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Pharmacological block of the slow component of the outward delayed rectifier current (I_{Ks}) fails to lengthen rabbit ventricular muscle QT_c and action potential duration

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1 The effects of I_{Ks} block by chromanol 293B and L-735,821 on rabbit QT-interval, action potential duration (APD), and membrane current were compared to those of E-4031, a recognized I_{Kr} blocker. Measurements were made in rabbit Langendorff-perfused whole hearts, isolated papillary muscle, and single isolated ventricular myocytes.

2 Neither chromanol 293B (10 μM) nor L-735,821 (100 nM) had a significant effect on QTc interval in Langendorff-perfused hearts. E-4031 (100 nM), on the other hand, significantly increased QTc interval ($35.6 \pm 3.9\%$, $n=8$, $P<0.05$).

3 Similarly both chromanol 293B (10 μM) and L-735,821 (100 nM) produced little increase in papillary muscle APD (less than 7%) while pacing at cycle lengths between 300 and 5000 ms. In contrast, E-4031 (100 nM) markedly increased (30–60%) APD in a reverse frequency-dependent manner.

4 In ventricular myocytes, the same concentrations of chromanol 293B (10 μM), L-735,821 (100 nM) and E-4031 (1 μM) markedly or totally blocked I_{Ks} and I_{Kr}, respectively.

5 I_{Ks} tail currents activated slowly (at +30 mV, $\tau = 888.1 \pm 48.2$ ms, $n=21$) and deactivated rapidly (at −40 mV, $\tau = 157.1 \pm 4.7$ ms, $n=22$), while I_{Kr} tail currents activated rapidly (at +30 mV, $\tau = 35.5 \pm 3.1$ ms, $n=26$) and deactivated slowly (at −40 mV, $\tau_1 = 641.5 \pm 29.0$ ms, $\tau_2 = 6531 \pm 343$, $n=35$). I_{Kr} was estimated to contribute substantially more to total current density during normal ventricular muscle action potentials (i.e., after a 150 ms square pulse to +30 mV) than does I_{Ks}.

6 These findings indicate that block of I_{Ks} is not likely to provide antiarrhythmic benefit by lengthening normal ventricular muscle QTc, APD, and refractoriness over a wide range of frequencies.

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Abbreviations: APA, action potential amplitude; APD, action potential duration; APD_{50} and APD_{90}, action potential durations at 50% and 90% of repolarization CL, cycle length; ECG, volume-conducted electrocardiogram; I_{Ca}, L-type calcium current; I_{Cl}, chloride current; I_K, delayed rectifier potassium current; I_{Kr}, rapid component of the delayed rectifier potassium current; I_{Ks}, slow component of the delayed rectifier potassium current; LQT, long QT syndrome; MDP, maximum diastolic potential

Introduction

Pharmacological lengthening of cardiac action potential duration (APD) is a well-recognized means of cardiac arrhythmia suppression (Singh & Vaughan Williams, 1970; Singh, 1988). Pharmacologic agents that target the membrane current recognized as most responsible for initiation of final action potential repolarization have therefore been intensely sought and developed over the past two decades as new antiarrhythmic agents. This current, the outwardly directed delayed rectifier potassium current (I_K) in most species (Noble & Tsien, 1969; Sanguinetti & Jurkiewicz, 1990; Follmer & Colatsky, 1990; Varró *et al*., 1993; Gintant, 1996; Salata *et al*., 1996b) including man (Li *et al*., 1996; Iost *et al*., 1998; 1999) consists of both a rapid (I_{Kr}) and slow (I_{Ks}) component. The two components of I_K differ from each other in terms of their drug sensitivity, current rectification, and kinetics (Sanguinetti & Jurkiewicz, 1990; Liu & Antzelevitch, 1995; Carmeliet, 1992; 1993; Gintant, 1996; Heath & Terrar, 1996b). Specific I_{Kr} blockers (e.g., d-sotalol, E-4031, and more recently dofetilide) greatly lengthen cardiac APD (Singh & Vaughan Williams, 1970; Strauss *et al*., 1970; Lathrop, 1985; Jurkiewicz & Sanguinetti, 1993) and several are well recognized as useful in ablating cardiac arrhythmias in man (Singh, 1988; Hohnloser & Woosley, 1994). All I_{Kr} blockers display 'reverse use-dependency' (Hondeghem & Snyders, 1990) characterized by greater increases in APD at long diastolic intervals than at

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short ones. Thus, when the time between initiation of successive action potentials is short and an APD increase is expected to provide the most antiarrhythmic benefit, the actual APD increase is least. Conversely, when the time between successive action potentials is long, I_{Kr} block produces a far greater increase in APD. Long APDs due to block of I_{Kr} at long diastolic intervals and slow heart rates are associated with induction of early after depolarizations believed to trigger Torsade de Pointes ventricular arrhythmias (Hohnloser & Woosley, 1994).

Selective I_{Ks} block, on the other hand, is hypothesized to increase APD and refractoriness in a frequency-independent manner (Jurkiewicz & Sanguinetti, 1993). Because of this, the search for selective I_{Ks} blockers has intensified as they may represent novel antiarrhythmic agents devoid of the risk of Torsade de Pointes arrhythmia induction. Propofol, thiopenthone (Heath & Terrar, 1996a) and indapamide (Turgeon *et al*., 1994) were first recognized as I_{Ks} blockers in guinea-pig ventricular myocytes. However, both these agents require concentrations greater than 100 μM to produce I_{Ks} block so that concerns over their selectivity and possible therapeutic usefulness have been justifiably raised. Three newer compounds, chromanol 293B (Busch *et al*., 1996; Varró *et al*., 2000), L-735,821 (Salata *et al*., 1996a; Cordeiro *et al*., 1998; Varró *et al*., 2000) and L-768,673 (Selnick *et al*., 1997; Lynch *et al*., 1999) have recently been reported to selectively block I_{Ks}. The effects of these compounds on QTc and cardiac action potential configuration, however, have not been characterized in rabbit, which represents a species widely used to determine the effects of new antiarrhythmic agents intended for use in man. Available published results of describing chromanol 293B and L-735821 effects, in fact, have often contradicted each other (e.g., Schreieck *et al*., 1997; Cordeiro *et al*., 1998; Anyukhovsky *et al*., 1999; Varró *et al*., 2000). These contradictory findings have caused us to question whether these differences may be due to species and regional variations in I_{Ks} (Varró *et al*., 2000).

Therefore, the major objective of this study was to characterize the effects of chromanol 293B and L-735,821 on whole heart QTc, papillary muscle APD, and isolated myocyte membrane current in rabbit; a species widely used for antiarrhythmic drug testing. For comparison, we compared the effects of the two I_{Ks} blockers to those of E-4031, a recognized I_{Kr} blocker.

Methods

All experiments were conducted in compliance with the *Guide for the Care and Use of Laboratory Animals* (U.S.A. NIH publication No 85-23, revised 1985). The protocols were approved by the review board of Committee on Animal Research of the Albert Szent-Györgyi Medical University (54/1999 OEj).

Preparation of the rabbit heart

New Zealand rabbits weighing 1.5–2.0 kg of either sex were used. Each animal was sacrificed by cervical dislocation after an intravenous injection of 400 IU kg^{-1} heparin. The chest was opened, the heart quickly removed and immediately immersed in oxygenated modified Locke's solution containing (in mM): NaCl 120, KCl 4, $CaCl_2$ 1.0, $MgCl_2$ 1, $NaHCO_3$ 22 and glucose 11. This solution had a pH ranging between 7.35 and 7.45 when saturated with 95% O_2 and 5% CO_2 at 37°C.

ECG measurements in Langendorff-perfused rabbit hearts

The hearts were after removal mounted on a modified, 60 cm high Langendorff column and perfused with oxygenated modified Locke's solution warmed to 37°C. After flushing blood from the coronary vasculature for 3–5 min, the heart was immersed in a tissue chamber filled with perfusion solution maintained at 37°C while continuing perfusion.

Volume-conducted electrocardiograms (ECGs) were obtained as previously described (Zabel *et al*., 1995). Briefly, four silver-silver chloride electrodes were positioned in a simulated Einthoven configuration with the reference and 'foot' electrodes situated beneath the heart and the 'arm' electrodes fixed to the upper walls of the tissue chamber to record the six bipolar ECG leads *I* through aVF. All leads were acquired by an ECG signal processing system (Cardiax 2.46, IMED Ltd., Budapest, Hungary) utilizing a 486-microprocessor based, IBM compatible personal computer. After analogue-to-digital conversion, the data were stored to hard disk and analysed off-line. Illustrated ECGs are displayed at 50 mm s^{-1}. After an 1 h equilibration period, baseline ECGs were obtained and a 40 min perfusion period was initiated with either chromanol 293B, L-735,821 or E-4031. ECG recordings were monitored continuously and compared to baseline measurements at the end of this period. QT intervals were always measured on lead *II* from QRS onset to the end of the T wave; biphasic T waves were measured to the time of final baseline return. These QT measurements and simultaneously recorded RR intervals were used to derive heart rate corrected QT intervals using Carlsson's formula (QTc = QT -0.175 (RR-300)) (Carlsson *et al*., 1993). ECG parameters were averaged from measures of three consecutive complexes and a single observer performed all analyses.

Conventional microelectrode measurements

Isolated right ventricular papillary muscle preparations were mounted individually in a tissue chamber (volume $\sim$40 ml) continuously superfused with modified Locke's solution warmed to 37°C while stimulated (Hugo Sachs Elektronik Stimulator Type 215/II, March-Hugstetten, Germany) at an 1000 ms constant cycle length (frequency = 1 Hz) using rectangular constant current pulses 2 ms in duration. These stimuli were isolated from ground and delivered through a bipolar platinum electrode in contact with the preparation. At least 1 h was allowed for each preparation to equilibrate after mounting before experimental measurements were initiated.

Transmembrane potentials were recorded using conventional 5–20 Mohm, 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 derived (Biologic Differentiator DV 140, Claix, France) and with transmembrane voltage continuously monitored on a dual beam storage oscilloscope (Tektronix 2230, 100 MHz Digital Storage Oscilloscope).

The maximum diastolic potential (MDP), action potential amplitude (APA) 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, Action Potential Evaluation System (APES)) running on a 386-microprocessor based, IBM compatible computer containing an ADA 3300 analogue-to-digital data acquisition board (Real Time Devices Inc.; State College, PA, U.S.A.) 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 while pacing cycle length was sequentially varied between 300 and 5000 ms. Twenty-five action potential were evoked at each cycle length and the cycle length was then changed so that 'quasi' steady-state frequency response relations could be rapidly generated. Each preparation was then superfused for 40–60 min with either chromanol 293B (Hoechst AG, Frankfurt, Germany), L-735,821 (Merck-Sharpe & Dohme Laboratories, West Point, PA, U.S.A.) or E-4031 (Institute for Drug Research, Budapest, Hungary) before repeating the pacing protocol. Attempts were made to maintain the same impalement throughout each experiment. If, however, an impalement became dislodged, adjustments were 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 arbitrary 5% limit was exceeded, the experiment was terminated and all data obtained excluded from analyses.

Patch-clamp measurements

Cell isolation Single ventricular myocytes were obtained by enzymatic dissociation of isolated New Zealand rabbit hearts. The chest was opened and the heart quickly removed and placed into cold (4°C) solution with the following composition (mM): NaCl 135, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, HEPES 10, $NaHCO_3$ 4.4, Glucose 10, $CaCl_2$ 1.8, (pH 7.2). The heart was then mounted on a modified, 60 cm high Langendorff column and perfused with oxygenated perfusate of the same composition warmed to 37°C. After 3–5 min of perfusion to flush blood from the coronary vasculature, the perfusate was switched to one having no exogenously added calcium (i.e., to one that was nominally Ca^{2+}-free) until the heart ceased contracting ($\sim$3 –4 min). Enzymatic digestion was accomplished by perfusion with the same, nominally Ca^{2+}-free solution with 0.33 mg ml^{-1} (90 U ml^{-1}) Collagenase (Sigma Type 1) and 0.02 mg ml^{-1} Pronase E (Sigma) in 0.1% Albumin added. Perfusion rate was maintained constant using a perfusion pump (flow rate $\sim$15 ml min^{-1}). After 15 min of the enzymatic perfusion, calcium was elevated to a concentration of 200 μM. Thirty to 35 minutes later the heart was removed from the aortic cannula and placed into enzyme free solution containing 1.8 mM $CaCl_2$ and 1% albumin warmed to 37°C for 10 min. Then, the tissue was minced into small chunks, and following gentle agitation myocytes were separated by filtering the resulting slurry through a nylon mesh. Myocytes were finally harvested by gravity sedimentation. Once the majority of individual myocytes had settled to the bottom of the container, the supernatant was decanted and replaced with Tyrode solution containing 1.8 mM $CaCl_2$ and the myocytes resuspended by gentle agitation. This procedure was repeated twice more and the resulting myocyte suspension was stored in HEPES buffered Tyrode solution at 10–14°C.

Experimental procedure, drugs and solutions One drop of cell suspension was placed in a transparent recording chamber mounted on the stage of an inverted microscope (TMS; Nikon, Tokyo, Japan) and at least 5 min were allowed for individual myocytes to settle and adhere to the chamber bottom before initiating superfusion. Myocytes were used that were rod shaped with clear striations. HEPES buffered Tyrode solution served as the normal superfusate in all experiments. 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.

Patch-clamp micropipettes were fabricated from borosilicate glass capillaries (Clark, Reading, U.K.) using a P-97 Flaming/Brown micropipette puller (Sutter Instrument Co, Novato, CA, U.S.A.). These electrodes had resistances between 1.5 and 2.5 Mohm when filled with pipette solution containing (in mM): K-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 by addition of KOH. Nisoldipine (1 μM) (Bayer AG, Leverkusen, Germany) in the external solution eliminated inward Ca^{2+} current (I_{Ca}) while the sodium current (I_{Na}) was inactivated during experiments by applying a holding potential of –40 mV. At this holding potential, transient outward current (I_{to}) was also largely inactivated. An Axopatch-1D amplifier (Axon Instruments, Foster City, CA, U.S.A.) was used to record membrane current in the whole-cell configuration of the patch-clamp technique. After establishing a high (1–10 Gohm) resistance seal by gentle suction, the cell membrane beneath the tip of the electrode was disrupted by further suction or application of 1.5 V electrical pulses applied for 1–5 ms. Cell capacitance (150.14 ± 0.14 pF, $n=73$) was measured by applying a 10 mV hyperpolarizing pulse from a holding potential of –10 mV and integration of the capacitive transient over time divided by the amplitude of the voltage step (10 mV). Series resistance was typically 4–8 Mohm prior to compensation (50–80%, depending on the voltage protocol utilized). Experiments where the series resistance was high, or where it increased substantially during measurement, were terminated and the data discarded. Membrane currents were digitized using a 333 kHz analogue-to-digital converter (Digidata 1200, Axon Instruments, with sampling rate ranging 0.4–50 kHz) under software control (pClamp 6.0, Axon Instruments). Analyses were performed using Axon (pClamp 6.0) software after low-pass filtering at 1 kHz. All patch-clamp data were collected at 37°C.

Solutions and the choice of drug concentrations Chromanol 293B was diluted at the time of use from a 10 mM stock solution containing 100% DMSO. DMSO at the resulting concentrations (0.1–0.3%) produced no discernible effect on QTc, APD, or the membrane currents assessed. L-735,821 was diluted from a 100 μM stock solution containing 10% DMSO. All stock solutions were prepared using HEPES buffered Tyrode solution as the solvent. E-4031 was diluted from a 1 mM aqueous stock solution at the time of each experiment. The concentrations of L-735,821 (100 nM) and chromanol 293B (10 μM) were comparable to those used by others and shown to block I_{Ks} in other species (Salata *et al.*,

1996a; Busch *et al*., 1996; Varró *et al*., 2000). We used 100 nM L-735,821 to assure completely block I_{Ks} during assessment of I_{Kn} and 1–5 μM E-4031 to fully block I_{Kr} during assessment of I_{Ks}. E-4031 concentrations were also similar to those used previously by us and others (Sanguinetti & Jurkiewicz, 1990; Salata *et al*., 1996b; Busch *et al*., 1996; Varró *et al*., 2000).

Statistical analyses

Results were compared using Student's *t*-tests for paired and unpaired data. When $P<0.05$, results were considered significant. Data are expressed as mean ± s.e.mean.

Results

Comparison of the effects of I_{Ks} and I_{Kr} block on QTc interval in isolated Langendorff-perfused rabbit hearts

Neither I_{Ks} blocker, chromanol 293B (10 μM) nor L-735,821 (100 nM) significantly lengthened QTc or increased RR interval after 40 min of exposure in isolated, Langendorff-perfused rabbit hearts after 40 min of exposure (Figure 1A, B, Table 1). The I_{Kr} blocker E-4031 (100 nM), significantly increased QTc under identical conditions (Figure 1C, Table 1). This E-4031 induced increase in QTc was associated with a significant increase in RR interval (420.5 ± 17.5 ms at baseline versus 463.5 ± 17.8 ms after E-4031, $n=8$, $P<0.05$).

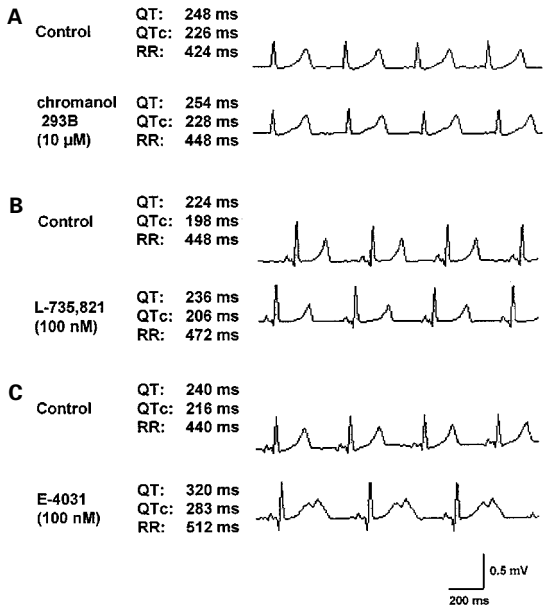

Figure 1 Effect of chromanol 293B (10 μM, A), L-735,821 (100 nM, B) and E-4031 (100 nM, C) after 40 min of perfusion on surface electrocardiogram recorded in spontaneously beating isolated Langendorff-perfused rabbit hearts. Note that chromanol caused only a 2 ms and L-735,821 only an 8 ms increase in the QTc interval. The I_{Ks} blockers chromanol 293B and L-735,821 increased spontaneous RR interval by 1.88 ± 2.93% ($n=7$) and by 3.12 ± 0.71*% ($n=7$, $*P<0.01$), respectively. The I_{Kr} blocker E 4031 increased QTc by 67 ms and prolonged spontaneous the RR interval by 10.61 ± 3.5*% ($n=8$, $*P<0.05$).

Effects of I_{Ks} and I_{Kr} block on ventricular action potential duration in isolated rabbit papillary muscle

Concentrations of chromanol 293B (10 μM) and L-735,821 (100 nM) reported to block I_{Ks} in other species failed to significantly affect rabbit papillary muscle APD while pacing at a constant pacing cycle length of 1000 ms (Figure 2A,B, Table 2). On the other hand, E-4031 (100 nM) markedly and significantly increased rabbit papillary muscle APD under identical conditions after 40 min of exposure (Figure 2C, Table 2). A similar difference in the effects of chromanol 293B (10 μM) and L-735,821 (100 nM) compared to E-4031 (100 nM) on APD were observed in rabbit ventricular muscle over a wide range of pacing cycle lengths (300–5000 ms) (Figure 3). Again, chromanol 293B and L-735,821 produced only small changes in APD over this entire range of pacing rates while E-4031 markedly lengthened rabbit papillary muscle APD in a reverse frequency-dependent fashion so that the increase in APD was greater at long cycle lengths than at short ones (Figure 3).

Table 1 Effect of chromanol 293B and L-735,821 on the QTc interval of the ECG recorded in isolated, Langendorff-perfused rabbit hearts compared to E-4031 induced change

Experimental condition	*Chromanol 293B* (10 μM) ($n=7$)	*L-735,821* (100 nM) ($n=7$)	*E-4031* (100 nM) ($n=8$)
Control (ms)	212.8 ± 7.5	212.4 ± 6.8	221.2 ± 5.0
Drug (ms)	223.2 ± 10.8	222.0 ± 7.7	299.1 ± 7.0*
Change (%)	4.7 ± 2.1	4.6 ± 2.1	35.6 ± 3.9*

Drug effect was measured after 40 min of exposure. $*P<0.05$ compared to control values.

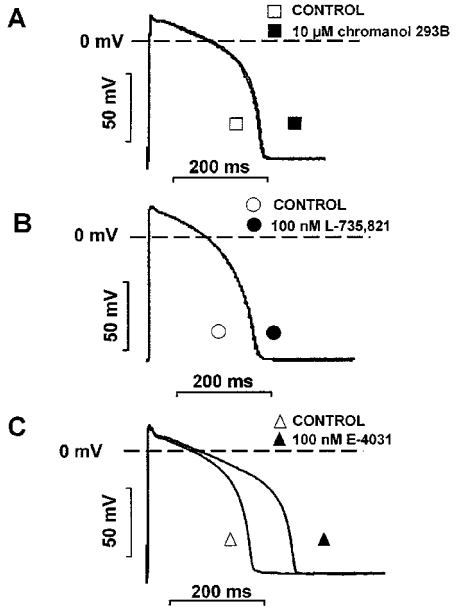

Figure 2 Rabbit ventricular papillary muscle action potential recordings before and after 40 min exposure to chromanol 293 B (10 μM, A), L-735,821 (100 nM, B), E-4031 (100 nM, C). Stimulation frequency was 1 Hz.

Table 2 Effect of chromanol 293B and L-735,821 on rabbit papillary muscle APD_{90} compared to that of E-4031

Experimental condition	*Chromanol 293B* (10 μM) ($n=7$)	*L-735,821* (100 nM) ($n=5$)	*E-4031* (100 nM) ($n=6$)
Control (ms)	191.9±12.4	171.8±9.7	196.3±8.4
Drug (ms)	193.1±13.1	172.8±9.2	272.8±11.45*
Change (%)	0.65±2.2%	0.7±1.4%	28.0±0.8%*

Drug effect was measured after 40 min of exposure. *$P<0.05$ compared to control values.

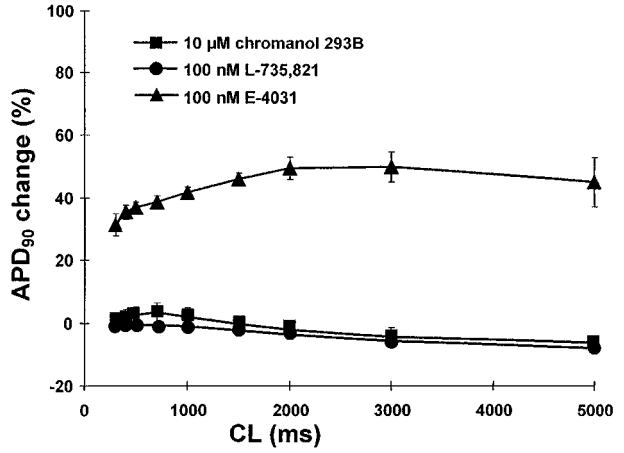

Figure 3 Frequency dependent effect of the I_{Ks} blockers chromanol 293B (10 μM, $n=7$) and L-735,821 (100 nM, $n=7$) on the action potential duration measured at 90% repolarization (APD_{90}) compared to that of the I_{Kr} blocker E-4031 (100 nM, $n=8$) in isolated rabbit ventricular papillary muscles. E-4031, the I_{Kr} blocker, lengthened APD in a reverse-frequency dependent manner while the two I_{Ks} blockers had little effect. On the abscissa CL = pacing cycle length in ms, and on the ordinate percentile change in APD_{90} is given. Bars represent ± s.e.mean.

Effect of I_{Ks} block in the presence of forskolin

Because I_{Ks} is modulated by changes in intracellular cyclic AMP, we also examined the effect of I_{Ks} block on APD in the presence of 1 μM forskolin to activate adenylcyclase and thereby increase intracellular cyclic AMP. Forskolin (1 μM) alone shortened APD in rabbit papillary muscle paced at cycle length of 1000 ms from 217.4±18.7 to 194.0±15.7 ms ($n=5$, $P<0.05$, Figure 4A). Addition of 10 μM chromanol 293B in the continuous presence of forskolin had little effect on APD (194.0±15.7 *versus* 190.8±12.9 ms, $n=5$, Figure 4A). Similar result was obtained with L-735,821 (100 nM, Figure 4B). These results show that selective I_{Ks} block does not alter APD substantially even in the presence of elevated intracellular cyclic AMP.

Effects of L-735,821 on I_{Ks} compared to that of E-4031 on I_{Kr} in isolated rabbit ventricular myocytes

The effects of L-735,821 (100 nM) on I_{Ks} tail currents following 5000 ms long test pulses to between 0 and +50 mV from and return to a holding potential of −40 mV in the presence of 1–5 μM E-4031 to inhibit I_{Kr} in isolated rabbit ventricular are illustrated in Figure 5A. As

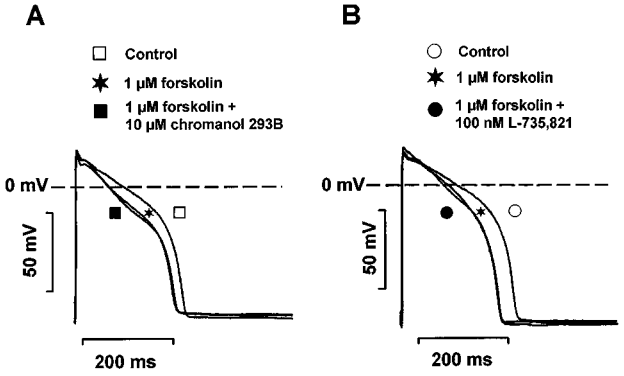

Figure 4 The effect of I_{Ks} block on the action potentials in the presence of adenylcyclase activation by forskolin (1 μM). (A) Action potential recorded from rabbit ventricular papillary muscle before and after forskolin (1 μM) and after administration of 10 μM chromanol 293B in the continuous presence of forskolin. (B) Result of a similar experiment using 100 nM L-735,821 to block I_{Ks}. Stimulation frequency was 1 Hz.

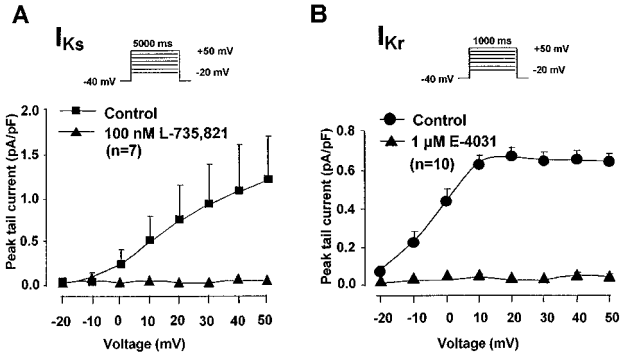

Figure 5 Effect of L-735821 (100 nM, A) and E-4031 (1 μM, B) on I_{Ks} and I_{Kr} current-voltage relations in rabbit ventricular myocytes. I_{Ks} was assessed by measuring peak outward tail current densities following a 5000 ms test pulses to between −20 and +50 mV from a holding potential of −40 mV in seven myocytes in the presence of E-4031 (5 μM) to totally block I_{Kr}. Pulse frequency was 0.1 Hz. Similarly, I_{Kr} was assessed by measuring peak outward tail current densities following a 1000 ms test pulses to between −20 and +50 mV from a holding potential of −40 mV in 10 myocytes in the presence of 100 nM L-735,821 to block I_{Ks}. Pulse frequency was 0.05 Hz. In all studies nisoldipine (1 μM) was used to block inward calcium current (I_{Ca}). Bars represent ± s.e.mean.

illustrated, L-735,821 (100 nM) completely abolished I_{Ks} (Figure 5A). In comparison, a greater concentration of E-4031 (1 μM) than used in examining its effects on rabbit QTc and APD was required to fully block I_{Kr} tails currents (Figure 5B). In these experiments, effects of E-4031 on I_{Kr} were examined in isolated rabbit ventricular myocytes using 1000 ms test pulses to between −20 and +50 mV from a holding potential of −40 mV (illustrated in the inset of Figure 5B).

I_{Ks} and I_{Kr} activation and deactivation kinetics in rabbit ventricular myocytes

We recently reported that in dog ventricular myocytes I_{Kr} activates rapidly during depolarizations to positive potentials but deactivates slowly (Varró *et al*., 2000). We also found I_{Ks}

to activate slowly at positive potentials in dog ventricular myocytes while it deactivates rapidly with respect to diastolic intervals encountered during physiologic heart rates. We speculated that in dog I_{Kr} and I_{Ks} kinetics such as these likely account for the unremarkable effect of both chromanol 293B and L-735,821 on ventricular muscle (and Purkinje strand) (Varró *et al*., 2000). Thus, we wished to determine and compare the kinetics of I_{Kr} and I_{Ks} activation and deactivation in rabbit ventricular myocytes during depolarization to +30 mV; a membrane voltage corresponding roughly to the rabbit ventricular muscle action potential plateau amplitude.

I_{Ks} kinetics were assessed in 22 rabbit ventricular myocytes using an envelope of tails protocol (Figure 6A) in the presence of 5 μM E-4031 to eliminate I_{Kr}. Under these conditions, I_{Ks} activation was slow ($\tau = 888.1 \pm 48.2$ ms, $n = 21$, at +30 mV) and I_{Ks} deactivation was fast ($\tau = 157.1 \pm 4.7$ ms, $n = 22$, at −40 mV).

Similarly, using incremental increases in test pulse duration while clamping the membrane potential to +30 mV from a holding potential of −40 mV in an envelope of tails protocol (Figure 6B) in the presence of 100 nM L-735,821 to block I_{Ks}, the activation time constant (τ) for I_{Kr} was 35.5 ± 3.1 ms ($n = 26$) and deactivation was slow and best fit as the sum of two exponentials; $\tau_1 = 641.5 \pm 25.0$ ms and $\tau_2 = 6531 \pm 343$ ms with amplitudes of $A_1 = 32.8 \pm 1.7$ pA and $A_2 = 42.4 \pm 2.1$ pA, respectively ($n = 35$).

To estimate the relative magnitude of I_{Ks} and I_{Kr} activated during rabbit ventricular action potential depolarizations, we compared the amplitudes of the L-735,821 sensitive (I_{Ks}) and E-4031 sensitive (I_{Kr}) currents at the end of an 150 ms long test pulse to +30 mV and their tail currents on return to a −40 mV holding potential. The +30 mV corresponds to a membrane voltage slightly more positive to that attained during the plateau of a normal action potential (Figure 2). The −40 mV was selected to represent a voltage level encounted during final action potential repolarization (phase III). I_{Ks} and I_{Kr} were measure 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 34.1 ± 4.2 pA ($n = 14$), or about 30% of the tail current amplitude measured after the voltage test pulse on returned to −40 mV (85.8 ± 9.2 pA, $n = 14$) (Figure 7A,C). 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 (Figure 7B,C). The magnitude of I_{Ks} during the test pulse was 13.27 ± 1.2 pA at +30 mV *versus* 6.6 ± 0.8 pA at −40 mV, ($n = 15$), approximately an order of magnitude less than the I_{Kr} tail current.

Discussion

Because the surface electrocardiogram remains the best clinical means of assessing antiarrhythmic drug therapy and monitoring development of proarrhythmic side effects, we determined the effects of two new, potentially beneficial antiarrhythmic agents, chromanol 293B and L-735,821, on QTc in isolated, Langendorff-perfused rabbit hearts. This experimental preparation allowed us to also determine the effect of the exact same drug concentrations on rabbit ventricular papillary muscle action potential configuration as well as the underlying membrane currents in isolated rabbit ventricular myocytes. We also compared the effects of these two reportedly selective I_{Ks} blockers to those of a recognized selective I_{Kr} blocker, E-4031. This comparison allowed direct assessment of the degree of QTc and action potential lengthening produced by complete, selective block of I_{Ks} *versus* I_{Kr} block in rabbit ventricular tissue. We found that chromanol 293B and L-735,821 did not substantially increase QTc in Langendorff-perfused rabbit hearts, nor did they increase isolated rabbit ventricular papillary muscle APD. L-735,821, however, did completely block I_{Ks} in isolated rabbit ventricular myocytes. In contrast, a concentration of E-4031 an order of magnitude less that which totally blocked I_{Kr} markedly increased QTc and rabbit ventricular muscle APD. Thus, if the basis of the ventricular antiarrhythmic effectiveness of I_{Kr} block by agents like E-

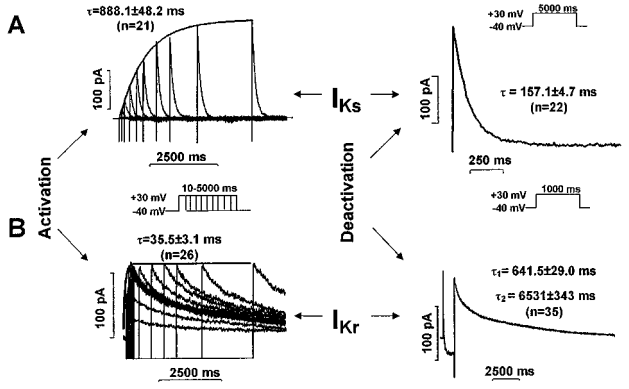

Figure 6 Activation and deactivation kinetics for I_{Ks} (A) and I_{Kr} (B) in rabbit ventricular myocytes. Activation kinetics (left) measured as peak tail currents at −40 mV after test pulses from a holding potential of −40 to +30 mV with sequentially increasing durations between 10 and 5000 ms. Deactivation kinetics (right) measured from outward tail current upon return to −40 mV after test pulse to +30 mV lasting for 5000 ms with I_{Ks} or for 1000 ms with I_{Kr}.

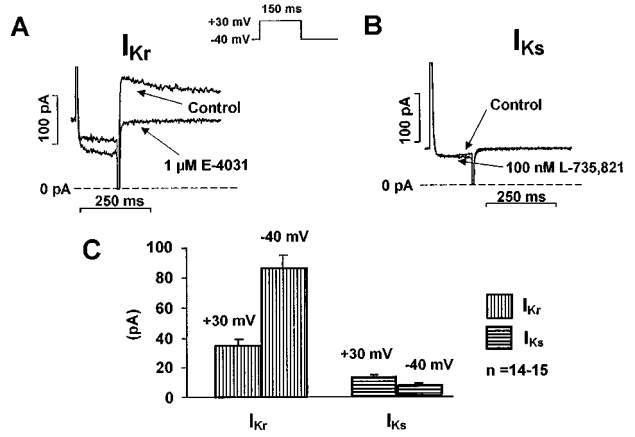

Figure 7 Membrane currents in rabbit ventricular myocytes observed during and after a 150 ms depolarizing pulse to +30 mV from a holding potential of −40 mV before and after I_{Kr} (1 μM E-4031, A) and I_{Ks} (100 nM L-735,821, B) block. (C) Bar graph shows mean±s.e.mean difference current magnitudes developed during depolarization to +30 mV and peak tail current on return to −40 mV after I_{Kr} (left) and I_{Ks} (right) block.

4031 is cardiac APD prolongation reflected as an increase in QTc, selective I_{Ks} block is unlikely to prove to be of antiarrhythmic benefit. Because the present studies were performed using normal rabbit hearts and recent reports do indicate antiarrhythmic benefit *in vivo* following I_{Ks} block (Billman *et al*., 1998a,b; Lynch *et al*., 1999) further electrophysiological studies are required to determine how pathophysiological situations may affect selective I_{Ks} block on QTc and APD.

Comparison of the results with earlier findings

Because selective I_{Ks} blockers have only recently become available (Salata *et al*., 1996a; Busch *et al*., 1996) relatively few descriptions of their pharmacological effects are yet to be found in the literature. Those studies reporting on the effects of I_{Ks} block on ventricular APD were performed in guinea-pig, dog, and human papillary muscle (Schreieck *et al*., 1997, Varró *et al*., 1999; 2000) as well as in isolated guinea-pig, dog, and human ventricular myocytes (Bosch *et al*., 1998; Varró *et al*., 2000). The effects of I_{Ks} block have also been examined in rabbit Purkinje fiber myocytes (Cordeiro *et al*., 1998). These published results at times contradict one another. Schreieck *et al*. (1997) using conventional microelectrodes, for example, did not observe significant guinea-pig papillary muscle APD increases using 10 μM chromanol 293B. Because I_{Ks} is enhanced by adrenergic stimulation (Bennett & Begenisch, 1987), some might argue that Schreieck *et al*'s. failure to induce an increase in APD with chromanol 293B resulted from likely endogenous catecholamine depletion (Schreieck *et al*., 1997). Bosch *et al*. (1998), on the other hand, reported in both current-clamped guinea-pig and human ventricular myocytes that chromanol 293B increased APD. Only 5–8 myocytes were examined in that study and baseline measurements were not observed in the same myocytes as those exposed to chromanol 293B. Thus, because APD measurements obtained in single, isolated myocytes usually display enormous beat-to-beat variability it is difficult to know how to interpret such results in comparison to other published results using different experiment techniques and perhaps better experimental controls. Our present results in isolated rabbit papillary muscles agree with those where Schreieck *et al*. (1997) found that 10 μM chromanol 293B did not substantially lengthen guinea-pig papillary muscle APD in the absence of forskolin as well as our own results in dog papillary muscle where both chromanol 293B and L-735,821 failed to notably affect APD either in the absence or presence of 1 μM forskolin to increase I_{Ks} in dog ventricle (Varró *et al*., 2000). Negligable (less than 5%) QT changes were reported also with L-768,673 in *in vivo* dog experiments (Lynch *et al*., 1999).

Although Cordeiro *et al*. (1998) found that I_{Ks} block with 20 nM L-735,821 markedly increased APD in isolated rabbit cardiac Purkinje fibre myocytes, we found no increase in rabbit papillary muscle using 100 nM L-735,821 which totally suppressed I_{Ks} in single ventricular myocytes. We can not provide an explanation for this discrepancy in findings; however, we have previously reported in isolated dog Purkinje strands that L-735,821 produces little effect on APD (Varró *et al*., 2000). Thus, it is unlikely that differences in I_{Ks} expression between ventricular muscle and Purkinje fibre myocytes explain the differences between our, and others, results compared to those of Cordeiro *et al*. (1998).

Rabbit QTc, papillary muscle APD, and block of I_{Kr} are in excellent agreement with those published by us and others (e.g., Lathrop, 1985; Sanguinetti & Jurkiewicz, 1990; Strauss, *et al*., 1970; Varró *et al*., 1985; 2000).

Although we have recently reported (Varró *et al*., 2000) that I_{Ks} block does not increase dog ventricular muscle APD, one might reasonably argue that because I_{Ks} is relatively weak in dog I_{Ks} block should not be expected to produce much response. Thus, the present findings are important as they extend our previous finding to another species, rabbit, where I_{Ks} is usually recorded as a large membrane current relative to other species (Salata *et al*., 1996b). In addition, it is important to note that rabbit is a frequently used species in evaluating the effects of new, potentially beneficial antiarrhythmic drugs. This is likely because in rabbit it is possible to directly compare the effects of equivalent investigational drug concentrations in the entire perfused heart to assess changes in QTc as well as in isolated multicellular papillary muscle and single isolated ventricular myocytes to determine effects on APD and transmembrane current.

Selectivity of I_{Ks} and I_{Kr} block

We intentionally chose to examine concentrations of chromanol 293B and L-735,821 and E-4031 expected to completely or nearly totally block either I_{Ks} or I_{Kr}, respectively. As indicated, the concentration of each drug examined was chosen on the basis of previously published reports of the effects of these compounds on cardiac myocytes membrane currents in other tissues (Cordeiro *et al*., 1998) and species (Salata *et al*., 1996b; Schreieck *et al*., 1997; Bosch *et al*., 1998). Our present findings therefore confirm that 100 nM L-735,821 completely blocked I_{Ks} and that 1 μM E-4031 blocked I_{Kr} in isolated, rabbit ventricular myocytes. This necessary confirmation of I_{Ks} and I_{Kr} block by L-735,821 and E-4031 also allowed us to examine the kinetics of I_{Ks} and I_{Kr} activation and deactivation in an attempt to provide an explanation for why total I_{Ks} block failed to affect QTc and APD in rabbit ventricle.

Kinetics of I_{Ks} activation and deactivation in rabbit ventricle compared to those of I_{Kr}: implications for prediction of pharmacological effect

We found that in rabbit ventricular myocytes I_{Ks} activated slowly ($\tau = 888.1 \pm 48.2$ ms, $n = 22$) and deactivated rapidly (157.1 ± 4.7, $n = 22$) in relation to the time of normal electrical diastole (193.8 ± 9.8, $n = 22$, calculate from baseline RR intervals–QT intervals in rabbit heart perfusion experiments) in rabbit ventricle. We also found that I_{Ks} activated rapidly as expected, but deactivated slowly. These findings are in good agreement with our findings in dog ventricle (Varró *et al*., 2000) where I_{Ks} also produced little effect on QTc and APD in comparison to I_{Kr} block that markedly and significantly lengthened QTc and APD. Gintant originally reported (1996) that in dog ventricular tissue I_{Ks} and I_{Kr} display similar activation and deactivation kinetics that with such kinetics, I_{Ks} accumulation was

unlikely at physiologic heart rates. This is unlike the situation in guinea-pig where I_{Ks} deactivates slowly (Jurkiewicz & Sanguinetti, 1993). Based on the earlier results in guinea-pig ventricular myocytes, selective I_{Ks} block was expected to increase APD without inducing reverse use-dependent lengthening as associated with I_{Kr} block because reduction in outward current due to I_{Ks} block would be greater at short diastolic intervals than at longer times between subsequent action potentials. The important question, therefore, is whether man more closely resembles guinea-pig or dog and rabbit in terms of I_{Ks} and I_{Kr} activation and deactivation kinetics. That is whether it is better to examine new antiarrhythmic drug effects in rabbit and dog ventricular preparation than in guinea-pig prior to initiating clinical studies in man. Our recent report that I_{Ks} deactivates rapidly in human myocytes (Iost *et al*., 1999) while I_{Kr} in human myocytes deactivates slowly (Iost *et al*., 1998; 1999) strongly suggests that either rabbit or dog would serve as better preclinical models for examining the effects of new antiarrhythmic agents than guinea-pig, but this question needs further studies, since it is still not satisfactory resolved (Sanguinetti & Jurkiewicz, 1990; Heath & Terrar, 1996a).

To demonstrate better this relation between I_{Ks} and I_{Kr} in rabbit ventricle, we compared the magnitude of both currents during and at the end of a 150 ms test pulse to +30 mV from a holding potential of −40 mV (inset for Figure 7) representing roughly the membrane voltage at the most positive part of the plateau and voltage at the beginning of rabbit ventricular muscle final repolarization. This comparison (Figure 7) illustrates that when both currents are measured at +30 and −40 mV, I_{Kr} is several times greater than I_{Ks} (2.6 times at +30 mV and 12.9 times at −40 mV, respectively). As such it is not surprising that complete I_{Ks} block had no marked effect on QTc and APD over a wide range of more rapid pacing rates while partial I_{Kr} block markedly and significantly increased both QTc and APD.

These findings explain the failure of I_{Ks} block by chromanol 293B and L-736,821 to substantially increase QTc and APD; however, the hereditary absence of channel proteins responsible for I_{Ks} is associated with the occurrence of one form of inherited Long QT syndrome, LQT1, in man (Keating *et al*., 1991). Previously we have addressed this apparent contradiction between the failure of acute pharmacological I_{Ks} block to increase QTc and the clinical finding of long QT often associated with hereditary lack of the channels responsible for generation of I_{Ks} (Varró *et al*., 2000). We believe that the absence of I_{Ks} in these individuals with LQT1 may hamper their ability to limit excessive APD lengthening due to other causes (e.g. hypokalaemia or bradycardia). This explanation would also help account for finding that penetrance (i.e., phenotype divided by genotype) is rather low with LQT1 compared to others forms of LQT (Swan *et al*., 1998; Priori *et al*., 1998).

Another issue not addressed by the studies conducted in this study is the known differences in anatomic distribution of the channels responsible for expression of I_{Ks} and I_{Kr}. These channels are known to be differentially distributed between atria and ventricles (Wang *et al*., 1994; Li *et al*., 1996) as well as within various regions of the ventricles (Cheng *et al*., 1999; Volders *et al*., 1999). Although we acknowledge that these tissue and regional differences in I_{Ks} are essential to understand, we only examined the effects of chromanol 293B and L-735,821 in isolated rabbit papillary muscle and ventricular myocytes dispersed from the entire ventricles. As such, we did not seek to examine the effects of these drugs on atrial tissue or whether there are regional differences in their effects within the ventricles.

Effect of I_{Ks} *block in the presence of forskolin*

In the present study neither chromanol 293B nor L-735,821 legthened repolarization in the presence of forskolin (Figure 4) i.e. when intracellular adenylcyclase was stimulated. This result was similar to that found earlier by us in dog (Varró *et al*., 2000) and different by Schreieck *et al*. (1997) in guinea-pig papillary muscle who found APD lengthening by 10 μM 293B in the presence of 10 nM isoproterenol. The deviation from the findings of Schreieck *et al*. (1997) might be due to the species differences since it is known that in guinea-pig ventricle I_{Ks} amplitude is relatively large compared with other species. Because other currents are also mediated by cyclic AMP (i.e. L-type calcium current (I_{Ca}), chloride current (I_{Cl}) and I_{Kr}) that also significantly affect APD (Walsh *et al*., 1989; Harvey & Hume, 1989; Heath & Terrar, 2000) the observation in the two studies may not be directly due I_{Ks} block alone.

Conclusion

In summary, our results clearly indicate that chromanol 293B and L-735,821 do not increase QTc in Langendorff-perfused rabbit hearts, nor do these compounds lengthen rabbit papillary muscle APD, while they do block I_{Ks} in isolated rabbit ventricular myocytes. The kinetics of I_{Ks} and I_{Kr} activation and deactivation in rabbit ventricular myocytes, which are different from those reported in guinea-pig and similar to those found in dog and man, serve to explain the results. These new findings suggest that rabbit is a good species for preclinical evaluation of new drugs believed to affect cardiac action potential repolarization.

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