Restricting Excessive Cardiac Action Potential and QT Prolongation
A Vital Role for $I_{Ks}$ in Human Ventricular Muscle

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Background—Although pharmacological block of the slow, delayed rectifier potassium current ($I_{Ks}$) by chromanol 293B, L-735,821, or HMR-1556 produces little effect on action potential duration (APD) in isolated rabbit and dog ventricular myocytes, the effect of $I_{Ks}$ block on normal human ventricular muscle APD is not known. Therefore, studies were conducted to elucidate the role of $I_{Ks}$ in normal human ventricular muscle and in preparations in which both repolarization reserve was attenuated and sympathetic activation was increased by exogenous dofetilide and adrenaline.

Methods and Results—Preparations were obtained from undiseased organ donors. Action potentials were measured in ventricular trabeculae and papillary muscles using conventional microelectrode techniques; membrane currents were measured in ventricular myocytes using voltage-clamp techniques. Chromanol 293B (10 μmol/L), L-735,821 (100 nmol/L), and HMR-1556 (100 nmol/L and 1 μmol/L) produced a <12-ms change in APD while pacing at cycle lengths ranging from 300 to 5000 ms, whereas the $I_{Ks}$ blockers sotalol and E-4031 markedly lengthened APD. In voltage-clamp experiments, L-735,821 and chromanol 293B each blocked $I_{Ks}$ in the presence of E-4031 to block $I_{Kr}$. The E-4031–sensitive current ($I_{ks}$) at the end of a 150-ms-long test pulse to 30 mV was 32.9±6.7 pA (n=8); the L-735,821–sensitive current ($I_{ks}$) magnitude was 17.8±2.94 pA (n=10). During a longer 500-ms test pulse, $I_{Ks}$ was not substantially changed (33.6±6.1 pA; n=8), and $I_{Ks}$ was significantly increased (49.6±7.24 pA; n=10). On application of an “action potential–like” test pulse, $I_{Ks}$ increased as voltage became more negative, whereas $I_{Ks}$ remained small throughout all phases of the action potential–like test pulse. In experiments in which APD was first lengthened by 50 nmol/L dofetilide and sympathetic activation was increased by 1 μmol/L adrenaline, 1 μmol/L HMR-1556 significantly increased APD by 14.7±3.2% (P<0.05; n=3).

Conclusions—Pharmacological $I_{Ks}$ block in the absence of sympathetic stimulation plays little role in increasing normal human ventricular muscle APD. However, when human ventricular muscle repolarization reserve is attenuated, $I_{Ks}$ plays an increasingly important role in limiting action potential prolongation. (Circulation. 2005;112:1392-1399.)

Key Words: arrhythmia ■ electrophysiology ■ ion channels ■ long-QT syndrome ■ potassium channels

A ction potential repolarization is an important phenomenon in the heart where it controls action potential duration (APD) and thus affects refractoriness and conduction of electrical impulses throughout the heart. Several pharmacological agents intended to abate cardiac arrhythmias specifically target mechanisms that regulate APD. In the ventricles, APD, in large part, determines dynamic changes in QT duration on a beat-by-beat basis.

See p 1376

The rapid component of the delayed rectifier potassium current ($I_{Ks}$) has been identified in several mammalian species, including humans, and pharmacological agents that selectively block $I_{Ks}$ (eg, E-4031, sotalol, and dofetilide) markedly increase APD, QT duration, and ventricular refractoriness. As such, high doses of these $I_{Ks}$ blockers are associated with induction of torsade de pointes. In addition, mutations in ion channel genes (eg, HERG and KCNE2) that suppress $I_{Ks}$ are associated with the inherited long-QT syndrome LQT2, which is linked to an increased incidence of sudden cardiac death. As such, $I_{Ks}$ plays a major role in regulation of action potential repolarization and is important in the maintenance of normal heart rhythms; loss of this current is highly arrhythmogenic.
The role of the slow delayed rectifier potassium current ($I_{Ks}$) in human ventricular muscle action potential repolarization, on the other hand, has been debated. As with $I_{Ks}$, $I_{Kux}$ has been identified in several mammalian species, including humans,6,12 and mutations in KCNQ1 and KCNE1, the α- and β-subunits of the $I_{Ks}$ potassium channel, are associated with another specific form of the inherited long-QT syndrome, LQT1.10 However, we have previously demonstrated that complete pharmacological block of $I_{Ks}$ by either chromanol 293B or L-735,821 has little effect on APD in isolated dog and rabbit ventricular muscle11,13 over a wide range of physiological pacing frequencies. These findings led us to speculate that $I_{Ks}$ normally plays little role in ventricular muscle action potential repolarization, but when APD is abnormally long, $I_{Ks}$ likely provides an important safety mechanism that, when removed, increases arrhythmic risk.11,13 Other investigators have confirmed these previous findings,14 and computer simulations supported speculation that $I_{Ks}$ plays a little role in adaptations of APD to changes in heart rate.15 The role of $I_{Ks}$ in human ventricular muscle, however, remains controversial despite our preliminary characterizations of $I_{Ks}$7 and $I_{Kux}$12 in isolated human ventricular myocytes that suggest that both currents behave similarly as in isolated dog2,11 and rabbit13 ventricular myocytes. Thus, the purpose of the present study was to confirm our initial findings while elucidating the role of $I_{Ks}$ in normal human ventricular muscle action potential repolarization in the absence of tonic sympathetic stimulation.

Preliminary results of this work were presented at the 1999 Annual Scientific Session of the American Heart Association.16

Methods

Patients
Hearts obtained from organ donors were explanted to obtain pulmonary and aortic valves for transplant surgery. Before cardiac explanation, organ donor patients did not receive medication except dobutamine, furosemide, and plasma expanders. The investigations conforms to the principles outlined in the Declaration of Helsinki (Cardiovascular Research 1997, 35:2 to 35:4), and all experimental protocols were approved by the Albert Szent-Györgyi Medical University Ethical Review Board (No. 51–57/1997 OEj). Proper consent was obtained for use of each individual’s tissue for experimentation.

Action Potential Measurements in Multicellular Preparations

Tissue Preparation
Action potentials were recorded in ventricular trabeculae and papillary muscle preparations (<2 mm in diameter; n=45) obtained from the right ventricles of 26 undiseased human donor hearts (from 17 men, 9 women; age, 41.7±4.1 years) using conventional microelectrode techniques. After explanation, each heart was perfused with cardioplegic solution and kept cold (4°C to 6°C) for 2 to 4 hours before dissection. Trabeculae and papillary muscles were then excised and mounted in a tissue chamber (volume, ~50 mL) perfused with oxygenated (95% O2, 5% CO2) modified Tyrode’s solution containing (in mmol/L) NaCl 115, KCl 4, CaCl2 1.8, MgCl2 1, NaHCO3 20, and glucose 11. The pH of this solution was 7.35 to 7.45 at 37°C.

Protocol
Initially, each preparation was stimulated at a basic cycle length of 1000 ms (frequency, 1 Hz) using 2-ms-long rectangular constant-voltage pulses isolated from ground and delivered via bipolar platinum electrodes in contact with the preparation using an EMG 4767 type of stimulator (Medicor Ltd). One hour or more was allowed for each preparation to equilibrate while continuously superfused with Tyrode’s solution warmed to 37°C. Transmembrane potentials were recorded using a conventional glass microelectrode filled with 3 mol/L KCl with a tip resistance of 5 to 20 mol/LΩ connected to an high-impedance electrometer (Bio-Logic VF102, CLAIX, F-38640) referenced to ground. The first derivative of transmembrane potential (V’mem) was electronically obtained using a Bio-Logic DV-140 (Claira, F-38640) differentiator designed and calibrated to have a linear response over the range of 10 to 1000 V/s. Amplifier outputs were digitized with an ADA 3300 analog-to-digital converter (Real Time Devices Inc) with a maximum sampling frequency of 50 kHz connected to an IBM-compatible personal computer. Data were stored and analyzed on a personal computer while being monitored on a dual-beam memory oscilloscope (Tektronix 2230). Resting membrane potential, action potential amplitude, and APD, measured at 50% and 90% repolarization (APD50 and APD90), were automatically measured with software developed in our laboratory (APES, Hugo Sachs Elektronik, March-Hugstetten). Stimulation pulses were also controlled by PC software providing constant-current pulses with programmed timing and amplitudes to the preparation via a EMG 47671 type of signal isolator (Medicor Ltd, Budapest).

In each experiment, baseline action potential characteristics were first obtained during superfusion with normal 37°C Tyrode’s solution during continuous pacing at a basic cycle length of 1000 ms, followed by a run of changing pacing cycle lengths (300, 400, 500, 700, 1000, 1500, 2000, 3000, and 5000 ms) sequentially applied for 25 beats each. This procedure allowed action potential parameters to be quickly obtained at each pacing cycle length after a “quasi-steady state” was established. Recordings were continuously monitored to confirm 1-to-1 activation throughout the procedure.

After baseline measurements were obtained, each preparation was superfused with Tyrode’s solution containing a single test drug diluted to the proper concentration for 40 to 60 minutes before measurements were repeated at 3-minute intervals in the continued presence of the test drug until a 5% change occurred in action potential characteristics between subsequent samples. When microelectrode impedance was lost, reimplantation was attempted. If action potential characteristics recorded with the new impalement deviated by >5% from the preceding ones, the experiment was terminated, and results were excluded from evaluation.

Drugs Used
The $I_{Ks}$ blockers d-sotalol (Bristol-Myers Squibb Co) and E-4031 (Institute for Drug Research Ltd) were prepared daily from aqueous stock solutions (30 and 10 mmol/L, respectively). Similarly, the $I_{Kux}$ blockers chromanol 293B (Aventis Pharma) and HMR-1556 (Aventis Pharma) plus L-735,821 (Merck-Sharpé & Dohme Co) were diluted from stock solutions (10 and 1 mmol/L, respectively) containing 100% dimethyl sulfoxide. This procedure resulted in a 0.1% dimethyl sulfoxide concentration when the effects of the drugs were examined. This and the lower dimethyl sulfoxide concentrations alone did not affect action potential characteristics in separate studies.

Transmembrane Current Measurements

Cell Isolation
Ventricular myocytes were enzymatically dissociated from 7 human hearts (3 male donors, 4 female donors; age, 41.55±5.7 years). After explanation, hearts were transported to the laboratory in cold (4°C) cardioplegic solution. A portion of the left ventricular wall was excised with an attached branch of the left descending coronary artery, which was cannulated and mounted on a modified 60-cm-high Langendorff perfusion apparatus. Each preparation was perfused with each of the following perfusates for the times indicated: (1) normal Tyrode’s solutions, 10 minutes; (2) Ca2+-free Tyrode’s solution, 10 minutes, and (3) Ca2+-free Tyrode’s solution containing collagenase (type I, 0.66 mg/mL, Sigma-Aldrich Fine Chemicals), elastase (type III, 0.045 mg/mL, Sigma-Aldrich Fine Chemicals), taurine (50 mmol/L, Sigma-Aldrich Fine Chemicals), and BSA (fraction V, fatty acid free, 2 mg/mL, Sigma-Aldrich Fine Chemi-
minimize the possible influence of the Na$^+$-dependent Ca$^{2+}$ entry, BAPTA rather than EGTA was used in the pipette solution to buffer excess Ca$^{2+}$ (Figure 1). The pH of the filling solution was adjusted to 7.2 by KOH. Myocytes in KB solution were stored at 4°C, and those stored in HEPES-buffered Tyrode's solution were used for recording. Although the yield varied greatly (from 5% to 70%) between each dissociation procedure, the ease of electrode-seal formation and the quality of the measurements obtained did not correlate with yield. HEPES-buffered Tyrode's solution was used as the normal superfusate. This solution contained (in mmol/L) as follows: (1) cardioplegic solution: NaCl 110, KCl 16, MgCl$_2$ 16, CaCl$_2$ 1.2, and NaHCO$_3$ 10; (2) normal Tyrode's solution: NaCl 135, KCl 4.7, NaHCO$_3$ 10, MgSO$_4$ 1.2, CaCl$_2$ 1.0 (pH 7.2 adjusted with NaOH); (3) KB solution: NaCl 135, KCl 4.7, KH$_2$PO$_4$ 1.2, MgSO$_4$ 1.2, HEPES 10, NaHCO$_3$ 4.4, and glucose 10 (pH 7.2 adjusted with NaOH); and (4) KB solution: KOH 90, l-glutamic acid 70, taurine 15, KCl 30, KH$_2$PO$_4$ 10, MgCl$_2$ 0.5, HEPES 10, glucose 11, and EGTA 0.5 (pH 7.3 adjusted with KOH).

**Cell Isolation Solutions**

The composition of each solution used for myocyte isolation was (in mmol/L) as follows: (1) cardioplegic solution: NaCl 110, KCl 16, MgCl$_2$ 16, CaCl$_2$ 1.2, and NaHCO$_3$ 10; (2) normal Tyrode's solution: NaCl 135, KCl 4.7, NaHCO$_3$ 4.4, KH$_2$PO$_4$ 1.2, MgSO$_4$ 1.2, HEPES 10, glucose 10, and CaCl$_2$ 1.0 (pH 7.2 adjusted with NaOH); (3) KB solution: NaCl 135, KCl 4.7, KH$_2$PO$_4$ 1.2, MgSO$_4$ 1.2, HEPES 10, NaHCO$_3$ 4.4, and glucose 10 (pH 7.2 adjusted with NaOH); and (4) KB solution: KOH 90, l-glutamic acid 70, taurine 15, KCl 30, KH$_2$PO$_4$ 10, MgCl$_2$ 0.5, HEPES 10, glucose 11, and EGTA 0.5 (pH 7.3 adjusted with KOH).

**Experimental Protocol**

One drop of the myocyte suspension was placed in a 0.4-mL transparent recording chamber mounted on the stage of an inverted microscope (TMS, Nikon UK Ltd), and at least 5 minutes was allowed for individual myocytes to settle and adhere to its bottom before superfusion was initiated. Only rod-shaped, clearly striated myocytes (average length, 100 μm; diameter, 20 μm; Cm, 210 μF) were used for recording. Although the yield varied greatly (from 5% to 70%) between each dissociation procedure, the ease of electrode-seal formation and the quality of the measurements obtained did not correlate with yield. HEPES-buffered Tyrode's solution was used as the normal superfusate. This solution contained (in mmol/L) NaCl 144, NaHPO$_4$ 0.33, KCl 4.0, CaCl$_2$ 1.8, MgCl$_2$ 0.53, glucose 5.5, and HEPES 5.0 at pH 7.4. Superfusion was maintained by gravity flow at ~2 to 2.5 mL/min. Micropipettes were fabricated from borosilicate glass capillaries (Clark Electromedical Instruments) using a microprocessor-controlled horizontal puller (Sutter Instruments). These electrodes had resistances between 1.5 and 2.5 MΩ when filled with a pipette solution containing (in mmol/L) K-aspartate 100, KCl 20, MgATP 5, K$_2$BAPTA 5, HEPES 10, and glucose 5. The pH of the filling solution was adjusted to 7.2 by KOH. BAPTA rather than EGTA was used in the pipette solution to minimize the influence of the Na$^+$/Ca$^{2+}$ exchanger current. The external solution contained 1 μmol/L nisoldipine to completely block the inward Ca$^{2+}$ current (I$_{Ca}$). The inward sodium current (I$_{Na}$) was inactivated using a ~40-mV holding potential, which also largely inactivated the transient outward current (I$_{to}$). Membrane currents were recorded with either an Axopatch-1D or an Axopatch 200B patch-clamp amplifier (Axon Instruments) in the whole-cell patch-clamp configuration. After a high- (1 to 10 GΩ) resistance seal was established by gentle suction, the cell membrane beneath the electrode tip was disrupted by additional suction or application of 1.5-V electrical pulses for 1 to 5 ms. Series resistance was typically 4 to 8 MΩ before compensation (usually 50% to 80%). When the initial series resistance was >10 MΩ or when series resistance increased substantially during measurement, the experiment was terminated, and the results were excluded from analyses. Membrane currents were digitized with a 333-kHz analog-to-digital converter and HEPES (10 mmol/L) and E-4031 (30 μmol/L) and d-sotalol (bottom right). Stimulation frequency was 1 Hz. (Digidata 1200, Axon Instruments) under software control (pClamp 6.0 and pClamp7, Axon Instruments), and results were analyzed with Axon software programs (pClamp 6.0 and plamp7.0, Axon Instruments) after low-pass (1-kHz) filtration.

**Statistical Analysis**

Student t tests for paired data were used to compare results. Results were considered significant at P<0.05.

**Results**

**Effects of I$_{Ks}$ and I$_{Kr}$ Blockade on Human Ventricular Muscle APD**

Concentrations of chromanol 293B (10 μmol/L), L-735,821 (100 nmol/L), and HMR-1556 (100 nmol/L and 1 μmol/L) reported to selectively block I$_{Ks}$ in cardiac ventricular muscle preparations$^{11-14,18,19}$ in other species produced a <9-ms (2.8%) change in human ventricular papillary muscle APD after 40 minutes of exposure during continuous pacing at a cycle length of 1000 ms (Figure 1, top and middle). In contrast, concentrations of d-sotalol (30 μmol/L) and E-4031 (1 μmol/L), expected to selectively block I$_{Kr}$$^{11}$, markedly and
significantly increased human ventricular muscle APD under identical conditions (Figure 1, bottom). This difference in the effects of chromanol 293 B (10 μmol/L), L-735,821 (100 nmol/L), and HMR 1556 (100 nmol/L, and 1 μmol/L) compared with d-sotalol (30 μmol/L) and E-4031 (1 μmol/L) on APD was observed in human ventricular muscle over a wide range of pacing cycle lengths (300 to 5000 ms) (Figure 2). Over this range of pacing cycle lengths, chromanol 293B (Figure 2), L-735,821, or HMR (100 nmol/L, and 1 μmol/L) produced a change of ±12 ms (3.2%) in APD, whereas d-sotalol and E-4031 each markedly lengthened human ventricular APD in a reverse frequency-dependent manner.

**I_{Kr} and I_{Ks} Characterization in Undiseased Human Ventricular Myocytes**

Figure 3A illustrates the effects of 100 nmol/L L-735,821 and 30 μmol/L chromanol 293B on I_{Ks} tail currents in isolated human ventricular myocytes after 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 μmol/L E-4031. E-4031 was added to completely inhibit I_{Ks}. L-735,821 (100 nmol/L) completely abolished I_{Ks}, whereas chromanol 293B (30 μmol/L) nearly did so. In other experiments, the selective I_{Ks} blocker E-4031 (1 μmol/L) alone completely blocked I_{Ks} tail currents (Figure 3B) elicited by 1000-ms-long test pulses to between −20 and 50 mV from and to return to a holding potential of −40 mV.

The relative magnitude of I_{Ks} and I_{Kt} activated during a standardized human ventricular action potential depolarization was estimated by comparing the amplitudes of the L-735,821–sensitive (I_{Kt}) and E-4031–sensitive (I_{Ks}) currents at the end of a 150-ms-long test pulse to 30 mV and their tail currents on return to a −40 mV holding potential. This test potential amplitude is slightly positive to the normal human ventricular muscle action potential plateau, whereas the holding potential used is representative of a voltage encountered during final action potential repolarization. Under these conditions, I_{Ks} and I_{Kt} were measured by subtracting membrane currents before and after 4 to 5 minutes of exposure to L-735,821 (100 nmol/L) or E-4031 (1 μmol/L), respectively. The E-4031–sensitive current (I_{Ks}) amplitude at the end of the 150-ms-long test pulse was 32.9 ± 6.7 pA (n = 8) or ∼27% of the tail current amplitude measured after the voltage test pulse returned to −40 mV (119.9 ± 16.6 pA; n = 8) (Figure 4A and 4C). Under identical voltage-clamp conditions, the L-735,821–sensitive current (I_{Kt}) during the test pulse to 30 mV was larger than its tail current on return to −40 mV (Figure 4B and 4C). The magnitude of I_{Kt} during the test pulse was 17.8 ± 2.94 pA at 30 mV compared with 6.7 ± 1.93 pA at −40 mV (n = 10) and approximately an order of magnitude less than the I_{Ks} tail current.

When I_{Ks} and I_{Kt} were individually measured after 500-ms-long test pulses to 30 mV and their values were compared with those obtained above after a 150-ms test pulse to the
same potential at the same frequency, $I_{K_r}$ was not substantially changed (33.6 ± 6.1 pA at 30 mV and 128.1 ± 17.4 pA at −40 mV; n = 8) (Figure 4D and 4F). This lack of effect of increasing test pulse duration on $I_{K_r}$ in human ventricular myocytes was similar to that which we previously reported in rabbit and dog. This failure to influence $I_{K_r}$ occurred because $I_{K_r}$ activated completely and quickly before the end of either the 150- or 500-ms test pulse. The magnitude of $I_{K_s}$, however, was significantly increased when the test pulse duration was increased from 150 to 500 ms (49.6 ± 7.24 pA at 30 mV and 16.4 ± 3.0 pA at −40 mV; n = 10). This increase in $I_{K_s}$ occurred because $I_{K_s}$ activated slowly so that it continued to activate beyond the shorter 150-ms test pulse duration (Figure 4E and 4F).

To determine the relative roles of $I_{K_s}$ and of $I_{K_r}$ under more physiological conditions, we also compared their magnitudes while applying an idealized ventricular muscle “action potential–like” test pulse. The test pulse used for these experiments was obtained by digitizing a representative human right ventricular action potential recorded using the conventional microelectrode technique during continuous pacing at a cycle length of 1000 ms. The waveform thus obtained arose from a diastolic potential of −85 mV and was 115 mV in amplitude with an APD$_{90}$ of 300 ms, which roughly corresponds to a normal human QT duration of 0.40 seconds at a heart rate of 70 bpm. A 40-ms-long prepulse to −40 mV was added (Figure 5, inset) to the beginning of this idealized action potential. When the $I_{K_s}$ difference current (ie, the E-4031–sensitive current) was measured during the idealized action potential plateau, applied as a voltage-clamp test pulse, it was small and increased in magnitude as the test voltage became more negative (Figure 5). In contrast, $I_{K_s}$, measured as the L-735,821–sensitive current, remained small throughout all phases of the action potential–like test pulse (Figure 5). Similar results were obtained in 4 additional human right ventricular myocytes prepared from 3 additional normal donor hearts. These results again indicate that the outward current normally carried by $I_{K_s}$ throughout the ventricular action potential is considerably larger than that carried by $I_{K_r}$ in the absence of sympathetic agonists. These results are consistent with our finding that $I_{K_s}$ block by chromanol 293B (10 μmol/L), L-735,821 (100 nmol/L), or HMR-1556 (100 nmol/L and 1 μmol/L) produces little effect on APD, whereas $I_{K_r}$ block by d-sotalol (30 μmol/L) or E-4031 (1 μmol/L) greatly increased APD (Figure 1).

**Effect of $I_{K_s}$ Block During Increased Sympathetic Activation After Attenuation of Repolarization Reserve**

The effect of 1 μmol/L HMR-1556 was also tested in preparations in the presence of 1 μmol/L adrenaline and 50 nmol/L dofetilide. In these experiments, HMR-1556–induced $I_{K_s}$ block significantly lengthened APD (14.7 ± 3.2%; Figure 4.

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**Figure 4.** E-4031– ($I_{K_r}$; A) and L-735,821– ($I_{K_s}$; B) sensitive currents in human ventricular myocytes after application of a short (150 ms) depolarizing test pulse to 30 mV from a holding potential of −40 mV in the absence of any sympathetic agonist. Recordings of E-4031– ($I_{K_r}$; D) and L-735,821– ($I_{K_s}$; E) sensitive currents after a long (500 ms) depolarizing test pulse to 30 mV from a holding potential of −40 mV. Average $I_{K_r}$ and $I_{K_s}$ currents at the end of short (150 ms; C) and long (500 ms; F) depolarizing test pulses to 30 mV, and peak tail current at −40 mV. Bars represent mean ± SEM.

**Figure 5.** E-4031– (1 μmol/L) sensitive ($I_{K_r}$) and L-735,821– (100 nmol/L) sensitive ($I_{K_s}$) difference currents recorded during an action potential–like test pulse in human ventricular myocytes in the absence of any sympathetic agonist. The action potential–like test pulse was obtained by recording a normal human ventricular action potential with a conventional microelectrode in a multicellular human papillary muscle preparation and adding a 50-ms prepulse from −80 to −40 mV. Similar results were obtained in 4 additional myocytes.
repolarization reserve was reduced by 50 nmol/L dofetilide. Columns and error bars indicate mean±SEM. *Significant changes (P<0.05, n=3) between the conditions represented by the bars.

Discussion

Our results indicate that in isolated, multicellular ventricular muscle obtained from normal, undiseased human hearts, chromanol 293B, L-735,821, and HMR-1556 did not markedly increase action potential over a range of pacing cycle lengths corresponding to heart rates of 12 to 200 bpm in the absence of a sympathetic agonist. In addition, our studies indicate that the concentrations of chromanol 293B and L-735,821 (10 µmol/L and 100 nmol/L, respectively) that failed to increase APD significantly blocked I_{Kr} in ventricular myocytes isolated from the same normal, undiseased human hearts. In contrast to these findings, we demonstrated that E-4031 (1 µmol/L) blocked I_{Kr} and dramatically increased normal human ventricular muscle APD, as did sotalol (30 µmol/L), another recognized I_{Kr} blocker that also dramatically increased human ventricular muscle APD under the same conditions in which chromanol 293B and L-735,821 failed.

The only study before this one to describe the effect of chromanol on human ventricular action potential characteristic was performed in right ventricular myocytes isolated from hearts explanted from patients with end-stage heart failure. In that study, 1 to 10 µmol/L chromanol 293B was reported to significantly increased APD. However, when Schreiek et al examined the effects of 10 µmol/L chromanol in guinea pig ventricular muscle preparations, they found no effect on APD in the absence of β-adrenocceptor stimulation. 19 When we previously examined the effects of chromanol 293B and L-735,821 ventricular papillary muscle preparations, 10 µmol/L chromanol 293B did not significantly increase APD. Our previous studies also demonstrated that the other I_{Kr} blocker, L-735,821 (100 nmol/L), did not significantly affect APD in the absence of a sympathetic agonist.

The explanation for these differences in results is unclear, although some investigators have attributed them to differences between single-cell and multicellular preparations. Sun et al, for instance, reported that higher chromanol concentrations (30 to 100 µmol/L) lengthened APD in perfused, multicellular dog ventricular muscle "wedge" preparations. From this finding, they speculated that chromanol is less able to diffuse into multicellular preparations than into single cells and that its effects are therefore less pronounced in multicellular preparations. 22 Thus, this group argues that higher concentrations of chromanol are necessary in multicellular ventricular preparations to achieve I_{Kr} block and to increase APD than are necessary to completely block I_{Kr} in isolated myocytes. Other investigators also suggested that, because of its physical and/or chemical properties, L-735,821 poorly penetrates multicellular preparations but easily enters single myocytes (Dr J.J. Salata, unpublished personal communication, 1998).

These explanations appear unlikely. In the present study in human and in our previous studies in rabbit and dog ventricular muscle, a concentration of L-735,821 and HMR-1556 that fully blocked I_{Kr} in single myocytes also failed to increase APD in multicellular preparations, although L-735,821 and HMR-1556 are reportedly more specific and potent than chromanol 293B. 23,24 In addition, the L-735,821 and HMR-1556 concentrations used in our experiments, 100 nmol/L and 1 µmol/L, respectively, are reported to be >10 times their EC_{50} for I_{Kr} block. 23,24 Furthermore, concentrations of E-4031 and d-sotalol that block I_{Kr} in isolated myocytes markedly increased APD in multicellular preparations; it appears unlikely that either E-4031 or sotalol has distinctly different abilities to penetrate multicellular preparations and single myocytes than L-735,821.

On the other hand, Stengl et al and Volders et al recently obtained results similar to our finding that I_{Kr} block
does not affect normal ventricular muscle APD in species other than guinea pig in the absence of sympathetic stimulation. They reported that, in both dog ventricular myocytes and papillary muscle preparations, HMR-1556 (a highly selective \(I_{Ks}\) blocker) failed to lengthen APD without prior sympathetic stimulation,\(^{14,25}\) even at high concentrations. These authors concluded that \(I_{Ks}\) block—induced repolarization lengthening requires an elevated degree of sympathetic tone as may occur in the setting of heart failure. Others\(^{26}\) have suggested that the sensitivity of APD shortening by \(I_{Ks}\) block is enhanced when phosphorylation is increased as expected during increased sympathetic nerve activity. Clearly, cAMP increases \(I_{Ks}\) and may alter activation and deactivation kinetics for \(I_{Ks}\). Thus, \(I_{Ks}\) is expected to have different effects on APD when sympathetic tone is increased. This relation between phosphorylation and the effects of \(I_{Ks}\) block on ventricular muscle APD needs further investigation to better elucidate the importance of sympathetic neural influences on electrogensis in normal and diseased human myocardium.

Nonetheless, our present findings clearly indicate that neither chromanol 293B nor L-735,821 and HMR-1556 markedly affect normal human ventricular muscle APD over a normal range of heart rates in the absence of sympathetic stimulation. Thus, \(I_{Ks}\) in the absence of sympathetic neural agonists plays little role in the repolarization of normal ventricular muscle action potentials. Rather, in normal human ventricular myocardium, \(I_{Ks}\) is the outward current most responsible for termination of the action potential plateau and initiation of final action potential repolarization. However, as we have previously speculated, \(I_{Ks}\) may play a vital role in normal myocardium when APD is prolonged as after a pause in rhythm, decreased levels of thyroid hormone, or hypothermia. Some investigators suggest that \(I_{Ks}\) thus provides a “repolarization reserve” when other outward repolarizing currents are reduced, eg, by remodeling of ion currents during heart failure progression.\(^{27,28}\) This role for \(I_{Ks}\) is supported by the finding that specific \(I_{Ks}\) block markedly lengthens APD after “repolarization reserve” is attenuated by \(I_{Ks}\) block with 50 nmol/L dofetilide. This observation is similar to that previously reported in dog ventricular muscle.\(^{11,29}\)

The lack of effect of \(I_{Ks}\) on normal APD now found in human ventricular tissue in the absence of sympathetic stimulation and previously reported in dog\(^{11,29}\) and rabbit\(^{13}\) occurs because \(I_{Ks}\) is relatively small compared with \(I_{Ks}\) over the range of voltages encountered during the time course of normal ventricular action potentials, and its activation is slow compared with that of \(I_{Ks}\) in these species. Indeed we have shown that, in normal human ventricular myocytes, \(I_{Ks}\) in the absence of a sympathetic agonist activates rapidly (\(\tau=31.0\pm7.4\) ms; \(n=6\)) during depolarizations to positive potentials (30 mV) but deactivates slowly at \(-40\) mV (\(\tau=599.9\pm53.9\) ms; \(n=8\)), whereas \(I_{Ks}\) activates slowly at positive potentials (\(\tau=1005\pm202.9\) ms at 30 mV; \(n=7\)) and deactivates comparatively rapidly (\(\tau=132.4\pm29.8\) ms at \(-40\) mV; \(n=7\)) with respect to diastolic intervals (300 to 700 ms) associated with physiological heart rates.\(^{7,12}\)

These kinetics for \(I_{Ks}\) and \(I_{Ks}\) activation and deactivation indicate that, when APD is abnormally long or repolarization reserves are attenuated and sympathetic activation is increased, \(I_{Ks}\) block should be expected to substantially lengthen APD while having little effect of normal APDs in the absence of sympathetic stimulation. Expectations were born out when 10 \(\mu\)mol/L chromanol 293B, 500 \(\mu\)mol/L HMR-1556, or 100 \(\mu\)mol/L L-735,821 was applied to normal dog ventricular muscle after APD was initially increased by exposure to E-4031 and veratrine\(^{11}\) or by application of 100 \(\mu\)mol/L isoproterenol, which enhanced \(\beta\)-receptor activation and levels of intracellular cAMP.\(^{25}\)

**Clinical Implications**

Inherited mutations in \(I_{Ks}\) channel protein encoding are associated with the occurrence of LQT1,\(^{11,30}\) yet our findings in normal human ventricular tissue in the absence of sympathetic stimulation indicate that pharmacological \(I_{Ks}\) block is not associated with an increase in APD, which is expected to correlate with an increase in long-QT duration. These 2 facts appear difficult to reconcile with one another, although it is known that penetration (ie, the number of individuals having a particular genotype relative to those displaying the associated clinical phenotype) is rather low\(^{31,32}\) in families with documented LQT1 mutations, histories of seizures, and sudden cardiac death. A far too simplistic explanation would be that individuals with mutated \(I_{Ks}\) channel proteins and elevated sympathetic tone lack the ability to compensate and limit excessive APD lengthening as a result of other causes such as extreme bradycardia, hypothyroidism, hypokalemia, changes in autonomic neural influences, or exposure to drugs affecting other repolarizing currents (eg, \(I_{Ks}\) blockers, \(I_{Kr}\), \(I_{K1}\)). Although this question is not directly addressed by the study conducted, the results obtained suggest that if an increase in QT duration is the basis for an increased risk of arrhythmia and sudden cardiac death in people suffering from long-QT syndrome, then therapeutic interventions that increase “repolarization reserve” by any means should be equally effective in reducing QT duration in all forms of the long-QT syndrome.\(^{11,33,34}\) An adequately powered, prospective clinical trial is required to address such speculation.

The findings reported in this study suggest that antiarrhythmic drugs that selectively block \(I_{Ks}\) are unlikely to affect ventricular arrhythmias in the absence of sympathetic neural stimulation. However, it must be recognized that sympathetic tone is forever fluctuating in the in situ human heart, and many believe that the selective \(I_{Ks}\) block in combination with \(\beta\)-adrenoceptor blockade should have antiarrhythmic benefit, citing the clinical antiarrhythmic effectiveness of amiodarone, which has both \(I_{Ks}\) and \(\beta\)-adrenoceptor blocking properties during chronic administration.\(^{35}\) Nonetheless, another little explored antiarrhythmic strategy might be to increase, rather than to block, \(I_{Ks}\). If \(I_{Ks}\) were increased (either pharmacologically or genetically), arrhythmia risk might be expected to be lowered; certainly, such an antiarrhythmic intervention would benefit patients with inherited or acquired long-QT syndrome. Therapeutic increases in \(I_{Ks}\) would increase repolarization reserve and possibly reduce the risk of sudden cardiac death during progression of heart failure when \(I_{Ks}\) and \(I_{Kr}\) expression are downregulated.\(^{27,28,33,34}\)
Conclusions
We earlier reported that in dog and rabbit ventricular myocytes, $I_{Ks}$ plays no obvious role in altering action potential repolarization and QT duration at normal heart rates. In the present study, in the absence of sympathetic stimulation, this finding is confirmed in isolated human ventricular preparations obtained from the hearts of individuals without heart disease. These findings should not be misconstrued as meaning that $I_{Ks}$ does not play an important role in the normal heart where sympathetic stimulation is ever present and fluctuating continuously. We also believe that $I_{Ks}$ is vitally important in the normal heart where it prevents excessive action potential prolongation in the setting of an elevated sympathetic tone after a single long diastolic interval after the compensatory pause that follows a premature ventricular depolarization or during bradyarrhythmia or when APD is prolonged by other means (eg, by unintentional $I_{Ks}$ block, hypothyroidism, or serum hypokalemia).

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References
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