Differential expression of KvLQT1 isoforms across the human ventricular wall

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The duration of repolarization is known to be heterogeneous across the depth of the ventricular wall, with a longer action potential duration in the midmyocardium (M region) compared with that in either the endocardium or epicardium (3, 9). Final repolarization of cardiac tissues strongly depends on the magnitude and kinetics of the delayed rectifier K+ current, which has been separated into rapidly and slowly activating components called \( I_{Kr} \) and \( I_{Ks} \), respectively (19, 20). Both components have been demonstrated in human ventricle (13). Furthermore, it has been shown that \( I_{Ks} \) contributes to human ventricular repolarization even at slow heart rates (5).

The heterogeneity of late repolarization across the ventricular wall has been explained in dog and guinea pig on the basis of a weaker \( I_{Ks} \) but a similar \( I_{Kr} \) amplitude in the midmyocardium compared with other layers (7, 11, 14).

The molecular basis of \( I_{Kr} \) and \( I_{Ks} \) channels has recently been elucidated: human ether-à-go-go (HERG) subunits coassemble to form \( I_{Kr} \) channels, whereas long Q-T mutant (KvLQT1) and its regulatory subunit \( I_{Ks} \) coassemble to produce \( I_{Ks} \) channels (4, 18). However, we and others have recently shown that the human ventricular \( I_{Ks} \) cardiac K+ channel is actually a complex protein assembly composed of KvLQT1 isoform 1 (the channel pore), KvLQT1 isoform 2 (an endogenous NH2-terminal truncated KvLQT1 splice variant), and \( I_{Ks} \) (the channel regulator) (8, 12). KvLQT1 isoform 2 exerts a strong dominant negative effect on the current generated by KvLQT1 isoform 1 expression (8, 12). Thus, in the heart, the amplitude of \( I_{Ks} \) depends on the expression level of KvLQT1 isoform 2. The present study was designed to characterize the relative expression of the genes encoding KvLQT1 isoforms 1 and 2 as well as \( I_{Ks} \) in ventricular epicardial, midmyocardial, and endocardial tissues from explanted human hearts. Our data show a comparable expression level of total KvLQT1 across the ventricular wall but a differential expression of KvLQT1 isoform 2 relative to isoform 1. Expression experiments demonstrate that differential expression of isoform 2 between the midmyocardium and other layers can account for the difference in \( I_{Ks} \) amplitude previously reported by others in the dog (11, 14). This study provides a possible molecular explanation for transmural repolarization heterogeneity in the human heart. This is also the first report of a physiological role assigned to an endogenously expressed dominant negative ionic channel isoform.

MATERIALS AND METHODS

Material. Human left and right ventricular tissues from 10 explanted human hearts were used. The patients were 10 males, ages 38–66 yr (mean 52.7), undergoing heart transplant for cardiomyopathy (ischemic: 4 patients; dilated: 3 patients; all patients were in New York Heart Association...
class III or IV) or cystic fibrosis (3 patients). Right auricular appendage biopsies were also obtained from four additional patients with coronary heart disease undergoing open-heart surgery. Slices of tissue were immediately dissected from endocardium (<2 mm from endocardial surface), midmyocardium, and epicardium (<2 mm from epicardial surface) of the lateral walls of the left and right ventricles in both apical and basal regions at a distance from the main coronary vessels.

Electrophysiological studies. After excision, the hearts were immediately immersed in oxygenated cold Tyrode solution containing (in mmol/l) 131 NaCl, 4 KCl, 0.5 MgCl2, 2.7 CaCl2, 5.5 d-glucose, 1.1 mannitol, 1.8 NaH2PO4, and 5 HEPES, with pH adjusted to 7.35 with Tris. The hearts were transported to the laboratory within <5 min. The experimental preparations consisted of transmural slices of the left ventricular wall [for detail, see Drouin et al. (9)]. They were placed in a tissue bath and superfused with oxygenated (95% O2–5% CO2) Tyrode solution (37 ± 0.5°C, pH 7.35) containing (in mmol/l) 131 NaCl, 4 KCl, 2.7 CaCl2, 0.5 MgCl2, 5.5 d-glucose, 1.1 mannitol, 1.8 NaH2PO4, and 18 NaHCO3. The flow rate in the tissue chamber was 12 ml/min, resulting in three changes of chamber volume per minute.

Tissues were impaled with 3 mol/l KCl-filled glass capillary microelectrodes. The electrodes were connected via an Ag/AgCl interface to an amplifier with high input impedance and input capacity neutralization (Biologic VF-102). Output was displayed on a digital storage oscilloscope (Gould 1604) coupled by a 488 IEEE-interface to a plotter (Gould Colorwriter 6300), a chart recorder (Gould 8188), and a modified digital audio processor (Sony PCM-501ES) coupled to a videotape recorder (JVC HR-D755S). The tissue chamber was connected to ground through a salt bridge (3 mol/l KCl) and an Ag/AgCl junction.

The preparations were allowed to equilibrate for 2 h while being paced at a cycle length of 1 s through Teflon-coated bipolar wire electrodes. Stimulus pulse duration was 1.5 ms, and amplitude was twice diastolic threshold. During the experiments, the action potentials were recorded at pacing cycle lengths (PCL) of 1, 2, 3, 4, 5, 6, and 10 s (always in this sequence). Action potential characteristics were measured at steady state for each PCL. We measured the resting potential, the amplitude of phase 0, the maximal rate of rise of phase 0 (Vmax), and the action potential duration at 90% of full repolarization (APD90). Only data from impalements that were maintained throughout the course of the experiment were analyzed. Data are expressed as means ± SE. Statistical analysis was performed using the Wilcoxon test for paired data and the nonparametric Mann-Whitney U test for unpaired data. A value of P < 0.05 was considered significant.

Molecular expression of KvLQT1 and Isk genes. Heart tissue samples were dissected out and quick-frozen in liquid nitrogen, and total RNA was then isolated separately from each sample using the classic guanidinium isothiocyanate method. RNA samples were quantified by spectrophotometric analysis. Lack of degradation of RNA samples was monitored by the observation of appropriate 28S-to-18S ribosomal RNA ratios as determined by ethidium bromide staining of the agarose gel.

For RNase protection assays, four different cDNAs were used as hybridization probes: 1) a human 274-bp KvLQT1 isoform 1 cDNA corresponding to the 5’–62 nucleotides upstream from the common part and 212 nucleotides common to both isoforms (Fig. 1); this cDNA contains 62 nucleotides specific for isoform 1 [for details, see Demolombe et al. (8)]; 2) a human 347-bp KvLQT1 isoform 2 cDNA corresponding to the 5’–27 nucleotides upstream and 320 nucleotides downstream from the start codon; this cDNA contains 33 nucleotides specific for isoform 2 [for details, see Demolombe et al. (8) and Fig. 1]; 3) a human 393-bp cDNA corresponding to the full-length Isk sequence (clone pRC-Isk); and 4) a human 103-bp cDNA encoding the cyclophilin gene, which was used...
PCL = 1 s

- - -

PCL = 10 s

Endo Mid Epi

Fig. 2. Action potentials recorded under steady-state conditions at pacing cycle lengths (PCL) of 1 and 10 s from endocardial (Endo), midmyocardial (Mid), and epicardial (Epi) cells from transmural preparations of left human ventricle. Voltage scale, 50 mV; timescale, 200 ms.

as an internal marker (Ambion, Austin, TX). All probes were sequenced to confirm their identity.

Antisense RNA probes were produced using appropriate polymerase (T3, T7, or SP6, Ambion) on linearized KvLQT1 isoform 1, KvLQT1 isoform 2, IsK, and cyclophilin templates in the presence of [α-32P]UTP (DuPont NEN). RNase protection assays (RPAs) were carried out as previously described (17) using the RPA II Kit (Ambion). For each tissue source, 15 µg of total RNA were hybridized to 0.5–1 x 10^6 counts/min of the RNA probe. The RNA was run after RNase digestion on a 5% polyacrylamide, 8 M urea gel. Sense KvLQT1 isoform 1 RNA was also hybridized and was run on the same gel to be used as a size marker (not illustrated) in addition to undigested RNA probes. Negative controls were run using yeast tRNA hybridized with the isoform 1 and isoform 2 probes.

Gels were exposed at −80° on X-ray films (Biomax-MS, Eastman Kodak) with an intensifying screen for 48 h. Former control experiments had shown that images obtained with such a delay with KvLQT1 probes were still in the linear range of signal intensity. Autoradiographs of RPAs were subjected to densitometric analysis using the ImageQuant program (Molecular Dynamics). Ratios of KvLQT1 isoform 1 and KvLQT1 isoform 2 mRNA levels were determined directly from specific signals as respective protected bands were hybridized with the same probe. The intensity of hybridization of RNAs to the cRNA probe for cyclophilin was used for quantification of overall KvLQT1 expression in the different tissues and for comparison with IsK gene expression. Data are expressed as means ± SE. Statistical analysis was performed using the nonparametric Mann-Whitney U test. A value of P < 0.05 was considered significant.

Heterologous expression in mammalian cells. The kidney-derived COS-7 cell line was obtained from the American Type Culture Collection. Cells grown on glass coverslips in petri dishes were microinjected into the nucleus with plasmids at day 1 after plating. The protocol to microinject cultured cells using the Eppendorf ECET microinjector 5246 system has been reported in detail elsewhere (16). Plasmids were diluted in a buffer made of (in mmol/l) 50 HEPES, 50 NaOH, and 40 NaCl, pH 7.4, supplemented with FITC-dextran 0.5%. Human cardiac KvLQT1 isoforms 1 and 2 as well as IsK cDNAs were similar to those used in previous experiments (8). A fourth plasmid, the green fluorescence protein plasmid (pGFP), was used as an inert plasmid to ensure that the total plasmid concentration injected was always 15 µg/ml.

Whole cell currents were recorded as previously described (16). The petri dish containing the cells was placed on the stage of an inverted microscope and continuously superfused with the standard extracellular solution containing (in mmol/l) 145 NaCl, 4 KCl, 1 MgCl2, 1 CaCl2, 5 HEPES, and 5 glucose, with pH adjusted to 7.4 with NaOH. Patch pipettes with a tip resistance of 2.5–5 MΩ were electrically connected to a patch-clamp amplifier (Axon Instruments). The intracellular medium contained (in mmol/l) 145 K-gluconate, 5 HEPES, 2 EGTA, 2 hemiMg-gluconate (0.1 free Mg2+), and 2 K-ATP, with pH adjusted to 7.2 with KOH, whereas the extracellular medium used to record pure K+ currents contained (in mmol/l) 145 Na-gluconate, 4 K-gluconate, 7 hemiCa-gluconate (1 free Ca2+), 4 hemiMg-gluconate (1 free Mg2+), 5 HEPES, and 5 glucose, with pH adjusted to 7.2 with NaOH. Free activities were calculated using software designed by G. L. Smith (University of Glasgow, Glasgow, UK). Stimulation, data recording, and analysis were performed through an analog-to-digital converter (Labmaster). A microperfusion system allowed local application and rapid change of the different experimental solutions warmed at 37°C. Patch-clamp measurements are presented as means ± SE. Statistical significance of the observed effects was assessed by means of the standard t-test. A value of P < 0.05 was considered significant.

RESULTS

Action potential recordings in human heart. Figure 2 shows typical action potentials recorded from endocardial, midmyocardial, and epicardial myocytes in a transmural slice of human ventricular free wall at two different cycle lengths (1 and 10 s). Table 1 summarizes the action potential characteristics from the three cell layers in human left ventricular preparations paced at a cycle length of 1 s. There was no significant difference in the resting potential between the cellular subtypes. The amplitude of phase 0 was greater in midmyocardial than in epicardial tissue. Vmax was significantly higher in midmyocardial cells than in epicardial and endocardial cells. Most importantly, APD90 was much longer in midmyocardial cells compared with other cell types. This pattern is comparable to that reported in a previous work from this laboratory [Drouin et al. (9)].
Table 1. Action potential characteristics from endocardial, midmyocardial, and epicardial left ventricular tissue layers

<table>
<thead>
<tr>
<th></th>
<th>Endocardium</th>
<th>Midmyocardium</th>
<th>Epicardium</th>
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<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>RP, mV</td>
<td>–87 ± 2</td>
<td>–86 ± 1</td>
<td>–86 ± 1</td>
</tr>
<tr>
<td>APA, mV</td>
<td>101 ± 2</td>
<td>106 ± 3†</td>
<td>95 ± 2</td>
</tr>
<tr>
<td>Vmax, V/s</td>
<td>231 ± 30</td>
<td>446 ± 46†</td>
<td>196 ± 20</td>
</tr>
<tr>
<td>APD90, ms</td>
<td>316 ± 31</td>
<td>432 ± 19*</td>
<td>324 ± 19</td>
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</table>

Values are means ± SE and were recorded at a pacing cycle length of 1 s. n, No. of experiments; RP, resting potential; APA, action potential amplitude; Vmax, maximal upstroke velocity of phase 0 of action potential; APD90, action potential duration at 90% of repolarization. *P < 0.005 vs. epicardium and endocardium; †P < 0.005 vs. epicardium.

RNA expression. KvLQT1 isoform expression was quantified in adult human cardiac muscle using an RPA. For isoform 1, the Rnase protection hybridization probe covered a 274-bp segment that comprised a 62-bp fragment of isoform 1-specific 5′ sequence and a 212-bp segment common to isoform 1 and 2 sequences [see Demolombe et al. (8)]. With this probe, two RNA fragments were Rnase protected: a 274-bp sequence that corresponded to isoform 1, and a 212-bp sequence that corresponded to non-isoform 1 transcripts (Fig. 3). The probe used for isoform 2 mRNA detection extended 347 bp and covered a portion of isoform 2-specific 5′ coding and noncoding sequence and also a 314-bp sequence common to isoform 1 and 2. Thus, with our isoform 2 probe, two RNA fragments were Rnase protected: a 347-bp sequence that specifically corresponded to isoform 2, and a 314-bp sequence that corresponded to non-isoform 2 transcripts. This approach allowed us to directly compare the expression level of the different isoforms in one tissue sample without the need for an internal marker. We also used cyclophilin gene expression to compare overall KvLQT1 gene expression. Iks gene expression was also determined in endocardial, midmyocardial, and epicardial tissues. Densitometric analysis revealed that overall expression of KvLQT1 gene was similar in the three tissue layers and not different between the right and left ventricles. Total expression of KvLQT1 gene represented, in left endocardial and epicardial tissues, 93.7 ± 7.2% and 91.9 ± 3.6% of left midmyocardial tissue expression, and in right endocardial and epicardial tissues, 91.3 ± 4.0% and 92.1 ± 10.5% of right midmyocardial tissue expression, respectively. None of these differences reached statistical significance. However, there was a significant difference in the ratio between KvLQT1 isoforms 1 and 2. Isoform 2 expression was higher in midmyocardial tissue compared with epicardial or endocardial tissue in the left and right ventricles. Isoform 2 represented 25.2 ± 2.3%, 31.7 ± 1.2%, and 24.9 ± 1.7% of total KvLQT1 expression in left ventricular endocardial, midmyocardial, and epicardial tissues, respectively (n = 10, P < 0.05, midmyocardium vs. endocardium and epicardium). Similar data were obtained from right ventricular samples, where isoform 2 represented 27.3 ± 1.9%, 33.9 ± 1.6%, and 27.7 ± 2.0% of total KvLQT1 expression in endocardial, midmyocardial, and epicardial tissues, respectively (n = 10, P < 0.05) (Figs. 3 and 4). Relative expression of isoform 2 in the auricle was 23.4 ± 3.4% (P < 0.05 vs. midmyocardial tissue); i.e., close to that measured in both the left ventricular endocardium and epicardium. Iks mRNA expression was not significantly different between epicardial, midmyocardial, and endocardial tissues (89.0 ± 16.6% and 94.2 ± 16.4% in endocardial and epicardial tissues, respectively, relative to the left midmyocardial tissue expression; not significant). From this set of experiments, we conclude that a modest albeit significant difference existed in the isoform 2-to-isoform 1 expression ratio between the midmyocardial layer and the endocardial and epicardial layers. The functional consequence of this difference on the amplitude of Iks was further assessed in expression experiments.

Functional expression. To determine the consequences of differential isoform 2 expression on Iks amplitude, patch-clamp experiments (Fig. 5) were conducted using the intranuclear plasmid injection tech-
nique. This technique allows accurate control of the relative expression of the different transgenes in mammalian cells. With the aim of investigating the effects related to different isoform 2-to-isoform 1 expression ratios, COS-7 cells were coinjected with KvLQT1 isoform 1 cDNA (5 µg/ml), IsK cDNA (5 µg/ml), and various KvLQT1 isoform 2 cDNA concentrations. Among these, we used a 2.7 µg/ml isoform 2 cDNA concentration leading to an isoform 2-to-isoform 1 ratio mimicking the mRNA expression ratio in the M region and a 1.25 µg/ml isoform 2 cDNA concentration leading to an isoform 2-to-isoform 1 ratio mimicking the mRNA expression ratio in the epicardium in RPA experiments. As shown in Fig. 5A and Table 2, cells injected with the “epicardium” isoform 2-to-isoform 1 ratio exhibited a voltage-activated $K^+$ current showing slow activation kinetics and slow deactivation kinetics. Cells coinjected with the “midmyocardium” isoform 2-to-isoform 1 ratio showed a $K^+$ current with similar activation and deactivation kinetics and a potential at half-maximal activation but with a markedly reduced amplitude (Table 2). Figure 5B also shows that the voltage-activated $K^+$ current density decreased in relation to the isoform 2 cDNA concentration in the injected medium. The amplitude of $I_{Ks}$ in cells expressing the midmyocardium isoform 2-to-isoform 1 ratio was only 25% of that of $I_{Ks}$ in cells expressing the epicardium ratio. Figure 5C shows that the activation curve was not different in cells expressing the midmyocardium or epicardium isoform 2-to-isoform 1 ratios. Our data demonstrate that small differences in the isoform 2-to-isoform 1 ratio as observed between the three layers produce dramatic effects on $I_{Ks}$ amplitude.

**DISCUSSION**

Our results show that 1) repolarization is heterogeneous along the endocardial, midmyocardial, and epicardial layers of the human ventricle, with APD being significantly longer in cells from the M region; 2) the KvLQT1 isoform 2-to-isoform 1 ratio is increased in midmyocardium compared with the endocardium or epicardium; and 3) a difference in the KvLQT1 isoform 2-to-isoform 1 ratio produced dramatic effects on $I_{Ks}$.
amplitude in comparison to an epicardium isoform 1 ratio produced a 75% reduction of the current amplitude because a midmyocardium isoform 2-to-1 ratio reproduced midmyocardial tissue conditions (plus 2.7 µg/ml pCI-KvLQT1 isoform 2 (i.e., 35% total KvLQT1), a typical cell coinjected with 5 µg/ml pCI-KvLQT1 isoform 1 plasmid.

Fig. 5. A: tail current-voltage curves for KvLQT1 current recorded in a typical cell coinjected with 5 µg/ml pCI-KvLQT1 isoform 1 plasmid plus 2.7 µg/ml pCI-KvLQT1 isoform 2 (i.e., 35% total KvLQT1), reproducing midmyocardial tissue conditions (■), or with 5 µg/ml pCI-KvLQT1 isoform 1 plasmid plus 1.25 µg/ml pCI-KvLQT1 isoform 2 (i.e., 20% total injected KvLQT1), reproducing epicardial tissue conditions (▲). Cells were coinjected with 5 µg/ml pRC-IsK. In these and following experiments, cells were also coinjected with a pGFP plasmid so that total plasmid concentration was always 15 µg/ml. Inset: typical current traces obtained in a COS-7 cell injected with “epicardium” (top) or “midmyocardium” isoform 2-to-1 isoform ratio (bottom). Superimposed current traces were obtained by voltage steps (20-mV increment) applied from −100 to +60 mV. Holding potential, −80 mV; current scale, 10 pA/pF; time scale, 500 ms. B: average whole cell K+ tail current density in COS-7 cells injected with KvLQT1 isoform 1 cDNA (5 µg/ml), IsK cDNA (5 µg/ml), and various concentrations of KvLQT1 isoform 2 cDNA as indicated. C: averaged KvLQT1 activation curves in cells injected with epicardial (▲, n = 5) and midmyocardial (■, n = 6) plasmid mixtures.

Electrophysiological data presented here are comparable to other previously published data (9, 21). Part of the repolarization characteristics encountered in midmyocardial cells has been attributed to a specific subpopulation of cells, called the M cells, that might represent as much as 30% of the cellular mass of the ventricle (14). M cells were first identified in dog heart (3). Drouin et al. (9) previously provided evidence for their existence in the human heart. M cell action potential has a longer duration, especially during bradycardia, and a larger maximal upstroke velocity of phase 0 (Vmax) compared with endocardial and epicardial cells. Characteristics of the delayed rectifier K+ current are different in canine M cells from those of cells isolated from the epicardial or endocardial regions because the contribution of the slowly activating component (Iks) is markedly reduced in the M region (14). Inversely, Iks amplitude does not differ between the endocardium and the epicardium (14). Regional variation in Iks electrophysiological properties does not apply to Ikr in the dog (11, 14), whereas it might apply in the ferret (6).

At the molecular level, a reduced Iks cannot be explained a priori by less abundant KvLQT1-IsK channels, because we did not observe any significant change in overall expression of KvLQT1 or IsK genes across the ventricular wall. In addition, an isolated reduction of KvLQT1 isoform 1 cannot account for the Iks decrease because the isoform 1 expression is reduced by <10% (present data), whereas Iks reduction in M cells in the dog heart reaches at least 50% (14). Demolombe et al. (8) previously showed that the amplitude of KvLQT1 current strongly depends on the expression of an NH2-terminal truncated KvLQT1 splice variant (isoform 2). Isoform 2 is endogenously expressed in the heart and exerts potent dominant negative effects on the full-length KvLQT1 protein (isoform 1). The present study provides evidence that the mRNA encoding the KvLQT1 isoform 2 is expressed in the different layers of the myocardium in both left and right ventricles. In addition, it shows that the isoform 2-to-1 mRNA expression ratio is higher in midmyocardial tissue than in endocardial and epicardial tissues. Interestingly, we found no difference in isoform 2 expression between endocardial and epicardial layers. We considered the possibility that tissues other than myocytes, such as blood vessels or neuronal cells, might be responsible for the observed differences. However, this possibility was judged unlikely because the amount

Table 2. Current characteristics from COS-7 cells coinjected with isoform 2-to-1 isoform 1 ratio approaching mRNA expression value observed in midmyocardium and epicardium

<table>
<thead>
<tr>
<th>Current density, pA/pF</th>
<th>Midmyocardium</th>
<th>Epicardium</th>
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<tr>
<td>$I_{\text{act}}$, ms</td>
<td>195 ± 33 (n = 8)</td>
<td>201 ± 25 (n = 9)</td>
</tr>
<tr>
<td>$I_{\text{deact}}$, ms</td>
<td>78 ± 11 (n = 6)</td>
<td>93 ± 10 (n = 8)</td>
</tr>
<tr>
<td>$V_{0.5}$, mV</td>
<td>14.0 ± 5.5 (n = 6)</td>
<td>10.3 ± 2.1 (n = 5)</td>
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Values are means ± SE; n values in parenthesis are no. of experiments. Time constant of activation ($I_{\text{act}}$) was measured at +40 mV; time constant of deactivation ($I_{\text{deact}}$) was measured at −40 mV. $V_{0.5}$, potential at half-maximal activation. *P < 0.02 vs. epicardium.
of KvLQT1 mRNA expression in these tissues is very low. Our data are in agreement with comparable $I_{Ks}$ amplitude between these two layers in the dog heart (14). Furthermore, Liu and Antzelevitch (14) observed no difference in $I_{Ks}$ activation or deactivation kinetics between the three ventricular layers. This observation is also compatible with an increased isoform 2 expression in the M region, because isoform 2 expression reduces $I_{Ks}$ amplitude but does not affect the current kinetics (8) (see also Table 2). The modest difference in isoform 2 expression between the M region and the epicardium and endocardium is critical in determining $K^+$ current amplitude, as demonstrated by expression experiments when KvLQT1 and IsK cDNAs are coexpressed in mammalian cells. As for other $K^+$ currents, the inward rectifier current magnitude does not differ among cells isolated from epicardial, midmyocardial, and endocardial tissues, and the gradient of transient outward current density present in the transmural ventricular wall cannot account for the longer M cell action potential (15). Thus, if we assume that there is a close relationship between mRNA levels of $K^+$ channel subunits and the current produced by these channel proteins, we can hypothesize that differential expression of KvLQT1 isoform 2 in epicardial, midmyocardial, and endocardial tissues is responsible for variation of $I_{Ks}$ amplitude across the ventricular wall and may contribute to the regional action potential heterogeneity observed at that level.

Clinically, the midmyocardium appears to be a critical region involved in arrhythmia genesis due to M cell repolarization characteristics. Early afterdepolarization-related triggered activity is a common feature in M cells in response to stimuli such as hypokalemia, slow driving rates, or the presence of action potential-prolonging drugs (2). This triggered activity may participate to initiate torsade de pointes while heterogeneous repolarization participates to maintain them. Because transmural heterogeneities in repolarization time are more important across the ventricular wall than those recorded along the surfaces of the heart, they may represent a more favorable substrate for the development of arrhythmias (1). This transmural heterogeneity may be partly explained by differential expression of KvLQT1 isoforms across the ventricular wall, which may therefore contribute to physiological Q-T dispersion. Indeed, conditions known to augment Q-T dispersion, including acquired long Q-T syndrome, lead to augmentation of transmural dispersion of repolarization in the wedge because of a preferential effect of the drugs to prolong the M cell action potential. On the other hand, antiarrhythmic agents known to diminish Q-T dispersion, such as amiodarone, also diminish transmural dispersion of repolarization by causing a preferential prolongation of APD in epicardium and endocardium (10, 22).

We propose that the relative expression level of KvLQT1 isoform 2 in the different ventricular layers is an important determinant of repolarization heterogeneity in the human ventricle. Increased isoform 2 expression in the midmyocardium may lead to an increased susceptibility to drugs that adversely prolong repolarization. At present, it is not known whether KvLQT1 isoforms 1 and 2 possess a common promoter or, alternatively, a different promoter, as is the case with insulin-like growth factor 2, for example, which has multiple promoters indicative of developmental and tissue dependency (23). Future studies should be directed to clarify this issue and also to investigate whether the isoform 2-to-isoform 1 expression ratio undergoes age-, sex- or other hormone-related variations.

Wethank Jacques Barhanin (Sophia-Antipolis, France) for providing KvLQT1 and IsK plasmids. We also thank Patricia Charpentier, Béatrice Le Ray, and Marie-Joseph Loubrat for expert technical assistance.

This work was supported by the Institut National de la Santé et de la Recherche Médicale (INSERM; PROGRES network).

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