Characterization and functional consequences of delayed rectifier current transient in ventricular repolarization

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Characterization and functional consequences of delayed rectifier current transient in ventricular repolarization. Am. J. Physiol. Heart Circ. Physiol. 278: H806–H817, 2000.—Although inactivation of the rapidly activating delayed rectifier current (I_{Kr}) limits outward current on depolarization, the role of I_{Kr} (and recovery from inactivation) during repolarization is uncertain. To characterize I_{Kr} during ventricular repolarization (and compare with the inward rectifier current, I_{K1}), voltage-clamp waveforms simulating the action potential were applied to canine ventricular, atrial, and Purkinje myocytes. In ventricular myocytes, I_{Kr} was minimal at plateau potentials but transiently increased during repolarizing ramps. The I_{Kr} transient was unaffected by repolarization rate and maximal after 150-ms depolarizations (+25 mV). Action potential clamps revealed the I_{Kr} transient terminating the plateau. Although peak I_{Kr} transient density was relatively uniform among myocytes, potentials characterizing the peak transients were widely dispersed. In contrast, peak inward rectifier current (I_{K1}) density during repolarization was dispersed, whereas potentials characterizing I_{K1} transients were widely dispersed. In summary, rapidly activating I_{Kr} provides a delayed voltage-dependent (and functionally time-independent) outward transient during ventricular repolarization, consistent with rapid recovery from inactivation. The heterogeneous voltage dependence of I_{Kr} provides a novel means for modulating the contribution of this current during repolarization.

arrhythmias; Purkinje fibers; human ether-à-go-go-related gene; atrial myocytes; canine myocardium

IN MOST MAMMALS (including humans), a rapidly activating component of delayed rectifier current (I_{Kr}) is postulated to play a prominent role in defining ventricular repolarization (8, 15, 22, 25). Indeed, most class III antiarrhythmic drugs (as well as some antihistamines and antibiotics with adverse cardiac effects) prolong the action potential duration and ventricular refractoriness, and they may promote torsade de pointes arrhythmias by blocking I_{Kr}. Despite the relatively rapid activation kinetics of I_{Kr}, its contribution during the action potential plateau is limited by inward rectification. Rectification of I_{Kr} has been attributed to rapid inactivation, which reduces outward current at depolarized potentials and contributes to the prolonged plateau phase of the ventricular action potentials (27, 28, 30). Studies of I_{Kr} in sinus node cells revealed that rapid recovery from inactivation (along with channel deactivation) occurs during repolarizing square-clamp pulses (26). The role that each process may play in defining I_{Kr} during a ventricular action potential is uncertain, because ventricular repolarization is slower than repolarizing square pulses and the gating characteristics of I_{Kr} vary between species.

Recent studies have highlighted differences in the densities of various potassium currents across the ventricular wall with implications regarding arrhythmogenesis and antiarrhythmic drug therapy. Heterogeneity of repolarization across the canine ventricular wall has been linked to regional variations in the density of the transient outward current (16) and the slowly activating component of delayed rectifier current (7, 17). Although pharmacological studies suggest that I_{Kr} plays a dominant role in defining the ventricular action potential duration, significant differences in ventricular I_{Kr} density have not been reported. In Purkinje fibers, direct measurements of I_{Kr} have proven difficult (4, 21), despite the fact that block of I_{Kr} causes dramatic prolongation of the action potential duration in these tissues (10). Detecting differences in I_{Kr} density or characteristics is difficult, because I_{Kr} is typically one of the smaller of the potassium repolarizing currents, and its characteristics may not be fully realized using traditional repolarizing square-clamp pulses.

To further characterize and evaluate the involvement of native I_{Kr} in ventricular repolarization, voltage-clamp protocols simulating the plateau and repolarization phases of the action potential were applied to isolated canine ventricular myocytes and Purkinje fibers. These results were also contrasted with those from atrial myocytes, allowing for a comparison of I_{Kr} characteristics from electrophysiologically distinct preparations and different sets of ionic currents. Results demonstrate that I_{Kr} provides a transient “pulse” of outward current later during the ventricular action potential plateau, consistent with its rapid reactivation over the time course of ventricular repolarization. The peak density of the I_{Kr} transient is unaffected by repolarization rates encountered physiologically and does not “accumulate” during rapid stimulation if the preceding plateau is sufficiently long to ensure I_{Kr} activation. Whereas the peak density of the I_{Kr} transient in ventricular myocytes is relatively constant, the voltage characterizing the peak I_{Kr} transient varies significantly. This variability provides a novel means
for modulating the contribution of $I_{Kr}$ during repolarization to affect electrical heterogeneity. In contrast, variability of inward rectifier current ($I_{Kr}$) density is the predominant means by which this current varies between ventricular myocytes. Action potential-clamp techniques (in which the recorded action potential serves as the command waveform) demonstrate the defining role of $I_{Kr}$ in the transition to phase 3 (terminal) ventricular repolarization and the activation of $I_{K1}$ to complete repolarization. Preliminary results have been reported in abstract form (9).

### METHODS

**Isolation of Ventricular and Purkinje Myocytes**

Cardiac myocytes were obtained after Langendorff perfusion of isolated canine hearts. Briefly, animals were anesthetized with pentobarbital sodium, and hearts were rapidly excised and placed in cold modified Tyrode solution (nominally calcium free) containing (in mM) 110 NaCl, 10 HEPES, 1.2 MgSO$_4$, 24 NaHCO$_3$, 6 KCl, and 11.1 glucose, supplemented with 0.5 mg/ml BSA, adjusted to pH 7.3 with 5 N NaOH, and aerated with 95% O$_2$-5% CO$_2$. The aorta was cannulated, and the heart was flushed with 100 ml of cold Tyrode solution and connected to a heated (37°C) Langendorff perfusion apparatus. After 10 min of additional flushing, the solution was switched to one containing 0.4 mg/ml collagenase (type II, Worthington). After 35 min of recirculation, the heart was removed from the apparatus. To isolate ventricular myocytes, we removed epicardial and endocardial layers, and the remaining left ventricular free wall was minced and agitated for an additional 20 min in an oxygenated shaker bath in enzyme-free Tyrode solution. Dispersed myocytes were subsequently filtered through stainless steel mesh and aliquoted into 15-ml test tubes containing modified Tyrode solution (without BSA). To isolate atrial myocytes, we removed the left atrium after recirculation, and it was minced and manually triturated until remaining chunks were dispersed. Myocytes were kept at room temperature until use (~10 h).

To isolate Purkinje cells, we removed larger free-running Purkinje fiber bundles from the heart after collagenase perfusion but before removal of the endocardium. Purkinje fibers were dissected free of endocardial tissues to prevent potential contamination by ventricular myocytes. Fibers were sliced, minced, and manually triturated for 5 min in modified Tyrode solution to free the Purkinje cells from the surrounding collagenase matrix. The distinct morphology of canine Purkinje myocytes (greater length than ventricular myocytes, minimal step-like projections along sides, and “crinkle-cut” surface undulations) confirmed their identity during electrophysiological studies. In a few instances slow, spontaneous, uniform contractions of some cells were observed when cells were placed in a HEPES-buffered solution [containing (in mM) 132 NaCl, 20 HEPES, 1.2 MgSO$_4$, 11.1 glucose, 4 KCl, and 2 CaCl$_2$, adjusted to pH 7.4 with HCl at 37°C], consistent with spontaneous phase 4 depolarization.

Aliquots of isolated myocytes were pipetted onto a heated (36–37°C) chamber on a Nikon invert scope as described previously (7). Myocytes were superfused with the HEPES-buffered solution. Only rod-like, relaxed myocytes (free from contraction bands, “bends,” and membrane “blebs”) with resting potentials more negative than −75 mV were studied.

### Voltage-Clamp Studies

“Traditional” square-clamp pulses and repolarizing ramp clamps. For voltage-clamp studies using traditional square-clamp pulses and repolarizing ramp clamps, whole cell patch-clamp techniques were applied to myocytes using an Axopatch 200A amplifier (Axon Instruments). Myocytes were accessed with the use of an intracellular solution containing (in mM) 125 K-aspartate, 20 KCl, 10 EGTA, 5 ATP (Mg salt), 1 MgCl$_2$, and 5 HEPES (free acid), adjusted to pH 7.3 with 5 N KOH. Extracellular solution contained nisoldipine or nimodipine (1 µM) to block L-type calcium current; contamination by Na$^+$ current ($I_{Na}$) during potassium current recordings was prevented by holding the membrane potential at −40 mV before depolarizations to the plateau range of potentials. Series resistance compensation was typically adjusted to values between 60 and 70%, and recorded potentials were offset negatively by 10 mV for junction potential as described previously (7).

Action potential clamps. For voltage-clamp studies using action potential waveforms (“action potential clamps,” see Ref. 5), perforated-patch techniques were used to minimize alterations of the intercellular milieu (11). Pipette solutions contained (in mM) 130 K-aspartate, 15 KCl, 5 HEPES (free acid), 10 NaCl, and 0.5 CaCl$_2$. 0.18 mg/ml amphotericin B was used as the pore-forming agent. To avoid problems inherent in recording action potential with standard patch-clamp head stages that result from input current in the current-clamp mode (19), an Axoclamp 2B amplifier was used to faithfully record transmembrane potentials (current-clamp mode); voltage clamp was performed as discontinuous single-electrode voltage clamp (or “switch” clamp; typical switching range 4–5 kHz). Action potential waveforms were applied to the same ventricular myocyte from which they were obtained.

### Data Interpretation and Analysis

$I_{Kr}$ was defined as drug-sensitive current blocked by the specific $I_{Kr}$-blocking agent E-4031, which was prepared from frozen aliquots of 5 mM aqueous stock solution. Except where noted, a 5 µM concentration of E-4031 was used to fully block native $I_{Kr}$ ($IC_{50} = 397$ nM, Ref. 25). The effects of lower E-4031 concentrations (0.5, 1, and 2.5 µM) were also evaluated in some experiments to assess possible concentration-dependent effects on the configuration of the drug-sensitive current. Because the effects of E-4031 were not readily reversible, drug-sensitive currents were evaluated immediately after bath equilibration (typically <5 min) to minimize the likelihood of nonspecific changes in membrane currents. In select studies, the identification of the E-4031-sensitive current as $I_{Kr}$ was confirmed by evaluating the effects of the $I_{Kr}$-blocking agents dofetilide (200 nM, Ref. 10) and sotalol (100 µM, Ref. 2). In additional experiments, extracellular potassium concentration ([K$^+$])$_o$ was reduced to 0 mM to reduce $I_{Kr}$ before exposure to E-4031 as a negative control (23).

$I_{Kr}$ was defined as current blocked by 20 mM CsCl (12). To minimize contamination from $I_{Kr}$ when $I_{K1}$ was being evaluated, we pretreated myocytes with E-4031 (to block $I_{Kr}$); contamination from slowly activating delayed rectifier K$^+$ current ($I_{K1}$) was minimized by using short (≤250 ms), moderately depolarizing (to ≤25 mV) square conditioning pulses to reduce activation of $I_{K1}$.

Lines were fit by linear regression, and curves were fit by nonlinear least-squares regression analysis (Origin, version 5.0, Microcal Software); t-Tests were used for unpaired samples to evaluate differences between two groups; comparisons between multiple groups were performed using ANOVA with
repeated measures followed by appropriate post hoc tests (Systat). Unless otherwise stated, results are presented as means ± SE.

RESULTS

An \( I_{Kr} \) Transient Is Present in Ventricular Myocytes

To assess the involvement of \( I_{Kr} \) during ventricular repolarization, we employed truncated ramp-clamp protocols to simulate the plateau and repolarization phases of the action potential. Figure 1A shows the typical voltage-clamp protocol used. From a holding potential of −85 mV, a 50-ms depolarizing pulse to −35 mV was applied to inactivate \( I_{Na} \). This step was immediately followed by a 250-ms pulse to +25 mV (grossly simulating the action potential plateau), followed by a repolarizing ramp (slope −1.2 V/s) simulating repolarization. Figure 1B shows typical membrane currents recorded from a ventricular myocyte in the absence (control) and presence of the specific \( I_{Kr} \)-blocking agent E-4031. E-4031 elicited a slight reduction of net outward current at +25 mV and much greater reduction of outward current early during the repolarizing ramp. Figure 1C shows the E-4031-sensitive “difference current” obtained by digital subtraction of membrane current in the absence and presence of E-4031. A small drug-sensitive current is rapidly attained during the step pulse to +25 mV, which transiently increases fourfold during repolarization, attaining a maximum value at a potential near −48 mV before declining at full repolarization. On the basis of the specificity of E-4031, we defined the drug-sensitive current transient as \( I_{Kr} \). The transient increase in \( I_{Kr} \) during repolarization occurs despite a continually decreasing electromotive force for potassium ions during the repolarizing ramp.

Three additional series of experiments confirmed the identity of the outward transient as \( I_{Kr} \). In one set of experiments, the effects of the \( I_{Kr} \)-blocking agent dofetilide (200 nM) on outward currents during repolarization were evaluated. In each of four experiments, a dofetilide-sensitive transient was observed analogous to that observed with E-4031. A similar drug-sensitive transient was also apparent when \( I_{Kr} \) was blocked with 100 µM sotalol. In a second set of experiments, the effects of E-4031 were evaluated in the presence of 0 mM [K+]o to eliminate \( I_{Kr} \) (23). Under these conditions, the E-4031-sensitive transient current was absent during repolarizing ramp clamps (n = 2 myocytes). Together, these results identified the drug-sensitive outward current transient during repolarization as \( I_{Kr} \), and not an artifact unique to E-4031. To examine the unlikely possibility that the \( I_{Kr} \) transient was caused by incremental block (and recovery) of \( I_{Kr} \) during repolarization, we compared the configuration of the transient during equilibration with lower (500 nM) or higher (5 µM) concentrations of E-4031 in the same myocytes. If the drug-sensitive transient had been caused by time-dependent block, the peak of the current transient would have been expected to shift toward more positive potentials as the drug concentration approached maximum blocking concentrations. In each of four experiments, the amplitude of the drug-sensitive transient increased with greater drug concentration, whereas the shape and voltage characterizing the peak of the transient remained unaltered (data not shown). The lack of effect of drug concentration on the \( I_{Kr} \) configuration suggests that incremental block of \( I_{Kr} \) during repolarization is not responsible for the \( I_{Kr} \) transient.

To determine the time course for activation of the \( I_{Kr} \) transient, we clamped ventricular myocytes to +25 mV for increasing durations before a constant repolarizing ramp. Clamp protocols were repeated in the absence and presence of E-4031, and \( I_{Kr} \) was measured as peak drug-sensitive current during repolarization for identical clamp sequences. Figure 2A shows the clamp protocol, consisting of a depolarizing step to −35 mV (to inactivate \( I_{Na} \)) and a second step to +25 mV ranging from 5 to 155 ms in duration (25-ms increments), followed by a repolarizing ramp (−1.21 V/s). Figure 2B shows E-4031-sensitive currents recorded from a typical myocyte (with currents offset by −75 pA for clarity). It is evident that the amplitude of the \( I_{Kr} \) transient...
increases with longer depolarizing step pulses. Figure 2 shows averaged results obtained from seven myocytes, with peak transients normalized as current density. After a 5-ms step pulse, a small $I_{Kr}$ transient is present that increases after progressively longer depolarizing pulses. The growth of the peak $I_{Kr}$ transient with progressively longer step pulses was fit to an exponential function with a time constant of 43.18 ms, attaining half-maximal values within 30 ms. This time constant may slightly overestimate the extent of $I_{Kr}$ activation during the step pulse, because activation likely continues during early portions of the repolarizing ramp. Despite the increasing current that follows longer depolarizations, the voltage of the peak $I_{Kr}$ transients was unaffected (data not shown).

In guinea pig myocytes, trains of conditioning depolarizing pulses do not enhance the amplitude of $I_{Kr}$ assessed during depolarizing ramps (13). To determine whether the $I_{Kr}$ transient is augmented by rapid electrical activity in canine ventricle, E-4031-sensitive currents were assessed during repolarizing ramps preceded by conditioning pulse trains; drug-sensitive currents were obtained using identical pulse protocols. Preliminary experiments showed that conditioning pulse trains had minimal effects on the $I_{Kr}$ transient when repolarizing ramps were immediately preceded by a 100-ms step pulse. These results reflect the nearly complete activation of $I_{Kr}$ during a 100-ms pulse (see Fig. 2). Thus, to examine possible modulation of the $I_{Kr}$ transient with rapid electrical activity, the duration of the activating pulse step was set to 12.5 ms. Figure 3A, top, illustrates the test waveform, whereas Fig. 3B, inset, illustrates the conditioning pulse protocol, consisting of trains of 0, 2, or 10 pulses applied before the start of the transient protocol. Trains consisted of 100-ms conditioning square pulses to +15 mV with 20-ms interpulse intervals; 13 ms elapsed between termination of the last conditioning pulse and the start of the clamp protocol. B: E-4031-sensitive currents (representing $I_{Kr}$) for plateau durations ranging from 5 (top) to 155 ms (bottom; zero current lines overlay each trace). Current traces have been offset by ~75 pA for clarity. Amplitude of $I_{Kr}$ transient increases with longer depolarizing pulses. C: summary of results from 7 myocytes. Growth of peak $I_{Kr}$ transient with progressively longer step pulses was fit to an exponential function with a time constant of 43.18 ms. Pulses were repeated once every 9 s. See text for further discussion.

Figure 2. Time course of activation of ventricular $I_{Kr}$ transient. A: clamp protocol. B: E-4031-sensitive currents (representing $I_{Kr}$) for plateau durations ranging from 5 (top) to 155 ms (bottom; zero current lines overlay each trace). Current traces have been offset by ~75 pA for clarity. Amplitude of $I_{Kr}$ transient increases with longer depolarizing pulses. C: summary of results from 7 myocytes. Growth of peak $I_{Kr}$ transient with progressively longer step pulses was fit to an exponential function with a time constant of 43.18 ms. Pulses were repeated once every 9 s. See text for further discussion.

Fig. 3. Conditioning pulse trains increase amplitude of ventricular $I_{Kr}$ transient after brief activating test pulses. A, top: test clamp protocol consisting of a 15-ms step pulse to ~35 mV (to inactivate $I_{Na}$), followed by a brief (12.5 ms) test pulse to +15 mV to initiate $I_{Kr}$ activation before a repolarizing ramp (~1.21 V/s). A, bottom: E-4031-sensitive currents obtained from 1 myocyte in absence of a conditioning pulse train (0 prepulses) and after a 10-pulse train (10 prepulses). Conditioning pulse train increased $I_{Kr}$ transient. Pulse sets were repeated every 12 s. Base of calibration represents ~40 pA. B: schematic (inset) of conditioning pulse train consisting of depolarizing square pulses (100-mV amplitude, 100-ms duration, 20-ms interpulse interval) applied from ~85 mV holding potential; 13 ms elapsed between last conditioning pulse and start of test pulse. Graph shows that density of peak $I_{Kr}$ transient is significantly enhanced after 2 conditioning prepulses, with less enhancement after 10 prepulses. Results represent averages from 11 myocytes. *P < 0.05.
I<sub>Kr</sub> transient test waveform. To maximize sensitivity when recording these small transients, we repeated conditioning pulse train/test pulse sequences three times (in the absence and presence of E-4031) and averaged the results before digital subtraction was performed. Figure 3A compares typical E-4031-sensitive currents preceded by either no prepulses or a train of 10 conditioning prepulses. The I<sub>Kr</sub> transient during repolarization is greater after the conditioning pulse train. Figure 3B summarizes results from 11 myocytes. The peak I<sub>Kr</sub> transient is enhanced after a 2-pulse conditioning train and further enhanced after a 10-pulse train. These results show that the amplitude of the I<sub>Kr</sub> transient is enhanced by repetitive electrical activity when the test waveform is sufficiently brief to prevent full activation of I<sub>Kr</sub>. In each of three additional experiments, no I<sub>Kr</sub> transient was observed with or without conditioning pulse trains when myocytes were superfused with 0 mM [K<sup>+</sup>].

To determine whether repolarization rate affected the I<sub>Kr</sub> transient, we compared E-4031-sensitive currents during repolarizing ramps of different slopes. I<sub>Kr</sub> transients were characterized on the basis of their peak amplitude and the potential at which the peak amplitude occurred. Experiments employed repolarizing ramps with slopes of −1.21, −0.607, and −0.406 V/s (encompassing rates encountered during ventricular repolarization). Figure 4A shows typical E-4031-sensitive currents obtained from a ventricular myocyte, with each ramp overlaying its respective I<sub>Kr</sub> transient. Figure 4B shows the peak density of I<sub>Kr</sub> transients obtained from 12 myocytes, along with their average value. Some variability in the density of the peak transient was apparent, with values ranging from 0.25 to 0.6 pA/pF (0.44 ± 0.10 pA/pF, mean ± SD). Altering the rate of repolarization did not affect the amplitude of the peak transient.

Figure 4C compares the potentials at which the peak transients occurred during repolarizing ramps. The average potential for the peak transients was slightly more negative for the faster versus slower ramp speeds (−53.9 vs. −50.1 mV); this difference did not achieve statistical significance. Significant heterogeneity was apparent in the voltages characterizing the peak transients, with values ranging from −33 to −64.7 mV. Heterogeneity was observed when myocytes from individual hearts as well as myocytes from different hearts were compared, demonstrating that variability in the cell isolation procedure is not responsible for the voltage heterogeneity.

Figure 4 demonstrates a wide voltage range characterizing the peak I<sub>Kr</sub> transients. To determine the origin of this variability, we initially compared the voltage describing the peak I<sub>Kr</sub> transients with those describing the remaining outward current peak after I<sub>Kr</sub> blockade, which occurs at more negative potentials (see Fig. 1). As a working hypothesis, we attributed the residual outward peak to the inward rectifier current (I<sub>K1</sub>), which is activated at more negative potentials and is responsible for terminal ventricular repolarization and the ventricular resting membrane potential. We reasoned that the potentials describing both the I<sub>Kr</sub> and I<sub>K1</sub> peaks would be shifted in the same direction along the voltage axis if the variability reflected nonspecific changes in individual myocytes. Experimental results were not consistent with this hypothesis. For the 12 myocytes studied in Fig. 4, the potential describing the peak I<sub>Kr</sub> transient ranged from −33 to −64.7 mV (−52.0 ± 7.17 mV, mean ± SD). In contrast, the peak outward current
remaining in the presence of E-4031 spanned a narrower range over more negative potentials (from −67.1 to −74.7 mV; −70.8 ± 2.3 mV, mean ± SD). These results suggest that the voltage heterogeneity characterizing \( I_{K1} \) transients is not shared with \( I_{K1} \).

To directly compare dispersion of the voltages describing the peak amplitudes of \( I_{Kr} \) and \( I_{K1} \), an additional series of experiments was conducted comparing the \( I_{Kr} \) transient with \( I_{K1} \) in the same ventricular myocytes. The experimental protocol sequentially recorded currents during repolarizing ramps 1) under drug-free (control) conditions, 2) during superfusion with E-4031, and 3) during superfusion with E-4031 and 20 mM CsCl; drug-sensitive currents (obtained by digital subtraction) were used to assess \( I_{Kr} \) (E-4031-sensitive current) and \( I_{K1} \) (Cs-sensitive current). Currents for each of three ramp slopes (−1.21, −0.607, or −0.406 V/s) for each myocyte were averaged and compared. Figure 5A shows E-4031-sensitive current (representing \( I_{Kr} \)) and larger, Cs-sensitive current (representing \( I_{K1} \)) recorded from a myocyte during a repolarizing ramp. Figure 5B displays current densities and corresponding potentials characterizing the peak transients for \( I_{Kr} \) transient and \( I_{K1} \) from 15 myocytes; individual as well as average values are represented. Results show that the average density of \( I_{Kr} \) is approximately fourfold less than that of \( I_{K1} \) and occurs 24 mV more positively than \( I_{K1} \). The variability of the \( I_{Kr} \) density is much less than that of \( I_{K1} \) (0.38 ± 0.05 vs. 1.46 ± 0.31 pA/pF, respectively, means ± SD). The greater variability is also reflected in the coefficient of variation for \( I_{Kr} \) versus \( I_{K1} \) (0.132 vs. 0.214, respectively), which considers relative variation normalized to mean values. Surprisingly, the voltage range characterizing the peak outward transients is much wider for \( I_{Kr} \) than \( I_{K1} \) (−51.7 ± 8.05 vs. −77.0 ± 1.44 mV, respectively, means ± SD). Thus, heterogeneity in the characteristics of \( I_{K1} \) and \( I_{Kr} \) can be ascribed to two different factors, variability in the voltage (but not density) of peak \( I_{Kr} \) transients and variability in the current density (but not voltage range) characterizing \( I_{K1} \).

An \( I_{Kr} \) Transient Is Present in Atrial Myocytes With Characteristics Similar to Those of Ventricular Cells

Results from ventricular cells were contrasted with those from atrial myocytes, allowing for a comparison of \( I_{Kr} \) characteristics from preparations with electrophysiologically distinct phenotypes and different sets of ionic current. Figure 6A shows currents recorded from an atrial myocyte in the absence and presence of E-4031. The experimental protocol sequentially recorded currents for ventricular myocytes, the rate of repolarization did not affect either the peak density or peak potential characterizing the \( I_{K1} \) transient. Whereas a greater range of densities is apparent when atrial versus ventricular myocytes are compared (compare Figs. 6B and 4B, respectively), the range of peak voltages for both myocyte types was similar (compare Figs. 6C and 4C).

\( I_{Kr} \) Transient Density Is Greater at More Positive Potentials

Because \( I_{Kr} \) is a potassium current, the peak amplitude of the \( I_{Kr} \) transient should be greater at potentials farther from the potassium equilibrium potential be-
cause of the greater driving force for potassium ions. To determine the relationship between the $I_{Kr}$ transient amplitude and the voltage characterizing its peak, these two variables were compared for atrial and ventricular myocytes. Results are shown in Fig. 7; values represent means ± SD derived using the ramp slope protocols from Figs. 4 and 6. For atrial (Fig. 7A) and ventricular (Fig. 7B) myocytes, transients occurring at more positive potentials typically have a greater $I_{Kr}$ density. Regression analysis predicts a 0.46 pA/pF increase in peak transient density per each 15-mV positive change in peak potential for atrial myocytes and a 0.24 pA/pF increase for ventricular myocytes.

Fig. 6. $I_{Kr}$ transient is present in atrial myocytes. A: representative experiment with an atrial myocyte, displaying the ramp-clamp protocol (top) and corresponding membrane current recorded in absence (control) and presence of E-4031 (middle). Bottom: E-4031-sensitive current shown on an expanded scale. An $I_{Kr}$ transient is apparent during repolarization (ramp slope −0.607 V/s). Note minimal $I_{K1}$ during repolarization (compare with Fig. 1 showing data for a ventricular myocyte). B: peak $I_{Kr}$ transient densities obtained from 10 atrial myocytes (■) along with average values (○) using repolarizing ramps of −1.21, −0.607, and −0.406 V/s. C: potentials for each peak transient (■) along with average values (○) from myocytes described in B. Rate of repolarization had no effect on peak current density or peak potential of atrial $I_{Kr}$ transients. Despite minimal $I_{K1}$, the heterogeneity characterizing peak potentials in atrial myocytes is similar to that in ventricular myocytes. Values are expressed as means ± SE. Atrial cells were obtained from 4 hearts.

Fig. 7. $I_{Kr}$ transient amplitude is greater at positive peak potentials. Mean values for peak $I_{Kr}$ densities are plotted against potentials characterizing maximal transients for atrial (A, n = 10) and ventricular (B, n = 12) myocytes. Linear regression results (curves representing 95% confidence limits) are superimposed on data, with equations and correlation coefficients indicated for each myocyte type. For either atrial or ventricular myocytes, peak transient density tends to be larger when transient occurs at more positive potentials. Data were obtained from cells described in Figs. 4 and 6 showing values obtained with repolarizing ramps imposed on each cell. Densities were weighted by error bars (SD) for regression analysis.
Atrial myocytes displayed a greater range of densities compared with ventricular myocytes (0.927 vs. 0.318 pA/pF, respectively) and greater mean current density (0.62 ± 0.08 vs. 0.44 ± 0.03 pA/pF, \( P = 0.04 \), unpaired t-test), with 50% of atrial myocytes showing a greater density than the greatest ventricular value. Despite the overall greater \( I_{Kr} \) density in atrial myocytes, the range of peak voltages of both myocytes was similar.

Direct Demonstration of \( I_{Kr} \) in Ventricular Repolarization: Action Potential Clamps

The action potential-clamp technique was used to demonstrate the role of the \( I_{Kr} \) transient during ventricular repolarization. With the use of this technique, action potentials recorded from individual myocytes under drug-free conditions (2-s stimulation rate) were used as the command waveforms in the presence of \( I_{Kr} \) blockade with E-4031; the difference in membrane current in the absence versus presence of E-4031 was calculated to demonstrate the contribution of \( I_{Kr} \) during ventricular repolarization. For this series of experiments, perforated-patch techniques were used to minimize alterations in the intracellular milieu, and a discontinuous “single-electrode switch-clamp” technique was used to faithfully record membrane potential, thereby preventing problems associated with action potential recordings obtained with patch-clamp head stages in the current-clamp mode (19). Results are shown in Fig. 8A, which shows 10 consecutive action potentials recorded in the absence (control) and presence of E-4031. E-4031 prolonged the average action potential by reducing the slope of the plateau phase without altering the configuration of the transition to terminal (phase 3) repolarization. Figure 8B shows the average E-4031-sensitive current \( (I_{Kr}) \) recorded using the action potentials as the command-clamp waveform. The E-4031-sensitive current appears as a linearly increasing outward current superimposed over the drug-free action potential-clamp waveform. \( I_{Kr} \) peaks at \(-46 \) mV, during the transition from phase 2 to phase 3 repolarization. Block of this small current (\(-50\)-pA peak amplitude) is responsible for the prolongation of the action potential shown in Fig. 8A. Similar results with E-4031 were observed in five Purkinje myocytes; a similar configuration was also observed after \( I_{Kr} \) block with \( 1 \times 10^{-4} \) M sotalol (\( n = 2 \) myocytes; data not shown).

\( I_{Kr} \) Transient Is Present in Purkinje Myocytes

\( I_{Kr} \) transient was also observed in isolated Purkinje myocytes. Figure 9A shows the voltage-clamp protocol (top) and corresponding membrane currents recorded from a Purkinje myocyte in the absence and presence of E-4031 (middle). Figure 9A, bottom, displays (on an expanded vertical axis) E-4031-sensitive current as a resurgent transient on repolarization. This configuration is consistent with that identified as \( I_{Kr} \) in ventricular myocytes. This myocyte also displayed reduced outward current on repolarization (consistent with a reduced \( I_{Kr} \) density in Purkinje vs. ventricular myocytes, see Ref. 21). Figure 9, B and C, summarizes the effect of repolarization rate on the \( I_{Kr} \) transient observed with five Purkinje myocytes. As described earlier for ventricular myocytes, the repolarization rate did not affect the amplitude (Fig. 9B) or potential (Fig. 9C) of the peak \( I_{Kr} \) transient. The average maximal density of the \( I_{Kr} \) transient from Purkinje myocytes (from all 3 ramp slopes) was \( 0.494 \pm 0.121 \) pA/pF (mean ± SD), which was not statistically different from that obtained in ventricular myocytes (\( P = 0.278 \), power = 0.28). Also, the average potential for the peak \( I_{Kr} \) transient in Purkinje myocytes (\(-57.5 \pm 17.4 \) mV, mean ± SD) was not statistically different from that in ventricular myocytes (\( P = 0.296 \), power = 0.69). These results confirm that the \( I_{Kr} \) transient is present in canine Purkinje myocytes and is comparable to that of ventricular myocytes. A more complete characterization of \( I_{Kr} \) in Purkinje myocytes was not attempted because of the limited number of isolated Purkinje myocytes that retained a relaxed morphology and stable holding current during recordings.

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Fig. 8. Action potential clamps: a direct demonstration of role of \( I_{Kr} \) in ventricular repolarization. A: 10 consecutive recordings of a ventricular action potential in absence (control) and presence of 5 \( \mu \)M E-4031 recorded using a conventional voltage follower and perforated-patch techniques. Control action potentials were digitally recorded and applied as voltage-clamp waveform. B: results obtained using action potential-clamp technique. Average action potential-clamp waveform has been superimposed over average membrane current \( (I_{Kr}) \) required to maintain the shorter action potential in presence of E-4031. \( I_{Kr} \) appears as an increasing outward current that peaks near transition between phase 2 and phase 3 (terminal) repolarization. Basic cycle length, 2 s. See text for further discussion.
**DISCUSSION**

**I_{Kr}** Provides a Transient, Late-Repolarizing Current

This study is the first to characterize an I_{Kr} transient in canine myocardium and directly demonstrate its role in ventricular repolarization. An outward current transient during repolarizing ramps was identified as I_{Kr} on the basis of the following evidence. 1) Similar transients are elicited during superfusion with three different I_{Kr}-blocking agents (E-4031, dofetilide, and sotalol). 2) The E-4031-sensitive outward transient is absent after prior reduction of I_{Kr} by superfusion with 0 mM [K⁺]o. The late outward transient is not an artifact resulting from intracellular dialysis because similar waveforms are observed when perforated-patch techniques are used with action potential clamps. Activation within the plateau range of potentials is sufficient to ensure that most of I_{Kr} is available during a 100-ms plateau (Fig. 2). Action potential clamps demonstrate that I_{Kr} provides a transient late outward current during the terminal portion of the action potential plateau, promoting the transition to phase 3 repolarization of the canine ventricular action potential. Thus, despite its name, the rapidly activating I_{Kr} current contributes substantial significant outward current later during the ventricular action potential as a resurgent outward current that precedes activation of I_{K1}.

Shibasaki (26) first suggested that voltage-dependent inactivation was responsible for inward rectification of I_{Kr} in rabbit sinoatrial nodal cells superfused in high [K⁺]o. Subsequent studies described I_{Kr} in guinea pig ventricular myocytes as a rapidly activating outward current that contributes minimal current at plateau potentials because of its inward rectification properties (2). Studies suggest that the human ether-a-go-go-related gene (HERG) encodes a potassium ion-selective channel that displays some electrophysiological and pharmacological characteristics similar to those of I_{Kr} (24, 29, 32). Studies of HERG expressed in HEK-293 cells or Xenopus oocytes support the role of voltage-gated fast inactivation in the rectification of I_{Kr} (27, 28). In these expression systems, rectification results from inactivation that proceeds at a rate faster than activation, limiting outward current on depolarization. I_{Kr} and HERG typically display a “hooked” configuration on step repolarization that has been attributed to rapid recovery from inactivation (relative to deactivation) immediately following a repolarizing square-clamp pulse.

On the basis of these reports, the configuration of the I_{Kr} transient during repolarization (in this study) is most easily explained by assuming rapid voltage-dependent recovery from inactivation along with slower deactivation kinetics. Because the amplitude and voltage characterizing the peak I_{Kr} transient are unaffected by repolarization rate (Figs. 4, 6, and 9), recovery from inactivation must be rapid relative to the physiological repolarization rates tested. The lack of effect of repolarization rate on I_{Kr} transient is also consistent with slow channel deactivation relative to repolarization. Prior studies of canine I_{Kr} demonstrated slow tail-current kinetics after step repolarizations under experimental conditions identical to those in this study (time constant of inactivation (τ) = 2–3 s at −40 mV, Ref. 7); two time constants have also been reported (τ values in range of 250–500 ms and 2–20 s, Ref. 17). Slow deactivation kinetics have also been reported for rabbit (0.8 s, Ref. 3) and cat (0.7 s, from Fig. 7A in Ref. 6).
ventricular myocytes and can be gleaned from studies of human ventricular myocytes ($r = 500$ ms at $-30$ mV, derived from Fig. 3 in Ref. 15); faster deactivation kinetics have been reported with guinea pigs ($t = 175$ ms near $-30$ mV, Ref. 25). The rapid recovery from inactivation and slow deactivation kinetics of canine $I_{Kr}$ provide a voltage-dependent (and functionally time-independent) outward transient during ventricular depolarization, analogous to that provided by $I_{K1}$ at more negative potentials during final repolarization.

In contrast to $I_{K1}$, greater heterogeneity dispersion is apparent in the potentials characterizing the peak $I_{Kr}$ transients (Figs. 4–6). The $I_{Kr}$ transient density is typically greater if it occurs at more positive potentials (Fig. 7). Thus the voltage of the peak transient modulates the contribution of $I_{Kr}$ during an action potential by two means, namely, 1) by affecting the timing of the transient and 2) by affecting the amplitude of the transient during depolarization. A more detailed kinetics analysis of $I_{Kr}$ inactivation in ventricular myocytes [using brief (a few ms) step-clamp protocols] is not technically feasible because of their large capacitance (mean value near 180 pF, unpublished observations) affecting temporal clamp fidelity and the presence of larger time-dependent currents present in this preparation. The use of repolarizing ramps enhances the resolution and characterization of native $I_{Kr}$ and provides an effective means to assess the effects of antiarrhythmic drugs and electrophysiological changes accompanying heart disease.

An outward $I_{Kr}$ transient is observed during repolarizing ramps (28) and with imposed action potential clamps (32) using heterologous expression systems expressing HERG. In oocytes, expressed peak HERG $I_{Kr}$ current increases with progressively slower repolarizing ramps (range $-0.1$ to $1$ V/s) and peaks at more negative potentials with faster repolarization (28). In contrast, native canine $I_{Kr}$ amplitude is not affected by ramp slopes (range $-1.21$ to $-0.4$ V/s, Fig. 4) and peaks at similar potentials as the amplitude grows after longer activating pulses (Fig. 2). It is uncertain whether these disparate results can be attributed to differences in experimental conditions, species, or differences in channel gating. A comparison of results obtained using native $I_{Kr}$ and heterologously expressed HERG requires caution because of the generally slower kinetics of HERG in heterologous expression systems, which are exaggerated at lower experimental temperatures (32). Computer simulations of guinea pig $I_{Kr}$ (assuming instantaneous recovery from inactivation) predict an outward $I_{Kr}$ transient during repolarization (31) but do not predict variability in the voltages describing the peak transients. This suggests that other factors are responsible for modulating native $I_{Kr}$ in ventricular myocytes.

The $I_{Kr}$ density in canine ventricular myocytes measured during repolarizing ramps (0.44 pA/pF, Fig. 4) is more than twofold greater than that measured during step repolarizing pulses to $-40$ mV under identical experimental conditions (0.2 pA/pF, Ref. 6). The greater current density with repolarizing ramps is present despite the reduced driving force for potassium ions (peak $I_{Kr}$ transients typically occurring between $-45$ and $-65$ mV, see Figs. 4 and 7). The lesser current density obtained using square repolarizing pulses is consistent with incomplete recovery from inactivation at $-40$ mV, which is likely reduced at more negative potentials during repolarizing ramps. Despite the greater canine $I_{Kr}$ density measured with repolarizing ramps, these values remain $\sim 50\%$ less than those used in simulations of guinea pig action potentials on the basis of experiments utilizing square-clamp pulses (1 pA/pF, Ref. 29, based on Ref. 25). The greater current density with guinea pig myocytes may contribute to the shorter action potentials and more rapid heart rates of this species. The mean peak transient current density was statistically greater in atrial versus ventricular myocytes (0.62 vs. 0.44 pA/pF, $P = 0.04$, Fig. 7). Whether this represents greater functional $I_{Kr}$ current in all atrial myocytes is uncertain, because the range of $I_{Kr}$ densities for atrial myocytes overlaps that of ventricular myocytes, and the shorter atrial action potential duration may limit the extent of $I_{Kr}$ activation. It remains to be determined whether the greater average transient density in atrial myocytes compensates for the shorter atrial action potential duration.

$I_{Kr}$ Also Provides a Transient, Late-Repolarizing Current in Purkinje Fibers

The present study is the first to demonstrate a delayed $I_{Kr}$ transient in Purkinje myocytes with characteristics qualitatively similar to those of ventricular myocytes (Fig. 9). Various $I_{Kr}$-blocking agents significantly prolong the action potential duration of Purkinje fibers (4, 10), suggesting that this current plays a prominent role in defining repolarization in this tissue. However, studies have reported minimal $I_{Kr}$ tail currents in canine and rabbit Purkinje myocytes (4, 21). We could detect no statistically significant difference in peak $I_{Kr}$ density of canine ventricular and Purkinje myocytes (compare Figs. 4 and 9). Although the possibility exists that small differences in $I_{Kr}$ density do exist that may be physiologically significant, the reduced levels of $I_{Kr}$ in Purkinje fibers (4) would ensure a more predominant effect of $I_{Kr}$ during depolarization, consistent with greater action potential prolongation generally observed with $I_{Kr}$ block in Purkinje versus ventricular myocytes.

Possible Mechanism of Heterogeneity of $I_{Kr}$ Inactivation

Significant dispersion is apparent in the potentials characterizing the peak $I_{Kr}$ transients in atrial, ventricular, and Purkinje myocytes. This wide voltage range describing the current-voltage ($I$-$V$) relationship of $I_{Kr}$ (Figs. 4–7 and 9) is a novel finding not readily apparent using traditional square-clamp pulses. Because similar variability was not observed for $I_{K1}$ in the same myocytes (Fig. 5), these differences reflect heterogeneity between myocytes (and not, for example, voltage offsets, which would affect measures of both potassium
currents). A number of arguments suggest that the heterogeneity characterizing the peak potential reflects real differences in the voltage dependence of $I_{Kr}$ (and not, for example, variability resulting from either measurement errors or the influence of contaminating currents). These arguments include the following findings. 1) The amplitude and voltage of the peak transient are easily determined from the transient configuration on an appropriate vertical scale. 2) The voltage of the peak $I_{Kr}$ transients during equilibration with either E-4031 or dofetilide remains unaffected despite the growing transient amplitude. 3) The peak potentials of growing $I_{Kr}$ transients that follow progressively longer activating pulses (Fig. 2) remain constant despite the increasing transient amplitude. 4) Standard deviations characterizing peak potentials are comparable for larger- and smaller-amplitude transients from different myocytes (Fig. 7). In addition, comparable voltage heterogeneity is observed in the peak potentials of $I_{Kr}$ transients of atrial and ventricular myocytes (Fig. 7). This heterogeneity remains despite the greater transient densities present in many atrial myocytes and the different ionic current profiles of these cell types. Because $I_{K1}$ is minimal in atrial (compared with ventricular) myocytes, the voltage heterogeneity of $I_{Kr}$ transients cannot be attributed to the influence of the larger $I_{K1}$ current in ventricle (which is also maximal at more negative potentials). Finally, because $I_{Kr}$ was assessed as drug-sensitive currents in these studies, arguments attributing voltage heterogeneity to contaminating currents require that these currents must also be affected by $I_{Kr}$-blocking agents and that the amplitude and voltage dependence of these additional drug-sensitive currents must be such that they “fuse” with $I_{Kr}$ to generate a smoothly configured transient; at present, there is no evidence for this effect. Together, these arguments support the (simpler) conclusion that heterogeneity in the voltage dependence of the peak transients reflects a characteristic of native $I_{Kr}$ in canine cardiac myocytes.

The voltage range characterizing the $I_{Kr}$ transients is most easily attributed to differences in the voltage dependence of $I_{Kr}$. A recent study has linked a mutation of HERG associated with long-QT syndrome to a shift in the voltage dependence of steady-state inactivation toward more negative potentials (20). In addition to enhancing rectification and reducing channel availability at positive potentials, this mutation would be expected to shift the peak potential of the $I_{Kr}$ transient to more negative potentials, further delaying repolarization. Studies have reported at least two isoforms of HERG in mouse ventricle (14, 18). The expression of these individual isoforms in Xenopus oocytes results in currents with kinetics either slower or faster than those of native $I_{Kr}$, whereas the expression of both isoforms results in intermediate kinetics (comparable to $I_{Kr}$) consistent with the coassembly of homomultimers. The voltage dependence of the I-V relationship of one of the proposed homomultimers (the relatively rare Merg1a*) was shifted by $+10$ mV compared with either of two other homomultimers or the heteromultimer Merg1a/1b. Although results with the mouse homomultimer likely would not account for the shifts in peak transient potentials observed in the present study, they do raise the possibility that some HERG isoforms may display altered voltage dependence. Whether such isoforms exist in canine myocardium is unknown. A recent study of HERG expressed in Xenopus oocytes showed that a small integral membrane protein [termed minK-related peptide 1 (MiRP1)] assembles with HERG and shifts the voltage dependence of activation in a positive direction without appreciably affecting rapid recovery from inactivation (1). Channels associated with MiRP1 also display reduced single-channel current and increased deactivation rate. The effects of MiRP1 on $I_{Kr}$ during repolarizing ramp clamps were not studied and are difficult to predict when oocyte studies (conducted at room temperature) are extrapolated to native channels and physiological temperatures. Thus, it is possible that HERG isoforms, accessory subunits, and/or metabolic factors may contribute toward the heterogeneous voltage dependence of $I_{Kr}$ transients; further studies combining cellular and molecular approaches with electrophysiological recordings are necessary to test these hypotheses in native tissues. Because ventricular myocytes in this study were derived from the entire left ventricular midwall, it is unknown whether the variability of $I_{Kr}$ is related to anatomic origin within the wall or how it may contribute to the electrophysiological distinctions between isolated epicardial, midmyocardial, and endocardial myocytes.

Functional Consequences of Heterogeneity of the $I_{Kr}$ Transient

Differences in the voltage dependence of $I_{Kr}$ (rather than differences in current density) represent the predominant means by which variations in $I_{Kr}$ contribute to ventricular heterogeneity. In contrast, differences in $I_{K1}$ density are largely responsible for heterogeneity of this current. The extent to which variations in the voltage dependence of $I_{Kr}$ are manifest in cardiac hypertrophy, electrical remodeling of the ventricle, and heart failure require further studies using ramp-clamp protocols. The variability of current density and voltage dependence of currents active during the plateau (where small current variations significantly affect the voltage trajectory) provide additional possibilities to explain regional and disease-related differences in action potential configurations.

In summary, this study demonstrates that, despite its rapid activation, $I_{Kr}$ provides a delayed outward current transient during repolarization of ventricular myocardium and Purkinje fibers. This resurgent current plays an increasingly prominent role later during the action potential plateau by promoting the initiation of terminal (phase 3) repolarization and activation of $I_{K1}$. Variability in the voltage dependence of the native $I_{Kr}$ transient and variability in the density of $I_{K1}$ provide two distinct mechanisms for modulating the role of these potassium currents during repolarization.
and contributing toward ventricular electrical heterogeneity.

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REFERENCES


