The role of the delayed rectifier component I_{Ks} in dog ventricular muscle and Purkinje fibre repolarization

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1. The relative contributions of the rapid and slow components of the delayed rectifier potassium current (I_{Kr} and I_{Ks}, respectively) to dog cardiac action potential configuration were compared in ventricular myocytes and in multicellular right ventricular papillary muscle and Purkinje fibre preparations. Whole-cell patch-clamp techniques, conventional microelectrode and *in vivo* ECG measurements were made at 37 °C.

2. Action potential duration (APD) was minimally increased (less than 7 %) by chromanol 293B (10 μM) and L-735,821 (100 nM), selective blockers of I_{Ks}, over a range of pacing cycle lengths (300–5000 ms) in both dog right ventricular papillary muscles and Purkinje fibre strands. D-Sotalol (30 μM) and E-4031 (1 μM), selective blockers of I_{Kr}, in the same preparations markedly (20–80 %) lengthened APD in a reverse frequency-dependent manner.

3. *In vivo* ECG recordings in intact anaesthetized dogs indicated no significant chromanol 293B (1 mg kg^{-1} I.V.) effect on the QTc interval (332·9 ± 16·1 ms before *versus* 330·5 ± 11·2 ms, $n = 6$, after chromanol 293B), while D-sotalol (1 mg kg^{-1} I.V.) significantly increased the QTc interval (323·9 ± 7·3 ms before *versus* 346·5 ± 6·4 ms, $n = 5$, after D-sotalol, $P < 0·05$).

4. The current density estimated during the normal ventricular muscle action potential (i.e. after a 200 ms square pulse to +30 mV or during a 250 ms long 'action potential-like' test pulse) indicates that substantially more current is conducted through I_{Kr} channels than through I_{Ks} channels. However, if the duration of the square test pulse or the 'action potential-like' test pulse was lengthened to 500 ms the relative contribution of I_{Ks} significantly increased.

5. When APD was pharmacologically prolonged in papillary muscle (1 μM E-4031 and 1 μg ml^{-1} veratrine), 100 nM L-735,821 and 10 μM chromanol 293B lengthened repolarization substantially by 14·4 ± 3·4 and 18·0 ± 3·4 % ($n = 8$), respectively.

6. We conclude that in this study I_{Ks} plays little role in normal dog ventricular muscle and Purkinje fibre action potential repolarization and that I_{Kr} is the major source of outward current responsible for initiation of final action potential repolarization. Thus, when APD is abnormally increased, the role of I_{Ks} in final repolarization increases to provide an important safety mechanism that reduces arrhythmia risk.

The delayed rectifier potassium current (I_K) is a major outward current responsible for ventricular muscle action potential repolarization (Carmeliet, 1993; Sanguinetti & Keating, 1997). This current was first described by Noble & Tsien (1969) using the two-microelectrode voltage-clamp technique in multicellular sheep cardiac Purkinje fibre strands. Since its discovery it has been examined in single isolated myocytes obtained from various regions of the heart in several mammalian species (Noble & Tsien, 1969; Sanguinetti & Jurkiewicz, 1990; Follmer & Colatsky, 1990; Varró *et al.* 1993; Gintant, 1996; Salata *et al.* 1996*a*). In most species, I_K can be separated into rapid and slow

components (I_{Kr} and I_{Ks}, respectively) that differ from one another in terms of their sensitivity to drugs, rectification characteristics, and kinetic properties (Sanguinetti & Jurkiewicz, 1990; Carmeliet, 1992; D. W. Liu & Antzelevitch, 1995; Gintant, 1996; Heath & Terrar, 1996*a*,*b*). Specific I_{Kr} blockers (e.g. D-sotalol, dofetilide and E-4031) greatly lengthen cardiac action potential duration (APD) (Singh & Vaughan Williams, 1970; Strauss *et al.* 1970; Lathrop, 1985; Jurkiewicz & Sanguinetti, 1993) and thus provide anti-arrhythmic benefit by increasing the refractory wavelength. However, these drugs also increase risk for development of bradycardia-induced polymorphic ventricular tachy-arrhythmias (Hohnloser & Woosley, 1994). The APD increase induced by selective I_{Kr} blockade displays 'reverse use dependency' (Hondeghem & Snyders, 1990) that is especially pronounced in Purkinje fibres (Varró *et al.* 1985). Thus, with a premature impulse, when the time between successive depolarizations is short and an increase in APD would provide the most anti-arrhythmic benefit, the actual APD increase due to I_{Kr} block is the least. Conversely, when the time between successive action potentials is long, as with slow heart rates, selective I_{Kr} block produces a far greater increase in APD. Long APDs such as these are often associated with the development of early after-depolarizations that are probably responsible for induction of Torsade de Pointes ventricular arrhythmias.

The absence of selective I_{Ks} blockers until recently has made it impossible to evaluate directly the physiological role of this current in determining action potential configuration. Nevertheless, selective I_{Ks} block has generally been assumed to increase APD and refractoriness in a frequency-independent manner. On this basis, there has been an effort to develop selective I_{Ks} blockers as potential anti-arrhythmic agents devoid of the risk of Torsade de Pointes arrhythmia induction. Propofol, thiopenthone (Heath & Terrar, 1996*a*) and indapamide (Turgeon *et al.* 1994) were first used as pharmacological tools to block I_{Ks} and thereby separate I_{Ks} from I_{Kr} in guinea-pig ventricular myocytes. These compounds, however, effectively block I_{Ks} at concentrations higher than 100 μM, which calls into question their I_{Ks} selectivity. Two compounds, chromanol 293B (Busch *et al.* 1996) and L-735,821 (Salata *et al.* 1996*b*; Cordeiro *et al.* 1998) have recently been reported to selectively block I_{Ks}, but their effects on cardiac action potential configuration have not been examined in detail. Moreover, available results obtained with chromanol 293B and with L-735,821 are contradictory. Cordeiro *et al.* (1998), for instance, found L-735,821 to markedly increase APD in single, isolated rabbit Purkinje fibre myocytes. Bosch *et al.* (1998) similarly found chromanol 293B to increase APD in guinea-pig and human ventricular myocytes. However, conventional microelectrode recordings in guinea-pig right papillary muscle showed that chromanol 293B only slightly lengthened APD in the absence of isoproterenol (isoprenaline) (Schreieck *et al.* 1997). These contradictory findings may have several explanations. For one, APD measurements in single myocytes inherently show relatively large beat-to-beat variations that make identification of the effects of selective ion channel block on action potential configuration uncertain at best. In addition, the relative expression of I_{Kr} and I_{Ks} exhibits considerable species variation (Jurkiewicz & Sanguinetti, 1993; Varró *et al.* 1993; Li *et al.* 1996; Salata *et al.* 1996*a*). Regional differences in ion channel expression within the ventricle (Antzelevitch *et al.* 1991; Bryant *et al.* 1998) probably also confound interpretation of results and lead to differences in the effects of selective ion channel blockade in myocytes isolated from whole hearts.

Because I_{Ks} activation occurs at around 0 mV and this voltage is more positive than the normal Purkinje fibre action potential plateau voltage, I_{Ks} block should not be expected to increase Purkinje fibre APD. Conversely, in ventricular muscle, action potential plateau voltage is more positive ($\sim$ +20 mV) allowing I_{Ks} to be substantially more activated. Thus I_{Ks} block in ventricular muscle would be expected to increase APD markedly. Such a difference in the effects of I_{Ks} block might, therefore, be expected to produce anti-arrhythmic benefit. This is because lengthening ventricular muscle APD with little or no change in Purkinje fibre APD would cause less drug-induced dispersion in repolarization and limit arrhythmogenesis.

The main goal of this study was to compare the magnitude and extent of changes in ventricular muscle APD produced by selective block of I_{Kr} and I_{Ks} with those effects produced in Purkinje fibres. The results from such studies would establish the role of I_{Ks} in producing normal cardiac action potential repolarization. Thus, we compared the effects of two purported I_{Ks} blockers (chromanol 293B and L-735,821) with the effects produced by two recognized, selective I_{Kr} blockers (E-4031and D-sotalol) in both single myocytes and multicellular cardiac preparations.

METHODS

All experiments were approved by the Hungarian National Research Foundation (OTKA) and conducted in compliance with the Guide for the Care and Use of Laboratory Animals (USA NIH publication No. 85-23, revised 1985).

Conventional microelectrode measurements

Adult mongrel dogs of either sex weighing 8–16 kg were used. Following anaesthesia induced by sodium pentobarbital (30 mg kg^{-1} I.V.), each heart was rapidly removed through a right lateral thoracotomy and immediately rinsed in oxygenated modified Locke's solution containing (mM): Na^+ 140, K^+ 4, Ca^{2+} 1·0, Mg^{2+} 1, Cl^- 126, HCO_3^- 25 and glucose 11. The solution pH ranged from 7·35 to 7·45 when gassed with 95% O_2–5% CO_2 at 37 °C. Purkinje strands obtained from either ventricle and right ventricular papillary muscle tips were mounted individually in a tissue chamber (volume ~40 ml). Each preparation was stimulated (HSE stimulator type 215/II) initially at a constant cycle length of 1000 ms (frequency 1 Hz) using rectangular constant current pulses of 2 ms in duration. The current pulses were isolated from ground

and delivered through bipolar platinum electrodes in contact with the preparations. At least 1 h was allowed for each preparation to equilibrate while continuously superfused with modified Locke's solution warmed to 37 °C before experimental measurements commenced. Transmembrane potentials were recorded using conventional 5–20 MΩ, 3 M KCl-filled microelectrodes connected to the input of a high impedance electrometer (Biologic Amplifier VF 102, Claix, France). In addition, the first derivative of transmembrane voltage with respect to time (V_{max}) was electronically obtained (Biologic Differentiator DV 140, Claix, France) and, along with the transmembrane voltage amplifier outputs, continuously monitored on a dual beam storage oscilloscope (Tektronix model 2230).

The maximum diastolic potential, action potential amplitude and action potential durations at 50% and 90% of repolarization (APD_{50} and APD_{90}) were automatically measured using software developed in our laboratory (Hugo Sachs Elektronik, March-Hugstetten, Germany; action potential evaluation system) running on a 386 microprocessor based, IBM compatible computer, containing an ADA 3300 analog-to-digital data acquisition board (Real Time Devices Inc., PA, USA) with a maximum sampling frequency of 40 kHz. In each experiment, baseline action potential characteristics were first determined during continuous pacing at 1 Hz, and then when pacing cycle length was sequentially varied from 300–5000 ms. The 25th action potential was measured at each cycle length, and the cycle length was then changed so that 'quasi' steady-state frequency response relations could be generated rapidly. Preparations were then superfused for 40–60 min with either drug before repeating the pacing protocol and measuring the same parameters. Attempts were made to maintain the same impalement throughout each experiment. If an impalement was, however, dislodged, electrode adjustment was attempted, and if the action potential characteristics of the re-established impalement deviated by less than 5% from the previous measurement, the experiment continued. When this 5% limit was exceeded, the experiment was terminated and all data were excluded from analyses.

Patch-clamp measurements

Cell isolation. Ventricular myocytes were enzymatically dissociated from hearts which were removed from mongrel dogs of either sex weighing 10–20 kg following anaesthesia (sodium pentobarbital, 30 mg kg^{-1} I.V.). The hearts were immediately placed in cold (4 °C) normal Tyrode solution. A portion of the left ventricular wall containing an arterial branch large enough to cannulate was then perfused in a modified Langendorff apparatus at a pressure of 60 cmH_2O with solutions in the following sequence: (1) normal Tyrode solution (10 min), (2) Ca^{2+}-free solution (10 min), and (3) Ca^{2+}-free solution containing collagenase (type I, 0·66 mg ml^{-1}, Sigma) and bovine serum albumin (fraction V, fatty acid free, 2 mg ml^{-1}, Sigma) (15 min). Protease (type XIV, 0·12 mg ml^{-1}, Sigma) was added to the final perfusate and another 15–30 min of digestion was allowed. Portions of the left ventricular wall judged to be well digested were diced into small pieces and placed either in Kraft-Brühe (KB) solution or in Ca^{2+}-free solution supplemented with $CaCl_2$ (1·25 mM) for 15 min. Next, these tissue samples were gently agitated in a small beaker to dislodge single myocytes from the extracellular matrix. All cell suspensions resulting from this dissociation procedure contained a mixture of subepicardial, midmyocardial and subendocardial myocytes. During the entire isolation procedure, solutions were gassed with 100% O_2 while their temperatures were maintained at 37 °C. Myocytes were allowed to settle to the bottom of the beaker for 10 min, and then half of the supernatant was replaced with fresh solution. This procedure was repeated three times. Myocytes placed in KB solution were stored at 4 °C; those placed in Tyrode solution were maintained at 12–14 °C prior to experimentation. Cells that were stored in KB solution or immediately placed in 1·25 mM calcium containing solution had the same appearance and there were no discernible differences in their characteristics.

Compositions of solutions used for cell isolation. Normal Tyrode solution (mM): NaCl 135, KCl 4·7, KH_2PO_4 1·2, $MgSO_4$ 1·2, Hepes 10, $NaHCO_3$ 4·4, glucose 10 and $CaCl_2$ 1·0 (pH 7·2 adjusted with NaOH). Ca^{2+}-free solution (mM): NaCl 135, KCl 4·7, KH_2PO_4 1·2, $MgSO_4$ 1·2, Hepes 10, $NaHCO_3$ 4·4, glucose 10 and taurine 20 (pH 7·2 adjusted with NaOH). KB solution (mM): KOH 90, L-glutamic acid 70, taurine 15, KCl 30, KH_2PO_4 10, $MgCl_2$ 0·5, Hepes 10, glucose 11 and EGTA 0·5 (pH 7·3 adjusted with KOH).

Experimental procedure, drugs and solutions. One drop of cell suspension was placed within a transparent recording chamber mounted on the stage of an inverted microscope (TMS, Nikon, Tokyo, Japan), and individual myocytes were allowed to settle and adhere to the chamber bottom for at least 5 min before superfusion was initiated. Only rod-shaped cells with clear striations were used. Cell capacitance (199·3 ± 13·7 pF, $n = 69$) was measured by applying 10 mV hyperpolarizing pulse from a holding potential of −10 mV. The capacity was measured by integration of the capacitive transient divided by the amplitude of the voltage step (10 mV). Hepes-buffered Tyrode solution served as the normal superfusate. This solution contained (mM): NaCl 144, NaH_2PO_4 0·33, KCl 4·0, $CaCl_2$ 1·8, $MgCl_2$ 0·53, glucose 5·5 and Hepes 5·0 at pH 7·4.

E-4031 (Institute for Drug Research, Budapest, Hungary) and D-sotalol (Bristol-Arzneimittel, Troisdorf, Germany) were diluted from a 1 mM or 10 mM aqueous stock solution, respectively, at the time of the experiment. Chromanol 293B (obtained as a gift from Hoechst AG, Frankfurt, Germany) was similarly diluted at the time of use from a 10 mM stock solution containing 100% DMSO. DMSO at this concentration did not produce discernible effects either on APD or measured currents. L-735,821 (obtained as a gift from Merck-Sharpe & Dohme Laboratories, Rathway, NJ, USA) was diluted in superfusate from a 100 μM stock solution containing 10% DMSO. Patch-clamp micropipettes were fabricated from borosilicate glass capillaries (Clark, Reading, UK) using a P-97 Flaming-Brown micropipette puller (Sutter Co., Novato, CA, USA). These electrodes had resistances between 1·5 and 2·5 MΩ when filled with pipette solution containing (mM): potassium aspartate 100, KCl 45, K_2ATP 3, $MgCl_2$ 1, EGTA 10 and Hepes 5. The pH of this solution was adjusted to 7·2 with KOH. Nisoldipine (1 μM) (obtained as a gift from Bayer AG, Leverkusen, Germany) was placed in the external solution to eliminate inward Ca^{2+} current (I_{Ca}), and sodium current (I_{Na}) was inactivated by applying a holding potential of −40 mV which also largely inactivated transient outward current (I_{to}). Membrane currents were recorded with an Axopatch-1D amplifier (Axon Instruments, Foster City, CA, USA) using the whole-cell configuration of the patch-clamp technique. After establishing a high (1–10 GΩ) resistance seal by gentle suction, the cell membrane beneath the tip of the electrode was disrupted by suction or by application of 1·5 V electrical pulses for 1–5 ms. The series resistance was typically 4–8 MΩ before compensation (50–80%, depending on the voltage protocols). Experiments where the series resistance was high, or substantially increased during measurement, were discarded. Membrane currents were digitized using a 333 kHz analog-to-digital converter

(Digidata 1200, Axon Instruments) under software control (pCLAMP 6.0, Axon Instruments). Analyses were performed using pCLAMP 6.0 software after low-pass filtering at 1 kHz. All patch-clamp data were collected at 37 °C.

ECG measurements in intact anaesthetized dogs

Adult mongrel dogs of either sex weighing 8–16 kg were anaesthetized using sodium pentobarbital (30 mg kg^{-1} I.V.) with subsequent bolus I.V. injections (6 mg kg^{-1}) administered as needed. These dogs were ventilated with room air at a rate and tidal volume sufficient to maintain arterial O_2, CO_2 and pH within normal limits (Végh *et al.* 1992). Catheters were inserted into the right and left femoral veins for drug and anaesthetic administration. The dose of each drug applied was 1 mg kg^{-1}. Drugs were administered slowly (over a period of 1 min) in a volume equivalent to 0·5 ml kg^{-1}. Surface electrocardiographic (ECG) leads I, II and III were continuously monitored and recorded after 1, 3 and 5 min and every subsequent 5 min during drug administration for up to 30 min. After completion of the experiments, animals were killed by I.V. overdose of pentobarbital.

Statistical analyses

Results were compared using Student's t tests for paired and unpaired data. When $P < 0{\cdot}05$, results were considered significant. Data are expressed as means ± S.E.M.

RESULTS

Effects of E-4031 and D-sotalol on I_{Kr}

The effects of E-4031 and D-sotalol on I_{Kr} were examined in isolated dog ventricular myocytes. Test pulses of 1000 ms in duration to between −20 mV and +50 mV were applied from a holding potential of −40 mV. The decaying tail current at −40 mV after the test pulse was assessed as I_{Kr}. L-735,821 (100 nM) or chromanol 293B (30 μM) were used to block I_{Ks} completely. Under these conditions, E-4031 (1 μM) completely abolished and D-sotalol (30 μM) attenuated (not shown) I_{Kr} tail currents (Fig. 1).

Effects of chromanol 293B and L-735,821 on I_{Ks}

The effects of chromanol 293B and L-735,821 on I_{Ks} were examined using long (5000 ms) test pulses to between 0 mV and +50 mV from a holding potential of −40 mV in the presence of 1–5 μM E-4031 to inhibit I_{Kr}. The decaying tail current at −40 mV following each test pulse was assessed as I_{Ks}. Chromanol 293B (10 μM) greatly reduced and L-735,821 (100 nM) completely abolished I_{Ks} (Figs 2 and 3).

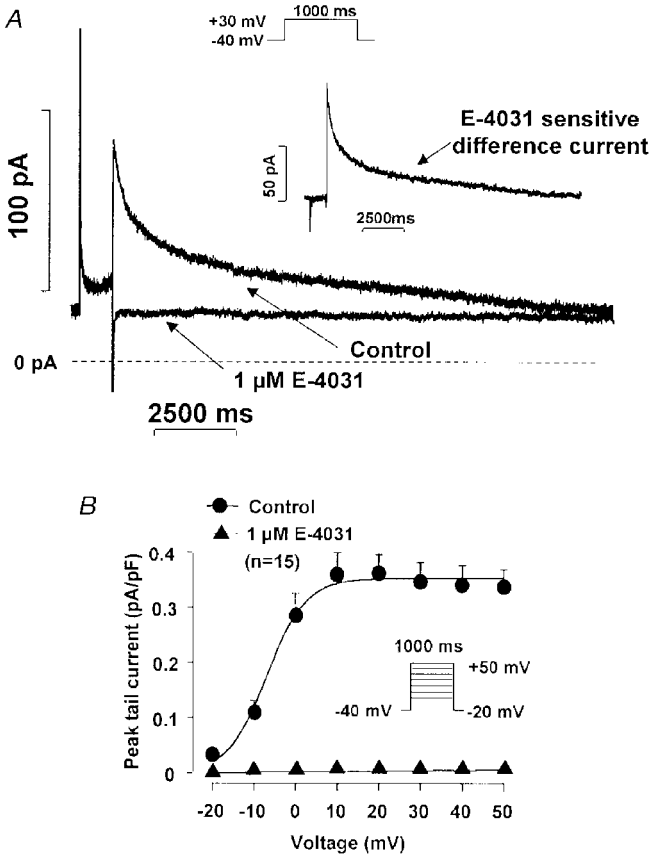

Figure 1. E-4031-sensitive current (I_{Kr}) in dog ventricular myocytes

A, recording of I_{Kr} in the absence and presence of 1 μM E-4031. The inset presents the E-4031 (1 μM)-sensitive difference current at +30 mV. *B*, the peak I_{Kr} tail current amplitude–voltage relationship in the absence and presence of 1 μM E-4031. Nisoldipine (1 μM) was used to block inward I_{Ca} and L-735,821 (100 nM) to block I_{Ks}. Holding potential (V_h) was −40 mV, pulse duration was 1000 ms, and pulse frequency was 0·05 Hz.

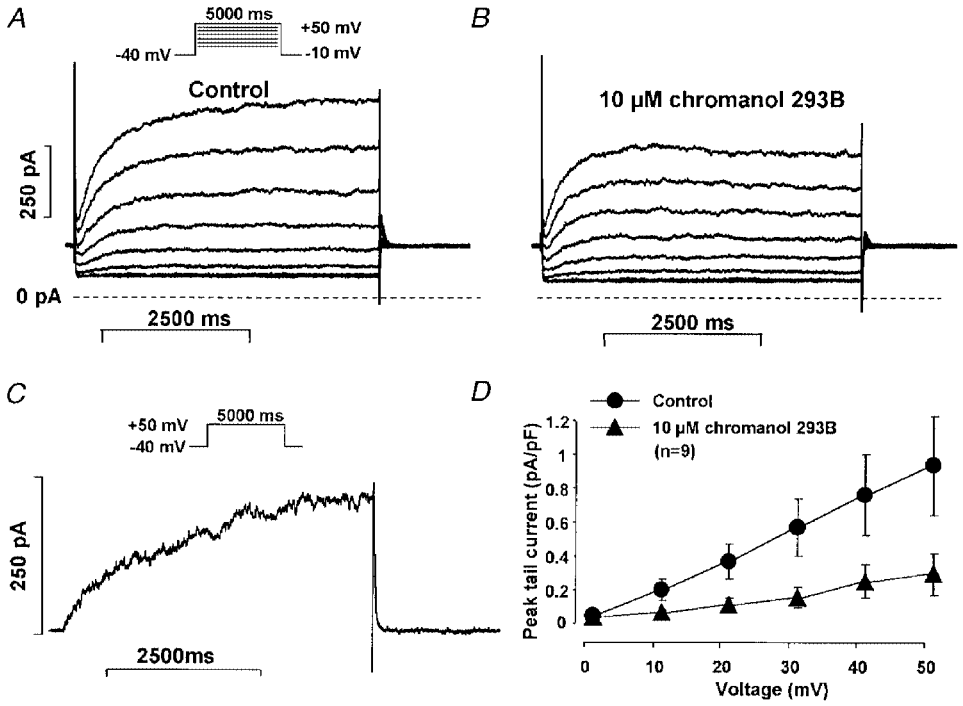

Figure 2. E-4031-insensitive current (I_{Ks}) in dog ventricular myocytes: effect of chromanol 293B
A and *B*, recordings in the absence and presence, respectively, of 10 μM chromanol 293B. *C*, the chromanol 293B (10 μM)-sensitive difference current at +50 mV. *D*, peak I_{Ks} tail current amplitude–voltage relationship in the absence and presence of 10 μM chromanol 293B. Nisoldipine (1 μM) was used to block inward I_{Ca} and E-4031 (5 μM) to block I_{Kr}. V_h was −40 mV, pulse duration was 5000 ms, and pulse frequency was 0·1 Hz.

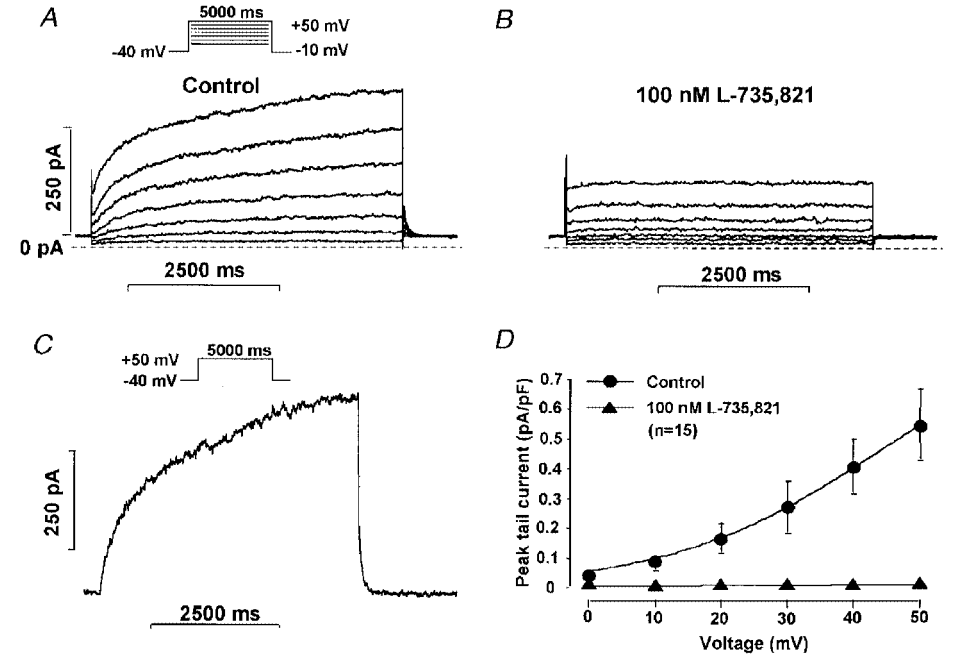

Figure 3. E-4031-insensitive current (I_{Ks}) in dog ventricular myocytes: effect of L-735,821
A and *B*, recordings in the absence and presence, respectively, of 100 nM L-735,821. *C*, the L-735,821 (100 nM)-sensitive difference current at +50 mV. *D*, peak I_{Ks} tail current amplitude–voltage relationship in the absence and presence of 100 nM L-735,821. Nisoldipine (1 μM) was used to block inward I_{Ca} and E-4031 (5 μM) to block I_{Kr}. V_h was −40 mV, pulse duration was 5000 ms, and pulse frequency was 0·1 Hz.

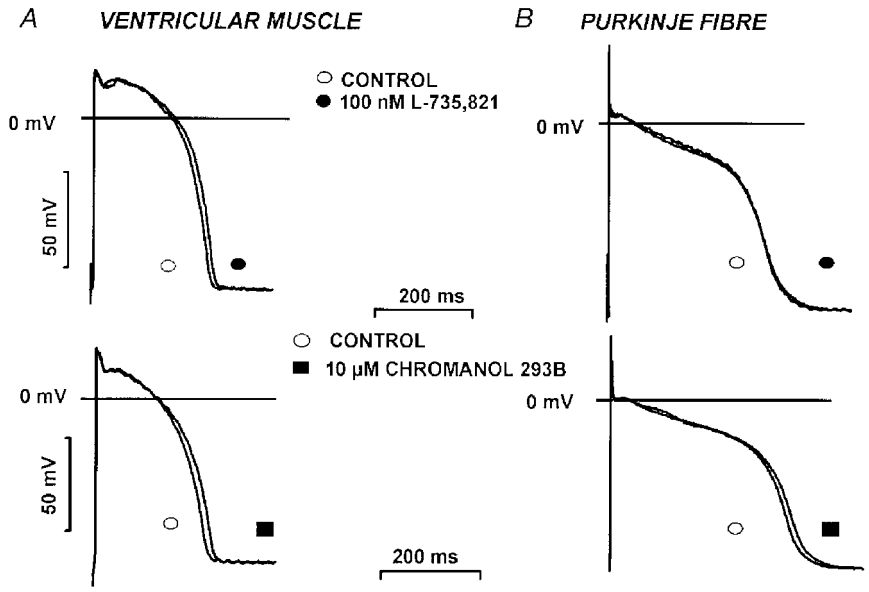

Figure 4. Effect of I_{Ks} block on the action potential in dog ventricular right papillary muscle and Purkinje fibre

Action potential recordings from canine ventricular papillary muscles (*A*) and Purkinje fibre strands (*B*) before and after 40 min superfusion with 100 nM L-735,821 (top) or 10 μM chromanol 293B (bottom). Stimulation frequency was 1 Hz.

Possible contribution of I_{Ks} and I_{Kr} to action potential repolarization in dog ventricular muscle and Purkinje fibres

The effects on dog ventricular muscle and Purkinje fibre action potential configuration produced by equipotent concentrations of chromanol 293B (10 μM) and L-735,821 (100 nM) that blocked I_{Ks} (Fig. 4) were examined and compared with those of D-sotalol (30 μM) and E-4031 (1 μM) that blocked I_{Kr} (Fig. 5). Conventional microelectrode techniques were used and the effects of these compounds that completely or markedly blocked either I_{Ks} or I_{Kr} were examined in both dog ventricular muscle and Purkinje fibre strands over a wide range of stimulation cycle lengths (300–5000 ms). Chromanol 293B and L-735,821 produced small changes in APD amounting to less than a 7 % increase over baseline measurements, and these unremarkable effects of I_{Ks} demonstrated little frequency dependence in both ventricular muscle and Purkinje fibre strands (Fig. 6). In

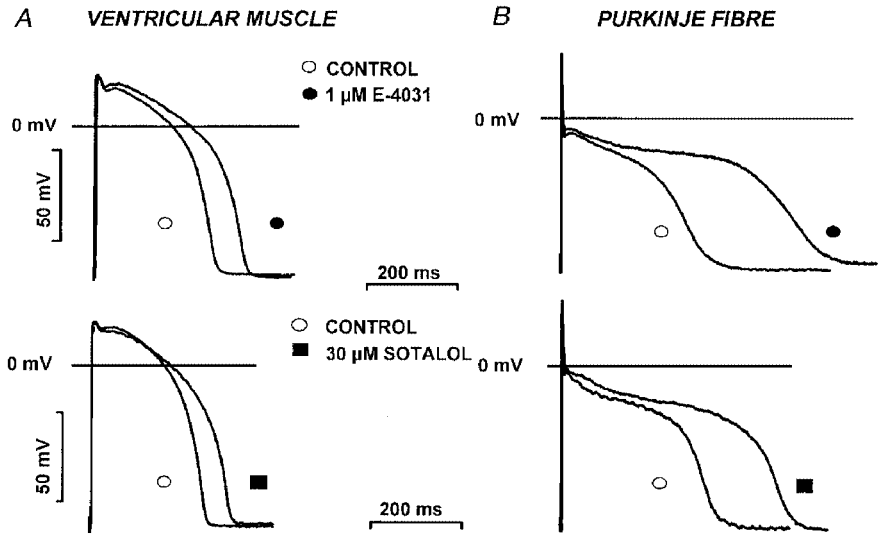

Figure 5. Effect of I_{Kr} block on the action potential in dog ventricular right papillary muscle and Purkinje fibre

Action potential recordings from canine ventricular papillary muscles (*A*) and Purkinje fibre strands (*B*) before and after 40 min superfusion with 1 μM E-4031 (top) or 30 μM D-sotalol (bottom). Stimulation frequency was 1 Hz.

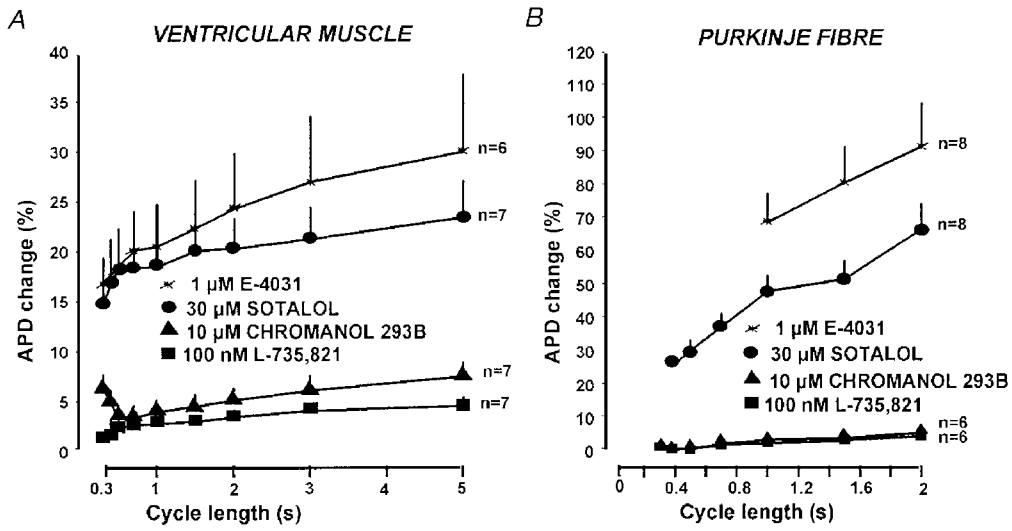

Figure 6. Frequency-dependent effect of I_{Kr} and I_{Ks} block on action potential duration
Frequency-dependent effect of I_{Kr} (by 1 μM E-4031 or 30 μM sotalol) and I_{Ks} block (by 10 μM chromanol 293B or 100 nM L-735,821) on action potential duration (APD) in canine ventricular papillary muscles (*A*) and Purkinje fibre strands (*B*). Pacing cycle length (1/frequency) is plotted on the abscissa and the ordinate indicates percentile changes in APD_{90}. Bars represent S.E.M.

contrast, D-sotalol and E-4031 markedly lengthened both dog papillary muscle and Purkinje fibre APD (Fig. 6). In addition, the increase in APD following I_{Kr} block occurred in a reverse frequency-dependent fashion so that the increase in APD was always greater at long cycle lengths than at short ones (Fig. 6). These results clearly show that I_{Kr} block lengthens APD greatly while selective I_{Ks} block in dog has little effect on normal cardiac APD in both ventricular muscle and Purkinje fibres.

Because I_{Ks} is modulated by changes in intracellular cAMP, we also examined the effects of I_{Ks} block on APD in the presence of 1 μM forskolin to activate adenylcyclase and increase intracellular cAMP. Forskolin (1 μM) alone ($n = 17$)

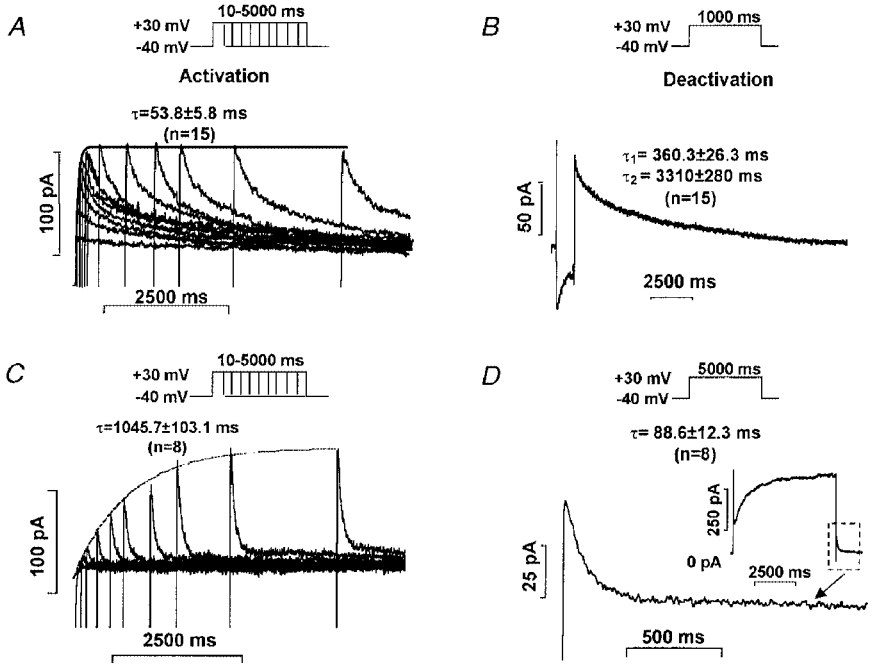

Figure 7. Activation and deactivation kinetics of I_{Kr} and I_{Ks} in dog ventricular myocytes
A and *C*, activation kinetics of I_{Kr} and I_{Ks}, respectively, measured as tail currents at −40 mV after test pulses to +30 mV with duration gradually increasing between 10 and 5000 ms. *B* and *D*, deactivation kinetics of I_{Kr} and I_{Ks} outward tail current, respectively, at −40 mV after a 1000 or 5000 ms, respectively, long test pulse to +30 mV. The inset in *D* shows I_{Ks} tail current at higher resolution.

markedly shortened APD in dog right papillary muscle paced at cycle lengths ranging between 300 and 5000 ms (i.e. from 190·2 ± 4·4 to 157·1 ± 3·3 ms and 258·2 ± 5·7 to 212·5 ± 4·2 ms at cycle lengths of 300 and 5000 ms, respectively). Addition of L-735,821 (100 nM) or chromanol 293B (10 μM) in the continuous presence of forskolin had little effect on APD (150·2 ± 2·2 *versus* 153·2 ± 2·6 ms and 207·5 ± 3·4 *versus* 209·0 ± 4·5 ms following L-735,821 and 164·1 ± 4·3 *versus* 176·0 ± 4·2 ms and 217·6 ± 5·1 *versus* 234·9 ± 9·1 ms following chromanol 293B at pacing cycle lengths of 300 and 5000 ms, respectively). These results again show that selective I_{Ks} block only slightly lengthened APD over a wide range of stimulation frequencies, even in the presence of elevated intracellular cAMP.

Estimation of I_{Ks} and I_{Kr} activation during the plateau phase of the action potential

Earlier results suggested that I_{Kr} activates rapidly during action potentials but deactivates slowly, while I_{Ks} activates slowly at more positive potentials (Gintant, 1996). In addition, I_{Ks} accumulation over successive depolarization is not likely since its deactivation is fast with respect to diastolic intervals occurring at physiological heart rates. I_{Kr} and I_{Ks} kinetics such as these may account for the small effect of chromanol 293B and L-735,821 on APD at concentrations that completely or markedly blocked I_{Ks} in the present study. To examine this phenomenon further, we carefully evaluated the kinetics of I_{Kr} and I_{Ks} at depolarized potentials (+30 mV) corresponding to the action potential plateau.

In our study, I_{Kr} indeed activated rapidly in dog ventricular myocytes (Fig. 7*A*). Using gradually increasing test pulse durations from a holding potential of −40 mV to +30 mV in the presence of 100 nM L-735,821 to block I_{Ks}, the activation time constant (τ) for I_{Kr} was 53·8 ± 5·8 ms ($n = 15$) with an amplitude (A) of 69·7 ± 6·4 pA ($n = 15$). Deactivation of I_{Kr} on return to −40 mV from +30 mV was slow (Fig. 7*B*), and it was best fitted by a double exponential relation where the parameters were: $\tau_1 = 360{\cdot}3 \pm 26{\cdot}3$ ms;

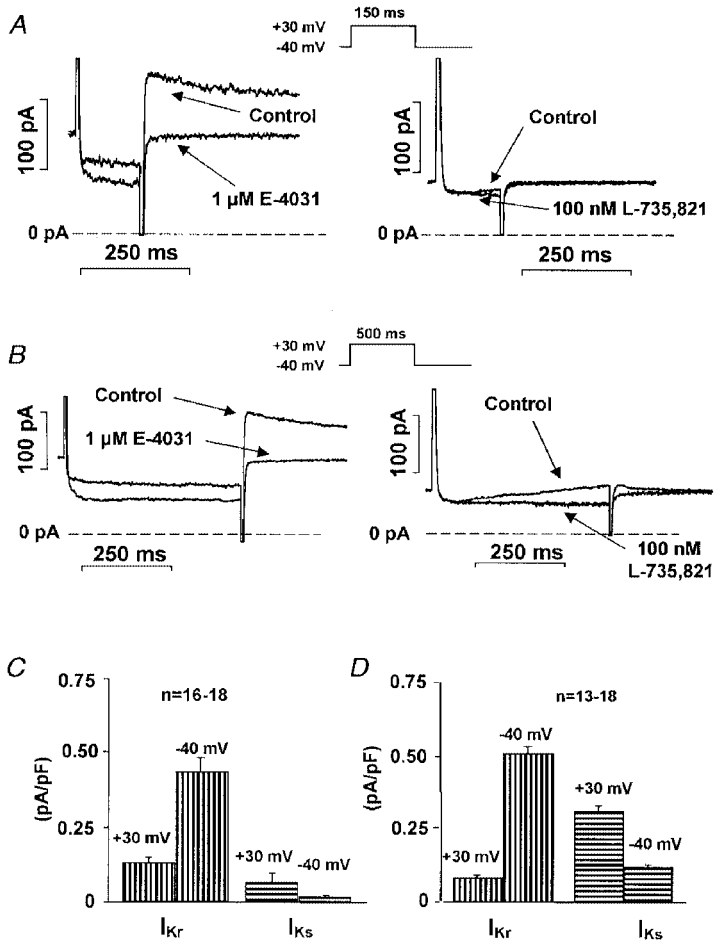

Figure 8. Comparison of the magnitude of I_{Kr} and I_{Ks} after short and long voltage pulses

A, recordings of E-4031 (I_{Kr}, left)- and L-735,821 (I_{Ks}, right)-sensitive currents after application of a short (150 ms) depolarizing test pulse to +30 mV from a holding potential of −40 mV. *B*, recordings of E-4031 (I_{Kr}, left)- and L-735,821 (I_{Ks}, right)-sensitive currents after a long (500 ms) depolarizing test pulse to +30 mV from a holding potential of −40 mV. *C*, average I_{Kr} and I_{Ks} currents at the end of a short (150 ms, right panel) and a long (500 ms, left panel) depolarizing test pulse to +30 mV, and peak tail current at −40 mV. Bars represent S.E.M.

$\tau_2 = 3310 \pm 280$ ms; $A_1 = 31{\cdot}8 \pm 0{\cdot}7$ pA and $A_2 = 34{\cdot}1 \pm 3{\cdot}14$ pA ($n = 15$).

I_{Ks} kinetics were also assessed but in the presence of 5 μM E-4031 to eliminate I_{Kr}. I_{Ks} activation under these conditions in dog ventricular myocytes was slow (Fig. 7*C*) ($\tau = 1045{\cdot}7 \pm 103{\cdot}1$ ms, $A = 61{\cdot}1 \pm 8{\cdot}3$ pA, $n = 8$). I_{Ks} deactivation in these myocytes was fast (Fig. 7*D*) ($\tau = 88{\cdot}6 \pm 12{\cdot}3$ ms, $n = 8$).

To estimate the magnitude of I_{Ks} and I_{Kr} activated during the cardiac action potential, we compared the amplitudes of the L-735,821-sensitive (I_{Ks}) and E-4031-sensitive (I_{Kr}) currents at the end of a 150 ms long test pulse to +30 mV and their tail currents on return to −40 mV. Using this protocol we assessed I_{Kr} and I_{Ks} at voltages corresponding to the plateau and repolarization phases of the action potential. Because deactivation of I_{Kr} is slow in comparison to its recovery from inactivation (Spector *et al.* 1996), tail currents measured at −40 mV do not accurately reflect the magnitude of I_{Kr} activated during the test pulse. The opposite situation may be expected with I_{Ks} because this current does not appear to inactivate and the driving force for K^+ is larger at positive than at negative voltages. We, therefore, measured I_{Ks} and I_{Kr} by subtracting membrane currents before and after 4–5 min of exposure to L-735,821 and E-4031, respectively. The E-4031-sensitive current (I_{Kr}) amplitude at the end of the 150 ms long test pulse was $25{\cdot}8 \pm 3{\cdot}2$ pA ($n = 16$), or about 29% ($86{\cdot}5 \pm 10{\cdot}5$ pA, $n = 16$) of the tail current amplitude measured after the test pulse returned to −40 mV (Fig. 8*A*, left panel). The L-735,821-sensitive current (I_{Ks}) during the test pulse to +30 mV was larger than its tail current on return to −40 mV (Fig. 8*A*, right panel). The magnitude of I_{Ks} tail current during the test pulse was $12{\cdot}5 \pm 0{\cdot}8$ pA at +30 mV *versus* $3{\cdot}5 \pm 0{\cdot}5$ pA at −40 mV ($n = 18$) still approximately an order of magnitude less than the I_{Kr} tail current.

We also compared I_{Ks} and I_{Kr} magnitudes during 'action-potential-like' test pulses. These test pulses were obtained by digitizing representative right ventricular dog action

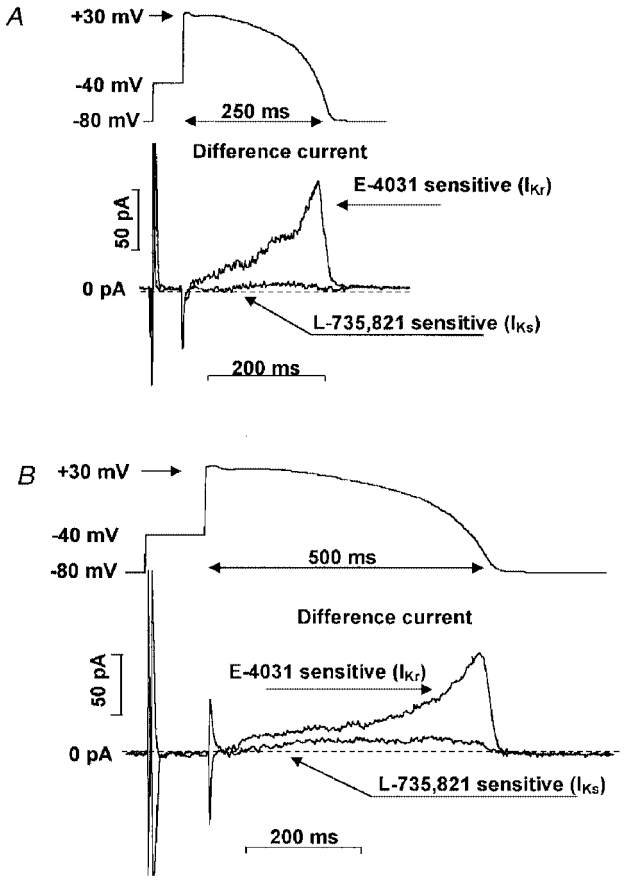

Figure 9. E-4031 (I_{Kr})- and L-735,821 (I_{Ks})-sensitive difference currents during short and long 'action potential-like' test pulse

A, E-4031-sensitive (1 μM) (I_{Kr}) and L-735,821-sensitive (100 nM) (I_{Ks}) difference currents recorded during an 'action-potential-like' test pulse in canine ventricular myocytes. The 'action-potential-like' test pulse was obtained by recording a normal canine ventricular action potential with a conventional microelectrode in a multicellular papillary muscle preparation and adding a 50 ms prepulse from −80 to −40 mV. *B*, recordings of E-4031-sensitive (I_{Kr}) and L-735,821-sensitive (I_{Ks}) currents when the action-potential-like test pulse duration was increased by a factor of 2 (i.e. to ~500 ms). Recordings in *A* and *B* were obtained in the same myocyte. Similar results to those illustrated were obtained in 4–7 additional myocytes.

Table 1. The effect of I.V. 1 mg kg^{-1} chromanol 293B and I.V. 1 mg kg^{-1} D-sotalol on the ECG interval durations in intact anaesthetized dogs

Interval durations	Chromanol 293B ($n = 6$)		D-Sotalol ($n = 5$)	
	Control	Chromanol 293B	Control	D-Sotalol
PP (ms)	463·3 ± 52·2	463·3 ± 39·4	360 ± 21·9	450·0 ± 19·5*
PQ (ms)	98·3 ± 10·5	95·0 ± 8·8	92·0 ± 10·2	96·0 ± 7·5
QRS (ms)	45·0 ± 2·2	48·3 ± 4·8	38·0 ± 2·0	42·0 ± 3·7
QT (ms)	223·3 ± 12·0	223·3 ± 9·5	194·0 ± 7·5	232·0 ± 3·7*
QTc (ms)	332·9 ± 16·1	330·5 ± 11·2	323·9 ± 7·3	346·5 ± 6·4*

QTc, QT was corrected using the Bazett equation. *$P < 0·05$.

potentials recorded with conventional microelectrodes. A 40 ms long prepulse to −40 mV was added at the beginning of the idealized action-potential-like test pulse (Fig. 9*A*). Under these conditions the I_{Kr} difference current (i.e. the E-4031-sensitive current) during the action potential plateau phase was small with its magnitude increasing as the test voltage became more negative (Fig. 9*A*). In contrast, the I_{Ks} difference current (i.e. the L-735,821-sensitive current) remained small throughout all phases of the action potential-like test pulse (Fig. 9*A*). These results indicate

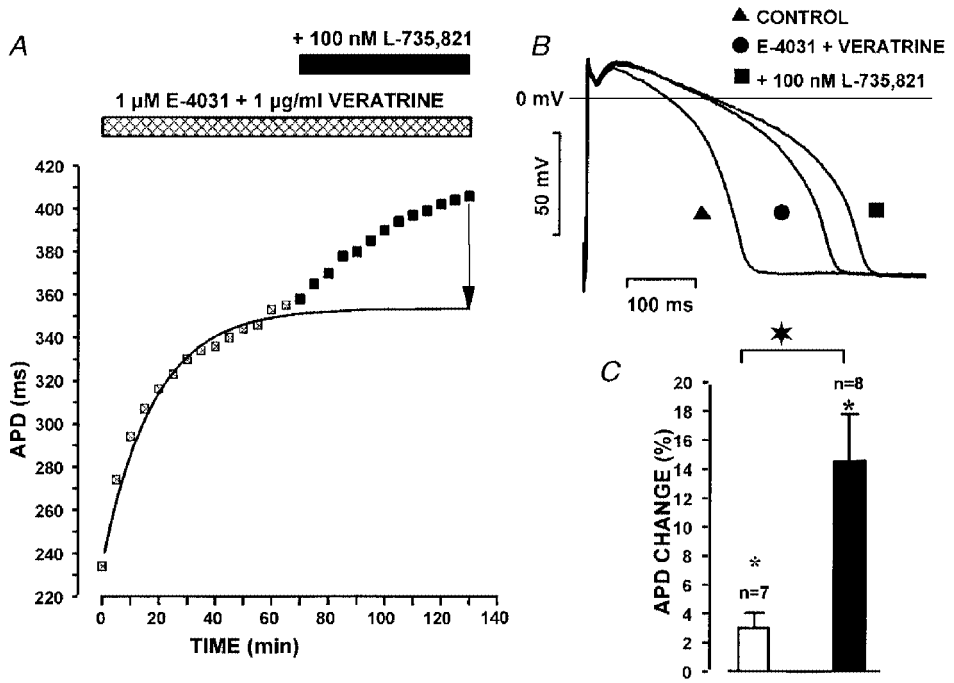

Figure 10. Effect of 100 nM L-735,821 on dog ventricular action potentials recorded in the presence of 1 µM E-4031 and 1 µg ml^{-1} veratrine

A, the time course of a representative experiment. At 0 min 1 µM E-4031 and 1 µg ml^{-1} veratrine were added and measurements were taken every 5 min until a 'quasi' steady state was achieved. Then 100 nM L-735,821 was added to the bath in the continuous presence of E-4031 and veratrine. The relation prior to addition of L-735,821 was fitted by the equation $Y = A + B\exp(-X/C)$ to estimate the time-dependent changes that would have occurred in the absence of the I_{Ks} blocker (continuous line) so that the magnitude of its effect at 140 min is indicated by the arrow. *B*, representative action potentials recorded at baseline (0 min), after exposure to E-4031 and veratrine alone (70 min), and following addition of L-735,821 (130 min). *C*, comparison of the effect of L-735,821 on 'short' (open bar) and on 'long' (filled bar) dog ventricular action potentials, respectively, recorded in the absence or presence of E-4031 and veratrine. Small asterisks represent significant changes from baseline measurements (i.e. at 0 min). The filled star represents significant changes between the bars ($P < 0·01$ in both cases). Columns and error bars indicate means and S.E.M.

that the outward current carried through I_{Kr} channels during the action potential is more than 10 times greater than through I_{Ks} channels (Figs 8*A* and 9*A*). These results agree well with the failure to increase ventricular and Purkinje fibre APD by blocking I_{Ks}, while I_{Kr} block caused marked lengthening.

Because we found I_{Ks} to have little role in normal action potential repolarization, we also examined its possible role when action potential duration was artificially increased. In these experiments we either applied long (500 ms) step-wise, rectangular test pulses to +30 mV (Fig. 8*B*) or an 'action-potential-like' test pulse having a duration of 500 ms (Fig. 9*B*). I_{Kr} was not substantially changed due to its fast activation when the duration of the test pulse was increased from 150 to 500 ms (I_{Kr} magnitude was $21{\cdot}3 \pm 1{\cdot}9$ pA at +30 mV and $101{\cdot}0 \pm 14{\cdot}7$ pA at −40 mV, $n = 13$) (Fig. 8*B*, left panel, and Fig. 9*B*). However, the magnitude of I_{Ks} was significantly increased to $61{\cdot}5 \pm 5{\cdot}7$ pA at +30 mV and $22{\cdot}7 \pm 2{\cdot}5$ pA ($n = 18$) when test pulse durations were increased to 500 ms (Fig. 8*B*, right panel, and Fig. 9*B*).

The effects of L-735,821 and chromanol 293B on pharmacologically lengthened action potentials

The effects of both L-735,821 and chromanol 293B were tested in dog ventricular papillary muscle action potentials, lengthened pharmacologically by exposure to 1 μM E-4031 (to block I_{Kr}) and 1 μg ml^{-1} veratrine (a recognized sodium channel agonist). In these experiments, performed while continuously pacing at 1 Hz, recordings were taken every 5 min after initiating superfusion with 1 μM E-4031 + 1 μg ml^{-1} veratrine until a 'quasi' steady-state was attained (Fig. 10*A* and *B*). Then, in the continued presence of E-4031 and veratrine that pharmacologically lengthened APD, the effects of I_{Ks} block were examined by either applying 100 nM L-735,821 or 10 μM chromanol 293B. L-735,821 markedly lengthened APD under these conditions from $383{\cdot}5 \pm 25{\cdot}2$ to $442{\cdot}1 \pm 32{\cdot}3$ ms ($P < 0{\cdot}01$, $n = 7$) (Fig. 10). This effect was in sharp contrast to the negligible effect of L-735,821 on normal APD (Figs 4 and 6). Comparable effects on APD were obtained with chromanol 293B in the continuous presence of E-4031 and veratrine (APD was $366{\cdot}1 \pm 13{\cdot}1$ ms before chromanol 293B *versus* $429{\cdot}5 \pm 23{\cdot}5$ ms after its addition, $P < 0{\cdot}01$, $n = 8$). These results indicate that the effect of I_{Ks} on APD is substantially increased when APD is abnormally lengthened.

Influence of I_{Ks} and I_{Kr} inhibitions on the QTc interval in anaesthetized closed chest dogs

To examine further whether I_{Ks} block lengthens APD and increases QT interval, we examined the effects of chromanol 293B (1 mg kg^{-1} I.V.) and D-sotalol (1 mg kg^{-1} I.V.) in anaesthetized dogs (Table 1). In agreement with our *in vitro* observations, chromanol 293B did not significantly affect QTc interval, while D-sotalol markedly lengthened it.

DISCUSSION

Summary of the main results

Our results indicate that both chromanol 293B and L-735,821, purportedly selective I_{Ks} blockers, did not substantially lengthen APD in either dog right ventricular papillary muscle or Purkinje fibre preparations. Equivalent concentrations of both compounds, however, substantially blocked I_{Ks} in isolated dog ventricular myocytes. Adenylcyclase stimulation by forskolin, known to increase I_{Ks} (Walsh *et al.* 1989), did not substantially enhance the small increase in APD induced by either chromanol 293B or L-735,821 in dog papillary muscle. In contrast, E-4031 and D-sotalol (recognized I_{Kr} blockers) markedly lengthened dog ventricular muscle and Purkinje fibre APD. In agreement with these *in vitro* results, QTc was increased *in vivo* by D-sotalol but not by chromanol 293B in anaesthetized dogs. However, in papillary muscle preparations where APD was prolonged by E-4031 and veratrine both chromanol 293B and L-735,821 increased repolarization considerably.

Choice of drug concentrations

The concentrations of drugs used in this study are comparable to those previously described in the literature (Lathrop, 1985; Sanguinetti & Jurkiewicz, 1990; Salata *et al.* 1996*b*; Busch *et al.* 1996). D-Sotalol at a concentration of 30 μM, inhibited I_{Kr} by 30–50%; 1 and 5 μM E-4031 caused complete block. This amount of I_{Kr} block made examination of the effects of D-sotalol and E-4031 on Purkinje fibre APD difficult. E-4031-induced Purkinje fibre APD lengthening was, for example, so pronounced that recordings at pacing cycle lengths shorter than 1000 ms could not be achieved because these stimuli fell within the total refractory period.

The concentrations of L-735,821 (100 nM) and chromanol 293B (10 μM and 30 μM) were also comparable to those used by others (Salata *et al.* 1996*b*; Busch *et al.* 1996). This L-735,821 concentration completely blocked I_{Ks} as previously reported (Salata *et al.* 1996*b*). Chromanol 293B at 10 μM blocked I_{Ks} by 70% in agreement with findings in guinea-pig ventricular myocytes (Busch *et al.* 1996). Higher chromanol 293B concentrations, however, notably affected other repolarizing currents (Bosch *et al.* 1998). Although the application of a higher concentration of chromanol 293B made interpretation of its effect on action potential repolarization uncertain at best; we used 30 μM chromanol 293B in order to block I_{Ks} completely, during the assessment of I_{Kr}. At this chromanol 293B concentration, we observed marked I_{to} depression in good agreement with earlier reports from Bosch *et al.* (1998). High chromanol 293B concentrations may also block I_{Kr}; however, the results of this current study do not address or confirm this speculation.

The 1 mg kg^{-1} I.V. dose of chromanol 293B in the *in vivo* experiments was chosen because both chromanol 293B and

D-sotalol have similar molecular weights (324·4 *versus* 309, respectively) and both compounds were assumed to have similar potencies for channel block. Although chromanol 293B proved to be a potent I_{Ks} blocker in the patch-clamp measurements, the possibility that the applied dose of 1 mg kg^{-1} I.V. chromanol 293B did not completely block I_{Ks} cannot be ruled out.

Comparison of the results with earlier findings

I_{Ks} and I_{Kr} are both generally accepted as having important roles during normal cardiac action potential repolarization (Sanguinetti & Jurkiewicz, 1990; D. W. Liu & Antzelevitch, 1995; Singh, 1998). However, selective I_{Ks} blockers have only recently been available (Salata *et al.* 1996*b*; Busch *et al.* 1996). With the development of such I_{Ks} blockers, it is possible to determine directly the effect of I_{Ks} on APD.

The few published studies that have examined the effect of I_{Ks} on cardiac APD were performed in guinea-pig papillary muscle (Schreieck *et al.* 1997) as well as in isolated guinea-pig and human ventricular myocytes (Bosch *et al.* 1998; Bryant *et al.* 1998) and in rabbit Purkinje cardiocytes (Cordeiro *et al.* 1998). The results obtained often contradict one another. Schreieck *et al.* (1997) for example using conventional microelectrodes, did not observe a significant APD increase after exposing multicellular guinea-pig papillary muscle preparations to 10 μM chromanol 293B. This lack of effect has been argued to result from the absence of adrenergic stimulation (Schreieck *et al.* 1997). In contrast, Bosch *et al.* (1998), using the whole-cell patch-clamp technique in single isolated guinea-pig and human myocytes, reported that APD increased following chromanol 293B exposure. In that study, a relatively small number of cells (5–8 cells) were examined and measurements in the absence or presence of chromanol 293B were made in different myocyte groups. It is also notable that APD measurements in single, isolated myocytes show enormous beat-to-beat variability probably due to loss of electrotonic influences among electrically coupled myocytes or the run-down of currents affecting repolarization. Nevertheless, our results using conventional microelectrode recordings in dog papillary muscles agree, in part, with those of Schreieck *et al.* (1997) in guinea-pig papillary muscle; i.e. 10 μM chromanol 293B did not lengthen APD in the absence of forskolin. However, in dog papillary muscle, we found no increase in APD after adenylcyclase stimulation as Schreieck *et al.* (1997) did following isoproterenol exposure in guinea-pig. This deviation from the findings of Schreieck *et al.* (1997) might be due to species differences. Certainly I_{Ks} amplitude is relatively large in the guinea-pig (Sanguinetti & Jurkiewicz, 1990) compared with that in the dog and other species (Gintant, 1996). In addition, we applied 1 μM forskolin while Schreieck *et al.* (1997) used 100 nM isoproterenol to activate adenylcyclase. Because other currents are also modulated by cAMP (e.g. I_{Ca} and I_{Cl}) that also affect APD (Harvey & Hume, 1989), the observations in the two studies may not be directly due to I_{Ks} block.

I_{Ks} block, in our study, produced substantially different effects in multicellular dog cardiac Purkinje fibre strands than previously reported by Cordeiro *et al.* (1998) using L-735,821 in four single, isolated rabbit cardiac Purkinje fibre cells. These investigators reported marked APD lengthening after superfusion with only 20 nM L-735,821. The reason for this discrepancy in findings is unknown. However, some investigators have suggested that because of its physical and/or chemical properties, L-735,821 poorly penetrates multicellular preparations but easily enters single myocytes (J. J. Salata, personal communication). Be that as it may, the action potential plateau voltage in the rabbit Purkinje fibre cells illustrated by Cordeiro *et al.* (1998, Fig. 9) is approximately −20 mV, while in the same study (Cordeiro *et al.* 1998, Fig. 11) these authors show that activation of the L-735,821-sensitive current (presumably I_{Ks}) occurs at voltages positive to 0 mV. These facts make it unlikely that the observed increase in APD reported by Cordeiro *et al.* (1998) was due to I_{Ks} block.

The effects of D-sotalol and E-4031 on Purkinje fibre APD in our study are in excellent agreement with those previously published (Strauss *et al.* 1970; Lathrop, 1985; Varró *et al.* 1986; Sanguinetti & Jurkiewicz, 1990).

Estimation of the amount of I_{Ks} and I_{Kr} activated during the action potential

We estimated I_{Kr} and I_{Ks} during normal ventricular action potentials. Currents measured during and after 200 ms rectangular and artificial action-potential-like test pulses indicated that I_{Kr} is several times greater than I_{Ks}. Consistent with these findings, the recent papers of Hancox *et al.* (1998) and Zhou *et al.* (1998) have also confirmed that I_{Kr} plays a crucial role in the action potential repolarization under physiological conditions. On the other hand, our finding suggests that I_{Ks}, unlike I_{Kr}, plays little role during normal action potential repolarization. Such a conclusion is well supported by the negligible effect of I_{Ks} block on isolated ventricular muscle and Purkinje fibre APD as well as on intact dog QTc.

When the duration of the rectangular or action potential-like test pulse was increased, however, I_{Ks} was more fully activated. Thus, I_{Ks} is expected to limit excessive APD lengthening when repolarization is abnormally lengthened. This speculation is supported by our experiments where APD was substantially increased pharmacologically by augmenting inward (I_{Na}) and decreasing outward (I_{Kr}) currents (Fig. 10).

Potential significance of the results

Prior to this study, I_{Ks} was believed vital to normal cardiac action potential repolarization. As such, I_{Ks} was thought to control normal APD and refractoriness (D. W. Liu & Antzelevitch, 1995; Sanguinetti & Keating, 1997; Singh, 1998). In addition, based on experiments performed in guinea-pig ventricular myocytes, selective I_{Ks} block was believed to increase APD without producing the undesired, reverse use-dependent APD lengthening which is

characteristic of I_{Kr} block (Jurkiewicz & Sanguinetti, 1993). This expectation was based on the finding that I_{Ks} deactivates slowly in guinea-pig so that reduction in outward current due to its block would be expected to be greater at fast heart rates (short diastolic intervals) than at slow heart rates or long intervals between subsequent action potentials. More recently, however, both in dog ventricular myocytes (Gintant, 1996) and human ventricular myocytes (Iost *et al.* 1998), I_{Kr} has been demonstrated to deactivate slowly while I_{Ks} deactivates relatively rapidly. This is quite unlike the situation in the guinea-pig and brings the speculation originally presented by Jurkiewicz & Sanguinetti (1993) into question. It is also notable that Heath & Terrar (1996*b*) have recently reported rather rapid deactivation of I_{Ks} also in guinea-pig myocytes. Our finding that I_{Ks} block does not remarkably increase APD in either normal dog ventricular muscle or Purkinje fibres over a wide range of pacing frequencies directly contradicts the Jurkiewicz & Sanguinetti (1993) hypothesis. Our findings, however, must be examined in perspective with other recent observations. Shimizu & Antzelevitch (1998) have, for example, recently reported that chromanol 293B lengthened APD in wedge-perfused canine left ventricular muscle preparations. In these experiments, 1–10 μM chromanol 293B produced only a slight increase in APD, as in the present study. However, in that study (Shimizu & Antzelevitch, 1998) chromanol 293B concentrations greater than 30 μM substantially increased APD. Such concentrations are greater than those required to block I_{Ks} fully, and these chromanol 293B concentrations probably affect other outward currents involved in the control of APD (Bosch *et al.* 1998).

I_{Ks} block in the presence of sympathetic stimulation is also believed to selectively prevent APD shortening associated with cAMP-dependent augmentation of I_{Ks} (Vanoli *et al.* 1995). Such an effect could potentially provide anti-arrhythmic benefit and represent an innovative approach to arrhythmia treatment. In support of this speculation L-768,673 (a structural analogue of L-735,821) provides anti-arrhythmic efficacy following coronary artery ligation and sympathetic stimulation (Billman *et al.* 1998*a*,*b*). Our results in the presence of forskolin do not support such a speculation. As such, additional research is needed to clarify the effect of sympathetic stimulation on I_{Ks} and their combined role in arrhythmogenesis.

Although I_{Ks} may have little role in normal action potential repolarization, it probably plays a vital role when cardiac APD is abnormally lengthened by other means (e.g. by reductions in I_{Kr} or I_{k1} or increases in I_{Na} or I_{Ca}). As such, pharmacological block of I_{Ks} might be expected to have severe detrimental consequences when this protective mechanism is eliminated. For example, if repolarization is excessively lengthened due to drug-induced I_{Kr} block, hypokalaemia, genetic abnormality, or bradycardia, the subsequent increase in APD would favour I_{Ks} activation and provide a negative feedback mechanism to limit further APD lengthening. Without such a mechanism, excessive APD lengthening might lead to enhanced regional repolarization dispersion (Surawicz, 1989) and increase propensity for development of early afterdepolarization (El-Sherif, 1992) associated with Torsade de Pointes induction. Such a role for I_{Ks} in limiting excessive APD lengthening was first postulated by Ito & Surawicz (1981), and if I_{Ks} plays such a role, anti-arrhythmic agents producing non-selective block of I_{Kr} and I_{Ks} (e.g. quinidine and azimilide) might be associated with a greater pro-arrhythmic risk than 'pure' (selective) I_{Kr} blockers (e.g. sotalol and dofetilide). In agreement with this speculation, Salata *et al.* (1998) have recently recommended I_{Ks} activation for prevention of pro-arrhythmic complications due to excessive potassium channel block.

Some forms of inherited long QT syndrome (LQT) probably represent situations where loss of the protective effect of I_{Ks} is detrimental. For example, LQT1 is an inherited disorder where fewer I_{Ks} channels are expressed than in normal individuals. Our results in dog indicating that I_{Ks} plays little role in normal action potential repolarization suggest that its absence alone would not result in a prolonged APD and a long QT interval. Thus, with the presence of the LQT1 phenotype in man associated with reduced I_{Ks} expression it is difficult to reconcile our findings. This discrepancy between observations may have two explanations: (1) I_{Ks} is more abundantly expressed in man than in dog, or (2) reduction in I_{Ks} in both dog and man increases the likelihood that reduction in other outward currents (or an increase in inward current) results in LQT. Preliminary results in man showing that I_{Ks} is similar to that in dog and that its block does not affect normal papillary muscle APD (Varró *et al.* 1999) supports the second possibility. Thus, it may be that the absence of I_{Ks} in these individuals simply limits their ability to restrict excessive APD lengthening due to other causes (e.g. hypokalaemia or bradycardia). This explanation would account for the recent finding that the penetrance of genetic defects involving reduction in I_{Ks} channel expression (LQT1) is rather low compared with other forms of LQT (Swan *et al.* 1998; Priori *et al.* 1998). Some of these authors report that only about 25% of patients with genetic defects encoding for I_{Ks} channels actually had abnormally long QT intervals (Priori *et al.* 1998).

Marked gender differences have recently been described in the prevalence of inherited and acquired LQT that may be due to differences in potassium current expression (X. K. Liu *et al.* 1998). This is an important area of research interest and significant differences may exist in I_{Ks} expression in males and females. However, in the present studies no attempt was made to differentiate between results obtained in myocytes or preparations isolated from animals of different gender.

D. W. Liu & Antzelevitch (1995) showed in isolated dog ventricular myocytes that M cells express a lower density of I_{Ks} channels than do subendocardial or subepicardial cells. These investigators postulate on this basis that the longer M cell APD was due to less repolarizing current

flowing through I_{Ks} channels. Our present data, however, indicate that an 80–100% I_{Ks} block failed to lengthen APD substantially in dog subendocardial papillary muscle; i.e. substantial I_{Ks} block did not cause subendocardial cells to resemble M cells. Thus, differences in other membrane currents probably account for the differences in M cell and subendocardial ventricular muscle cell action potential configurations. Differences in endocardial and M cell sodium window currents (or slowly inactivating I_{Na}) density, for example, may help account for APD differences in these two cell types.

Conclusions

This study indicates that in normal dog ventricular muscle I_{Ks} plays a minor role in control of APD. This current, however, could provide an important means of limiting excessive APD lengthening when action potentials are increased beyond normal by other mechanisms.

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