Idiosyncratic Gating of HERG-like K⁺ Channels in Microglia

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ABSTRACT A simple kinetic model is presented to explain the gating of a HERG-like voltage-gated K⁺ conductance described in the accompanying paper (Zhou, W., F.S. Cayabyab, P.S. Pennefather, L.C. Schlichter, and T.E. DeCoursey. 1998. J. Gen. Physiol. 111:781–794). The model proposes two kinetically distinct closing pathways, a rapid one favored by depolarization (deactivation) and a slow one favored by hyperpolarization (inactivation). The overlap of these two processes leads to a window current between −50 and +20 mV with a peak at −36 mV of ~12% maximal conductance. The near absence of depolarization-activated outward current in microglia, compared with HERG channels expressed in oocytes or cardiac myocytes, can be explained if activation is shifted negatively in microglia. As seen with experimental data, availability predicted by the model was more steeply voltage dependent, and the midpoint more positive when determined by making the holding potential progressively more positive at intervals of 20 s (starting at −120 mV), rather than progressively more negative (starting at 40 mV). In the model, this hysteresis was generated by postulating slow and ultra-slow components of inactivation. The ultra-slow component takes minutes to equilibrate at −40 mV but is steeply voltage dependent, leading to protocol-dependent modulation of the HERG-like current. The data suggest that “deactivation” and “inactivation” are coupled through the open state. This is particularly evident in isotonic Cs⁺, where a delayed and transient outward current develops on depolarization with a decay time constant more voltage dependent and slower than the deactivation process observed at the same potential after a brief hyperpolarization.

KEY WORDS: gating kinetics • ion channels • deactivation • inactivation • erg

INTRODUCTION

In the previous paper (Zhou et al., 1998), we described in a microglial cell line, MLS-9, a K⁺ conductance resembling that generated by the human ether-à-go-go-related gene (HERG)† in most respects. Two notable differences include an almost complete absence of outward current during depolarizing pulses in symmetrical K⁺ salts and the existence of very slow gating around −40 mV. Here we describe a simple kinetic model that describes the data reasonably well.

The model postulates two kinetically distinct closing pathways, one favored by hyperpolarization leading to closed states that equilibrate slowly with the open state, and the other favored by depolarization that equilibrates rapidly with the open state. Because the slowly equilibrating closed states behave like classical absorbing inactivated states, it is convenient to consider these channels to be in a resting state at depolarized potentials and to activate and then inactivate upon hyperpolarization. Overlap in the voltage dependence of these two closing pathways leads to a standing window current between −50 and +20 mV that may be important for microglial biology. In addition, equilibration of inactivated states appears to take minutes at potentials around the peak of the window current yet occurs much more rapidly at more positive and negative potentials. This gating behavior leads to steady state levels of HERG-like current that are not simply voltage dependent but also dependent on prior voltage history. Our model thus predicts that oscillations in microglial membrane potential can have frequency- or use-dependent effects if the frequency of oscillation is faster than the slow gating steps (see MacDonald et al., 1991; Jassar et al., 1993).

The predictions of our sequential model are contrasted with those of an uncoupled model that assumes independent activation and inactivation. Although such a model can account for steady state behavior and rapid gating of the current, under certain conditions the two models diverge and the experimental data supports the coupled sequential model. This is particularly evident in isotonic Cs⁺, where a delayed and transient outward current develops on depolarization with a decay time constant more voltage dependent and slower than the deactivation process observed at the same potential after a brief hyperpolarization.

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† Abbreviation used in this paper: HERG, human ether-à-go-go-related gene (erg) and its product.
We also compare our model to another coupled sequential model developed recently by Wang et al. (1997) to describe gating of HERG channels expressed in oocytes. Although steady state inactivation appears similar in the two models, steady state activation in oocytes appears to have a half-maximal potential that is 40 mV more positive. This difference accounts for the substantially greater outward current component observed in asymmetrical K+ salines with HERG expressed in oocytes compared with the HERG-like current in microglia. A hybrid model constructed with activation kinetics modified to generate a 40-mV shift in the voltage–activation curve and inactivation kinetics identical to the model of Wang et al. (1997) predicts steady state currents that overlap reasonably well with our observed data. However, this hybrid model does not predict the observed slow gating phenomena such as hysteresis in the availability curves.

**Materials and Methods**

**Experimental results.** Experimental results reported here were obtained with the same cells and experimental techniques described in the previous paper (Zhou et al., 1998).

**Simulations.** The simulated responses were generated using a commercially available software package called Axon Engineer (Axon Software, Madison, WI). Details are described elsewhere (Pennefather and DeCoursey, 1994).

**Theory**

Various terminologies have been used to describe HERG and HERG-like K⁺ currents. In describing our results (Zhou et al., 1998), we define activation as the fast onset of current with hyperpolarization, and inactivation as the slower closing that follows this opening. The term, deactivation, is used to describe the fast closing that occurs at depolarized potentials. We will show below that our data is well described by Scheme I. Scheme I postulates two kinetically distinct pathways of channel closing: a rapidly equilibrating pathway leading to Cᵢ, a closed state favored by depolarization, and a slowly equilibrating pathway leading sequentially to slowly and ultra-slowly equilibrating closed states (Cₛ and Cᵤ) favored by hyperpolarization. At −80 mV, most of the channels reside in the slowly gating closed states that behave functionally like inactivated states. On depolarization after inactivation, they revert back to the open state from which they rapidly deactivate to state Cᵢ. As a result, little or no tail current is generated during the depolarizing pulse. However, on repolarization, those channels that have had an opportunity to convert to state Cᵢ activate rapidly before slowly converting back to state Cᵢ.

\[
\begin{align*}
Cᵢ & \xrightarrow[k₁₀]{k₀₁} \text{OPEN} \xleftarrow[k₁₀]{k₀₁} Cᵢ & Cₛ & \xrightarrow[k₂₃]{k₂₃} Cᵤ
\end{align*}
\]

rapid \quad slow \quad ultra-slow (scheme I)

In showing that Scheme I adequately describes our data, we have not engaged in systematic parameter optimization strategies. Rather, we have simply used our experimental data to suggest approximate values for the rate constants and their voltage dependence and have shown that these nonoptimized parameters predict responses that are close to what are observed. The rate constants used in our simulations of Scheme I and the experimental measurements used in constraining them are listed in Table I. The rate constants k₀₁, k₁₀, k₁₂, and k₂₃ are anchored by current relaxations at a voltage range where the model predicts that the major determinant of the current relaxation after a voltage jump is one of those rate constants. The voltage dependence of the change in measured time constant of the current relaxation in that voltage range (determined as described in Zhou et al., 1998) is used to extrapolate the rate constant into ranges of potential where the particular rate constant is not the prime determinant of gating kinetics.

The information enclosed in brackets beside the rate equations listed in Table I indicate the relaxation time constant, the range of potentials, and the data set used to define the rate equations. These first four rate constants (k₀₁, k₁₀, k₁₂, k₂₃) are the prime determinants of gating observed with standard protocols used to define activation and deactivation (see Fig. 2 A), and inactivation and recovery from inactivation (see Fig. 2 B) of the HERG-like current. The rate constants of ultra-slow inactivation are based on the hysteresis observed in measuring normal inactivation. These rates, k₃₂ and k₄₅, were established by adjusting them so that simulated results roughly matched the experimental observations.

For comparison, we have considered a gating model (Scheme II) in which deactivation and inactivation are independent. In

<table>
<thead>
<tr>
<th>Table I</th>
<th>Rate Constants Used in Simulation of Schemes I–III</th>
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<tbody>
<tr>
<td>Scheme I (coupled model)</td>
<td>k₀₁ = 110 \exp[-1.0(F/RT) \left(V + 120\right)] s⁻¹</td>
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<tr>
<td></td>
<td>(τᵢᵢᵢ, 120 to −80 mV; see Fig. 7, Zhou et al., 1998)</td>
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<tr>
<td></td>
<td>k₁₀ = 66/[1.0 + \exp[-0.70(F/RT) \left(V + 20\right)]] s⁻¹</td>
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<td></td>
<td>(τᵢᵢᵢᵢ, 20 to 80 mV; see Fig. 7, Zhou et al., 1998)</td>
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<tr>
<td></td>
<td>k₁₂ = 10 \exp[-1.5(F/RT) \left(V + 120\right)] s⁻¹</td>
</tr>
<tr>
<td></td>
<td>(τᵢᵢᵢᵢᵢ, −120 to 80 mV; see Fig. 8, Zhou et al., 1998)</td>
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<tr>
<td></td>
<td>k₂₃ = 4 \exp[1.0(F/RT) \left(V − 40\right)] s⁻¹</td>
</tr>
<tr>
<td></td>
<td>(τᵢᵢᵦᵦ, 40 to 0 mV; see Fig. 8, Zhou et al., 1998)</td>
</tr>
<tr>
<td></td>
<td>k₃₂ = 0.005 \exp[-1.7(F/RT) \left(V + 30\right)] s⁻¹</td>
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<td>(use dependent inactivation, hysteresis)</td>
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<tr>
<td></td>
<td>k₄₅ = 0.005 \exp[3.0(F/RT) \left(V + 30\right)] s⁻¹</td>
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<tr>
<td></td>
<td>(use dependent recovery, hysteresis)</td>
</tr>
<tr>
<td>Scheme II (independent model)</td>
<td>k₀₁, k₁₀, k₁₂, k₂₃, k₄₅ (same as Scheme I)</td>
</tr>
<tr>
<td>k₂₁ = 4 \exp[1.0(F/RT) \left(V + 1\right)] s⁻¹</td>
<td></td>
</tr>
<tr>
<td>Scheme III (modified from Wang et al., 1997)</td>
<td>k₀₁ (same as Scheme I)</td>
</tr>
<tr>
<td></td>
<td>Kₑ₁ = 2.930.8266 \exp[0.64(F/RT) \left(V + 1\right)] s⁻¹</td>
</tr>
<tr>
<td></td>
<td>(double values used by Wang et al., 1997)</td>
</tr>
<tr>
<td></td>
<td>(All other rate equations shifted by −5 mV)</td>
</tr>
<tr>
<td></td>
<td>Kₑ₁ = 0.0689 \exp[-1.1(F/RT) \left(V + 5\right)] s⁻¹</td>
</tr>
<tr>
<td></td>
<td>Kₑ₂ = 13.733 \exp[1.0(F/RT) \left(V + 5\right)] s⁻¹</td>
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<tr>
<td></td>
<td>Kₑ₅ = 36.778 s⁻¹</td>
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<tr>
<td></td>
<td>Kₑ₂₃ = 23.761 s⁻¹</td>
</tr>
<tr>
<td></td>
<td>Kₑ₃₄ = 47.092 \exp[-1.6(F/RT) \left(V + 5\right)] s⁻¹</td>
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<tr>
<td></td>
<td>Kₑ₄₅ = 22.348 \exp[0.5(F/RT) \left(V + 5\right)] s⁻¹</td>
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V is membrane potential in millivolts, F is Faraday’s constant, R is the gas constant, T is temperature.
that case, the open probability is defined as the product of the
proportion of channels that are neither deactivated ($N$) nor inac-
tivated ($H$) and the time course of current relaxation at a particu-
lar potential reflects whichever transition process is rate limiting at
that potential.

We use the same rate equations to define Scheme II as Scheme
I, except that $k_{21}$ is shifted by 40 mV to compensate for the lack
of coupling between activation and inactivation implicit in Scheme I,
giving $k'_{21}$ (see Table I). The appropriateness of this modification
is shown in Fig. 1, where the steady state proportions of inacti-
vated, open, and deactivated channels predicted by Schemes I–III
are plotted. The shift in $k_{21}$ to $k'_{21}$ in Scheme II allows the curve
describing steady state inactivation to superimpose almost exactly
with the curve predicted by Scheme I.

Scheme III is based on kinetic parameters derived by Wang et
al. (1997) to describe HERG currents expressed in Xenopus oo-
cytes. The behavior of HERG-like currents in microglia could be
mimicked with fairly minor adjustments to their parameters, with
the exception of the opening rate $k_{01}$. Apparently, the steady state
activation curve is shifted negatively ~40 mV in microglia, prima-
}

**Figure 1.** Simulation of steady state inactivation, activation, and
deactivation predicted by the three kinetic schemes. The curves
that decline with depolarization represent the proportion of chan-
nels ($N$) that at steady state are open, in the absence of inactiva-
tion. The curves that ascend represent the proportion of channels
($H$) that at steady state are not in inactivated ($C_i$ states), and are
available to open. The bell-shaped curves, which are significant be-
tween ~50 and +20 mV, represent the steady state open probabil-
ity that is manifested as window current. The lines leading from
the numeral 3 point to curves predicted by Scheme III.

\[
\begin{align*}
(C_r & \xrightarrow{k_{01}} N \xleftarrow{k_{10}} ) \times (H \xrightarrow{k_{12}} C_s \xrightarrow{k_{31}} C_{us}) \\
\text{rapid} & \quad \quad \quad \text{slow} \quad \text{ultra-slow} \\
\text{(scheme ii)} & \\end{align*}
\]

The rate equations describing this scheme are listed in Table I.

**Figure 2.** (A) Simulation of the experiment illustrated in Fig. 5
A of Zhou et al. (1998). A brief 25-ms step from a holding potential
of 0 to $-120$ mV is followed by 500-ms steps to test potentials that
increased in 20-mV increments from $-100$ to $+80$ mV (omitting 0
mV). (B) Simulation of the experiment illustrated in Fig. 7 of
Zhou et al. (1998). A 300-ms step from 0 to $-120$ mV is followed at
increasing intervals by an identical test step. The recovery of the re-
sponse to the second step reflects recovery from inactivation.
et al. (1998). The K⁺ conductance was activated by a brief pulse to −120 mV from holding potential, V_hold = 0 mV, followed by a step to a range of potentials. The test current at most potentials decayed rapidly as channels closed, in terms of our model, predominantly into state Cr. The time constant of decay, \( \tau \text{tail} \), was moderately voltage dependent, becoming faster at large positive potentials. At moderately negative potentials, the current no longer decayed completely, consistent with a window current existing in this voltage range. At larger negative potentials, the current decayed anomalously slowly, and the simulations show that this is due to channels entering the inactivated or slowly equilibrating Cs states, rather than the Cr or resting state. The turn-on of current during the brief hyperpolarizing step defines \( \tau \text{act} \), this becomes faster as the hyperpolarizing step is made more negative, but the size of the outward tail seen upon repolarization is not increased since activation is maximal by −120 mV (data not shown).

The simulations illustrated in Fig. 2 B are driven by the protocol used to generate the data in Fig. 7 of Zhou et al. (1998). A hyperpolarizing pulse to −120 mV from 0 mV is paired with a second pulse of the same type with an incrementing interval. The decline of the current during the 300-ms hyperpolarizing pulse reflects inactivation of the channels that activated rapidly after the voltage step. The time constant of this inactivation (\( \tau \text{i} \)) increases with hyperpolarization and at −120 mV is determined primarily by \( k_{12} \). That the channels are inactivated in the classic sense defined by Hodgkin and Huxley (1952) and not simply resting is demonstrated by the fact that little current can be activated by the second hyperpolarizing pulse after short delays. The time constant of recovery from inactivation (\( \tau \text{recovery} \)) is monitored by the increase in activatable current with increasing delays between the paired pulses. At 0 mV, this recovery time course is dominated by \( k_{21} \).

Because the rate equations defining ultra-slow inactivation were based on limited types of data, we explored how sensitive the simulated currents were to changes in these parameters. In addition, it is useful to know how the existence of an ultra-slow inactivation mechanism would manifest itself in experimental data. Arbitrarily multiplying \( k_{23} \) and \( k_{32} \) by a factor of four had little detectable effect on simulations driven by the protocols illustrated in Fig. 2 (data not shown). However, a second ultra-slow inactivation state must then be postulated to account for hysteresis and use dependence observed in certain protocols (see below). There is little difference in the predictions of the coupled or the independent models of activation and inactivation (Schemes I and II) for protocols such as are illustrated in Fig. 2 (data not shown). However, differences become apparent in the presence of Cs⁺, and under other conditions that accentuate the idiosyncratic gating properties of HERG-like currents.

**Gating in the Presence of High [Cs⁺]o**

The peculiar gating behavior previously observed in Cs⁺ solutions for HERG channels exogenously expressed in *Xenopus* oocytes (Schönherr and Heinemann, 1996) also occurs in microglia cells. When \( V_{\text{hold}} \) was 0 mV, small time-dependent inward Cs⁺ currents were seen in isotonic Cs⁺ saline, which were ~5–10%...
of the amplitude of K⁺ currents in the same cell in K⁺
saline (data not shown). This suggests that Cs⁺ perme-
ability is 10% that of K⁺, a conclusion supported by the
observed reversal potential with 160 mM Cs⁺ outside
and 160 mM K⁺ inside. As a result of this change in re-
versal potential, outward currents are more apparent.

Fig. 3 A shows that when Vhold was −80 mV, outward
currents were observed at positive potentials, evidently
reflecting K⁺ efflux from the cell. These outward cur-
rents develop with a voltage-dependent delay and show
a steeply voltage-dependent decay phase. Both the ris-
ing and falling phases become markedly faster at more
positive potentials. By the end of the 1-s depolarizing
pulses, most of the channels had closed. After a brief
step to −80 mV to reopen a large proportion of chan-
nels, steps back to positive potentials elicited normal
tail currents, which decayed much more rapidly than
did the currents during the first depolarization.

Fig. 3 B shows that this behavior is well described by
the coupled gating model (Scheme I). The presence of
the weakly permeant Cs⁺ was modeled by reducing ex-
tracellular K⁺ to 10 mM and the rates of activation (k_{01})
and deactivation (k_{10}) were reduced by a factor of two
while retaining the same voltage dependence, as was
found experimentally (data not shown); otherwise, the
same parameters were used as in the previous simula-
tions. A small leak current is included to facilitate com-
parison with the real data in Fig. 3 A.

In terms of Scheme I, the rapid deactivation of the
second transient outward current is a simple tail cur-
rent reflecting conversion from state O to Cr and is
dominated by rate constant k_{10}. The decay phase of the
first transient outward current is a convolution of the
latency for channel recovery from inactivation and k_{10}
(see Aldrich et al., 1983). Entry into the deactivated Cᵣ
d state occurs in a coupled sequential fashion such that
the channel must pass through several intermediate
states (including the open state) while recovering from
inactivation. As a result, there is a delay in the develop-
ment of the transient outward current during the first
pulse, and the outward current decays much more
slowly than expected from τtail measured at the same
potential (i.e., during the second pulse).

In our simulations, this delayed transient outward
current was prominent only when conversion between
the inactivated states (Cs, Cus) and the resting state (Cr)
was constrained to pass through the open state (i.e., a
linear-coupled system). If rapid closing and slow closing
were assumed to be uncoupled and independent
(Scheme II; compare Faravelli et al., 1996), the transient
outward current was also observed but showed little de-
lay and had a final rate of decay that was simply domi-
nated by k_{10} much like the decay of the second pulse
(Fig. 3 C). Because the slower gating process is more
steeply voltage dependent than the faster one, cou-
pling imparts this steep voltage dependence to the rate
at which outward current decays during the first pulse
(Fig. 3, A and B). The uncoupled model, Scheme II,
predicts that this decay rate will exhibit the same mod-
est voltage dependence of the fast process (Fig. 3 C).
Therefore, in subsequent simulations we consider only
coupled sequential models (Schemes I and III).

Use Dependence of Current Availability

The experiment depicted in Fig. 4 illustrates the neces-
sity of postulating a second inactivated state and sug-
gests an explanation for hysteresis observed in the avail-
ability measurements (Fig. 4 E; Zhou et al., 1998). Iden-
tical test pulses to −120 mV were applied from different
V_{hold}, as labeled. When V_{hold} was initially 0 mV (Fig. 4 A)
or more positive, the conductance was fully available;
i.e., all channels were in the rapidly equilibrating resting
state Cr and the test current during the pulse to −120
mV was maximal. 1 min after changing V_{hold} was changed to
a moderately negative potential (−40 mV), the test cur-
rent evoked by stepping to −120 mV was still large (Fig.
4 B). During subsequent pulses (Fig. 4, C and D), the test
current was attenuated by >80%. These four records are
superimposed on the right (Fig. 4 F). In contrast, when
V_{hold} was initially −80 mV where all of the channels were
in inactivated states Cs and Cus (Fig. 4 G), the test current
1 min after changing V_{hold} to −40 mV was quite small
(H). During a subsequent pulse, the test current in-
creased somewhat (Fig. 4 I). Again, the four records are
superimposed at the end of the row in Fig. 4 J.

The key observation is that when V_{hold} was changed to
−40 mV from the positive voltage range, in which Cs;
predominate, there was very little decrement of avail-
ability even after 1 min (Fig. 4, A vs. B). There are two
implications: (a) conversion to Cs states (i.e., inactiva-
tion) proceeds exceedingly slowly at −40 mV (see also
Fig. 6 in Zhou et al., 1998); and (b) inactivation devel-
ops in a sequentially coupled fashion from the open
state (conversion from Cr to Cus occurs through O and
Cs). Although some channels are open at −40 mV, as
can be seen from the distinct inward window current in
Fig. 4 B, the open probability is low. The observed time
constant of equilibration under these circumstances
will be slowed by a factor approximately equal to the in-
verse of the open probability (i.e., P_{open}⁻¹) (Bernasconi,
1976; MacDonald et al., 1991). The same argument
holds for equilibration between Cs and Cus. A single hy-
perpolarizing test pulse opens many channels, “short-
circuiting” this slow equilibration so that a pseudo-equili-
librium can be reached much more rapidly.

The predictions of Scheme I for this protocol are
shown in Fig. 5. The model reproduces the use depend-
ence fairly accurately. At least two Cs states were needed
to reproduce the very slow equilibration observed at −40
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mV, as well as the kinetics and voltage dependence observed at more negative and positive potentials. Indeed, the interaction between test pulse frequency and the establishment of the steady state response places important constraints on the rate equations defining the equilibration between \( C_s \) and \( C_{us} \). Subtle differences remain between experimental and simulated results (for example, in Fig. 5 B the first response in the train is slightly bigger than subsequent responses while the reverse is true in the experimental results), suggesting that there may be more than two inactivated states. Nevertheless, the simple model described by Scheme I is remarkably robust in predicting the responses of the HERG-like current to diverse voltage protocols.

During standard tail current measurements, anomalously slow closing at large negative potentials was observed (Fig. 5 A in Zhou et al., 1998). The idea that this slow decay was due to inactivation is explored in the experiment depicted in Fig. 6. A brief 20-ms command to \(-120 \text{ mV}\) from a holding potential of 0 mV, followed by a 180-ms command to a given test potential is repeated with a frequency of 1 Hz. When the tail current was measured at \(-100 \text{ mV}\) (where it decayed anomalously slowly, see Fig. 2 A), there was a use-dependent build up of inactivation during repeated pulses (Fig. 6 A). In contrast, when the pulse sequence eliciting a tail current at \(+40 \text{ mV}\) was repeated at \(\approx 1 \text{ Hz}\) (Fig. 6 B), there was little or no accumulation of inactivation. When the protocol in Fig. 6 A was repeated with a longer interval between pulses (10 s), the use dependence was greatly reduced (Fig. 6 D). The use dependence seen in Fig. 6 A is mimicked by our model (Fig. 6 C), as is the lack of use dependence for the protocols in Fig. 6, B and D (data not shown).
Hysteresis in Steady State Availability and Window Current Measurements

The voltage dependence and magnitudes of the rates governing ultra-slow inactivation were deduced from the use-dependent protocols (Figs. 4 and 6). Scheme I, incorporating these parameters, predicted the observed hysteresis of availability measurements obtained with incrementing or decrementing conditioning commands 20 s in duration. Scheme I, incorporating these parameters, predicted the observed hysteresis of availability measurements obtained with incrementing or decrementing conditioning commands 20 s in duration. The voltage commands are plotted below the current traces. In A, $V_{\text{hold}} = 0$ mV. After a 60-s step to $-40$ mV, availability is tested by a 300-ms step to $-120$ mV. This test step is repeated four times at 30-s intervals. The membrane voltage is then returned to 0 mV, and 10 s later a command to $-120$ mV measures maximal availability. In B, $V_{\text{hold}} = -80$ mV. Again, after 60 s at $-40$ mV, availability is tested by a 300-ms step to $-120$ mV, which is repeated four times at 50 s intervals. The membrane voltage is then returned to 0 mV, and 10 s later a command to $-120$ mV measures maximal availability.

Figure 5. Simulation of history dependence of availability. Scheme I predicted results (A and B) that were quite similar to those shown in Fig. 4, E and F, respectively. On the left, the full protocol is shown, while on the right the responses to the test pulses are superimposed and plotted on an expanded time base. The voltage commands are plotted below the current traces. In A, $V_{\text{hold}} = 0$ mV. After a 60-s step to $-40$ mV, availability is tested by a 300-ms step to $-120$ mV. This test step is repeated four times at 30-s intervals. The membrane voltage is then returned to 0 mV, and 10 s later a command to $-120$ mV measures maximal availability. In B, $V_{\text{hold}} = -80$ mV. Again, after 60 s at $-40$ mV, availability is tested by a 300-ms step to $-120$ mV, which is repeated four times at 50 s intervals. The membrane voltage is then returned to 0 mV, and 10 s later a command to $-120$ mV measures maximal availability.

Figure 6. Use-dependent inactivation. A voltage pulse protocol (above each set of records) like that used to elicit tail currents in Fig. 2 A (and Fig. 5 A in Zhou et al., 1998) was repeated and the first five current records are plotted. With a test pulse to 100 mV repeated at 0.91 Hz, there was substantial accumulation of inactivation (A). This result is qualitatively mimicked in our simulations (C). When the rapid 0.91-Hz rate was used with a test pulse to $+40$ mV, no accumulation of inactivation was observed (B). In D, the voltage protocol in A was repeated at a slower rate (0.1 Hz) and no build up of inactivation was observed. This result was also mimicked by simulations using Scheme I (not shown). The calibration bars in D also apply to A and B. The peak test currents from these simulations are plotted in Fig. 7, C and D (Schemes I and III, respectively), for comparison with the actual data in Fig. 4 C of Zhou et al. (1998). On the decrementing course of this protocol, both schemes predict similar results. Availability remains constant until the command potential drops below 0 mV and becomes negligible by the time the steps reach $-80$ mV. However, the predictions of the two schemes diverge for the increasing limb. With Scheme III, no hysteresis is seen, while Scheme I predicts hysteresis comparable with that observed experimentally. For Scheme I, the midpoint of a Boltzmann distribution ($V_{1/2}$) was $20$ mV more positive on the way up and the slope was somewhat steeper than on the way down.

The calculated window currents, derived from $P_{\text{open}}$ at the end of each 20-s sojourn at $V_{\text{hold}}$ (including a linear leak to facilitate comparison with Fig. 4 D) are plotted in Fig. 7, E and F (Schemes I and III, respectively). Once again, on the decrementing course of this protocol, both schemes predict similar results: the apparent
steady state current at $V_{\text{hold}}$ increases to a peak at $-40$ mV and disappears at $-80$ mV. On the way up, Scheme I predicted substantial hysteresis, and with Scheme III there was no hysteresis.

The parameters used in our simulations to obtain this match were derived by trial and error. However, our simulations predict that greater constraints on ul-
when depolarizing pulses elicit little or no observable outward current.

**HERG-like currents in microglia are influenced by an ultra-slow inactivation process.** Comparison of our model with others revealed another difference. To explain several aspects of our data, it was necessary to postulate the existence of an ultra-slow inactivation process. Manifestations include incomplete recovery from inactivation and pronounced hysteresis in the measurement of quasi–steady state inactivation and window current. The existence of an absorbing closed state with extremely slow equilibration in the voltage range near or slightly positive to a normal resting potential range would have significant effects on the physiological behavior of these channels. The slow kinetics of the inactivation process effectively introduces a lag in the feedback between voltage and gating. At a large negative potential, all of the channels are inactivated and recover very slowly with moderate depolarization. However, the steep voltage dependence of the recovery kinetics means that a strong depolarization would greatly enhance the availability of HERG-like channels and facilitate repolarization. The slow and incomplete inactivation at moderately negative potentials would permit sustained K⁺ current that would persist tens of seconds even at physiological membrane potentials, which in these cells appears to be \( \sim -40 \text{ mV} \).

**Nomenclature**

The HERG channel has been described either as a depolarization-activated K⁺ channel with anomalously rapid inactivation at positive potentials (Shibasaki, 1987; Trudeau et al., 1995; Spector et al., 1996; Smith et al., 1996), or a channel that activates and then inactivates upon hyperpolarization (Bauer et al., 1990; Dousmanis and Pennefather, 1992; Arcangeli et al., 1995; Trudeau et al., 1995; Bauer et al., 1996; Ho et al., 1996; Hu and Shi, 1997; Weinsberg et al., 1997). The former terminology stems from the finding that HERG underlies a component of \( I_{Kr} \) (Sanguinetti et al., 1995), a “delayed rectifier” current in cardiac muscle. As pointed out by Faravelli et al. (1996), these two viewpoints are speculative—they use different terms to describe identical phenomena, hence the choice of nomenclature is semantic. The fast gating mechanism opens the channels upon hyperpolarization. The slow mechanism opens channels upon depolarization. In the steady state, the channels close at either extreme of voltage. In their original description of the phenomena of activation and inactivation, Hodgkin and Huxley (1952) defined “inactivation” as the slower gating process. Because the Gestalt of HERG-like currents in symmetrical K⁺ solutions is of a channel conducting large inward currents and only small outward currents, HERG-like currents in various cells have invariably been described as inactivating inward-rectifier currents. Nevertheless, several rationales have been presented for describing the rapidly equilibrating closed state as the inactivated state and designating HERG channels as outward rectifiers.

**Analogy of properties.** Tetraethylammonium (TEA⁺) effects can be used to distinguish between N- and C-type inactivation (Choi et al., 1991), the former inhibited by internal and the latter by external TEA⁺. Because external TEA⁺ slows fast closing of HERG channels at positive potentials, this closing has been considered analogous to C-type inactivation (Smith et al., 1996). However, internal quaternary ammonium ions interfere with the closing of delayed-rectifier channels at negative potentials (Armstrong, 1969); thus, the effect of external TEA⁺ is precisely what one would predict if HERG, like KAT1 (Anderson et al., 1992; Cao et al., 1995; Hoshi, 1995), were a K⁺ channel with functionally inverted gating machinery. The slowing of HERG closing by increased \([K⁺]_o\) (Wang et al., 1996) is at first reminiscent of both delayed rectifiers and inward rectifiers, in which closing is slowed by increased \([K⁺]_o\). However, because both closing and inactivation of depolarization-activated delayed-rectifier K⁺ channels are slowed by elevated \([K⁺]_o\), this property cannot be used to distinguish between these gating processes.

HERG channels lack an inactivation process like other K⁺ channels. The possibility of N-type inactivation has been ruled out by mutagenesis studies (Schönherr and Heinemann, 1996; Spector et al., 1996; Smith et al., 1996). A hallmark of C-type inactivation of K⁺ channels (as well as Na⁺ channels) is that inactivation is essentially voltage independent and derives its apparent voltage dependence from coupling to activation. Both gating processes of HERG channels are distinctly voltage dependent (Wang et al., 1996; Zhou et al., 1998) and, therefore, neither can reasonably be described as C-type inactivation. These differences from known mechanisms of inactivation suggest that a distinct mechanism exists for HERG channels, and that terminology may as well be based on function.

**Which gating process is more labile?** Traditionally, inactivation is thought of as being more labile than activation. The rapid channel closing at positive potentials (here called deactivation) can be removed by substitution of a single amino acid presumed to be in the outer vestibule of the pore (Schönherr and Heinemann, 1996). Similarly, the stability of the open state of the closely related eag channel is greatly enhanced by a single amino acid substitution, resulting in an effectively voltage independent (and open) channel (Tang and Papazian, 1997). Both gating mechanisms therefore exhibit molecular lability.

The inactivation (slow closing) of HERG-like channels at negative potentials is labile in its native molecu-
lar state as well. Arcangeli et al. (1995) remarked on the heterogeneity in the steady state inactivation of HERG-like currents in neuroblastoma cells, with $V_{1/2}$ ranging from $-40$ to $0$ mV. We observed lability in this process as well, with a few cells exhibiting much slower and less pronounced inactivation during hyperpolarizing pulses. Complete removal of extracellular divalent cations removes inactivation of HERG-like currents in neuroblastoma cells (Faravelli et al., 1996) and slows inactivation of $I_{K_r}$ in rabbit SA node cells (Ho et al., 1996). Both gating mechanisms of HERG-like channels are rather labile, and thus this property is not particularly enlightening.

Structural comparisons. There are some similarities in the primary amino acid sequence and proposed secondary structure of HERG and depolarization-activated K$^+$ channels. However, there is just 15% homology with Shaker channels and slightly higher with KAT1 (Warmke and Ganetzky, 1994). Both HERG channels and depolarization-activated K$^+$ channels have six putative membrane-spanning domains, but so does the hyperpolarization-activated inward-rectifier K$^+$ channel (KAT1) in plants (Anderson et al., 1992; Cao et al., 1995). Although clearly distinct from the animal inward rectifier family, HERG functionally resembles plant inward rectifiers. Regardless of structural considerations, there is no unique molecular definition of activation and inactivation beyond simply whatever mechanism is found to be responsible for gating processes that were already named on the basis of function. Several radically different mechanisms have been found to account for inactivation. Likewise, activation may arise from a variety of molecular mechanisms in different types of channels. In the absence of compelling reasons to do otherwise, we prefer to use the classical Hodgkin-Huxley definitions and describe HERG-like channels in microglia as existing in a resting state at depolarized potentials, and as activating and inactivating on hyperpolarization.

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Notes Added in Proof. Two recent papers have shown that fast and slow closing (what we have called deactivation and inactivation) can be modified apparently independently of one another. Zhou et al. (Zhou, A., Q.P. Xu, and M. Sanguinetti. 1998. A mutation in the pore region of HERG K$^+$ channels expressed in Xenopus oocytes reduces rectification by shifting the voltage dependence of inactivation. J. Physiol. (Camb.), 509:129–137) showed how a point mutation in the pore region of the channel (S631A) shifts the voltage range over which deactivation occurs without affecting inactivation. Ho et al. (Ho, W.-K., I. Kim, C.O. Lee, and Y.E. Earm. 1998. Voltage-dependent blockade of HERG channels expressed in Xenopus oocytes by external Ca$^{2+}$ and Mg$^{2+}$. J. Physiol. (Camb.), 507:631–638) showed that reducing divalent cation levels could slow inactivation without affecting deactivation. Nevertheless, we find that both effects can be well described by modified versions of Scheme I, the coupled model (results not shown). As pointed out above, both independent and coupled models can predict similar findings, but only a coupled model can account for the results shown in Fig. 3 A. Additional evidence that the structure of HERG differs radically from Kv channels is its coassembly with minK, which may in fact line the conduction pathway (Tai, K.-K., and S.A.N. Goldstein. 1998. The conduction pore of a cardiac potassium channel. Nature. 391:605–608).

REFERENCES


rent in rabbit sino-arterial node cells by external Ca\textsuperscript{2+} and Mg\textsuperscript{2+}. J. Physiol. (Camb.). 494:727–742.


