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Articles

Two Components of Delayed Rectifier Current in Canine Atrium and Ventricle

Does I_{Ks} Play a Role in the Reverse Rate Dependence of Class III Agents?

Gary A. Gintant

Abstract

Abstract Because the number and characteristics of delayed rectifier K^+ current (I_K) components vary between species, the role of each component in the action potential and modulation by class III agents is uncertain. To address these issues, I_K was assessed in adult isolated canine ventricular and atrial myocytes by using whole-cell and perforated-patch techniques. I_K components were characterized by using two complementary approaches: a kinetic approach (based on biexponential fits to deactivating tail currents) and a pharmacological approach (using the methanesulfonanilide compound E-4031). In ventricular myocytes, two exponential tail current components were distinguished; these components differed in the voltage and time dependence of activation and the effect of lower $[K^+]_o$. Both kinetic components contributed equally to peak tail current amplitude (measured at −35 mV) after a single 300-ms pulse to 5 mV, simulating an action potential. By use of E-4031, rapidly and slowly activating components of I_K (I_{Kr} and I_{Ks}, respectively) that were analogous to tail components described kinetically were identified. The activation kinetics and rectification properties of canine I_{Kr} and I_{Ks} are qualitatively similar to those described previously for guinea pigs. In contrast, canine I_{Kr} and I_{Ks} deactivation kinetics differed markedly from those found in guinea pigs, with canine I_{Kr} deactivating slowly (time constant τ, 2 to 3 s near −35 mV) and I_{Ks} deactivating rapidly (τ, 150 ms near −35 mV and decreasing to 30 ms near −85 mV). E-4031 elicited reverse rate-dependent effects (greater drug-induced prolongation of the action potential at slower stimulation rates); this effect is inconsistent with the hypothesis attributing reverse rate dependence to incomplete I_{Ks} deactivation during rapid stimulation (due to rapid deactivation of canine I_{Ks}). Two I_K components with characteristics comparable to those found in ventricular myocytes were also observed in atrial myocytes. In conclusion, (1) I_{Kr}- and I_{Ks}-like components of I_K are present in canine atrial and ventricular myocytes, with deactivation kinetics strikingly different from those found in guinea pigs, and (2) the rapid deactivation kinetics of canine I_{Ks} do not support its role in reverse rate dependence with class III agents in this species.

Key Words:
delayed rectifier K^+ currents
E-4031
reverse rate dependence
ventricular myocytes
atrial myocytes

It is generally accepted that I_K provides outward current during the cardiac action potential to facilitate repolarization.[1] Recent pharmacological studies have

identified two I_K components in guinea pig atrial and ventricular myocytes based on the effects of the prototypic class III antiarrhythmic agent E-4031.[2 3 4] These two components have been termed I_{Kr} (described as a rapidly activating and deactivating component blocked by E-4031 that displays inward rectification) and I_{Ks} (an E-4031-resistant component that activates and deactivates slowly and displays minimal rectification). The number of I_K components appears to differ when different species are compared. In contrast to findings in guinea pigs, pharmacological evidence suggests that one I_K component is present in rabbit[5] and feline[6] ventricles; I_K is reportedly present[7] or absent[8 9] in adult rat ventricles. In canine ventricles, two I_K components have recently been reported.[10 11] The characteristics of I_K also appear to differ across species. For example, I_{Kr} deactivates rapidly in guinea pig myocytes[2 3 4] but more slowly in rabbit myocytes.[5] Although two I_K components have also been reported in atrium from guinea pigs, a description of atrial I_K in other animal models has not been reported.

Most class III antiarrhythmic agents cause greater prolongation of the APD at slow versus rapid rates of stimulation. This effect, which has been termed reverse use dependence[12] or reverse rate dependence,[13] has been implicated in the bradycardia-dependent proarrhythmic effects of various class III agents. Reverse rate dependence has been demonstrated with various I_{Kr} blocking agents, including the structurally related methanesulfonanilides E-4031, dofetilide, sotalol, and WAY-123,398.[9 14 15] In guinea pigs, reverse rate dependence has been attributed to the "accumulation" of I_{Ks} during rapid stimulation (resulting from the incomplete deactivation of this current), which minimizes the effects of I_{Kr} block at faster stimulation rates.[13]

Because of the importance of I_K in modulating repolarization and its possible role in defining the heterogeneity of electrical activity across the ventricular wall[1 16 17] and because of its use as a potential focus for modulation by class III agents with antiarrhythmic as well as arrhythmogenic potential,[12 18] the characteristics of I_K were studied by using isolated myocytes derived from the canine left ventricular free wall and atrium. An earlier study identified two components of canine ventricular I_K.[10] In this study, both components were characterized kinetically and compared with those identified pharmacologically by using E-4031 to avoid possible confounding effects of voltage- and time-dependent block by this agent. With either approach, two I_K components were identified, with each component contributing comparable repolarizing current after 300-ms clamp pulses to the plateau range of potentials. Two I_K components were also identified in canine atrial myocytes, with characteristics similar to those found in the ventricle. Although the activation kinetics of canine I_{Kr} and I_{Ks} were found to be comparable to those found in guinea pigs, their deactivation kinetics were distinctly different, with canine I_{Kr} deactivating slowly and I_{Ks} deactivating more rapidly. Despite the rapid deactivation of canine I_{Ks}, block of I_{Kr} by E-4031 caused reverse rate-dependent effects on the canine ventricular action potential, an effect inconsistent with the postulated role of I_{Ks} in reverse rate dependence. These results suggest that the mechanisms responsible for reverse rate dependence by antiarrhythmic agents require reevaluation in this widely used animal model for electrophysiological studies as well as in other species.

Materials and Methods

Isolation of Canine Ventricular Myocytes

Ventricular myocytes from adult male mongrel dogs were isolated by using techniques described previously[19 20] and as approved by the institutional animal care and use committee. Briefly, hearts were removed from anesthetized heparin-

pretreated dogs and placed in cold nominally Ca^{2+}-free Tyrode's solution containing (mmol/L) NaCl 118.5, KCl 2.8, $NaHCO_3$ 14.5, KH_2PO_4 1.2, $MgSO_4$ 2.7, and glucose 11.1 and aerated with 95% O_2/5% CO_2. Wedges of left ventricular free wall supplied by the left anterior descending coronary artery were excised, cannulated, and subsequently flushed with 40 mL warm Tyrode's solution supplemented with 0.1% BSA (fraction V, protease-free, Sigma Chemical Co). The cannulated wedge was then mounted in a warmed jacketed Langendorff perfusion system and perfused with nominally Ca^{2+}-free Tyrode's solution supplemented with 0.04% collagenase (type IV, Worthington Biochemical Co), 1% BSA, and 1.5 mmol/L $MgSO_4$ (37°C). After <20 minutes of perfusion, the wedge was removed, and epicardial and endocardial layers (minimum, 1 mm thick) were removed by shaving with a dermatome. Chunks of remaining midmyocardial tissue were then minced and immersed in HEPES-buffered "base" solution containing (mmol/L) NaCl 132, HEPES 20, $MgSO_4$ 1.2, glucose 11.1, and KCl 4.0, supplemented with 1.5% BSA, 0.04% collagenase, 2.0 mmol/L $MgSO_4$, and 0.3 mmol/L $CaCl_2$ (pH 7.2, termed "digest solution") and aerated with 100% O_2 in a shaker bath (37°C). At 10-minute intervals, digests were filtered through nylon mesh, centrifuged, and resuspended in base solution supplemented with 0.5 mmol/L $CaCl_2$, 2.0 mmol/L $MgSO_4$, and 3% BSA (termed "storage" solution). Larger tissue fragments were returned to fresh digest solution for further dissociation, typically for 20 to 30 additional minutes. Cells resuspended in the storage solution (at room temperature) were used within 12 hours; some cells were "washed" by passage through a Percoll gradient to remove excess debris.

Isolation of Canine Atrial Myocytes

Hearts were removed, placed in a Langendorff apparatus, and perfused (37°C) with a solution containing (mmol/L) KCl 80, KH_2PO_4 30, $MgSO_4$ 4, HEPES 20, glucose 10, taurine 20, creatine 5, succinate 5, and EDTA 1, along with 0.1% BSA (pH 7.2). After a 10-minute rinse, perfusion was switched to a fresh solution supplemented with collagenase (type II, 125 U/mL, Worthington) and recirculated for an additional 30 minutes. A portion of the left atrium was removed and triturated, and myocytes were placed in the recording chamber.

Experimental Apparatus and Solutions

Myocytes were allowed to settle on the bottom of a Peltier-based temperature-controlled perfusion bath mounted on an inverted microscope. Cells were superfused (0.8 mL/min) with HEPES-buffered Tyrode's solution containing (mmol/L) NaCl 132, $MgSO_4$ 1.2, HEPES 20, glucose 11.1, KCl 4, and $CaCl_2$ 2 (pH 7.4 with HCl). Bath temperature was monitored with a small thermistor probe (0.35-mm diameter, NBD Enterprises) located on the bath bottom and positioned within 2 mm of the myocyte under study. Whole-cell experiments were conducted at 36°C to 37°C; bath solutions were exchanged within ≈30 s. Only rodlike quiescent (when not stimulated) myocytes with uniform sarcomeric appearance and nonrounded or contracted edges were chosen for study. Most myocytes studied were isolated from the midventricular free wall to minimize differences in I_{to} density.[20]

E-4031 was prepared fresh daily as a 5 mmol/L aqueous stock solution. A final bath concentration of 5 μmol/L was chosen on the basis of studies showing that this concentration totally blocked I_{Kr} in guinea pig myocytes.[2] Nisoldipine was prepared fresh daily in 30% ethyl alcohol and used in a darkened room. The possible effects of ethyl alcohol were not studied.

Electrical Recordings

Membrane currents were obtained by using the whole-cell configuration of the patch-clamp technique.[21] Borosilicate glass patch electrodes were coated with either Sylgard or dental wax and heat-polished immediately before filling with a standard high-K^+ solution containing (mmol/L) potassium aspartate 125, KCl 20, EGTA 10, ATP (magnesium salt) 5, $MgCl_2$ 1, and HEPES-free acid 5, adjusted to 7.3 with 5N KOH; electrode resistances typically ranged from 1 to 2.5 MΩ. The potential was adjusted for a 10-mV junction potential just before sealing. After formation of a gigaseal and compensation of electrode capacitance, the patch was ruptured either electronically or by negative pressure. Analog series resistance compensation was then adjusted to values typically 60% to 70%. An additional initial check of ventricular cell viability was routinely provided by assessing the N-shaped steady state current-voltage characteristics recorded with a 100-mV depolarizing ramp pulse.

Where indicated, perforated-patch recordings of whole-cell ventricular I_K were obtained by using techniques adopted from Horn and Marty.[22] These experiments examined I_K with minimal alterations of the intracellular milieu (which would be expected if whole-cell patch techniques were used). The tip of a freshly coated heat-polished pipette was filled with a solution containing (mmol/L) potassium aspartate 125, KCl 20, Na_2-ATP 5, $MgCl_2$ 1, and HEPES 5. Subsequently, 20 μL of freshly prepared amphotericin solution (Sigma A-4888, 6 mg/100 μL dimethyl sulfoxide) was added to 5 mL of filling solution and used to backfill the pipette. After formation of a gigaseal, the diffusion of amphotericin to the pipette tip and its partitioning into the cell membrane established electrical continuity between the pipette and the cell interior through newly formed pores. Typical access resistances of 10 MΩ were obtained within 20 minutes of establishing a gigaseal.

For studies of reverse rate dependence with E-4031, changes in the ventricular APD were assessed by using standard microelectrode techniques (as in Reference 19).

Data Acquisition and Analysis

Currents were low pass-filtered (500 Hz), digitized (typically 166 Hz), stored, and analyzed on an 80386-based computer by using PCLAMP software (version 5.5.1, Axon Instruments). Cell capacitance was determined at the resting membrane potential (negative to −80 mV) after cell rupture by methods that account for the series resistance (typically 10 MΩ) and input resistance (typically 15 to 25 M) for canine ventricular myocytes as described previously.[10] Values of series resistance (R_S) and cell input resistance (R_{in}) were calculated from the current response to a 5-mV hyperpolarizing pulse from the zero current potential according to the following equation:

$$Cm=(\tau/R_s)\times[1+(R_s/R_{in})]$$

where Cm is cell capacitance and τ is the time constant of the "off transient." R_{in} in the range of −40 mV (holding potentials for I_K studies) was estimated to be ≥200 MΩ for ventricular myocytes on the basis of the slope of the steady state current-voltage relations (see above).

The kinetics of I_K deactivation were determined by using nonlinear least-squares regression analysis applied to tail currents, with two exponential components (I_{Ke1} and I_{Ke2}) adequately described the decaying tail currents (see Fig 1⇓; also, Reference 10). In nearly all cases, two exponential components adequately reproduced the tail currents; fits with greater than two exponential components did not significantly enhance descriptions of the time-dependent current, as judged by

residual analysis. The amplitude of each tail current component was obtained by extrapolation of fits to the onset of repolarization. Tail currents of 8- to 12-s duration were typically fit to ensure adequate resolution of the slower tail current component; data tracings were truncated in most figures to enhance visual comparisons of the current time course. Because of the typical small amplitude of tail currents, experiments were analyzed and subject to curve fitting only when stable baseline currents were maintained as monitored continuously during experiments and detected from recorded data tracings (see Figs 1⇓, 3⇓, 5⇓, 8⇓, and 10⇓). Depolarizing test pulses were applied once every 20 to 30 s (held constant in any individual experiment) to allow the slower tail component to fully decay.

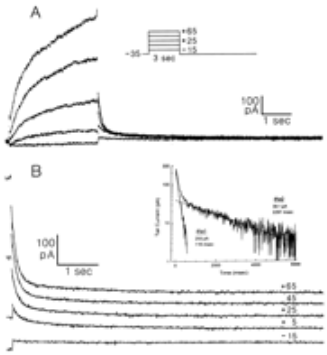

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Figure 1.

Two exponential components of I_K (I_{Ke1} and I_{Ke2} [iKe1 and iKe2 on figure]) in isolated canine ventricular myocytes. A, Membrane currents recorded during 3-s depolarizing test pulses to −15, 5, 25, 45, and 65 mV and upon repolarization (holding potential, −35 mV). Pulses were applied once every 25 s to ensure deactivation of I_K tail currents. B, Tail currents (and superimposed fits) after pulses to indicated potentials are illustrated on expanded scale and offset for clarity. Currents were fit to the sum of two exponential components and a constant. The peak amplitudes of tail current components were 5.4, 54.8, 126, 200, and 255 pA for I_{Ke1} and 12.8, 34.7, 38.8, 37.0, and 39.1 pA for I_{Ke2} (ascending test pulse potentials). Only the first 7.5 s of tail currents is shown. Inset, Tail currents and superimposed fits for depolarizing pulse to +65 mV plotted on semilogarithmic coordinates. Subtraction of the slower exponential component (I_{Ke2}) from the current tracing revealed the faster exponential component (I_{Ke1}). See text for further discussion.

The two exponential tail current components were termed I_{Ke1} and I_{Ke2} (see Fig 1⇑). Studies of I_K in guinea pig myocytes have used the terms I_{Kr} and I_{Ks} (r referring to rapidly activating and s referring to slowly activating in Reference 2) to describe two I_K components based upon pharmacological blockade by E-4031. The I_{Ke1}/I_{Ke2} nomenclature was used to describe the present kinetic-based studies, since the I_{Ke1}/I_{Ke2} nomenclature follows earlier descriptions of I_K in cardiac Purkinje fibers, and kinetic distinctions described are independent of possible voltage- and time-dependent effects of drug block.

I_{Kact} was defined as the time-dependent current during depolarizing test pulses measured from the minimum outward current at the onset of depolarization and the maximum outward current immediately before repolarization. The initial time course of current activation is complex and generally does not fit well as two exponential components; this is consistent with a number of native cardiac K^+ channels (eg, see Reference 23).

Records characterizing baseline I_K were typically obtained within 10 minutes of cell break-in to minimize possible current rundown. Although possible time-dependent changes in whole cell I_K were not systematically investigated, biexponential tail currents were observed immediately after clamp "tune-up" (requiring <3 minutes after patch rupture) as well as after 30 minutes of maintained access. The effects of reducing $[K^+]_o$ and E-4031 were assessed by comparing currents immediately before and after equilibration, typically within 5 minutes. Where appropriate, data are expressed as mean±SEM.

Isolation of Canine I_K

The confident evaluation of I_K depends on the removal of overlapping contaminating ionic currents, such as inward Na^+ and Ca^{2+} currents and I_{to}. Holding potentials ranging from −35 to −40 mV were used to inactivate T-type Ca^{2+} current and fast Na^+ current; L-type Ca^{2+} current was blocked by using nisoldipine (1 μmol/L) under darkened conditions. In contrast to an earlier report,[24] nisoldipine had no discernible effect on tail currents at the lower concentration used (n=3; data not shown), in agreement with other studies (see References 2 and 25). These results suggest that I_K deactivation is not influenced by transmembrane-dependent Ca^{2+} influx via L-type Ca^{2+} channels. Possible contamination from electrogenic Na^+-Ca^{2+} exchange should be minimized by the high EGTA concentrations used in the patch pipettes.

The activation of canine I_K likely overlaps the 4-aminopyridine-sensitive I_{to}, which is minimized (but not fully inactivated) with holding potentials used in this study.[20] 4-Aminopyridine was not used to block I_{to}, since preliminary studies showed that it blocked I_K in canine myocytes (as has been reported in guinea pig preparations[26]), and block of I_{to} may be complex.[27] As I_{to} rapidly inactivates, the time course of I_K activation for only the first 10 to 20 ms is likely affected. Estimates of the amplitude of I_K activation upon depolarization (I_{Kact}, above) likely represent a minor underestimation of total I_K amplitude because of the rapid kinetics of I_{to} inactivation compared with slower I_K activation at positive test potentials.

Results

Two I_K Components Can Be Distinguished Kinetically in Canine Ventricular Myocytes

Fig 1⇑ details typical I_K obtained from an isolated canine ventricular myocyte. Panel A illustrates membrane currents during 3-s depolarizing test pulses and tail currents after repolarization to the holding potential of −35 mV. Panel B illustrates the family of tail currents (offset on an expanded scale) labeled according to the preceding test pulse potential. Superimposed on each tail is a biexponential fit. Tail currents were well described as the sum of two exponential components and a constant according to the following equation: $I(t)=A1\cdot\exp(-t/\tau_1)+A2\cdot\exp(-t/\tau_2)+Ao$, where τ_1 and τ_2 represent the more rapid and slower exponential components, A1 and A2 represent the amplitude of each respective component, and Ao represents the baseline current. The two components were termed I_{Ke1} and I_{Ke2}, with subscripts e1 and e2 referring to the faster and slower exponential tail current components, respectively. A similar approach was previously used to define regional differences in I_K components in canine myocytes.[10]

The biexponential nature of the tail currents is further illustrated in the inset, which plots (on semilogarithmic coordinates) the tail current after a depolarizing pulse to +65 mV. For this tail current, the time constants for I_{Ke1} and I_{Ke2} were 116 and 2297 ms, respectively, with the peak amplitude of I_{Ke1} 6.5-fold greater than that of

I_{Ke2}. Time constants for I_{Ke1} were typically 15 to 20 times faster than those for I_{Ke2} at the −35-mV holding potential. In this myocyte, the time constants for I_{Ke1} and I_{Ke2} ranged from 116 to 140 ms and from 1969 to 2317 ms, respectively. In four myocytes in which perforated patch-clamp technique was used to maintain the intracellular milieu, tail currents were also well described by biexponential fits similar to those used for myocytes by whole-cell patch methods (data not shown). These results demonstrate that the biexponential currents observed by whole-cell recording techniques cannot be attributed to intracellular dialysis.

Fig 2⇓ highlights differences in the voltage-dependent activation of I_K over a wide range of test pulse potentials. Panel 2A summarizes differences in the amplitude of each tail component after 3-s test pulses, with peak values of I_{Ke1} and I_{Ke2} normalized to those after a test pulse to +65 mV in the same myocyte. Panel A shows that the amplitude of the I_{Ke1} component continues to increase with more positive test pulse potentials up to +65 mV. (In two additional experiments, I_{Ke1} showed no indication of saturation with a +75-mV test pulse.) In contrast, the peak amplitude of I_{Ke2} is attained with test pulses to 0 mV. When test pulses to +65 mV were used, the density of the I_{Ke1} tail component (1.04 pA/pF) was much greater than that of I_{Ke2} (0.188 pA/pF). The effect of test pulse potential on I_K activation (I_{Kact}) during depolarizing pulses is illustrated in Panel B. As before, current density was normalized to maximum I_{Kact} measured during a +65-mV test pulse. The current-voltage relation for I_K was described as an increasing curvilinear function of test pulse potential; the average maximum density of I_{Kact} was 2.38 pA/pF (at +65-mV test potential).

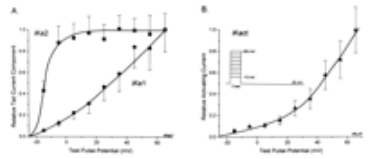

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Figure 2.

Voltage dependence of I_K components. A, For each myocyte, the peak amplitude of each tail component after a 3-s test pulse was normalized to that obtained after a test pulse to +65 mV. I_{Ke1} (iKe1) amplitude (•) continued to increase over the range of test pulse potentials examined; in contrast, I_{Ke2} (iKe2, ▪) attained maximal values after test potentials near 0 mV. The density of I_{Ke1} (1.04 pA/pF) was approximately fivefold greater than that for I_{Ke2} (0.188 pA/pF). B, Current-voltage relation for I_K is shown. The amplitude of I_{Kact} during test pulses (iKact, ▴) was normalized to that obtained during a +65-mV test pulse. I_{Kact} continues to increase over the voltage range examined. The density of I_{Kact} (after a +65-mV test pulse) was 2.38 pA/pF. Inset, Voltage-clamp protocol used. Data for panels A and B were obtained from the same nine myocytes; each data point represents a minimum of seven determinations. Curves were drawn by eye. Holding potential was −35 mV; pulses were applied once every 25 s.

To assess the time course of activation of each tail component, the growth of each component was determined from fits following depolarizing pulses of selected durations. Fig 3⇓ shows averaged results obtained from five myocytes by use of test pulses to 60 mV (−40-mV holding potential). When this protocol was used, the amplitude of the slowly decaying exponential component (I_{Ke2}) after 50-ms pulses was equivalent to those obtained after 650-ms pulses. This result demonstrates

rapid activation of the slowly deactivating tail component. In contrast, the amplitude of the rapidly decaying exponential component (I_{Ke1}) continues to increase after longer depolarizing pulses. The inset shows results from a typical experiment; I_K during depolarization (I_{Kact}) and the I_K tail current continue to increase with prolonged depolarizing pulses, consistent with the hypothesis that I_{Ke1} represents the majority of I_K activated during strong prolonged depolarizing pulses. Tail currents following pulses shorter than 50 ms were not characterized because of the difficulty in fitting these smaller currents and possible overlap with inactivation of I_{to}.

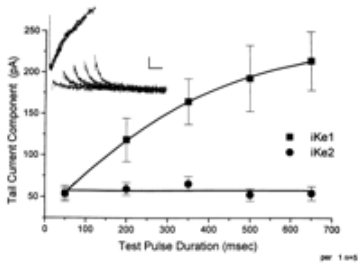

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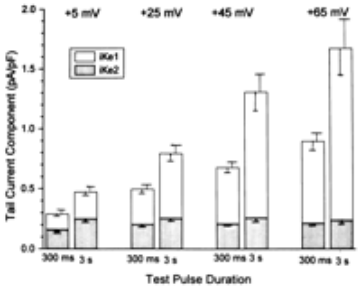
Figure 3.

Different activation kinetics of tail current components I_{Ke1} and I_{Ke2} (iKe1 and iKe2, respectively). To determine the time course of activation of the tail components, currents following the depolarizing pulses were subjected to biexponential fits, and amplitudes of each component were plotted. For each of five myocytes, activation of I_{Ke2} (•) was rapid, reaching a plateau within a 50-ms depolarizing pulse. In contrast, I_{Ke1} activation (▪) continued to increase through the range of test durations studied. Test pulse potential was 60 mV; holding potential, −40 mV. Inset, Typical recording of membrane currents. Biexponential fits were superimposed on family of tail currents. Calibration was 200 ms (horizontal) and 60 pA (vertical).

The time course of activation of each I_K component at other test potentials was not systematically examined. However, tail currents were compared by using 300-ms and 3-s test pulses for select potentials in the same myocyte, providing an indication of the contribution of each I_K component during prolonged versus short depolarizations (the later resembling a single action potential). Results from 13 myocytes are summarized in Fig 4⇓. For each test potential, the total tail current density (represented by bar height) is greater after the longer test pulse, largely because of an increase in the more rapid (I_{Ke1}) tail component (open bars). A slight increase in the slower tail component (I_{Ke2} [shaded bars]) was noted by comparing 300-ms versus 3-s depolarizing test pulses, which did not achieve statistical significance. This result confirms that I_{Ke2} activation is largely complete within 300 ms for all test potentials examined and does not show evidence of slow inactivation. The figure also confirms that (1) for each test pulse duration, I_{Ke1} increases after stronger depolarizing pulses, and (2) for each test potential, I_{Ke1} increases with longer depolarization. As a consequence, for test potentials ≥25 mV, I_{Ke1} is the predominant tail current component. However, the amplitudes of I_{Ke1} and I_{Ke2} are similar for 300-ms pulses in the range of potentials encountered during ventricular repolarization (<+25 mV), suggesting that both components provide comparable repolarizing current before termination of the plateau of a single action potential.

Figure 4.



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Tail current components I_{Ke1} and I_{Ke2} (iKe1 and iKe2, respectively) compared after prolonged vs short depolarizing pulses to different test potentials. For each of 13 myocytes, the amplitudes of the tail components were compared after 300-ms (left partners) vs 3-s (right partners) pulses to selected test potentials (indicated above). The amplitude of I_{Ke1}, the more rapid component, is represented as open area of bar; that of I_{Ke2}, the slower component, is represented as the shaded area of the bar. For potentials encountered during the ventricular action potential plateau (+5 to +25 mV), the amplitudes of I_{Ke1} and I_{Ke2} are comparable after 300-ms pulses (mimicking a single action potential). Holding potential was −35 mV. Standard error bars for I_{Ke1} and I_{Ke2} are indicated by attached downward flags; upward error bars represent standard error for the sum of I_{Ke1} and I_{Ke2} from each myocyte.

The two tail components were differentially affected by eliminating $[K^+]_o$. Typical results are illustrated in Fig 5⇓, which shows superimposed membrane currents recorded in 4 mmol/L (solid circles) and 0 mmol/L (open circles) potassium Tyrode's solution. Reducing $[K^+]_o$ greatly reduces the amplitude of I_{Ke2} while it increases the amplitude of I_{Ke1} (fit parameters indicated in figure). The time constants of either component were unaffected. Similar results were observed in four analogous experiments. Reducing $[K^+]_o$ to 0 mmol/L also increased the amplitude of I_K activated upon depolarization and caused a reduction in net outward current at the holding potentials used (range, −35 to −50 mV).

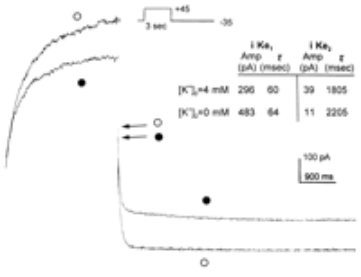

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Figure 5.

Lowering $[K^+]_o$ differentially affects tail components. Typical membrane currents following a depolarizing test pulse in the presence of 4 mmol/L $[K^+]_o$ (•) and after 5 minutes of exposure to 0 mmol/L $[K^+]_o$ (○) are shown. Removing extracellular K^+ (1) reduced the holding current, (2) reduced I_{Ke2} (iKe2), the slower tail current component (note diminished slope of tail current), and (3) enhanced the amplitude (Amp) of the remaining I_{Ke1} (iKe1) (compare arrows indicating peak tail currents in 4 vs 0 mmol/L $[K^+]_o$). The Amp of I_{Kact} (iKact) upon depolarization was also increased by reducing $[K^+]_o$. Inset, Table⇓ indicates fitting parameters for I_{Ke1} and I_{Ke2} in the presence and absence of $[K^+]_o$. Holding potential was −35 mV; test potential, 45 mV.

The voltage dependence of I_K deactivation was assessed by using two different protocols. In one series of experiments (summarized in Fig 6⇓), tail currents following 3-s depolarizing pulses were fit with two exponentials. Despite variability of the derived time constants for each component, the components clustered into a faster (I_{Ke1}) and slower (I_{Ke2}) range through −20 to −60 mV. In most cases, the time constant for deactivation of the faster tail component appeared to decline at more negative potentials.

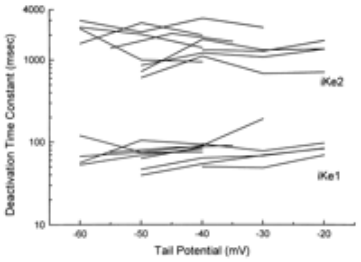

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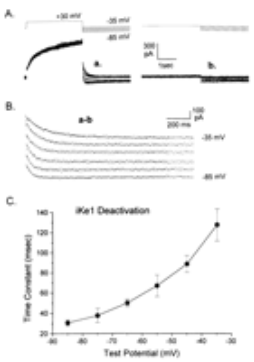
Figure 6.

Voltage dependence of deactivation of I_K tail components I_{Ke1} and I_{Ke2} (iKe1 and iKe2, respectively). The figure illustrates time constants of the two tail current components obtained at selected repolarizing potentials. For each of 10 myocytes examined, two distinct ranges of time constants could be distinguished over the voltage range examined (−20 to −60 mV). Each set of lines represents results obtained at different potentials from a single myocyte. Depolarizing pulse duration was 3 s; a test pulse potential of either +25 or +40 mV was used (constant for each myocyte).

To further examine the voltage-dependent kinetics of I_{Ke1}, two-step clamp protocols were applied in the presence of 0 mmol/L $[K^+]_o$. This strategy (1) minimized variability inherent in biexponential fits (by eliminating the slower I_{Ke2} component), (2) increased the amplitude of I_{Ke1}, thereby allowing for a wider range of potentials to be examined, and (3) minimized contamination by I_{K1}. The protocol used is illustrated in Fig 7A⇓. To eliminate contamination of time-dependent currents sometimes present during hyperpolarization alone, tail currents without prepulses (Fig 7A⇓, a) were subtracted from those with a depolarizing prepulse (Fig 7A⇓, b). The resultant difference currents (Fig 7B⇓, a−b) were well fit by using a single exponential (shown superimposed on calculated tracings) at all potentials. Fig 7C⇓ summarizes the deactivation kinetics of I_{Kr} obtained from five myocytes; the time constant for I_{Ke1} deactivation decreased with hyperpolarization, approaching a value of 30 ms at resting potentials near −85 mV. Similar results were obtained in two experiments in the presence of 4 mmol/L $[K^+]_o$ with the slower (I_{Kr} component; see below) blocked by E-4031 (data not shown). The voltage dependence of deactivation of the E-4031-sensitive current component was not evaluated because of the smaller amplitude of this current.

Figure 7.

Voltage-dependent deactivation of I_{Ke1} (iKe1). A, In the presence of 0 mmol/L $[K^+]_o$, currents were recorded at selected test potentials with (tracings at a) and without (tracings at b) a 3-s pulse to activate I_K. B, I_{Ke1} deactivation was assessed by fits to tracings obtained by subtracting currents without prepulse from currents preceded by prepulse (a−b). Shown are derived currents and superimposed



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monoexponential fits. Currents were offset for clarity. C, A summary of the voltage dependence of deactivation of I_{Ke1} obtained from five myocytes is shown. I_{Ke1} (I_{Ks}) deactivation is more rapid at negative potentials. Curve was drawn by eye.

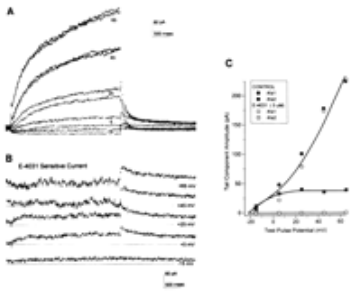
Two I_K Components Distinguished Pharmacologically in Canine Ventricular Myocytes

Earlier studies with guinea pig myocytes demonstrated the presence of two I_K components on the basis of the effects of a prototypic class III antiarrhythmic agent E-4031.[2] In guinea pig myocytes, the E-4031-sensitive component of I_K (I_{Kr}) rapidly activates and deactivates and displays inward rectification; the E-4031-insensitive current (I_{Ks}) activates and deactivates more slowly and shows minimal inward rectification. An initial study had suggested that I_{Ke1} was analogous to I_{Ks}, and I_{Ke2} was analogous to I_{Kr}.[10] In the present study, the effects of E-4031 were further characterized, including its effects on I_K activation.

Representative effects of E-4031 on I_K are illustrated in Fig 8⇓. Panel A overlays current recordings during and after 3-s depolarizing pulses in the absence and presence of E-4031. In general, E-4031 reduces outward current during moderately depolarized test pulses (−15 to +25 mV) to a greater extent than at strongly depolarized potentials. The time course of I_K is parallel in the absence and presence of E-4031 during the later 2.5 s of the pulse. During the first 300 ms of a depolarizing pulse, E-4031 alters the time course of I_K activation. This effect is further illustrated in panel B, which shows E-4031-sensitive currents obtained by digital subtraction of currents in the absence versus presence of the compound (ie, drug-free control minus E-4031). With moderately depolarizing pulses (test potentials of +5 to +25 mV), the E-4031-sensitive current is an increasing outward current, which reaches a plateau within 300 ms. Beyond 300 ms, the E-4031-sensitive current is time independent, accounting for the parallel currents observed later during depolarizing pulses in panel A. Upon repolarization to −35 mV, the drug-sensitive tail current is larger than that activated upon depolarization, consistent with the inwardly rectifying properties of I_{Kr}. The amplitude of the E-4031-sensitive tail component is similar when test pulse potentials >+5 mV are used, consistent with the characteristics of voltage-dependent activation of the kinetically defined I_{Ke2} component. During strongly depolarized test pulses, E-4031-sensitive current is harder to resolve, consistent with the rectification properties of this current and the small amplitude of this current relative to the larger E-4031-insensitive (I_{Ks}-like) current.

Figure 8.

Effects of E-4031 on canine I_K. A, Membrane currents during and after



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test pulses to −15, 5, 25, 45, and 65 mV (as indicated) in the absence (upper tracings of each pair) and presence (lower tracings of each pair) of 5 μmol/L E-4031. E-4031 reduced I_K activation at modestly depolarized test potentials. Note also the reduction of the slow tail current component and reduced outward holding current with E-4031. B, E-4031-sensitive current obtained by digital subtraction (control minus drug). Drug-sensitive currents (and zero lines) were offset upward for clarity. E-4031-sensitive current was an outward current that attained steady values within 500 ms. Drug-sensitive tail currents were larger than currents upon depolarization, demonstrating inward rectification of E-4031-sensitive current. C, Effects of E-4031 on I_K tail components I_{Ke1} and I_{Ke2} (iKe1 and iKe2, respectively) after a wide range of test pulse potentials. E-4031 abolished I_{Ke2} for all test pulse potentials while leaving I_{Ke1} essentially unaffected. Similar results were obtained in five additional experiments. Since I_{Kr} and I_{Ks} are defined as E-4031-sensitive and -insensitive I_K tail current components, respectively, I_{Ke2} is analogous to I_{Kr}, and I_{Ke1} is analogous to I_{Ks}.

Because of the small amplitude of E-4031-sensitive current relative to total I_K during depolarization and concerns that small nonspecific changes in membrane current could be incorrectly interpreted as drug effects, an additional series of five experiments was performed by comparing the effects of E-4031 on I_K during moderate versus strongly depolarizing pulses. For these experiments, a sequence of two depolarizing pulses (to 5 and 45 mV; 3-s duration; holding potential, −35 mV) was sequentially applied before and during equilibration with E-4031, and drug-sensitive currents were measured. Time-dependent E-4031-sensitive current was greater at the 5-mV test potential in one experiment, greater at 45 mV in two experiments, and equal in the remaining two experiments. It could not be determined whether the approach to the plateau of the E-4031-sensitive current was any more rapid at either test potential. However, in each of the five experiments, E-4031-sensitive current upon repolarization was greater than during depolarization, consistent with the rectification apparent in Fig 8B⇑. Subsequent experiments (see Table⇓) demonstrate no significant effect of E-4031 on I_K activation at a test potential of 55 mV. Thus, the E-4031-sensitive (I_{Kr}) component of I_K activation in canines displays rapid activation and inward rectification, similar to guinea pigs.

Table 1.

Effects of E-4031 on Tail Current Components

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The voltage-dependent activation of canine I_{Kr} and I_{Ks} was assessed from the amplitude of individual tail current components in the absence and presence of

E-4031. Fig 8C⇑ illustrates typical effects over a wide range of test pulse potentials. E-4031 consistently blocked the slower (I_{Ke2}) tail component (squares), while minimally affecting the more rapid (I_{Ke1}) component (circles). These results confirm that I_{Kr} (defined as E-4031–sensitive current) is analogous to the slowly deactivating tail component (I_{Ke2}) defined kinetically and that I_{Ks} (defined as E-4031–resistant current) is analogous to the rapidly deactivating tail component (I_{Ke1}). Results obtained from seven similar experiments are summarized in the Table⇑. E-4031 consistently blocked I_{Ke2} without affecting the amplitude or kinetics of I_{Ke1}. Thus, despite similar I_{Kr} activation between canines versus guinea pigs, the kinetics of deactivation are strikingly different.

E-4031 consistently reduced net outward current at holding potentials ranging from −35 to −45 mV (see Table⇑ and Figs 8⇑ and 10⇓) by values approaching 50% of the amplitude of the fully activated I_{Kr} tail component. A number of observations suggest that this effect is not due to block of sustained I_{Kr}. First, earlier kinetic-based results (Fig 2⇑; also author's unpublished data, 1995) demonstrated a threshold for I_{Kr} activation near −35 mV, which is positive to the holding potentials used. Second, E-4031 affected the holding current when myocytes were bathed in 0 mmol/L $[K^+]_o$ (which reduced I_{Kr} and electrogenic Na^+-K^+ pump current). Under these experimental conditions, holding current was reduced in four of five myocytes coincident with drug equilibration; no change in holding current was noted in the fifth experiment. If one assumes that I_{Kr} activation occurs negative to −35 mV, reduced net outward current with E-4031 could reflect an incremental block of I_{Kr} resulting from depolarizing clamp pulses used to monitor drug equilibration. This interpretation is incorrect, because holding current shifted inward when the membrane potential was maintained at −40 mV and depolarizing pulses were withheld during drug equilibration (three experiments).

To further study the steady state effects of E-4031 over a wider range of membrane potentials, 5-s depolarizing ramps were applied to approximate steady state current-voltage relations (holding potential, −85 mV; peak potential, +15 mV; slope, 20 mV/s). Currents obtained using 5-s and 3-s ramps were superimposable, demonstrating that longer ramps were of sufficient duration to produce a "quasi-steady state" current-voltage relation. E-4031 reduced outward currents during these depolarizing ramps only at more positive potentials. E-4031–sensitive current was first observed at "threshold" potentials between −27 and −15 mV (−21.4±4.4 mV [mean±SD], n=5) and was apparent up to the ramp peak (+15 mV) but absent at more negative potentials. This observation is consistent with the threshold potential for I_{Kr} activation determined using square clamp pulses but inconsistent with decreased outward current when the membrane potential is maintained at −40 mV for prolonged periods. This discrepancy suggests that maintained depolarization near −40 mV is necessary to elicit this steady state component of E-4031–sensitive current. Further studies are necessary to resolve the basis for this drug-sensitive component.

Possible use-dependent block of I_{Kr} by E-4031 was assessed by using trains of 200-ms depolarizing pulses applied at frequencies of 2.5, 1.0, and 0.25 Hz (21, 21, and 6 pulses per train, respectively; holding potential, −40 mV; pulse potential, +20 mV). Pulse trains were terminated with a standard 300-ms depolarizing test pulse, and the amplitude of fast and slow tail current components was subsequently evaluated. In each of five experiments, 5 μmol/L E-4031 abolished I_{Kr} irrespective of the pulse train frequency. Thus, block of canine I_{Kr} by E-4031 is frequency independent under these experimental conditions.

E-4031 Produces Reverse Rate-Dependent Effects on Canine Ventricular Action

Potential

In numerous tissues, class III agents including the methanesulfonanilides dofetilide and E-4031 (see "Discussion") elicit a greater prolongation of the APD at longer versus shorter stimulation rates, an effect that has been termed "reverse use dependence"[12] or "reverse rate dependence."[13] In guinea pigs, this effect has been attributed to the accumulation of I_{Ks} at rapid stimulation rates (due to the slow deactivation kinetics of I_{Ks} in this species), which mitigates the effects of I_{Kr} blockade at rapid rates.[13] To determine if the rapid deactivation kinetics of canine I_{Ks} affected reverse rate dependence, the effects of E-4031 on the APD-rate relation were examined. For these studies, standard microelectrode techniques were used to minimize alterations to the intracellular milieu. Fig 9⇓ illustrates the typical effects of E-4031 on an action potential stimulated at basic cycle lengths of 800 ms (panel A) and 2 s (panel B). E-4031 prolonged the APD by reducing the slope of phase 2 repolarization; neither early nor late repolarization was appreciably affected. E-4031 caused a greater prolongation of the APD at the slower cycle length despite the fact that at either cycle length the diastolic interval was long enough to ensure full deactivation of I_{Ks}. Panel C further illustrates reverse rate dependence, with E-4031 observed with three midmyocardial myocytes over a wider range of stimulation rates. Because APD showed variations on a beat-to beat basis, three action potentials were recorded, and durations were averaged for each stimulation rate. E-4031 caused prominent reverse rate dependence in each of the myocytes. Reverse rate dependence was also observed in three additional experiments in which myocytes were accessed by using perforated-patch techniques (data not shown).

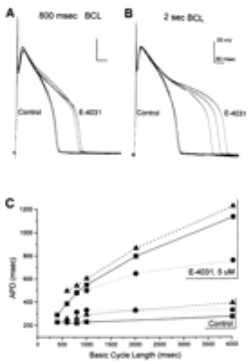

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Figure 9.

Reverse rate-dependent effects of E-4031 in canine ventricular myocytes. A and B, Typical effects of E-4031 on a midmyocardial myocyte stimulated at basic cycle lengths (BCLs) of 800 ms and 2 s. Each panel shows three consecutive action potentials. E-4031 caused greater prolongation of APD at the slower BCL. The effects of E-4031 were rapid (coincident with bath equilibration) and partially reversible (tested in two experiments). Zero potential line is depicted as the bottom of the calibration square. C, Effect of E-4031 on the APD-rate relation of midmyocardial myocytes. Illustrated are relations for three individual myocytes in the absence (lower set) and presence (upper set) of E-4031. E-4031 elicited reverse rate-dependent effects by causing greater prolongation of the APD at longer vs shorter BCLs. APD measured from the upstroke to 5 mV before full repolarization, with each measurement representing the mean of three consecutive action potentials. Standard microelectrode techniques were used.

Two I_K Components Are Present in Canine Atrial Myocytes

The characteristics of I_K in canine left atrial myocytes were assessed to determine (1) whether two components of I_K were present in atrial myocytes and (2) whether I_K deactivation kinetics of atrial myocytes were comparable to ventricular myocytes. Typical results are presented in Fig 10⇓. Panel A displays currents representing I_K activation and deactivation that qualitatively resembled those of ventricular myocytes (compare with Fig 1⇑). During 3-s pulses, I_K attained steady state values with modest depolarization but continued to increase throughout stronger depolarizing test pulses. Similarly, I_K deactivation was well described when two exponential components were used; the inset illustrates a biexponential fit after the depolarizing pulse to +25 mV, which was fit with time constants of 128 and 2088 ms. Two tail components were also observed with shorter (300-ms) depolarizing test pulses; by use of this protocol, the amplitudes of the fast and slow tail components were comparable for test pulses to 5 mV; with more depolarized test pulses, the faster component predominated (data not shown). Panel B illustrates the effects of E-4031 on atrial I_K: as with ventricular myocytes, E-4031 (1) reduced the holding current, (2) abolished the slower tail current component, and (3) had minimal effects on I_K activation at more positive depolarized potentials. Panel C shows the voltage dependence of activation of each tail current component. As in ventricular myocytes, I_{Ke1} was fully activated after test pulses to −10 mV, whereas the amplitude of I_{Ke2} continued to increase with progressively stronger depolarizations. Thus, the number and characteristics of the atrial I_K components resembled I_K described in ventricular myocytes.

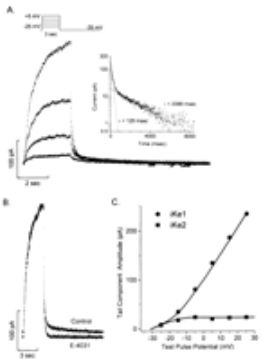

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Figure 10.

Two I_K components in canine atrial myocytes. A, Typical currents recorded from left atrial myocytes were similar to those recorded from ventricular myocytes (compare with Fig 1⇑). Inset, Tail current after depolarizing pulse to +5 mV plotted on semilogarithmic coordinates and fit to time constants of 128 and 2088 ms. Voltage protocol is illustrated above. B, Effects of E-4031 on atrial I_K. E-4031 altered the deactivation kinetics by eliminating the kinetically slower tail current component (τ, 1972 ms; amplitude, 21 pA) without affecting the kinetics (τ, 136 ms) or amplitude (147 pA [control] vs 155 pA [E-4031]) of I_{Ks}; E-4031 also reduced the holding current from 26 to 12 pA. Holding potential was −35 mV; test potential, +5 mV. Similar results were obtained in three additional myocytes. C, Voltage dependence of activation of I_K tail components (I_{Ke1} and I_{Ke2} [iKe1 and iKe2, respectively]) was obtained by using 3-s depolarizing test pulses. The slower tail component (I_{Ke2} or I_{Kr}) was maximally activated at potentials near −10 mV, whereas the amplitude of the faster component (I_{Ke1} or I_{Ks}) continued to increase after stronger depolarizing conditioning pulses. Results were comparable to ventricular myocytes (compare with Fig 2⇑). Figures for all panels were obtained from the same atrial myocyte.

Discussion

Two I_K Components Are Present in Canine Ventricle and Atrium

The present study concludes on the basis of independent kinetic and pharmacological evidence that two components of I_K are present in canine atrial and ventricular myocytes. Two tail current components can be distinguished kinetically on the basis of (1) two exponential components describing I_K deactivation, (2) differences in the voltage and time dependence of activation of each tail component, and (3) the differential sensitivity of each component to reduced $[K^+]_o$. I_K components described kinetically (I_{Ke1} and I_{Ke2}) were subsequently compared with components identified pharmacologically by using E-4031. The voltage dependence, activation kinetics, and rectification properties of E-4031-sensitive current (typically defined as I_{Kr}) were analogous to I_{Ke2} (defined kinetically), whereas E-4031-insensitive current (I_{Ks}) was analogous to the more rapid I_{Ke1} in atrial and ventricular myocytes. These similarities argue against the possibility that pharmacologically defined deactivation of I_{Kr} is dependent on the blocking characteristics of E-4031.

Two dominant exponential components for canine I_K deactivation are readily identified during control conditions, and a single dominant exponential component remains after removal of I_{Kr} (by either lowering $[K^+]_o$ or E-4031). Parameters derived from exponential fits are dependent on (1) adequate isolation of the current under study and (2) the accuracy of the dissociation of overlapping components, which may not be easily resolved for components of similar time constants and/or peak amplitudes. Complicated multistate (multiexponential) gating schemes[23] [28] [29] have described I_K (or I_K components) as a single conductance with multiple closed states, which were not considered in the present study. In addition, concluding that a kinetic component represents an individual conductance relies on the implicit assumption that the component deactivates as a monoexponential function. Despite these limitations, the effects of E-4031 and low $[K^+]_o$ on canine I_K are consistent with two functional I_K components, with each component most readily interpreted as a conductance displaying different pharmacological sensitivity and rectification properties.

The presence and characteristics of I_{Kr} and I_{Ks} in working ventricular myocardium are species dependent; I_{Kr} appears to be essentially lacking in adult rat ventricle (References 8 and 9, but see Reference 7), but an I_{Kr}-like component is the predominant (sole?) component in feline[6] myocytes. In rabbit ventricular myocytes, I_{Kr}[5] (or perhaps both I_{Kr} and I_{Ks}[30]) is present. Although direct I_K measures suggest that delayed rectifier current is minimal in human ventricular cells,[31] indirect evidence suggests that both I_{Kr} and I_{Ks} are present in human ventricle; E-4031[32] and dofetilide[33] (a blocker of I_{Kr}[5]) prolong ventricular repolarization, and mRNA for an I_{Ks}-like current is present in human myocardium,[34] which expresses an I_{Ks}-like current in oocytes.[35] In atrial myocytes isolated from guinea pig[3] and humans,[36] two I_K components have been distinguished. Knowledge of the number, characteristics, and amplitude of individual I_K components is essential to understanding the role of I_K in repolarization and the effects of class III agents.

In canine and guinea pig ventricular myocytes, I_{Ks} tail current density is greater than that of I_{Kr}. For canine ventricular myocytes, I_{Ks} density is approximately fivefold greater than that of I_{Kr} (1.04 versus 0.188 pA/pF; see legend, Fig 2⇑) measured from tail currents at −35 mV after 3-s test pulses to +65 mV. This value is about half that reported in guinea pig myocytes (measured at −40 mV after 7.5-s test pulses to +60 mV [Fig 9⇑, Reference 2]) because of the greater I_{Kr} density in guinea pigs. The relative proportion of I_{Kr} to I_{Ks} is likely to remain despite regional

variations in I_{Ks} density in canine ventricle.[10,11] In canine ventricular myocytes, a single depolarizing pulse ≤300 ms in duration (to potentials encountered during the action potential plateau) elicited equal contributions from I_{Kr} and I_{Ks} measured at −35 mV (potentials typically encountered near termination of the plateau). Thus, the contribution of I_{Ks}, the slowly activating component, after a short depolarization is comparable to I_{Kr}, the fully activated component, because of the greater I_{Ks} density (Fig 4⇑). During strong and prolonged depolarizations, I_{Ks} becomes increasingly dominant because of (1) its greater activation at more positive potentials, (2) the greater driving force for K^+ ions, and (3) the reduced I_{Kr} due to inward rectification. Further experimental studies will be necessary to determine the contribution of each component to repolarization at different rates of activity. Recent computer simulations (based on guinea pig I_K kinetics) stressed the importance of the relative densities of I_{Kr} and I_{Ks} in repolarization and arrhythmogenesis.[37]

Two exponential components of I_{Kr} deactivation have been reported in guinea pig ventricular myocytes[2] and AT-1 cells.[38] In feline myocytes (which display predominantly I_{Kr}[5,6]), two exponential components of I_K deactivation are present in an external solution deficient in Na^+ and K^+.[17] In the present study, one exponential component adequately described the deactivation kinetics of canine I_{Kr}. If present, two kinetic components of canine I_{Kr} would be difficult to resolve because of the small amplitude and slow decay of this drug-sensitive current. In all three species, I_{Kr} saturates at potentials in the range of 0 to +40 mV and displays inward rectification. I_{Kr} activation in guinea pigs and dogs appears similar, with rapid activation (within 300 ms) at potentials near 0 mV, full activation near 0 mV, and a half-activation voltage ranging between −10 to −20 mV. I_{Kr} activates slightly slower in AT-1 cells (400 ms time constant at 0 mV), with a component of slow inactivation apparent at strongly depolarized potentials not present in other tissues.

I_{Kr} was originally defined as an E-4031-sensitive current with rapidly activating and deactivating kinetics and I_{Ks} as a slowly activating and deactivating current insensitive to E-4031.[2] Although I_{Kr} activation is qualitatively similar in dogs and guinea pigs (see above), the deactivation kinetics of canine I_{Kr} are strikingly slower (with time constants of 2000 to 3000 ms near −40 mV) compared with the slowest deactivation time constant of 630 ms[4] and an undetermined time constant slower than 200 ms sometimes present in guinea pig myocytes (mentioned in Reference 2). The slow deactivation kinetics of canine I_{Kr} are revealed by using either E-4031 or the antihistamine terfenadine,[39] which blocks I_{Kr} in feline myocytes.[40] In AT-1 cells, the slowest time constant for I_{Kr} deactivation (400 ms) occurs near −40 mV; at similar potentials, I_{Kr} deactivation in rabbits[38] and cats[5] appears intermediate compared with either guinea pigs or dogs. Correspondingly, although I_{Ks} activation in canine and guinea pig myocytes is qualitatively similar, the deactivation kinetics of canine I_{Ks} are much more rapid (τ, 100 ms at −40) than observed in guinea pig myocytes (τ, 775 ms; Reference 4). It is unknown whether the same channels are responsible for I_{Kr} and I_{Ks} in these preparations or whether species-specific isoforms, differences in levels of expression, modulatory subunits, or other factors account for the kinetic differences. I_{Kr} and I_{Ks} subcategories (based on distinctions in deactivation kinetics) would be useful when comparing each I_K component in native membranes and heterologous expression systems.

Block of I_{Kr} by dofetilide in guinea pigs[13] and rabbits[5] is independent of frequency because of the slow recovery from block, which is further slowed at potentials near the resting membrane potential.[5] However, the methanesulfonanilides E-4031 and dofetilide have been shown to have reverse rate-dependent effects on the guinea

pig action potential.[9 41 42] These observations were resolved by attributing reverse rate dependence to the accumulation of I_{Ks} at rapid stimulation rates resulting from the slow deactivation kinetics of guinea pig I_{Ks}.[13] According to this hypothesis, the increased contribution of I_{Ks} during rapid pacing is responsible for mitigating the effect of I_{Kr} block, thereby causing reverse rate dependence. Reverse rate dependence with E-4031 in canine ventricular myocytes is inconsistent with this hypothesis, because the rapid deactivation kinetics of canine I_{Ks} (τ, 30 ms at −85 mV; Fig 7⇑) predicts that I_{Ks} would accumulate only at very rapid rates. However, reverse rate dependence was observed at rates slower than 1 Hz and with diastolic intervals sufficiently long to ensure full deactivation of I_{Ks}. Two other reports provide information refuting the role of I_{Ks} in reverse rate dependence: (1) In cat ventricular myocytes (a preparation that reportedly lacks I_{Ks}), the selective I_{Kr} blocking agent WAY 123,398 causes reverse rate dependence.[15] (2) Reverse rate-dependence has been observed in canine ventricular myocytes when both I_{Kr} and I_{Ks} were reduced with the class III agent azimilide[43] ; however, the specificity of I_K block by azimilide requires further study. It is unlikely that E-4031 block of canine I_{Kr} is modulated by stimulation rate, because 5 μmol/L E-4031 consistently blocked all I_{Kr}s (as judged from tail currents), and block of canine I_{Kr} (assessed with a holding potential of −40 mV) was frequency independent. Similar results have been reported with other species (see above). Thus, the present results suggest that currents other than I_{Ks} play a role in reverse rate dependence in canine ventricle. The same arguments apply to canine atrial myocytes; reverse rate dependence with E-4031 has been demonstrated in canine atrium in vivo[44] despite the rapid deactivation kinetics of I_{Ks} in atrium as in ventricle.

Presumably, increased net outward current with rapid stimulation, resulting from ionic current(s) other than I_{Ks}, reduces the effect of I_{Kr} block to promote reverse rate dependence. Further studies are necessary to identify these currents. Alternatively, increased net outward current during rapid stimulation might not be required for reverse rate dependence if the membrane conductance during the plateau is sufficiently increased so as to minimize the role of I_{Kr} in defining the action potential configuration at faster rates.

In canine myocytes, E-4031 consistently reduced the outward holding current when the holding potential was maintained near −40 mV. This effect appears to require a sustained depolarization, because it is not observed at comparable potentials when slowly depolarizing ramps are used. The ionic basis for this effect in canines is still uncertain. It is presently unknown whether this effect in canines is specific to E-4031 or shared with other class III agents. An effect of E-4031 on holding current was not reported in guinea pig atrial or ventricular myocytes.[2 3]

In summary, the present study demonstrates that I_K in canine ventricular and atrial myocytes can be functionally considered as two distinct components. In ventricular myocytes, both components participate in repolarization by contributing outward current at slow stimulation rates. Although the activation and voltage dependence of I_{Kr} and I_{Ks} in dogs and guinea pigs are comparable, their deactivation kinetics are distinctly different, with canine I_{Ks} deactivating as rapidly as guinea pig I_{Kr}. Despite these kinetic differences, reverse rate dependence with E-4031 is apparent in both species, suggesting that the incomplete deactivation of I_{Ks} is not necessary for reverse rate dependence by class III agents in canine myocytes. These results indicate that further studies are necessary to elucidate the ionic mechanism responsible for reverse rate dependence, which has been implicated in the potentially life-threatening proarrhythmic effects of class III agents, including excessive prolongation of the QT interval at slow rates and torsade de pointes.

Selected Abbreviations and Acronyms

τ	=time constant
APD	=action potential duration
E-4031	=1-[2-(6-methyl-2-pyridyl)ethyl]-4-(4-methylsulfonyl-aminobenzoyl)piperidine
I_K	=delayed rectifier K^+ current
I_{K1}	=inward rectifier K^+ current
I_{Kact}	=activating I_K
I_{Ke1}	=faster exponential tail current component of I_K
I_{Ke2}	=slower exponential tail current component of I_K
I_{Kr}	=rapidly activating component of I_K
I_{Ks}	=slowly activating component of I_K
I_{to}	=transient outward K^+ current

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Footnotes

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