = V at which a' and b' have a value equal to u in different K levels

V_{20ms} = $V_{a'=u}$ where $u = 1/20$ ms

V_{mid} = V for half maximal activation of I_{K1}

S = slope factor defining steady state voltage activation curve

$\beta = F/RT$

= Faraday's constant/(gas constant)(absolute temperature)

= 1/24.4 mV under our conditions

z = effective gating charge governing the binding of external K

x, y = effective gating charge governing opening and closing isomerization, respectively

z' = effective gating charge governing isomerization between open and closed states

$= x + y$

z'' = effective gating charge governing channel blockade by internal Mg

METHODS

Methods are described in detail in previous papers (Cohen et al., 1989; Oliva et al., 1990). Briefly, canine Purkinje myocytes were dispersed after exposure to collagenase by titration. Whole-cell recordings were made from those cells using patch electrodes of 2–4 MegaOhms. Electrodes contained in mM: 120 KCl, 5 Na_2 ATP, 1 $MgCl_2$, 5 Hepes, 5 EGTA neutralized to pH 7.2 with ~25 mM KOH. Free Mg was estimated to be 26 μM (see Oliva et al., 1990). External solution with 5 mM K contained in mM: 137 ChCl, 0.5 $CaCl_2$, 0.1 $CdCl_2$, 1 $MgCl_2$, 5 Hepes, neutralized to pH 7.2 with 2 mM KOH. Replacement of external Na by choline reduced inactivation of I_{K1}. Three other levels of external KCl were examined: 12, 42, and 82 mM. In those solutions, 7, 37, and 77 mM KCl was substituted for equal amounts of ChCl. Internal K was lowered by omitting all but 25 mM K in the patch filling solution and replacing it with choline. Dialysis of intracellular K was slow and incomplete so that after 30 min, when records were taken, internal K (estimated from the K reversal potential) was reduced only to ~40 mM. We believe that intracellular Mg equilibrated with pipette Mg at an even slower rate. Matsuda et al. (1987) showed that in cells well dialysed by this pipette solution, instantaneous rectification was abolished. Yet, we continued to see considerable instantaneous rectification even after 1 h of dialysis (see Oliva et al., 1990). All records were made at 9–10°C. Cooling served to slow the kinetics of I_{K1} gating. Unless noted otherwise, all means are expressed as mean ± S.D.

Examples of raw data and of the protocols used in this study can be found in Cohen et al., (1990). In general, membrane potential was held roughly 10 mV positive to V_K. Membrane potential was controlled using an Axopatch 1B (Axon Instruments, Burlingame, CA) voltage clamp amplifier. Activation curves and rates of activation were determined using a two step voltage clamp protocol. The first step was to a range of potentials, this was followed by a step to a fixed potential generally 30 mV negative to V_K. The rate of development of the current during the first step provided information about the kinetics of activation. The magnitude of the time-dependent current during the second step provided information about the degree of steady-state activation attained at the end of the first pulse. Deactivation was measured using a three step protocol. The first step was to a potential 30 mV negative to V_K to activate I_{K1}. The second step was to the potential of interest and was of variable duration. The third step was back to the same potential as the first step. The amount of time-dependent current evoked by the third step relative to the first step indicated the degree of deactivation that occurred during the second step. Deactivation could not be measured directly because outward current through I_{K1} channels was small due to instantaneous rectification.

RESULTS

Number of K ion involved in gating I_{K1} channels

Fig. 1 summarizes the effects of voltage and of potassium on the opening rate constant, a'. This parameter was estimated by dividing the steady-state fractional activation, at a given voltage and K level, by the time constant of the increase in current associated with a voltage step to that voltage (Eq. 2A). Increasing external K shifts the mid point of activation (V_{mid}, *squares*) to more positive potentials without changing the voltage dependence of a'. A very similar situation is observed when internal K is lowered. However, now the voltage associated with a given value of a' (e.g., 1/20 ms, *dashed line*) is much further away from V_{mid}. The lines drawn through the points have a slope of 1/14.6 mV with normal K_i and 1/12.8 mV when K_i is low. These values were estimated by normalizing all values of a' to that at V_{mid} then combining values for all levels of external K.

The relations described in Fig. 1 give a measure of the voltage dependence of a' and suggest that this is independent of the level of external K; increasing external K decreases the opening rate a' without changing its voltage sensitivity. In principle, it should be possible to determine how many K ions cooperate to activate I_{K1} channels (i.e., m) from the relation between the a', and $[K]_o$ at a given voltage. In practice, a' can be accurately measured only in a relatively narrow range of potentials, between $V_K - 5$ mV and $V_K - 40$ mV. Eq. 8 shows how the parameter m can be derived by consider-

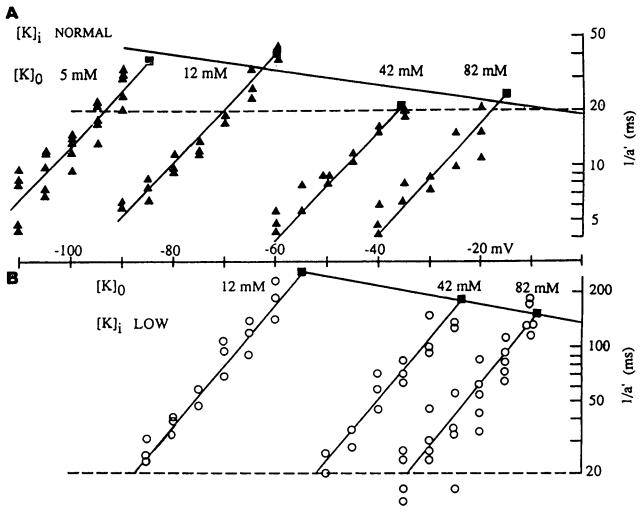


FIGURE 1. Voltage dependence of the rate of opening (a') of I_{K1} channels at different level external K. (A) Normal internal K. The value of a' at a given voltage was calculated from the fraction of I_{K1} channels activated (P_g) at the voltage and the time constant (τ) of the current relaxation associated with I_{K1} activation ($a' = P_g/\tau$). The inverse of a' was plotted against the voltage at which it was recorded. The solid lines drawn through the points have a slope of 1/14.6 mV (see Fig. 3). The filled squares represent the predicted value of a' at the estimated V_{mid}, the voltage for half maximal activation of I_{K1}. The solid line through the squares is a least squares regression with a slope of 1/106 mV. The dashed line indicates the voltages at which the value a' equals 1/20 ms. Values of a' were determined over a range of voltages in four levels of external K: 5, 12, 42, and 82 mM. (B) Low internal K. The same as A, except that the slope of the line through the values of a' was 1/12.8 mV (see Fig. 3) and the slope of the line through the values of a' at V_{mid} was 1/87 mV. The values of a' were determined over a range of voltages in three levels of external K: 12, 42, and 82 mM. Note that a' equals 1/20 ms at a voltage much more negative to V_{mid} when I_i is low than when K_i is normal.

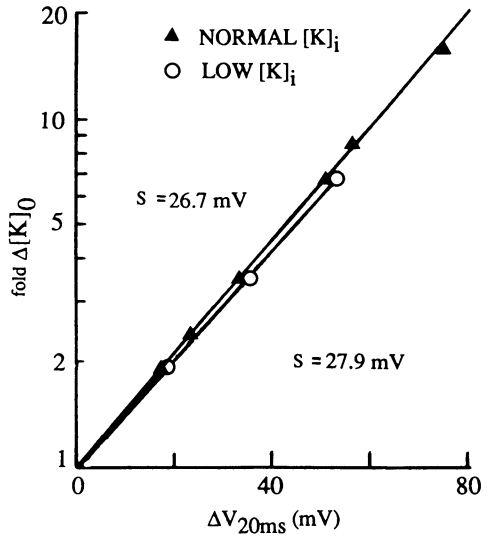


FIGURE 2. Shift in the voltage dependence of a' with changes in external K. Changing external K did not appear to affect the slope of the relation between a' and voltage. This parallel shift was assessed by plotting the change in V_{20ms} (the voltage required to produce a value of $a' = 1/20$ ms) against the fold change in K levels. When K_i is normal, V_{20ms} shifts 26.7 mV per e-fold change in external K. When K_i is low the shift is 27.9 mV per e-fold change in K. Note that some of the larger values in this plot are linear summations of smaller values but are included for completeness.

ing the change in a' with voltage and the change in voltage required to attain a given a' as $[K]_o$ is increased. The ratio of the slopes of those two relations gives the change of a' with $[K]_o$ and is equal to m, the number of K ions that cooperate in opening I_{K1} channels.

The shift in voltage required to obtain a given value of a' (ΔV_{20ms}) caused by changes in external K is plotted against Δ ln (K) in Fig. 2. The relation is almost identical for both low and normal K_i with slopes of 1/26.7 mV and 1/27.9 mV respectively. For normal and low internal K, the ratio of this slope with that describing the relation ln (a') vs V is 1.83 and 2.18, respectively. Both ratios are reasonably close to 2. We will assume therefore, in subsequent calculations that two K ions must bind to the channel before it can open and that this number is not influenced by lowering $[K]_i$.

Steady-state activation curves were calculated as described by Cohen et al. (1989). The mean slope factor, S, obtained by pooling values obtained at all levels of external K when K_i was normal was 5.1 ± 1.6 mV (n = 14). Fig. 3 shows the relation between several critical voltages and external K. Both V_K and V_{mid} shift in parallel as external K is increased. Therefore dV_K/dV_{mid} is equal to 1. In the Theory section, we show that $n + m$ can be predicted if S and dV_K/dV_{mid} are known. Using Eq. 11 we obtain $n + m = 24.4/5.1 = 4.8$. In subsequent

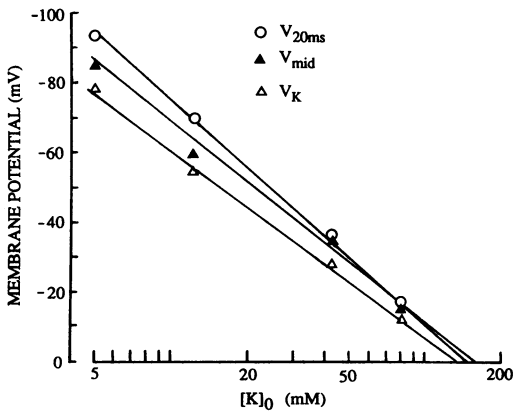


FIGURE 3. Relation between three critical voltages, V_{20ms}, V_{mid}, and V_K, and the concentration of external K. V_{mid} and V_K are mean values. V_{20ms} was determined graphically using the data in Fig. 2.

calculations we will assume that $n + m = 5$. Because we have calculated that $m = 2$, this implies that n, the number of K ions that cooperates to hold the channel open, is equal to 3. However, given the fact that S is not well defined, our data is consistent with values of n ranging from 2 to 5. The slope factor describing the activation curve when K_i is low (6.9 ± 1.8 mV; see Cohen et al., 1989) is greater than when K_i is normal. As will be discussed below, this may arise from a distortion of b' due to an increased sensitivity to channel blockade by internal Mg, rather than as the result of a change in n. We believe that neither n nor m are influenced by lowering $[K]_i$.

In the Theory section, we point out that it is impossible with our data to determine the origin of the voltage dependence of I_{K1} activation. The voltage dependence could arise entirely from direct voltage dependence of K binding, it could arise entirely from voltage dependence of the isomerization between a closed state with a low affinity for K and an open state with a high affinity for K or, it could arise from the combined voltage dependence of those two processes. Some voltage dependence of K binding must exist if the approximation assumed in Eq. 3 is to hold despite 80 mV shifts in V_{mid} and V_K as is observed (see Fig. 1). The observation that the activation rate of V_{mid} decreases as V_{mid} becomes more positive with increasing $[K]_o$ suggests that there is at least some voltage dependence to the isomerization step. The relation between $\ln(a'[V_{mid}])$ vs V_{mid} is predicted by our model to have a slope equal to $\beta(my - x)/(n + n)$ (see Eq. A17). If there were no voltage dependence to the isomerization step and the effective gating charges x and y governing isomerization were therefore equal to zero, that slope should also equal zero. In fact we find the slope to equal 1/106 mV. Substituting in Eq. A17 and assuming that $m = 2$ and $n = 3$, this slope implies that $y = (0.6 + \frac{3}{2}x)$ and therefore y is 0.6 when $x = 0$. Maximal values of x and y can be calculated by considering the situation if there were no voltage dependence to the binding of K (e.g., if $z = 0$). Then, the slope of the relation between $\ln(a')$ and V would equal $-\beta x$ (Eq. 7) giving a maximal value of ~1.8 for x. From Eq. 9 we know that if $z = 0, z' = x + y = n + m$ so that a maximal value of y will be ~3.3. In this way we can estimate that the range of possible values of x and y that are consistent with are data to be 0–1.8 and 0.6–3.3, respectively. Because the $dV_K/dV_{mid} = 1 = z + z'/(n + m)$ (Eq. 9) we can estimate that $z = (0.9 - x/2)$ or $(1.1 - y/3)$ and the range of possible values of z will be 0–0.9. Thus, the values of z, z', x, and y are interdependent; assigning a value to any one of these parameters fixes the values of the other corresponding parameters.

Effects of lowering internal K on the rate of activation of I_{K1}

It is clear from Fig. 1 that lowering internal K slows the rate of activation of I_{K1}. The model proposed in the theory section can be used to explore the origin of this effect. The action does not appear to arise from a change in the degree of voltage or K dependency of I_{K1} gating because both the parameter m and the slope of the relation $\ln(a')$ vs voltage appear little affected by lowering internal K. We believe that the effect arise because of a shift in the voltage dependence of either K binding or channel isomerization.

By definition, $a'(V_{mid}) = b'(V_{mid})$. We can predict from Fig. 1 that when $V_{mid} = 0$ mV $a'(0) = b'(0) = 1/19$ ms when K_i is normal and $a'(0) = b'(0) = 1/140$ ms when K_i is low. From Fig. 3 we can predict that for V_{mid} to equal 0 mV, K_o must be 162 mM when K_i is normal. Similar data indicates that K_o must be 117 mM when K_i is low and $V_{mid} = 0$ mV. Using Eq. 3c our data suggests that $a'(0) = a(0)[K/L(0)]^2$ when $V_{mid} = 0$ mV. Therefore, $L(0)/[a(0)]^{1/2} = 706$ mM $ms^{1/2}$ when K_i is normal and 1,384 mM $ms^{1/2}$ when K_i is low. Similarly, $b'(0) = b(0)[T(0)/K]^3$ so that $T(0)[b(0)]^{1/3} = 61$ mM/$ms^{1/3}$ when K_i is normal and 22 mM/$ms^{1/3}$ when K_i is low. We cannot distinguish between the effects of lowering K_i on isomerization or on binding of K_o but, it is clear that the changes in the parameters governing those processes need not be large to account for the effects of lowering K_i.

Although, we do not know the precise origin of the voltage dependence of I_{K1}, it is instructive to consider two extremes. If the binding of K is not directly voltage dependent (e.g., $z = 0$) and the effect of lowering internal K is due entirely to a shift in the voltage dependence of the opening and closing isomerization rate constants a and b, then $a_N/a_L = 3.8$ and $b_N/b_L = 19.4$. The subscript N and L refer to normal and low intracellular K respectively. Above, we have argued that if $z = 0$, then $x = 1.8$ and $y = 3.3$ In that case then, the change in opening rate when $[K]_i$ is lowered could be accounted for by a +18 mV (e.g., (ln (3.8)/1.8) 24.4 mV) shift in the relation between a and voltage. The change in closing rate could be accounted for by a −22 mV shift in the relation between b and voltage. If only the binding of K is voltage sensitive (e.g., $z' = 0$), then $L_N/L_L = 0.5$ and $T_N/T_L = 2.8$. Under this condition $z = 1$, because V_{mid} shifts in parallel with V_K. The change in L then could be caused by a + 17 mV (e.g., ln [2.0]24.4 mV) shift in the voltage dependence of this dissociation constant. The change in T could be caused by a −24 mV shift. Therefore, with both extreme situations of voltage insensitive isomerization or voltage insensitive binding of K_o, similar shifts in voltage dependence can account

for the change in kinetics. This suggests that similar shifts could account for the changes observed if both binding of K and isomerization were voltage dependent.

It should be noted, that the shifts are unlikely to result from a simple charge screening effect on the membrane field because the V_{mid} is only slightly affected by lowering internal K. In the appendix (Eq. A12), we show how that shift in V_{mid} can be predicted. Using the values derived above, a + 8 mV shift is predicted. The recorded shift is ~ +6 mV (see Cohen et al., 1989). V_K, on the other hand, shifted ~25 mV in the positive direction. There is thus, a dissociation of the shift in V_{mid} and the shift in V_K when K_i is lowered.

Possible influence of internal Mg on the deactivation rate

Cohen et al., 1989 showed that when internal K is normal I_{K1} turns off very quickly following voltage commands positive to V_K. The rate of closing increased with increasing depolarization. However, when K_i is reduced to ~40 mM, the rate of closing at potentials positive to V_K is slow and becomes slower with depolarization (see Fig. 4, and Cohen et al., 1989). In the Theory section, we have shown how this situation can arise as the result of blockade of the I_{K1} channel by internal Mg, provided that the level of blockade of the channel bores by magnesium increases with voltage more steeply than the rate of closing in the absence of magnesium. In terms of our model parameters this will occur when $rz'' > n$. The data in Fig. 4 is consistent with a value of $rz'' = 4.5$. The work of others has suggested that the number of divalent cation binding sites on the channel, r, is equal to 3 (Matsuda, 1988) or 4 (Mazzanti and DiFrancesco, 1989). The parameter z'' predicted by our model thus is consistent with evidence presented by others that Mg binds between 75 and 60% of the way through inwardly rectifying K channels (see Matsuda, 1988; Horie and Irasawa, 1987, 1989).

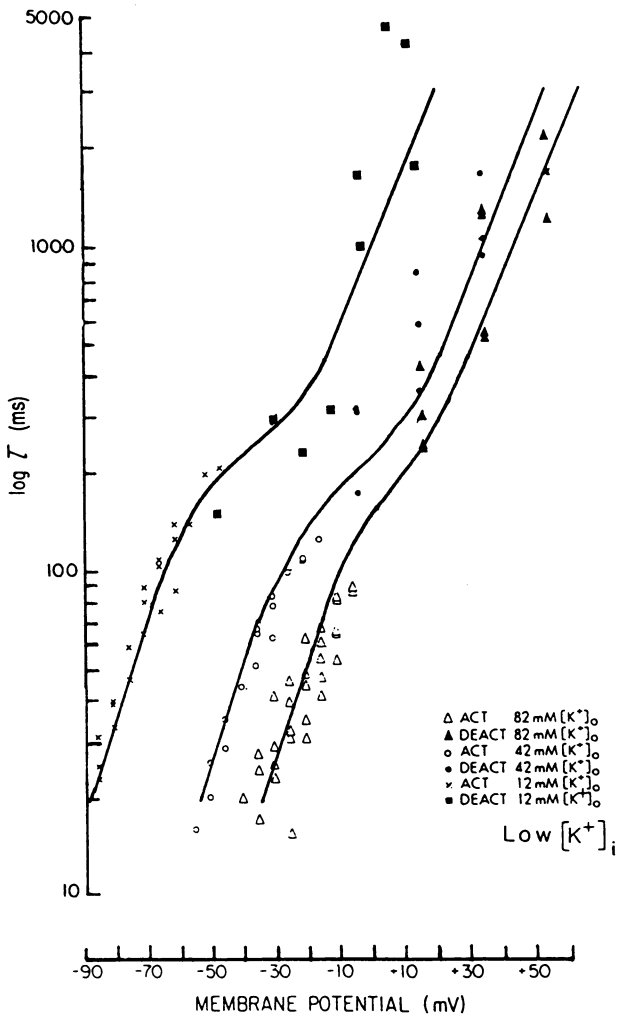


FIGURE 4. Time constants (τ) of current relaxations determined from activation and deactivation protocols with low K_i. τ's were plotted against the voltage at which they were recorded. The pipette filling solution contained 25 mM K and the level of intracellular K was estimated to be around 40 mM. Measurements were made over a range of voltages in three different levels of external K, 12, 42, and 82 mM. Solid lines are values of τ predicted by the model described in 5 using the following parameters. $L(0)/[a(0)]^{1/2}$ and $T(0)[b(0)]^{1/3}$ are as described in the text. We have used $b'(0) = a'(0) = 1/120$ ms when $V_{mid} = 0$ mV to improve the fit rather than 1/140 ms, indicated by Fig. 1. This discrepancy probably reflects the fact that p is not insignificant at V_{mid} when K_i is low. We have set $a(0) = 10a_2(0)$ and $b(0) = 10b_2(0)$ (see Eq. A7*a*, *b*). We derived p(0 mV, 12 mM) using Eq. 12 and found it to equal 0.05 in low K_i. We then used Eq. B1*a* to calculate that p(0 mV, 42 mM) = 0.27 and p(0 mV, 82 mM) = 0.47.

DISCUSSION

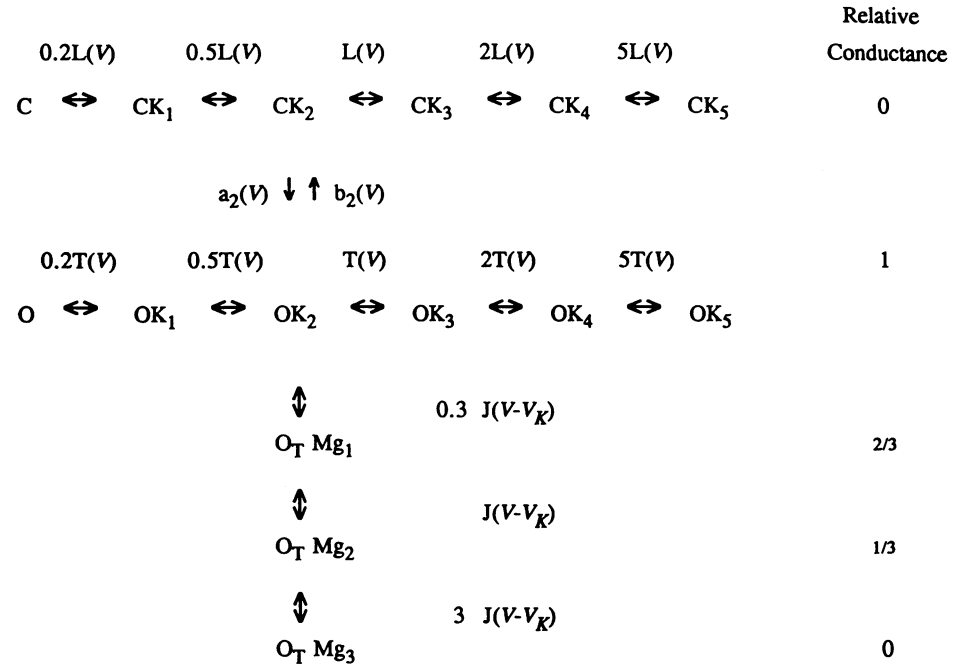
Our data suggests that occupancy of several (~2) K binding sites are required before an I_{K1} channel will open and binding of several more (~3) K ions to the open channel will slow the rate of closing of that channel. Our model says nothing about whether those two sets of K binding sites are physically equivalent to one another or represent distinct domains on the channel. Whatever the case, our results do suggest that the affinity of all binding sites for K on open channels is higher than that for closed channels since binding of K promotes the open state.

With regards to the binding of K_o to open channels it is of interest to consider the "occupancy hypothesis" which proposes that the association of the permeating ion with a binding site within the channel can slow the

closing of that channel. Such effects of permeating ions or channel duration have been observed for a variety of channels (see Ascher et al., 1978; Chesnoy-Marchais, 1985; Matteson and Swenson, 1986; Zilberter et al., 1988). It is notable that Moczydlowski et al. (1985) have shown that activation kinetics of calcium-activated K channels are affected by changes of surface charge to the same degree as a channel conductance suggesting that the regulatory binding site for calcium on that channel experiences a field similar to that experienced by the pore.

Fig. 5 summarizes the model that we have used to interpret our results and lists the parameters of the model that are consistent with our results. This model combines aspects of both K-activated K channel models of inward rectification and of blocking particle models. In keeping with the conclusion of Oliva et al. (1990), the model suggests that Mg blockade has the paradoxical

MODEL



PARAMETERS

	Normal $[K]_i$		Low $[K]_i$
m	2		
n	3		
r	3		
z	0.9	0	
z', (x+y)	0.6	5.0	
x	0	1.7	
y	0.6	3.3	
nz+y	3.3		
z+z'/(n+m)	1.0		
z''	1.5		
$L(0)/a_2(0)^{1/2}$	706 mM $ms^{1/2}$		1260 mM $ms^{1/2}$
$T(0)b_2(0)^{1/3}$	61 mM/$ms^{1/3}$		23 mM/$ms^{1/3}$
p(0mV, 12 mM)	0.22		0.05

FIGURE 5. Kinetic scheme describing the model. Abbreviations are defined in the text and in the glossary. Numbers on the left give the relative conductance of the various states of the I_{K1} channel.

result of interfering with the closure of I_{K1} channels promoted by depolarization. Thus, an enhancement of Mg blockade as the result of lowering internal K can account for the reversal of the voltage dependence of I_{K1} deactivation observed when K_i is lowered. We estimate that a five-fold increase in the potency of Mg for blocking the channel when K_i is lowered combined with the changes in opening and closing rates at 0mV could account for the pronounced changes in the voltage dependence of deactivation of I_{K1}.

It is known that lowering K_i will reduce the maximal conductance of inwardly rectifying K channels (see Hagiwara and Yoshi, 1979). If the reduced K conductance arises because single-channel K conductance is decreased, this could explain the apparent enhancement of Mg blockade when K_i is reduced. Also, the apparent shifts in the relations between voltage and the opening and closing rates suggest that a shift in the voltage dependence of the dissociation constant for Mg blockade also is possible. If z'' had a value of 1.5, a shift in the voltage dependence of J by 26 mV would cause a five-fold change in J at a given voltage. Experiments involving single-channel measurements where the potency of Mg for channel blockade can be determined directly will be required before these issues can be resolved.

Our data, as they stand, do not allow us to distinguish the degree to which the voltage dependence of activation is due to voltage dependent isomerization or voltage dependent binding of K ions to sites that regulate isomerization. Therefore, a range of values for the parameters that govern those functions in our model are consistent with our data. In principle, however, it should be possible to narrow that range of possible values. For example, in the case of the calcium activated K channel, at extremes of voltage, the rates of channel opening and closing reach limiting values that are independent of voltage and calcium levels. At these extremes of voltage, the calcium binding sites are saturated and isomerization rates dominate the opening and closing processes. Such results for calcium activated K channels, suggest that voltage dependent binding of calcium to regulatory sites entirely accounts for the voltage dependence of channel gating (see Moczydlowski and Latorre, 1983). It is of note that Kurachi (1985) found that the opening and closing rates of I_{K1} channels also approached limiting values 50 mV away from V_K.

The postulated influence of Mg on I_{K1} channels allow us to account for a number of puzzling results concerning the kinetic and steady state properties of I_{K1}. For example, Saigusa and Matsuda (1988) reported that lowering K_i had no apparent effect of I_{K1} gating properties other than those predicted from the change in V_K. Neither the τ at V_{mid} nor the slope factor of the steady-state activation curve appeared to be affected much by lowering K_i. In contrast, we (Cohen et al., 1989; this study) found dramatic effects of lowering K_i. However, it is clear that in the study by Saigusa and Matsuda (1988), who studied small ventricular myocytes, internal Mg levels were greatly reduced by dialysis whereas in our studies using large purkinje myocytes, we were unable to affect the cell Mg levels by buffering pipette Mg with ATP (see also Oliva et al., 1990). We still have no explanation for why they did not observe the decrease in I_{K1} activation rate observed here upon lowering K_i. In support of our observation, it is of note that, Hagiwara and Yoshi (1979) also observed a decrease in activation rate of the inward rectifier of sea urchin eggs upon lowering internal K.

Our results are consistent with recent observations by Ishihara et al. (1989), who showed that increasing levels of intracellular Mg can lead to open channel blockade of I_{K1} channels and reduced rates of deactivation of I_{K1} at positive potentials. The influence of K_o on the potency of Mg blockade is essential for our model to account for our data. This property has been observed previously for the ATP sensitive inward rectifier of heart cells (see Horie et al., 1987) and for I_{K1} channels (see Oliva et al., 1990; Matsuda, 1991*a*,*b*). It will be important to verify the influence of internal and external K on Mg blockade directly, using single-channel analysis.

Recently, Matsuda (1991*b*) has reported that lowering K_i had no effect on the potency of Mg to block I_{Ki} channels. Resting levels of Mg in myocytes are thought to be around 0.5 mM (Murphy et al., 1989). If one accepts this value as the level found in the purkinje myocyte studied here, the p(0 mV, 12 mM) values of 0.22 and 0.05 estimates for high and low K_i conditions (see Fig. 5) correspond to K_D's of 140 and 28 μM, respectively, for binding of intracellular Mg to bores of the I_{K1} channel. A K_D for intracellular Mg of ~25 μM was estimated by Ishihara et al., 1990 for I_{K1} channels recorded under the conditions of V_m = 0mV, K_o = 14 mM, K_i = 140 mM and Mg_i = 0.5 mM. As expected from this high potency for Mg and in contrast to what is observed in more intact preparations (see above), they observed that under those conditions the rate of deactivation of I_{K1} decreased with depolarization despite the normal levels of K_i. The results of Matsuda (1991*b*) suggested that Mg was even more potent at blocking I_{K1} channels than observed by Ishahara et al. (1990). In both studies, access to the intracellular compartment was achieved by crushing one end of the myocyte. Possibly, factors as yet to be determined that regulate the sensitivity of I_{K1} channels to Mg_i and K_i are lost during the very effective dialysis of myocyte contents associated with that method.

Silver and Decoursey (1990) have shown that pulmo-

nary artery endothelial cells express a K current very similar to I_{K1}. They showed that lowering intracellular Mg had small but significant effects on the gating of the inward rectifier K current in those cells. However, the changes that they did see when Mg was lowered, such as increased rate of deactivation and reduced instantaneous current, are consistent with our model. They further confirmed that Mg in fact increases outward current through I_{K1} channels. Our model predicts that much more dramatic effects of lowering Mg would have been observed if intracellular K was also low.

Deactivation by depolarization positive to V_K ensures that the I_{K1} conductance will contribute little to K accumulation that can occur in the restricted extracellular spaces of the myocardium. Nevertheless, the gating of I_{K1} remains very effective at stabilizing the cardiac diastolic potential close to V_K (Pennefather and Cohen, 1990). Disturbances in the gating of I_{K1} are likely therefore, to be arrythmogenic. In this paper we have proposed that levels of K_o, K_i, Mg_i and Ca_i all can interact in modulating the time and voltage dependence of I_{K1} gating. Changes in the levels of these ions will therefore lead to changes in I_{K1} gating. It is well known that levels of K_o can vary during cardiac activity due to K accumulation in restricted extracellular spaces (see Cohen and Kline, 1982). Substantial reductions of K_i have been reported during ischemia (Dresdner et al., 1987) or hypoxia (Baumgarten et al., 1981). Local levels of Ca_i can vary between 50 nM and 50 μM (Smith and Augustine, 1989). There have been reports that levels of Mg_i may be regulated by hormones (Romani and Scarpa, 1990). All of these changes can occur independently of one another and are of sufficient magnitude to affect I_{K1}.

To appreciate the physiological and pathophysiological significance of these influences, a model is needed. The model presented here, originally proposed by Matsuda (1988) and elaborated upon by Oliva et al. (1990) and Ishihara et al. (1989) seems to be consistent with most observations on I_{K1} kinetics that have been made to date. However, to use a model to interpolate between observations and to generate testable hypotheses, constraints must be placed on the parameters defining the model. In this paper, we have shown how such constraints can be developed by simply examining the influence of K_o and voltage on the time course of current relaxations associated with voltage jumps. Using this approach, we have shown also that the dramatic effects of lowering K_i on I_{K1} kinetics (Cohen et al., 1989, this paper) can be explained by relatively small changes in a number of the model parameters. The next step will be to use more direct measurements of model parameters (e.g., single-channel analysis; measurements of intracellular Mg) to confirm the validity of our conclusions.

APPENDIX A

General derivation

Scheme 2 in the theory section predicts that

$$CK_i(L_1^*L_2^*\cdots{}^*L_i) = (C_0)(K^i) \qquad (A1a)$$

$$OK_i(T_1^*T_2^*\cdots{}^*T_i) = (O_0)(K^i), \qquad (A1b)$$

where C_0 and O_0 are states of the channel not associated with K and L_i and T_i are dissociation constants for the i^{th} gating ion of K that binds to form CK_i and OK_i respectively.
Therefore,

$$C_T = C_o + C_oK/L_1 + \cdots + C_oK^{m+n}/L_1^*L_2^*\cdots{}^*L_{m+n} \qquad (A2a)$$

and,

$$O_T = O_o + O_oK/T_1 + \cdots + O_oK^{m+n}/T_1^*T_2^*\cdots{}^*T_{m+n}. \qquad (A2b)$$

Therefore,

$$\frac{CK_i}{C_T} = \frac{K^i/(L_1^*L_2^*\cdots{}^*L_i)}{1 + K/L_1 + \cdots + K^{n+m}/(L_1^*L_2^*\cdots{}^*L_{n+m})} \qquad (A3a)$$

$$\frac{OK_i}{O_T} = \frac{K^i/(T_1^*T_2^*\cdots{}^*T_i)}{1 + K/T_1 + \cdots + K^{n+m}/(T_1^*T_2^*\cdots{}^*T_{n+m})}, \qquad (A3b)$$

where C_T and O_T refer to the sum of all closed and open states respectively.

Now, if the $m + n$ binding sites are equivalent and noninteracting with microscopic dissociation constants L and T in the closed and open states respectively, then because of statistical considerations,

$$L_i = \frac{iL}{m + n + 1 - i} \qquad (A4a)$$

and

$$T_i = \frac{iT}{m + n + 1 - i}. \qquad (A4b)$$

For example, if $i = 4$ and $m + n = 5$ then $i/(m + n + 1 - i) = 4/2$. This makes sense because there are four ways for the channel to lose one K ion and there are two ways for the fourth K ion to associate with the channel. Therefore, from Eq. A2,

$$C_T = C_o(1 + K/L)^{m+n} \text{ and } O_T = O_o(1 + K/T)^{m+n} \qquad (A5a)$$

$$CK_m = C_o\left[\frac{(m + n)K}{1L} * \frac{(m + n - 1)K}{2L} * \frac{(m + n - 2)K}{3L} * \cdots * \frac{(n + 1)K}{mL}\right] \qquad (A5b)$$

$$= C_o\frac{[(m + n)!]}{n!m!} * (K/L)^m. \qquad (A5c)$$

Similarly,

$$OK_m = C_o\frac{[(m + n)!]}{n!m!} * (K/T)^m. \qquad (A5d)$$

Scheme 2 predicts that the macroscopic rates of opening, a', and closing b' will be such that

$$a' = a_m C K_M / C_T \tag{A6a}$$

and

$$b' = b_m O K_m / O_T. \tag{A6b}$$

Combining Eqs. A5 and A6, one obtains,

$$a' = \frac{a}{(1 + K/L)^n (1 + L/K)^m} b' = \frac{b}{(1 + K/T)^n (1 + T/K)^m} \tag{A7a, b}$$

where $a = a_m N$ and $b = b_m N$ and $N = [(m + n)!]/m!n!$

also,

$$b'/a' = (b/a)(T/L)^n[(L + K)/(T + K)]^{n+m}. \tag{A7c}$$

Now for K to activate the channel, $L > T$. Also, for many millivolts positive and negative to V_{mid}, the voltage where current is activated half maximally, a' and b' are very sensitive to external K, a' increases as external K is increased and b' decreases. This implies that $a' \ll a$ and $b' \ll b$. Also, that $(L[V_{mid}]/K) \gg 1$ and $(T[V_{mid}]/K) \ll 1$. Therefore, for voltages around V_{mid}.

$$a'(V) = a(V)[K/L(V)]^m \text{ and } b'(V) = b(V)[T(V)/K]^n \tag{A7d, e}$$

also,

$$b'(V)/a'(V) = [b(V)/a(V)][T(V)^n]\,[L(V)^m]/K^{n+m}. \tag{A7f}$$

Number of K_o was involved in promoting activation

From Eq. A7*d* we know that

$$\frac{d \ln (a')}{dV} = \frac{d \ln (a)}{dV} - \frac{nd \ln [(L + K)/L]}{dV} - \frac{md \ln [(L + K)/K]}{dV}$$

$$= \frac{d \ln (a)}{dV} + \frac{nd \ln (L)}{dV} - \frac{nd \ln (L + K)}{dV} - \frac{md \ln (L + K)}{dV}$$

$$= \frac{d \ln (a)}{dV} + \frac{nd \ln (L)}{dV} - \frac{(m + n)d(L)}{(L + K)dV}$$

$$= \frac{d \ln (a)}{dV} + \frac{nd \ln (L)}{dV} - \left\{\frac{(n + m)L}{L + K}\right\} \frac{d \ln (L)}{dV}$$

$$= \frac{d \ln (a)}{dV} + [n - (n + m)L/(L + K)] \frac{d \ln (L)}{dV}.$$

Combining with Eqs. 4a and 5a

$$\frac{d \ln (a')}{dV} = \beta(-x + z[n - (n + m)L/(L + K)]) \tag{A8}$$

if $L \gg K$

$$\frac{d \ln (a')}{dV} = -\beta(x + zm). \tag{A8}$$

Similarly, for a given value of a' equal to an arbitrary value u, where $a'(V_1, K_1) = a'(V_2, K_2) = u$ at different levels of $[K]_o$:

$$a(0)K_1^m \exp [(x + mz)\beta V_1] = a(0)K_2^m \exp [(x + mz)\beta V_2],$$

therefore, $m\Delta \ln (K) = -\Delta\beta V_{a'=u}(x + mz)$ combining with Eq. A8,

$$m = [d \ln (a')/dV]/[\Delta \ln (K)/\Delta V_{a'=u}] \tag{A9}$$

Relation between voltage for half activation (V_{mid}) and [K]

At the half activation voltage, $a'(V_{mid}) = b'(V_{mid})$. Combining this equality with Eqs. 4, 6, and A7*f* we obtain:

$$\ln (M/(K)^{n+m}) = -\beta V_{mid}(z(n + m) + z') \tag{A10}$$

where

$$M = [b(0)/a(0)][T(0)]^n[L(0)]^m,$$

therefore;

$$(n + m) \ln (K) = \beta V_{mid}(z[n + m] + z') + \ln (M),$$

subtracting $(n + m) \ln (K_i)$ from both sides gives

$$(n + m)\beta V_K = \beta V_{mid}(z[n + m] + z') + \ln (M/(K_i)^{n+m})$$

and

$$V_K/V_{mid} = z + z'/(n + m) + \ln (M/(K_i)/\beta(n + m),$$

therefore;

$$dV_K/dV_{mid} = z + z'/(n + m) = [dV_K/d \ln (K)]/[dV_{mid}/d \ln (K)] \tag{A11}$$

Eq. A10 suggests that V_{mid} is independent of internal [K]. However, if V_{mid} does change when K_i is changed in such a way that z, z' and $n + m$ remain unchanged then

$$(z[n + m] + z')\beta\Delta V_{mid} = \Delta \ln (M) = \Delta(\ln [b(0)/a(0)][T(0)]^n[L(0)]^m \tag{A12}$$

If the isomerization constant or the K dissociation constant are unaffected by lowering $[K]_i$ there should be no change in V_{mid}. For V_{mid} to change in parallel with V_K when $[K]_i$ is changed, where $\beta\Delta V_K = \Delta \ln (K_i)$, then M would have to change in such a way that $\Delta \ln (M) = [z(n + m) + z']\Delta \ln (K_i)$.

Number of K ions involved in steady-state activation

We know that $P_g = (1 + b'/a')^{-1}$, therefore, $(1/P_g) - 1 = b'/a'$. Again, combining Eqs. 6 and A7*f* we obtain:

$$\ln (b'/a') = \ln (M/[K]^{n+m}) + \beta V(z[n + m] + z'). \tag{A13}$$

A regression of $\ln([1/P_g] - 1)$ versus voltage will have a slope, equal to the inverse of the slope factor, S, of the voltage activation curve.
therefore,

$$1/S = \beta(z[n + m] + z'). \quad \text{(A14)}$$

Combining Eqs. A14 and A11 we obtain,

$$1/(n + m) = S\beta(dV_K/dV_{mid}). \quad \text{(A15)}$$

Relation between activation rate at the half maximal activation and V_{mid} when K_o is changed

From Eq. A7*d*, we know that

$$\frac{d \ln (a'[V_{mid}])}{dV_{mid}} = \frac{md \ln (K)}{dV_{mid}} - \frac{md \ln (L)}{dV_{mid}} + \frac{d \ln (a[V_{mid}])}{dV_{mid}},$$

$$= \frac{md \ln (K)}{dV_{mid}} - \frac{x\beta dV_{mid}}{dV_{mid}} - \frac{-mz\beta dV_{mid}}{dV_{mid}},$$

because $\beta d V_K = d \ln (K)$;

$$\frac{d \ln (a'[V_{mid}])}{dV_{mid}} = m\beta dV_K/dV_{mid} - \beta x + mz\beta, \quad \text{(A16)}$$

combining Eqs. A16 and A11 we obtain,

$$\frac{d \ln (a'[V_{mid}])}{dV_{mid}} = \beta[m(z + z'/[n + m]) - x - mz],$$

recalling that $z' = (x + y)$

$$d \ln (a'[V_{mid}])/dV_{mid} = \beta(my - nx)/(n + m). \quad \text{(A17)}$$

APPENDIX B

Influence of magnesium on channel gating

Let P be the probability that an open bore of the I_{K1} channel is not blocked by Mg. If p is a function of $(V - V_K)$ and reflects occupancy of a binding site for Mg where the binding step is controlled by an effective gating charge z'', then,

$$1/p = 1 + Mg/\{J(V_K) \exp [-z''\beta(V - V_K)]\}, \quad \text{(B1)}$$

where $J(V_K)$ is the value of the Mg dissociation constant, J, for that site at a given level of $[K]_o$. Because, $\beta V_K = \ln (K_o/K_i)$,

$$J(V_K) \exp (-z''\beta[V - V_K]) = J(V_K) \exp (-z''\beta V)(K_o/K_i)^{z'}.$$

If we define $J(V, K) = J(V_K) \exp (-z''\beta V)$ then,

$$1/p = 1 + Mg/\{J(V, K)(K_o/K_i)^{z'}\}. \quad \text{(B1}a\text{)}$$

When $p \ll 1$,

$$p = (K_o/K_i)^{z'}[J(V, K)/Mg], \quad \text{(B1}b\text{)}$$

substituting Eq. B1*b* into Eq. 12 of the Theory section one obtains,

$$b'(V, K) = [J(V, K)/Mg]^r b(V)T(V)^n(K_o)^{(rz''-n)}/(K_i)^{rz''}. \quad \text{(B2)}$$

Thus, when $p \ll 1$, the voltage dependence of b' can be reversed provided the change of $(J[V, K])^r$ with voltage is greater than that of $b'(V)T(V)^n$. This will occur when $rz'' > y + nz$ because,

$$d \ln (b')/dV = rd \ln [J(V, K)]/dV + d \ln [b(V)]/dV + nd \ln [T(V)]/dV$$

$$= (-rz'' + y + nz)\beta. \quad \text{(B3)}$$

Also, provided that $J(V_K)$ is the same at all levels of $[K]_o$, or in other words that the relation between p and voltage shifts exactly in parallel with V_K, then it can be shown in a manner similar to the derivation of Eq. A9 that,

$$d \ln (b')/dK = [d \ln (b')/dV]/[\Delta \ln (K_o)/\Delta V_{b'=u}] = rz'' - n. \quad \text{(B4)}$$

Finally, it should be noted, that the apparent dependence of p on $(V - V_K)$ may be secondary to J being a function of K_o as the result of an allosteric interaction rather than an direct interaction between K and Mg in the pore.

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