

Fast Inactivation Causes Rectification of the I_{Kr} Channel

PETER S. SPECTOR,* MARK E. CURRAN,*[†] ANRUO ZOU,*[‡] MARK T. KEATING,*^{§||} and MICHAEL C. SANGUINETTI*[‡]

From the *Cardiology Division, [†]Eccles Program in Human Molecular Biology and Genetics, [§]Department of Human Genetics, ^{||}Howard Hughes Medical Institute, University of Utah, Salt Lake City, Utah 84112

ABSTRACT The mechanism of rectification of HERG, the human cardiac delayed rectifier K^+ channel, was studied after heterologous expression in *Xenopus* oocytes. Currents were measured using two-microelectrode and macropatch voltage clamp techniques. The fully activated current-voltage (I - V) relationship for HERG inwardly rectified. Rectification was not altered by exposing the cytoplasmic side of a macropatch to a divalent-free solution, indicating this property was not caused by voltage-dependent block of outward current by Mg^{2+} or other soluble cytosolic molecules. The instantaneous I - V relationship for HERG was linear after removal of fast inactivation by a brief hyperpolarization. The time constants for the onset of and recovery from inactivation were a bell-shaped function of membrane potential. The time constants of inactivation varied from 1.8 ms at +50 mV to 16 ms at -20 mV; recovery from inactivation varied from 4.7 ms at -120 mV to 15 ms at -50 mV. Truncation of the NH_2 -terminal region of HERG shifted the voltage dependence of activation and inactivation by +20 to +30 mV. In addition, the rate of deactivation of the truncated channel was much faster than wild-type HERG. The mechanism of HERG rectification is voltage-gated fast inactivation. Inactivation of channels proceeds at a much faster rate than activation, such that no outward current is observed upon depolarization to very high membrane potentials. Fast inactivation of HERG and the resulting rectification are partly responsible for the prolonged plateau phase typical of ventricular action potentials. Key words: cardiac • HERG • long QT syndrome • potassium

INTRODUCTION

The duration of cardiac action potentials is determined primarily by the magnitude of outward currents activated upon membrane depolarization. Ventricular action potentials of most mammals have a prolonged depolarized state, the plateau phase. Outward currents during the plateau phase are small because repolarizing K^+ currents either inactivate rapidly (e.g., transient outward current, I_{to}), preferentially conduct inward rather than outward current (e.g., inward rectifier, I_{K1}),¹ or activate slowly (e.g., delayed rectifier, I_K). In many mammals, including humans, I_K is the sum of two distinct currents, I_{Ks} and I_{Kr} (Sanguinetti and Jurkiewicz, 1990; Wang et al., 1994). I_{Ks} activates very slowly and has an ohmic current-voltage (I - V) relationship; I_{Kr} also activates relatively slowly, but more rapidly than I_{Ks} , and

the fully activated I - V relationship inwardly rectifies (Sanguinetti and Jurkiewicz, 1990). It was proposed that rectification of I_{Kr} results from rapid inactivation (Shibasaki, 1987; Sanguinetti and Jurkiewicz, 1990). Thus, I_{Kr} is characterized by all three properties (rapid inactivation, inward rectification, and slow activation) that reduce macroscopic conductance at plateau potentials.

A decrease in I_{Kr} by class III antiarrhythmic drugs can cause long QT syndrome and induce the arrhythmia, torsade de pointes (Roden, 1988). Long QT syndrome can also be inherited. We recently showed that chromosome 7-linked LQT is caused by mutations in *HERG* (Curran et al., 1995), a gene that encodes the human cardiac I_{Kr} channel (Sanguinetti et al., 1995). Thus, a decrease in I_{Kr} magnitude, either by block with drugs or mutations in *HERG*, can prolong cardiac repolarization and increase the risk of arrhythmias. These findings demonstrate the pivotal role of I_{Kr} in normal repolarization of human ventricular myocardium.

The I - V relationship of I_{Kr} in myocytes, and HERG channels expressed in oocytes, has a negative slope conductance at positive membrane potentials. The mechanism of I_K rectification was first studied in iso-

Address correspondence to Michael Sanguinetti, Ph.D., University of Utah, EIHC, Bldg. 533, Room 4220, Salt Lake City, UT 84112. Fax, (801) 585-3501; E-mail, mike.sanguinetti@genetics.utah.edu

¹Abbreviations used in this paper: I_K , delayed rectifier K^+ current; I_{Kr} , rapid delayed rectifier K^+ current; I_{Ks} , slow delayed rectifier K^+ current; I_{K1} , inward rectifier; IRK , inward rectifier K^+ .

lated rabbit atrial node cells (Shibasaki, 1987). Shibasaki suggested that rectification results from rapid, voltage-gated inactivation that occurs at a rate faster than channel activation. The evidence for this mechanism was indirect: an initial rapid increase in current before the slower decline of deactivating tail currents. The hook in these tail currents was proposed to represent recovery from channel inactivation that occurred during the preceding membrane depolarization. Similar hooks were observed in tail currents of HERG expressed in oocytes (Sanguinetti et al., 1995; Trudeau et al., 1995). The onset of current inactivation has not been measured. Rectification of I_{Kr} could also result from block of outward current by intracellular molecules, such as Mg^{2+} or polyamines, as demonstrated for inward rectifier K^+ channels (Vandenberg, 1987; Ficker et al., 1994; Lu and MacKinnon, 1994), and as proposed for a component of I_K in chick atrial cells (Shrier and Clay, 1986).

In this study, we investigate the mechanism of rectification of HERG, the human I_{Kr} channel, expressed in *Xenopus* oocytes. We show that rectification of HERG is caused by an intrinsic property, voltage-gated inactivation, and not voltage-dependent block by a soluble intracellular molecule.

MATERIALS AND METHODS

Molecular Biology

The HERG cDNA expression construct in the pSP64 transcription vector (Promega Corp., Madison, WI) and synthesis of complementary RNA (cRNA) was as previously described (Sanguinetti et al., 1995).

An NH_2 -terminal deletion mutant of HERG was prepared. A forward primer, 5'-TTC TTG AAG CTT CTC AGG ATG GAC CGT GAG ATC ATA GCA CCT A-3', containing a HindIII site, 6 basepairs of 5' untranslated sequence, an ATG, and 22 basepairs of HERG coding sequence (amino acids 355–361), was synthesized and used in PCR reactions with reverse primer, 5'-GTC CGC GCC CAG GGA CAG GAC-3', to generate a fragment containing unique HindIII and BstEII restriction sites. This fragment was digested with HindIII and BstEII, gel purified, and ligated into HindIII/BstEII-digested wild-type (WT) HERG in the pSP64 expression vector. The resulting construct codes for a protein lacking amino acids 2–354 of HERG.

cRNA Injection and Voltage Clamp of Oocytes

Isolation and maintenance of *Xenopus* oocytes and injection with cRNA was performed as described (Sanguinetti et al., 1995). Stage V and VI oocytes were injected with 50 nl of cRNA encoding HERG (0.125 ng/nL). Currents were recorded (2–4 d after injection) with an amplifier (TEV-200; Dagan Corp., Minneapolis, MN) using standard two-microelectrode voltage clamp techniques as described (Sanguinetti et al., 1995). For most experiments, oocytes were bathed in a solution containing the following (in mM): 94 NaCl, 4 KCl, 2 $MgCl_2$, 0.1 $CaCl_2$, and 5 HEPES, pH 7.6. To record macropatch currents, pipettes were filled with

a solution containing the following (in mM): 40 KCl, 54 NaCl, 2 $MgCl_2$, 0.1 $CaCl_2$, and 5 HEPES, pH 7.4; the bathing solution contained 120 KCl, 10 HEPES, and 5 EGTA, pH 7.4. Specific voltage pulse protocols are described in Results and in the figure legends.

Data Analyses

pCLAMP software (version 6.2; Axon Instruments, Foster City, CA) was used to measure current amplitudes and fit current tracings to exponential functions. Exponential fits to current (I) traces were performed using the Chebyshev technique to determine the time constants (τ) and amplitudes (A) for single ($I = A_0 + A_1 e^{-I/\tau}$) or biexponential ($I = A_0 + A_1 \cdot \exp^{-I/\tau_1} + A_2 \cdot \exp^{-I/\tau_2}$) functions. The voltage dependence of the time constants for activating and inactivating macroscopic current are given by $(\alpha + \beta)^{-1}$, where $\alpha = \alpha_0 \exp[\alpha_1(V - V_0)]$, and $\beta = \beta_0 \exp[-\beta_1(V - V_0)]$. All data are expressed as the mean \pm SEM.

RESULTS

Rectification of HERG

The $I-V$ relationship for HERG expressed in *Xenopus* oocytes was bell shaped when determined using a standard protocol in which test pulses were applied in 10-mV steps from a negative holding potential. The graph in Fig. 1 *A* is a plot of peak outward currents and instantaneous currents, measured after settling of the initial capacitance spike. Currents slowly activated during depolarization to potentials positive to -60 mV and reached a maximum value at -10 mV when assessed with 4-s pulses. Peak current was progressively decreased when activated at potentials > -10 mV. Test pulses at potentials negative to -60 mV did not elicit time-dependent current. This is in marked contrast to typical inward rectifier K^+ (IRK) channels, where large inward currents are activated during hyperpolarizing pulses. Activation of inward current (tails) was only observed with hyperpolarization if applied after a depolarizing prepulse (Fig. 1 *B*). The fully activated $I-V$ relationship for HERG, determined from peak tail currents after a prepulse to $+40$ mV, exhibited weak inward rectification (Fig. 1 *B*), similar to ROMK1 expressed in oocytes (Lu and MacKinnon, 1994; Wible et al., 1994) and I_{Kr} in cardiac myocytes (Sanguinetti and Jurkiewicz, 1990; Sanguinetti and Jurkiewicz, 1991). Thus, HERG is a depolarization-activated current. The standard $I-V$ determined with 4-s depolarizations has a region of negative slope conductance, and the fully activated $I-V$ exhibits inward rectification.

Instantaneous I-V Relationship of HERG Is Linear when Measured after Removal of Fast Inactivation

We and others have proposed that rectification of I_{Kr} and HERG results from voltage-dependent inactivation of channels that proceeds at a faster rate than channel activation (Shibasaki, 1987; Sanguinetti and Jurkiewicz,

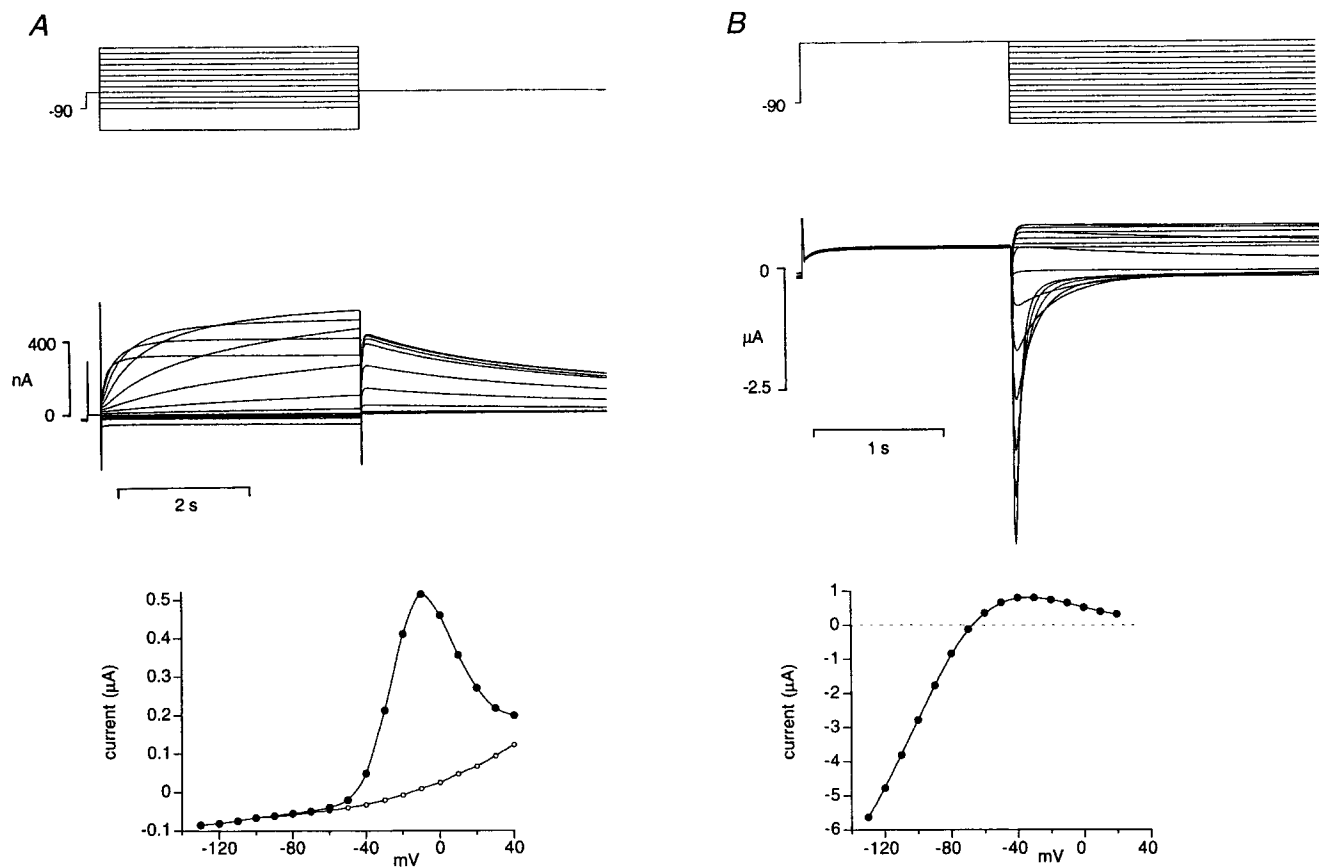


FIGURE 1. Current–voltage relationships for HERG. (A) I - V relationship determined with a standard pulse protocol. 4-s pulses were applied to test potentials of -120 to $+50$ mV in 10-mV steps from a holding potential of -90 mV after a brief prepulse to -60 mV. Test pulses were applied once every 15 s. The lower panel is a plot of current amplitude measured at the end of the 4-s test pulse (●) and instantaneous (leak) current (○). (B) Fully activated I - V relationship determined in the same oocyte. A 1.6-s prepulse to $+20$ mV was followed by test pulses to potentials ranging from -130 to $+20$ mV. The lower panel is a plot of peak currents recorded during the test pulse.

1990; Sanguinetti et al., 1995; Trudeau et al., 1995). According to this hypothesis, outward current during depolarizing steps is small because channels either inactivate directly from a closed state ($C \rightarrow I$) or immediately after activation ($C \rightarrow O \rightarrow I$). It was proposed that recovery from inactivation after membrane repolarization was via the open state ($I \rightarrow O \rightarrow C$). This later transition was measured as a hook in the deactivating tail currents upon repolarization (Shibasaki, 1987; Sanguinetti et al., 1995). Because inactivation apparently develops much faster than activation, the time course of inactivation cannot be quantified using a simple voltage-step protocol. We used a dual-pulse protocol to permit the recovery of channels from fast inactivation before applying a test pulse (Fig. 2 A, inset). This protocol allows quantification of the subsequent onset of fast inactivation. Currents were activated (and inactivated) by a 300-ms pulse to $+40$ mV. The membrane was then hyperpolarized to -110 mV for 25 ms before applying a second pulse to a variable test potential. Recovery from fast inactivation at -110 mV proceeds with a time

constant of 6 ms. Therefore, the interpulse interval of 25 ms was sufficient to allow $\sim 90\%$ recovery from inactivation but too short for detectable deactivation ($\tau = 182$ and 720 ms). The instantaneous current activated by the second depolarization was large and rapidly decreased in magnitude during the pulse. This decay of current after the capacitance spike was fit with a single exponential function. The amplitude of instantaneous current was obtained by extrapolating the fit to the beginning of the test pulse. The instantaneous I - V relationship determined with this pulse protocol was linear, in contrast to the inwardly rectifying I - V relationship determined without a hyperpolarizing interpulse (Fig. 2 B). These data indicate that the negative slope conductance of the standard I - V relationship (Fig. 1 A) and inward rectification of the fully activated I - V relationship (Fig. 1 B) is caused by channel closure that occurs faster than channel activation.

The time course of recovery from fast inactivation was determined as described previously (Sanguinetti et al., 1995). After a 300-ms depolarization to $+40$ mV,

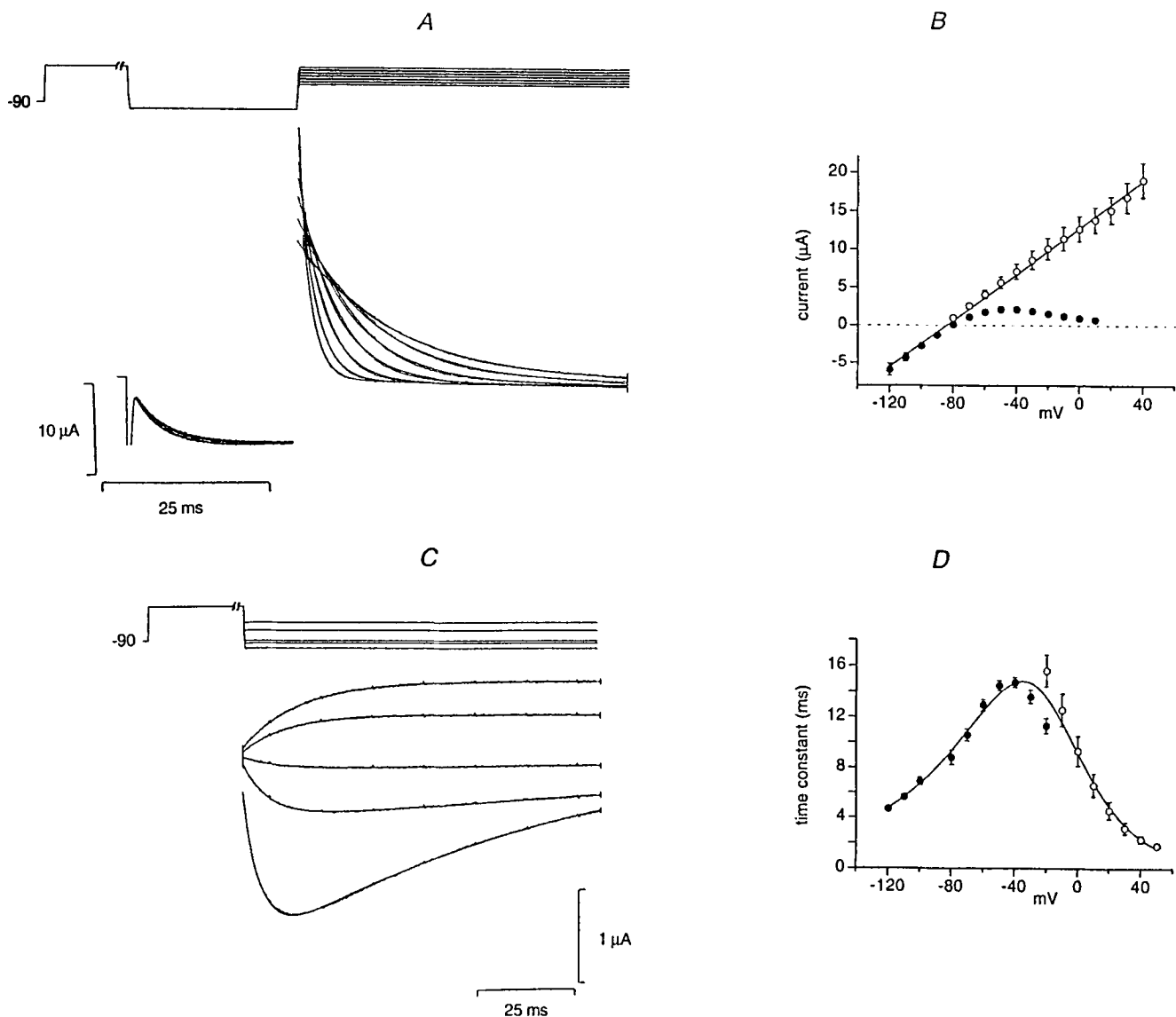


FIGURE 2. Inward rectification of fully activated I - V relationship for HERG is caused by fast inactivation. (A) Onset of fast inactivation. HERG was activated and inactivated with a 300-ms pulse to +40 mV. A 25-ms interpulse to -110 mV was used to permit recovery from inactivation, followed by a test pulse. Test pulses were applied once every 15 s. Currents shown were recorded at test potentials of -20 to +40 mV, applied in 10-mV increments. The decay of current during the test pulse is superimposed by fits of the data to a single exponential function. Capacity transients have been blanked. (B) Fully activated I - V plots determined with the standard protocol (●, as in Fig. 1 B) or after recovery from fast inactivation (○, as in Fig. 2 A); $n =$ six oocytes. (C) Recovery from fast inactivation. HERG was activated by a 300-ms pulse to +40 mV, followed by a test pulse to a variable potential. Pulses were applied once every 10 s. Currents shown were recorded at -120, -100, -90, -50, and -20 mV. Currents were fit with either a single- or double-exponential function as described (Sanguinetti et al., 1995) and the fitted trace superimposed over the current trace. Capacity transients have been blanked. (D) Voltage-dependent kinetics of onset (○), and recovery from (●), fast inactivation of HERG; $n =$ six oocytes. The smooth curve corresponds to $1/(\alpha + \beta)$, where $\alpha = 0.085 \exp[0.04(V - 0.36)] \text{ ms}^{-1}$; $\beta = 0.026 \exp[-0.017(V - 0.36)] \text{ ms}^{-1}$.

HERG tail currents were fit with single- or double-exponential functions (Fig. 2 C). At membrane potentials ≤ -80 mV, tail currents were fit with a double-exponential function to account for the rapid increase in current caused by recovery from channel inactivation and the much slower decrease in current caused by channel

deactivation. At membrane potentials ≥ -70 mV, deactivation was slow enough that the much faster phase of recovery from inactivation could be fit with a single-exponential function. The time constants describing the onset and recovery from fast inactivation were a bell-shaped function of voltage with a maximum near

-40 mV (Fig. 2 D), similar to the relationship describing the kinetics of activation and deactivation of HERG measured under identical conditions (see Fig. 5). Thus, the bell-shaped relationship describing the voltage-dependent kinetics of HERG inactivation resembles that of channel activation.

Fast Inactivation of HERG Is an Intrinsic Gating Process

To determine if rapid inactivation of HERG results from voltage-dependent block by intracellular molecules (e.g., Mg^{2+} , Ca^{2+} , or polyamines) as reported for cardiac inward rectifier K^+ channels (Vandenberg, 1987; Mazzanti and DiFrancesco, 1989) and heterologously expressed IRK1 and ROMK1 channels (Ficker et al., 1994; Lu and MacKinnon, 1994; Wible et al., 1994; Fakler et al., 1995), we compared $I-V$ curves of HERG current recorded in cell-attached macropatches with those obtained after excision of the patch (inside-out configuration) into a divalent-free solution containing either 10 mM EDTA or 5 mM EGTA. To activate HERG, the membrane was depolarized to +40 mV for 400 ms, and then ramped to -120 mV. As a basis for comparison, we first measured whole-cell HERG currents. In the presence of 40 mM $[K^+]_e$, HERG exhibited strong inward rectification using this voltage clamp protocol (Fig. 3 A). A similar $I-V$ relationship was obtained when HERG was measured in a cell-attached macropatch in another oocyte (Fig. 3 B), and 5 min after the same macropatch was excised and exposed to a Ca^{2+} - and Mg^{2+} -free bath solution (Fig. 3 C). After patch excision, currents were recorded every 10 s for a total of ~5 min. During this time, the bath was continuously perfused with the divalent-free solution at a rate of 3 ml/min (0.5 ml chamber volume). The properties of rectification were not altered after patch excision. Similar results were obtained in an additional eight experiments performed with EDTA to chelate Mg^{2+} , and in five experiments using EGTA to chelate Ca^{2+} . These data indicate that rectification of HERG channels is an intrinsic process and, unlike IRK channels, does not result from block by Mg^{2+} , Ca^{2+} , polyamines, or other soluble intracellular molecules.

Fast Inactivation Is Not Mediated by the NH_2 -terminal Region of HERG Channels

The sequence of HERG is 49% homologous to mouse EAG, a delayed rectifier K^+ channel that has a linear $I-V$ relationship (Bruggemann et al., 1993). A major difference between these two K^+ channels is that the NH_2 terminus of HERG is nearly twice as long as the same region in EAG (396 vs 206 amino acids). Fast inactivation of some *Shaker* K^+ channels is mediated by binding of the NH_2 -terminal region to a receptor site on the channel (Hoshi et al., 1990). We determined if a similar mechanism underlies inactivation of HERG.

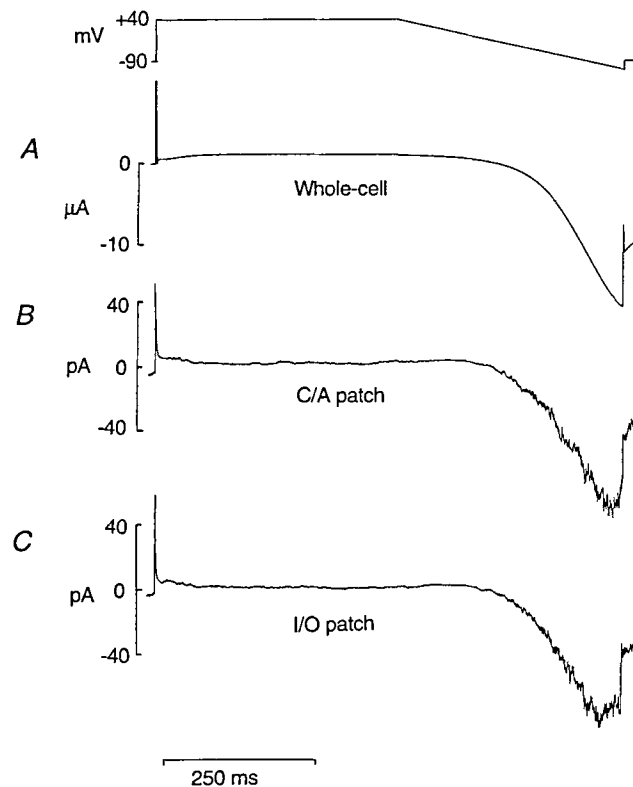


FIGURE 3. Rectification of HERG $I-V$ relationship is not altered in inside-out macropatches. (A) Whole-cell HERG current recorded in an oocyte bathed in a solution containing the following (in mM): 40 KCl, 58 NaCl, 5 HEPES, 0.1 $CaCl_2$, and 1.2 $MgCl_2$. (B) HERG current measured in a cell-attached (C/A) macropatch. The bathing solution contained (in mM) 120 KCl, 10 HEPES, and 10 EDTA, and the pipette solution was the same as the extracellular solution used to record whole-cell currents described above. (C) Same patch as in B, 5 min after excision to form inside-out (I/O) configuration. In all cases, currents were elicited by a 400-ms step to +40 mV, followed by a 375 ms ramp to -120 mV. Note that HERG inwardly rectifies in both C/A and I/O patches.

The properties of rectification for N-truncated HERG channels were assessed using similar protocols to those shown in Fig. 2. The only difference was that the onset of HERG inactivation were assessed after a 7.2-ms interpulse to -90 mV (Fig. 4 A). This shorter interpulse interval was necessary to avoid channel deactivation that developed much faster for N-truncated than WT HERG. The fully activated $I-V$ relationship for N-truncated HERG exhibited inward rectification, and the instantaneous $I-V$ relationship determined after recovery from fast inactivation was linear (Fig. 4 B). The extent of inward rectification of N-truncated HERG was less than WT HERG. For example, the ratio of fully activated/instantaneous current at -20 mV was 0.12 for WT HERG, compared with 0.52 for N-truncated HERG. This difference in rectification is accounted for by the

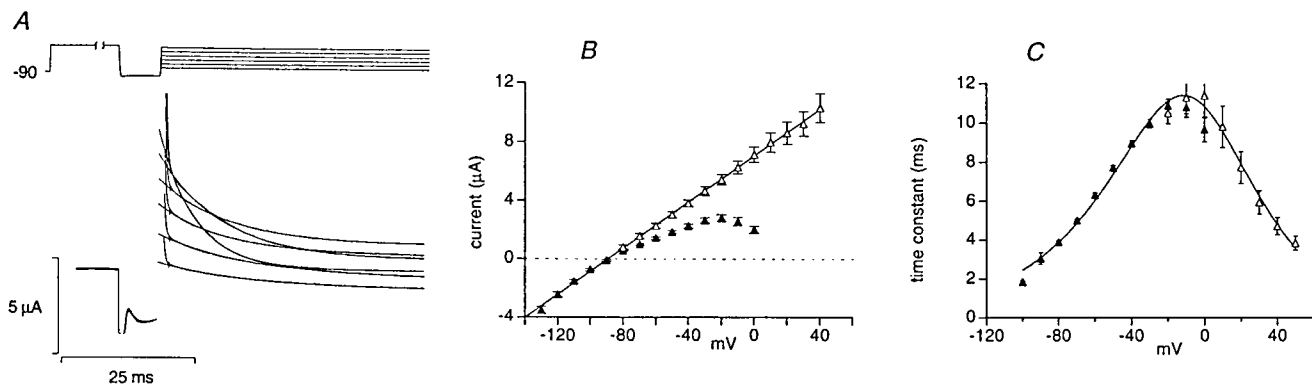


FIGURE 4. Rectification of HERG is not mediated by N-type inactivation. (A) Onset of fast inactivation for N-truncated HERG. HERG was activated and inactivated with a 300-ms pulse to +40 mV. A 7.2-ms interpulse to -110 mV was used to permit recovery from inactivation, followed by a test pulse to a variable potential. Currents shown were recorded at test potentials of -70 to +30 mV, applied in 20-mV increments. Test pulses were applied once every 15 s. Decay of current was fit with single-exponential function, and fitted trace superimposed over the current trace. Capacity transients have been blanked. (B) Fully activated I - V plots for N-truncated HERG determined with standard pulse protocol (\blacktriangle), or after recovery from fast inactivation (\triangle); $n =$ seven oocytes. (C) Voltage-dependent kinetics for onset (\triangle) and recovery from (\blacktriangle) fast inactivation of N-truncated HERG; $n =$ five oocytes. Smooth curve corresponds to $1/(\alpha + \beta)$, where $\alpha = 0.047 \exp[0.031(V + 7.6)] \text{ ms}^{-1}$; $\beta = 0.044 \exp[-0.024(V + 7.6)] \text{ ms}^{-1}$.

+30-mV shift in the voltage dependence of inactivation of N-truncated HERG compared with WT HERG. The fully activated I - V relationship peaked at -50 mV for WT HERG (Fig. 2 C) and -20 mV for N-truncated HERG (Fig. 4 B). The onset of and recovery from fast inactivation were rapid and best fit with a single exponential function (Fig. 4 C). The relationship between time constants and membrane potential peaked at -10 mV, 30 mV positive to the peak measured with WT HERG. These data indicate that the NH_2 -terminal region does not mediate channel inactivation, but its deletion alters the voltage dependence of channel gating.

The time constants of current activation for N-truncated HERG were similar to WT HERG but shifted to more positive membrane potentials (Fig. 5). In contrast, the rate of HERG deactivation was much faster for N-truncated than WT HERG, even when the shift in voltage-dependent gating was taken into consideration (Fig. 5). These data suggest that the NH_2 -terminal region of HERG modulates the rate of channel closure.

Physiologic Relevance of HERG Rectification

HERG was measured during voltage ramps to estimate the extent of channel rectification during a voltage transition like the repolarization phase of a cardiac action potential. In the example shown in Fig. 6, an oocyte was voltage clamped at a holding potential of -81.5 mV, the reversal potential for HERG current in this cell. Depolarization to +39 mV was followed by a ramp of membrane potential back to the holding potential. A voltage ramp was applied once every 5 s, and the duration varied from 1.2 to 0.2 s in 0.1-s decrements (Fig. 6 A), and from 200 to 75 ms in 25-ms decre-

ments. The peak amplitude of HERG current varied as a function of ramp duration (Fig. 6 B). The integral of current was a linear function of ramp duration between 0.2 and 1.2 s (Fig. 6 C). Outward HERG current reached a maximum at -50 mV for voltage ramps with a duration >200 ms (Fig. 6 D). The peak of the HERG I - V relationship was less negative when assessed with shorter ramps. For example, HERG peaked at -30 mV when measured with a 125-ms voltage ramp. Similar results were obtained in seven additional oocytes. This experiment demonstrates that rectification significantly reduces outward HERG current at potentials typical of the plateau phase of cardiac action potentials.

DISCUSSION

Rectification of HERG is an intrinsic property caused by fast inactivation. Rectification was not affected by exposure of the inside face of excised patches to a divalent-free bath solution, indicating that fast inactivation is an intrinsic gating process and not due to voltage-dependent block by intracellular Mg^{2+} , Ca^{2+} , or polyamines as reported for I_{K} channels (Ficker et al., 1994; Lu and MacKinnon, 1994; Wible et al., 1994; Fakler et al., 1995). Shibasaki (1987) first proposed that rectification of I_{K} in rabbit atrial pacemaker myocytes was due to rapid channel inactivation, and a similar mechanism was later proposed for I_{Kr} in guinea pig ventricular myocytes (Sanguinetti and Jurkiewicz, 1990) and HERG heterologously expressed in oocytes (Sanguinetti et al., 1995; Trudeau et al., 1995). However, the onset of inactivation was not directly measured in any of these previous studies. Rapid channel inactivation was inferred from the presence of hooked tail cur-

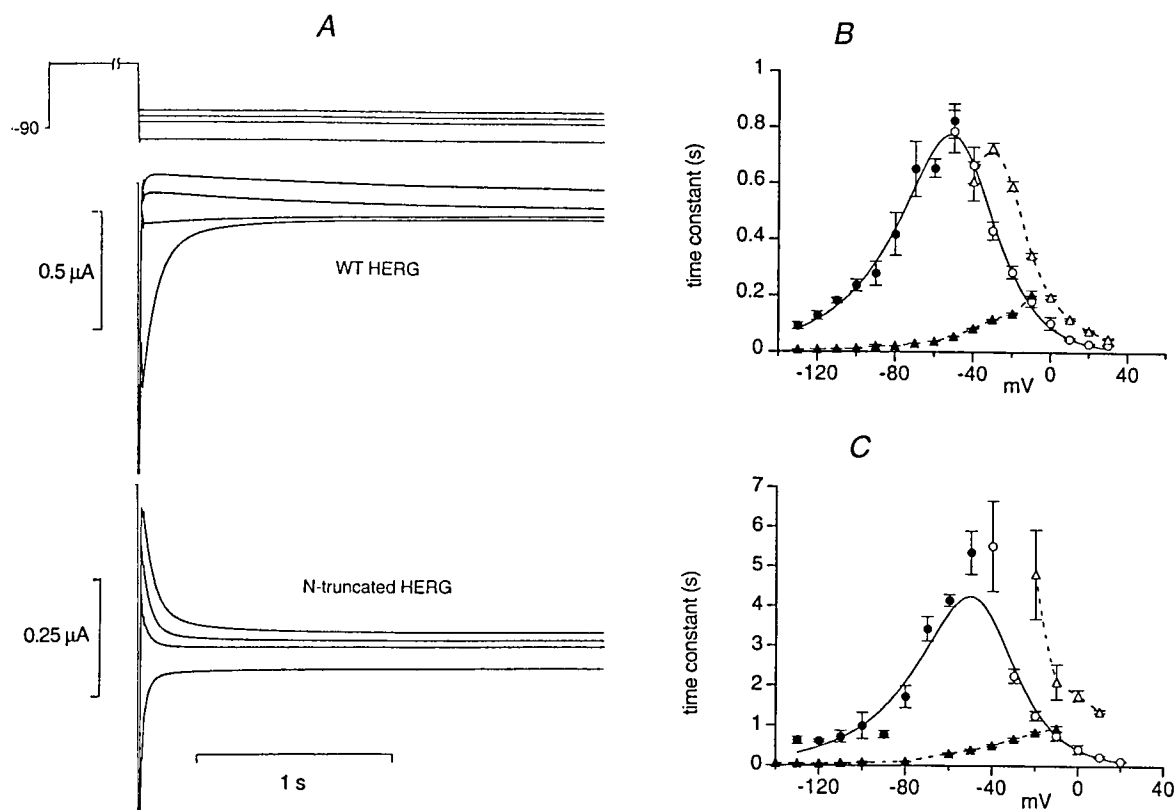


FIGURE 5. N-truncated HERG activates normally but deactivates more rapidly than WT-HERG. (A) Pulse protocol and representative currents for WT HERG and N-truncated HERG. Currents shown were recorded at test potentials of -110 , -80 , -70 , and -60 mV. (B and C) Time constants describing time course of fast (B) and slow (C) components of activation (\circ , Δ) and deactivation (\bullet , \blacktriangle) for WT HERG (\circ , $n = 5$) and N-truncated HERG (Δ , $n = 11$). Smooth curves correspond to $1/(\alpha + \beta)$, where $\alpha = 1.07 \exp[0.061(V + 38)] \text{ ms}^{-1}$; $\beta = 0.516 \exp[-0.034(V + 38)] \text{ ms}^{-1}$ (B), and $\alpha = 0.68 \exp[0.066(V + 19.3)] \text{ ms}^{-1}$; $\beta = 0.045 \exp[-0.038(V + 19.4)] \text{ ms}^{-1}$ (C).

rents. These hooks were interpreted to result from the reopening of channels upon recovery from fast inactivation. In the present study, we used a dual-pulse protocol to measure directly the onset of fast inactivation. After a depolarizing pulse, the membrane was hyperpolarized briefly to permit recovery from inactivation. This pulse was immediately followed by a second (test) depolarization to allow detection of reopened channels before the onset of inactivation. The magnitude of instantaneous currents measured during the second depolarization was a linear function of test potential, implicating voltage-dependent inactivation as the cause of HERG rectification. Unlike most other K^+ channels, inactivation of I_{Kr} channels develops faster than activation. The extent of HERG channel inactivation increases with membrane depolarization and proceeds at a rate 10–50 times faster than the fast component of activation. This results in a progressive decrease in steady-state current amplitude at membrane potentials > -50 mV.

HERG is a depolarization-activated channel with properties more like typical delayed rectifier than inward rectifier K^+ channels. A recent study of HERG

channels expressed in oocytes concluded that this channel was an inward rectifier (Trudeau et al., 1995). While it is true that the I - V relationship of this channel can exhibit inward rectification, this property is only apparent if currents are elicited from a holding potential positive to that required for channel activation. HERG is not activated by hyperpolarizations applied from a holding potential negative to that required for channel activation. By contrast, IRK channels are activated by hyperpolarization from any holding potential. When measured under physiologic conditions, including activation by depolarizations applied from a negative holding potential in the presence of normal external $[\text{K}^+]$ (e.g., 4 mM), the properties of HERG current are very similar to I_{Kr} in myocytes. Under these conditions, the I - V relationship determined from test depolarizations has a region of negative slope conductance. The voltage at which the slope conductance becomes negative depends on the pulse duration. The I - V peaked at -10 mV when currents were measured with 4-s pulses but peaked at -50 mV when measured after full activation. In these experiments, oocytes were bathed in a solution containing 0.1 mM CaCl_2 to suppress en-

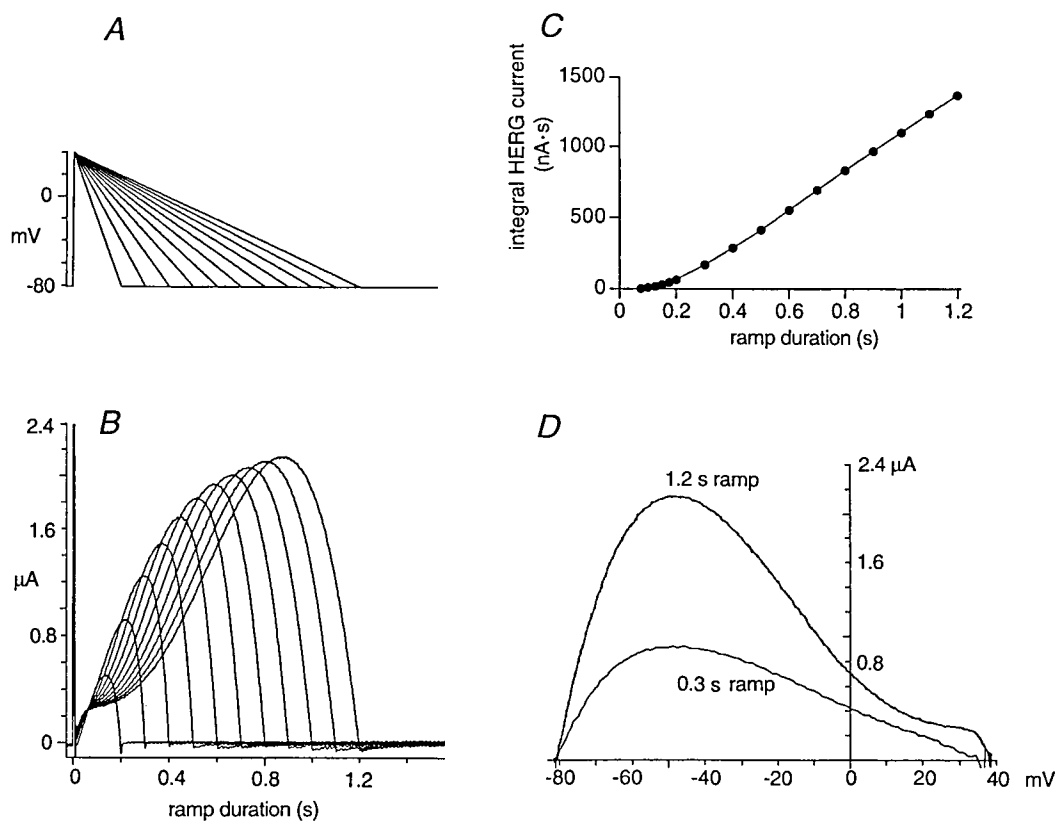


FIGURE 6. HERG currents activated by voltage ramps. (A) Voltage ramp protocol: From a holding potential of -81.5 mV, the membrane was pulsed to $+39$ mV, followed by a voltage ramp that varied in duration from 1.2 to 0.2 s (0.1-s decrements). (B) HERG currents recorded during voltage ramps shown in A. (C) Integral of HERG currents obtained with voltage ramps. (D) I - V relationship for HERG current recorded during a 0.3- and a 1.2-s voltage ramp.

ogenous Ca^{2+} -activated Cl^- current. Under more physiologic conditions (e.g., 1.8 mM $[\text{Ca}^{2+}]_e$), the peak of the I - V relationship would be more positive because of screening of negative surface charge by external Ca^{2+} (Frankenhaeuser and Hodgkin, 1957). Therefore, HERG only exhibits inward rectification if quantified using a depolarizing prepulse.

Rectification is also an intrinsic channel property of KAT1 (Hoshi, 1995; Schroeder, 1995), a K^+ channel expressed in higher plants such as *Arabidopsis* (Anderson et al., 1992), with sequence homology to HERG. Unlike HERG, KAT1 channels are activated by hyperpolarization but not by depolarization. This property is similar to IRK channels cloned from animals. However, unlike animal IRK channels, which have only two transmembrane domains, the KAT1 channel has six transmembrane domains and an S4 region with several positively charged residues similar to animal delayed rectifier K^+ channels. Chimeric channels constructed between KAT1 (NH_2 terminus to the S5 linker) and a *Xenopus* delayed rectifier K^+ channel (H5 to the COOH terminus) retained the rectification properties of KAT1 (Cao et al., 1995). Thus, the structural determinants of KAT1 rectification are contained somewhere between the

NH_2 -terminal and S4-S5 linker regions of the channel. Despite similar primary sequence, HERG and KAT1 channels are functionally distinct.

Our data indicate that inactivation of HERG is not mediated by an N-type (ball and chain) mechanism as previously demonstrated for *Shaker* K^+ channels (Hoshi et al., 1990). The voltage dependence of HERG channel gating was shifted $+30$ mV by NH_2 -terminal deletion, but the process of rectification was not affected. In addition, the N-truncated channels deactivated at a much faster rate than WT channels. The role of C-type inactivation must be explored. We attempted to examine the role of the COOH terminus in HERG rectification. However, the COOH-terminal truncated HERG construct did not express functional channels (data not shown).

In summary, our data indicate that fast inactivation of HERG channels causes rectification. This property reduces I_{Kr} during the plateau phase of cardiac action potentials. Rectification of I_{Kr} , a delayed rectifier, and I_{K1} , the inward rectifier, results from fundamentally different mechanisms, but the physiologic consequences are similar: reduced outward conductance at positive membrane potentials and delayed repolarization.

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