Molecular basis of species-specific expression of repolarizing K⁺ currents in the heart

Stephen Zicha,1,2 Isaac Moss,3 Bruce Allen,1 Andras Varro,3 Julius Papp,3 Robert Dumaine,4 Charles Antzelevich,4 and Stanley Nattel1,2

1Department of Medicine and Research Center, Montreal Heart Institute and University of Montreal, Montreal H3T 1C8; 2Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada H3G 1Y6; 3Department of Pharmacology and Pharcamotherapy, University of Szeged, H-6701, and Research Unit for Cardiovascular Pharmacology, Hungarian Academy of Sciences, Szeged H-6701, Hungary; and 4Masonic Medical Research Laboratories, Uitca, New York 13501-1787

Submitted 26 January 2003; accepted in final form 16 June 2003


The molecular basis of species-specific repolarizing K⁺ current profiles has not been established. The pore-forming α-subunits of IK, and the slow component of IK (IKs) are formed by subunits encoded by the ether-a-go-go-related gene (ERG1) mRNAs in rabbits, guinea pigs, and humans. KvLQT1 mRNA was present in rabbits but undetectable in guinea pigs. MinK mRNA concentration in guinea pigs was almost threefold greater versus humans and 20-fold versus rabbits. MinK protein expression in guinea pigs was almost twofold that in humans and sixfold that in rabbits. KvLQT1 mRNA concentration was greatest in humans, and protein expression in humans was increased by ~2- and ~7-fold compared with values in rabbits and guinea pigs, respectively. The ether-a-go-go-related gene (ERG1) mRNAs were more concentrated in humans, but ERG1 protein expression could not be compared across species because of epitope sequence differences. We conclude that important interspecies differences in cardiac K⁺ channel subunit expression exist and may contribute to the following: 1) lack of a transient outward current in the guinea pig (α-subunit transcription absent in the guinea pig heart); 2) small slow delayed rectifier current and torsades de pointes susceptibility in the rabbit (low-level minK expression); and 3) large slow component of the delayed rectifier current in the guinea pig (strong minK expression).

arrhythmia; ion channels; electrophysiology; ECG; antiarrhythmic drugs; proarrhythmia

ELECTROPHYSIOLOGICAL STUDIES have demonstrated differences in the K⁺ current profiles responsible for cardiac action potential (AP) repolarization among different species, including the guinea pig, rabbit, and human. The transient outward K⁺ current (Iₒ) plays an important role in rabbit (9, 21) and human (7) cardiac AP repolarization; however, it is believed to be absent in the guinea pig (21). On the other hand, the delayed rectifier current (IK) is very prominent in the guinea pig (13, 16, 21) but smaller in the rabbit (9, 13, 16, 21) and human (13, 22). These ionic current profiles are associated with distinct AP properties in each species (21). There are species-dependent particularities in sensitivity to class III drug-induced early afterdepolarizations (EADs) and long QT syndromes (LQTSs), with rabbits being particularly susceptible to EAD and LQTS induction by blockers of the rapid component of IK (IKr) (5, 15).

The molecular basis of species-specific repolarizing K⁺ current profiles has not been established. The pore-forming α-subunits of IKr and the slow component of IK (IKs) are formed by subunits encoded by the ether-a-go-go-related gene (ERG1) and KvLQT1 gene, respectively (2, 19, 20). MinK is an essential β-subunit for IKs formation (2, 19). It has been suggested that minK-related peptide-1 (MiRP1) is essential for the formation of IKr (1), but the precise role of MiRP1 in IKr has been questioned (26). Ikᵣ is formed by voltage-gated K⁺ channel subunits (Kᵥ1.4, -4.2, and -4.3) in rabbits and by Kv4.3 subunits in humans (6, 23). The present study was designed to assess the following: 1) whether the absence of Ikᵣ in the guinea pig heart can be attributed to lack of cardiac expression of the relevant subunits; 2) whether the Ikᵣ-encoding subunit expression differences between the rabbit and guinea pig are consistent with their current and EAD sensitivity profiles; and 3) how Ikᵣ subunit expression in human hearts compares with rabbit and guinea pig hearts.

MATERIALS AND METHODS

RNA purification. New Zealand White rabbits (1.8–2.2 kg) or Dunkin-Hartley guinea pigs (500 g) were euthanized by cervical dislocation. The left ventricular free wall was separated and frozen in liquid nitrogen. The left ventricular free wall from the basal region of undiseased human tissues were obtained from five general organ donor patients (3 women and 2 men) under procedures approved by the Ethical Review Board for Human Experimentation at the Montreal Heart Institute.

http://www.ajpheart.org 0363-6135/03 $5.00 Copyright © 2003 the American Physiological Society H1641
Board of the Medical Center of the University of Szeged. These tissues were stored in cardioplegic solution composed of (in mM) 110 NaCl, 16 KCl, 1.2 CaCl₂, and 5 NaHCO₃ and kept at 4°C for ~6–8 h before being frozen in liquid nitrogen. Total RNA was isolated from 0.5- to 1.0-g samples with the use of TRIzol reagent (Invitrogen), followed by chloroform extraction and isopropanol precipitation. Genomic DNA was eliminated by incubation in DNase I (0.1 U/μl, 37°C) for 30 min, followed by acid phenol-chloroform extraction. RNA was quantified by spectrophotometric absorbance at 260 nm, purity was confirmed by the A260/A280 ratio, and integrity was evaluated by ethidium bromide staining on a denaturing agarose gel. RNA samples were stored at ~80°C in RNasesecure resuspension solution (Ambion).

PCR primers. Degenerate primers for initial RT-PCR were designed based on published cDNA sequences for Kv1.4, Kv4.2, Kv4.3, KvLQT1, ERG1, minK, and MiRP1. Highly conserved and specific sequences were selected and primer pair specificity was confirmed by comparison with the GenBank database with the use of the Basic Local Alignment Search Tool. α-Actin was used as a positive control for RT-PCR. In preliminary studies, MiRP1 signals were extremely weak and further quantification was not performed. Competitive RT-PCR was used to obtain the absolute mRNA concentrations essential for interspecies comparison. Species-specific gene-specific primers (GSPs) for competitive RT-PCR were based on previously published sequences, or, when a sequence was not available, the DNA product of PCR with degenerate primers was sequenced for nested primer design (Table 1). Chimeric primer pairs for RNA-mimic synthesis were constructed with a human cardiac α-actin sequence flanked by GSPs. An eight-nucleotide sequence, GGC-CGGCG, corresponding to the 3'-end of the T7 promoter, was conjugated to the 5'-end of each forward chimeric primer.

Synthesis of RNA mimic. First-strand cDNA synthesized by RT with ventricular mRNA samples was used as a template for subsequent PCR amplification steps with chimeric primer pairs. The resulting cDNA mimic contains a 460-bp α-actin sequence flanked at the 5'-end by the sense GSP sequence and an 8-bp T7-promoter sequence and at the 3'-end by the antisense GSP sequence. Products were gel purified with the QIAquick gel extraction kit (Qiagen). The RNA mimic (internal standard) was created with the use of an in vitro transcription kit (mMESSAGE Machine, Ambion). The product was incubated with RNase-free DNase I (30 min, 37°C) to eliminate cDNA contamination, followed by phenol-chloroform extraction and isopropanol precipitation. Mimic size and concentration were determined by migration on a denaturing RNA gel alongside predetermined RNA concentrations to create a standard curve.

Competitive RT-PCR. RNA mimic samples of serial 10-fold dilutions were added to reaction mixtures containing 1 μg total RNA. RNA was denatured at 65°C (15 min). RT was conducted in a 20-μl reaction mixture containing reaction buffer (10 mmol/l Tris-HCl, pH 8.3, and 50 mmol/l KCl), 2.5 mmol/l MgCl₂, 1 mmol/l 2-deoxynucleotide 5'–triphosphate (Roche), 3.2 μg random primers p(dN)₆ (Roche), 5 mmol/l dithiothreitol, 50 units of RNase inhibitor (Promega), and 200 units of Moloney murine leukemia virus reverse transcriptase (GIBCO-BRL). First-strand cDNAs were synthesized at 42°C (1 h) and the remaining enzymes were heat deactivated (99°C, 5 min).

First-strand cDNA from the RT step was used as a template in 25-μl reaction mixtures, including 10 mmol/l Tris-HCl, pH 8.3, 50 mmol/l KCl, 1.5 mmol/l MgCl₂, 1 mmol/l 2-deoxynucleotide 5'–triphosphate, 0.5 μmol/l GSPs, 0.625 mmol/l DMSO, and 2.5 units of Taq polymerase (GIBCO-BRL). Reactions were hot started at 93°C for 30 s (denaturing), followed by 30 amplification cycles (93°C, 30 s (denaturing); 55–58°C, 30 s (annealing); 72°C, 30 s (extension)). A final 72°C extension step was performed for 5 min. RT-

### Table 1. Primers for RT-PCR

<table>
<thead>
<tr>
<th>Current</th>
<th>Clone</th>
<th>Primer Pair</th>
<th>Position, bp</th>
<th>Size, bp</th>
<th>T, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{to}$</td>
<td>Kv1.4</td>
<td>F: AACACTGACATGCTTATG</td>
<td>3–390</td>
<td>388</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: TAGTAAACACCTCGCTCCTC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kv4.2</td>
<td>F: GTGGATACACTCGGATTC</td>
<td>1,377–1,756</td>
<td>378</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: TTCCGAGTGCTCAGGAG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kv4.3</td>
<td>F: GCAGGAAAAGGAGGCTG</td>
<td>1,381–1,832</td>
<td>452</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GGCGAGGAGGATATG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$I_{Kr}$</td>
<td>HKvLQT1</td>
<td>F: CCAACCTAAAGGCTATCG</td>
<td>1,114–1,461</td>
<td>348</td>
<td>54.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: CTTTGGTCTTCTACTGCTTCA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RKvLQT1</td>
<td>F: GCCGCGAAGATATGGTG</td>
<td>86–402</td>
<td>317</td>
<td>57.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: CTTTCAGAGGAGTACAGA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GPKvLQT1</td>
<td>F: GTGCTGCCACATTGACCTCA</td>
<td>66–362</td>
<td>297</td>
<td>56.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GGACACCTTGGGGCTGAA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HminK</td>
<td>F: AGCCCGTTTCTTCTGACAGC</td>
<td>28–255</td>
<td>228</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: ATCGAGGCTATGGTAAACG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RminK</td>
<td>F: CGTCGATGCCTTCTTGACG</td>
<td>23–285</td>
<td>263</td>
<td>58.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: TACGCCCTCCTGGCTTCTG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GPMiNK</td>
<td>F: CGGCGACCTTAAACCAAGAT</td>
<td>54–330</td>
<td>277</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: TTCAGAGGCCAGCAATG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$I_{Cr}$</td>
<td>HERG1</td>
<td>F: TGCCGCGACATCATCTCAA</td>
<td>1,466–1,846</td>
<td>381</td>
<td>57.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: AAGCGCGCGTGGACATCTTT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RERG1</td>
<td>F: TACGCGAGGAGAGATGGATT</td>
<td>2,577–2,890</td>
<td>314</td>
<td>62.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: TCCTGGACCTCGGACTTGA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GPERG1</td>
<td>F: TGCGGACCTCCTCGACTGG</td>
<td>13–232</td>
<td>220</td>
<td>55.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GCTTGGACCTTGCTCCTTG</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$I_{to}$, transient outward current; $I_{Kr}$, slow component of the delayed rectifier current ($I_{Kr}$); $I_{Cr}$, rapid component of $I_{Kr}$; Kv1.4–4.3, voltage-gated K⁺ channel subunits; bp, base pair; T, temperature; H-, R-, and GPKvLQT1, human, rat, and guinea pig KvLQT1 gene; H-, R-, and GPMiNK, human, rat, and guinea pig minK gene; ERG, ether-a-go-go-related gene; F and R, forward and reverse primers; LQT1, long QT syndrome 1.

AJPH-Circ Physiol • VOL 285 • OCTOBER 2003 • www.ajpheart.org
negative controls were obtained to exclude genomic contamination for all RT-PCR reactions.

PCR products were visualized under UV light with ethidium bromide staining in 1.5% agarose gels. The images were captured with a Nighthawk camera, and band density was determined with Quantity One software. A DNA mass marker (100 ng) was used to determine the size and quantity of DNA bands and to create a standard curve in each experiment for absolute quantification. Natural logarithm plots $\ln([\text{target}]/[\text{mimic}])$ versus $\ln([\text{mimic}])$ were fit by linear regression to determine the absolute concentration of target mRNA as previously described (23, 25, 28).

**Western blot studies.** Membrane protein was extracted with 5 mmol/l Tris-HCl (pH 7.4), 2 mmol/l EDTA, 5 μg/ml leupeptin, 10 μg/ml benzamidine, and 5 μg/ml soybean trypsin inhibitor, followed by tissue homogenization. All procedures were performed at 4°C. Membrane proteins were fractionated on either 8% (ERG1, KvLQT1) or 12% (minK) SDS-polyacrylamide gels and transferred electrophoretically to Immobilon-P polyvinylidene fluoride membranes (Millipore) in 25 mmol/l Tris base, 192 mmol/l glycine, and 5% methanol at 0.09 mA for 18 h (ERG1, KvLQT1) or 65 V for 20 min (minK). Membranes were blocked in 5% nonfat dry milk (Bio-Rad) in 50 mmol/l Tris-HCl, 500 mmol/l NaCl (pH 7.5), and 0.05% Tween 20 (TTBS) for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature) and then incubated with primary antibody (1:200 dilution) in 5% nonfat dry milk in TTBS for 2 h (room temperature). Membranes were washed three times in TTBS, reblocked in 5% nonfat dry milk in TTBS (10 min), and then incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (1:5,000, for Santa Cruz ERG1) or donkey anti-goat IgG secondary antibody (1:10,000, for Santa Cruz KvLQT1, minK) in 5% nonfat dry milk in TTBS (10 min), and then incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (1:5,000, for Santa Cruz ERG1) or donkey anti-goat IgG secondary antibody (1:10,000, for Santa Cruz antibodies) in 5% nonfat dry milk in TTBS (40 min). They were subsequently washed three times in TTBS and once in TBS. Signals were obtained with Western Lightning Chemiluminescence Reagent Plus (Perkin Elmer Life Sciences). Band densities were determined with a laser scanner (model 420oe, PDI) and Quantity One software (PDI). Protein loading was controlled by probing all Western blots with anti-GAPDH antibody (RDI) and by normalizing ion channel protein band intensity to that of GAPDH.

**Data analysis.** All data are expressed as means ± SE. Each determination was performed on an individual heart; n values represent the number of hearts studied. Western blot band intensities are expressed quantitatively as arbitrary optical density (OD) units, which correspond to the laser densitometric K+ channel subunit membrane protein band intensity after background subtraction divided by the GAPDH signal intensity for the same sample. Statistical comparisons were performed with ANOVA and Student’s t-test with Bonferroni’s correction. A two-tailed $P < 0.05$ indicated statistical significance.

**RESULTS**

$I_{\alpha_\text{o}}$-encoding subunit mRNA expression. Figure 1 shows RT-PCR signals for Kv1.4, -4.2, and -4.3 in the rabbit heart (lanes 3, 5, and 7). In contrast, no mRNA encoding these subunits could be identified in the guinea pig heart (lanes 4, 6, and 8). Lanes 1 and 2 demonstrate the presence of α-actin in both rabbit and guinea pig mRNA samples, indicating their intactness. Lane 9 shows the presence of two bands corresponding to Kv4.3 mRNA in the guinea pig brain, representing the previously identified long and short splice variants. Thus the absence of Kv4.3 in the guinea pig heart is due to a lack of transcription rather than absence in the guinea pig genome.

**Results of competitive RT-PCR.** Figure 2, A–C, shows examples of gels obtained from human, rabbit, and guinea pig KvLQT1 competitive RT-PCR reactions. In all cases, lane 0 contains 100 ng of DNA mass ladder to create the standard curve for each gel. Lanes 1–6 were obtained with serial dilutions of the RNA mimic along with 1 μg total RNA. The top bands represent the internal standard PCR product, whereas the bottom bands are the target KvLQT1 bands coamplified with the mimics in the same reaction tube. As the mimic concentration decreases from left to right, the target band becomes stronger, demonstrating the competition between mimic and target. For each experiment, the sample KvLQT1 mRNA concentration was calculated on the basis of the target and mimic band intensities at all dilutions, as described in MATERIALS AND METHODS. Figure 2D shows the mean data for each dilution in each species. The average absolute amounts of KvLQT1 mRNA in human, rabbit, and guinea pig hearts were

![Fig. 1. RT-PCR products for α-actin (positive control), voltage-gated K+ channel subunit (Kv)1.4, -4.2, and -4.3 in rabbit (R) and guinea pig (GP) heart and guinea pig brain.](http://ajpheart.physiology.org/)

AJP-Heart Circ Physiol • VOL 285 • OCTOBER 2003 • www.ajpheart.org
15.5 ± 2.7, 8.6 ± 3.3, and 5.3 ± 1.4 amol/μg total RNA, respectively.

Figure 3, A–C, shows representative gels from minK competitive RT-PCRs. Particularly small mimic concentrations had to be used for rabbit hearts because of the weak minK expression and the point of equivalence was well to the right of the gel, corresponding to low mRNA concentrations. The mean data for all minK competitive RT-PCRs are shown in Fig. 3D. They confirm that for any mimic concentration, the highest target/mimic band intensities were observed in guinea pig hearts and the lowest in rabbit hearts, with human hearts being intermediate. Mean calculated mRNA concentrations were 34.1 ± 12.5, 1.5 ± 0.3, and 12.7 ± 3.6 amol/μg total RNA for guinea pig, rabbit, and human hearts, respectively.

Examples of ERG1 competitive RT-PCR gels are shown in Fig. 4, A–C. Mean data are provided in Fig. 4D. The target/mimic ratios are largest in the human, followed by the rabbit and then guinea pig. Mean calculated ERG1 concentrations averaged 18.1 ± 4.0,
20.2 ± 7.1, and 92.8 ± 19.4 amol/µg total RNA for the guinea pig, rabbit, and human, respectively.

Figure 5 shows comparisons between mRNA concentrations of various subunits among species. There was an almost twofold difference in mean KvLQT1 mRNA concentrations between human and rabbit hearts (Fig. 5A), and rabbit heart concentrations were slightly higher than in guinea pig hearts. There were major differences among species in the expression of minK mRNA (Fig. 5B). MinK expression was about threefold stronger in guinea pig hearts, but weaker in rabbit hearts than in human hearts. ERG1 mRNA was significantly more strongly expressed in human hearts than in the other species (Fig. 5C). Because KvLQT1 and minK coassemble to form $I_{Ks}$ (2, 19), the ratio of the concentrations of these subunits may be important in determining subunit assembly and channel formation. Figure 5D shows mean minK-to-KvLQT1 mRNA concentration ratios based on calculations for both subunits in each heart. The ratio was highest in the guinea pig hearts, lower in humans, and lower still in the rabbit.

Fig. 4. Ether-a-go-go-related gene (ERG1) competitive RT-PCR. A–C: representative gels. Mimic dilutions for both human and guinea pig samples were the following: 64 and 6.4 ng and 640, 320, 64, and 6.4 pg. For the rabbit, mimic concentrations in the initial reaction tubes were 4.5 ng and 453, 227, 113, 45.3, and 4.5 pg, respectively. D: means ± SE data (n = 5 hearts/determination).

Fig. 5. Means ± SE mRNA concentrations for KvLQT1 (A), minK (B), and ERG1 (C). *P < 0.05 vs. rabbit. D: means ± SE KvLQT1-to-minK concentration ratio. *P < 0.05 vs. guinea pig. Inset: Human (H) and rabbit values on larger scale.
Western blot studies. Figure 6A shows representative KvLQT1 protein bands detected at the expected molecular mass (∼75 kDa, with the band in guinea pigs having a slightly smaller molecular mass). The KvLQT1 signal was suppressed by preincubation with antigenic peptide (Fig. 6A, last three lanes). Corresponding GAPDH signals to which KvLQT1 bands were normalized are shown in Fig. 6B. Figure 6C shows that humans had a significantly greater amount of KvLQT1 protein (3.1 ± 0.5 arbitrary OD units) compared with both guinea pigs (0.4 ± 0.2) and rabbits (1.5 ± 0.4, n = 5/group, P < 0.05).

Figure 7A shows a typical minK Western blot. Signals were detected in all species at ∼27 kDa. An additional, very faint, band was detected at 24 kDa in guinea pigs. Preincubation with antigenic peptide suppressed the minK signal (+control antigen, Fig. 7A, last three lanes). MinK band intensity was clearly strongest in guinea pig.
For each group, P pig samples as a single band at log no. APC-016, Alomone) detected ERG1 in guinea pig (Fig. 8). An antibody raised against a rat epitope (commercially available antibodies had been raised against a different human ERG1 epitope from that shown in A) gave no signal in human samples. An antibody raised against a different human ERG1 epitope (Santa Cruz) was used and detected a single 165-kDa band in rabbit samples (Fig. 8C). As expected, a band was detected with human samples as well. Because three different antibodies had to be used to properly detect ERG1 in humans, rabbits, and guinea pigs, and because the epitope sequences varied, meaningful band intensity comparisons could not be made.

**DISCUSSION**

In the present study, we examined the expression of \( K^+ \) channel subunits underlying time-dependent repolarizing currents in various species. We noted clear species-dependent differences in subunit expression that parallel and shed potential light on the mechanisms of differences in \( K^+ \) current profiles.

*Species-specific time-dependent \( K^+ \) currents and molecular basis.* Time-dependent \( K^+ \) currents play an important role in governing cardiac repolarization, thereby determining the occurrence of a broad range of arrhythmias and mediating a wide variety of antiarrhythmic and proarrhythmic drug actions (11, 17, 18). Animal models have been essential for an appreciation of the determinants of repolarization of APs and of the mechanisms underlying cardiac arrhythmias. One limitation in using animal models to understand the determinants of repolarization and arrhythmias in humans has been a lack of information about how the relative distributions of ion channel subunits in different species compare with each other and with the ion channel subunit distribution in humans.

There are well-recognized differences in \( I_K \) between rabbit and guinea pig hearts (9, 13, 16, 21). Guinea pig \( I_K \) is much larger and shows slower kinetics than in the rabbit (16). Our observation that minK is much more strongly expressed in the guinea pig than the rabbit at both the mRNA (Fig. 5) and protein (Fig. 7) levels provides a possible molecular basis for the differences. In the absence of minK, KvLQT1 is known to form small, rapidly activating currents, whereas expression of KvLQT1 and minK carries robust currents with the typical properties of \( I_K \) (2, 19). Therefore, the relative lack of minK in rabbits is a plausible explanation for their small and more rapidly activating \( I_K \).

It has been difficult to record \( I_K \) in human cardiac myocytes (3, 7, 13, 22). This has led to the suggestion that \( I_K \) may be quantitatively less important in human hearts than in other species. Our data suggest a strong \( I_K \) subunit expression in the human heart, with evidence for stronger KvLQT1 and ERG1 expression in humans than in rabbits or guinea pigs and minK expression that is intermediate between guinea pigs and rabbits. These results suggest that \( I_K \) is likely of the same order of importance in human hearts as in other species, and that the difficulties reported in recording \( I_K \) in human hearts may be related to the sensitivity of \( I_K \) to cell isolation (27) and the fact that human tissue preparations are never available for cell isolation under the conditions achievable for animal models rather than to lesser importance of \( I_K \) in the human heart.

It is well known that guinea pig cardiac \( I_K \) density is particularly large (13, 16, 21). Our findings suggest...
that strong expression of \( I_{Ks} \) subunits (Fig. 7), particularly \( \text{minK} \), accounts at least in part for the large guinea pig \( I_K \). The rabbit is known to have small \( I_K \) (9, 13, 16, 21) and to be particularly prone to class III drug-induced EADs and torsades de pointes (5, 15). There is evidence that \( I_{Ks} \) acts as a safety mechanism against excessive AP duration prolongation with \( I_K \) inhibition and that in circumstances in which \( I_{Ks} \) is reduced, \( I_K \) inhibition produces enhanced repolarization delays and a greater risk of EADs (4, 10, 29). Thus the relatively low level of \( \text{minK} \) expression in the rabbit heart, resulting in small \( I_{Ks} \), may provide the molecular basis for the sensitivity of the rabbit to \( I_K \) blocking drug-induced repolarization abnormalities and related arrhythmias. The rabbit may thus provide a natural model analogous to human LQTS associated with relative \( \text{minK} \) deficiency; however, further pharmacological and electrophysiological studies are needed to evaluate this notion.

The rabbit and guinea pig have long been recognized to be at opposite ends of the repolarizing current and AP profile spectrum, with the rabbit showing large \( I_{to} \) and small \( I_K \) (9, 13, 16, 21) and the guinea pig showing large \( I_K \) and little or no \( I_{to} \) (13, 16, 21). In fact, there has been some controversy about the presence or absence of \( I_{to} \) in the guinea pig. Although many investigators (8, 12, 14, 24) have reported a lack of \( I_{to} \) in guinea pig hearts, the sensitivity of guinea pig AP repolarization to 4-aminopyridine, and the recording of rapidly inactivating depolarization-induced outward currents, has led to some doubt the possible expression of \( I_{to} \) in the guinea pig heart. Our results show that the transcripts corresponding to \( I_{to} \) K\(^+\) channel \( \alpha \)-subunits Kv1.4, -4.2, and -4.3 are lacking in guinea pig hearts and provide molecular confirmation for the conclusions of recent experimental studies indicating that no \( I_{to} \) is present in the guinea pig heart (8). We did not compare \( I_{to} \) subunit distribution in the rabbit versus human, because this has been the object of a previous detailed publication (23), which showed that rabbit cardiac \( I_{to} \) reflects the presence of Kv1.4, -4.2, and -4.3, whereas Kv4.3 is predominant in humans.

**Potential limitations.** It is apparent that differences in repolarization properties between species may be due to species-specific differences in the expression of Kv subunits. However, it is also well known that these channels are modulated by a variety of signaling mechanisms and regulatory factors, so that differences in such modulation between species could also contribute to the observed electrophysiological differences. The much smaller \( \text{minK} \) expression in rabbits compared with guinea pigs is an appealing explanation for the smaller rabbit \( I_{Ks} \) density, but we cannot exclude a contribution from other factors, such as regulatory differences and the role of other unidentified subunits that might contribute to \( I_{Ks} \).

A particular advantage of competitive RT-PCR is that it provides absolute quantification of transcript concentration, allowing for comparisons in the expression of each \( K^+ \) channel subunit across different species as well as comparisons between the expression of different subunits within a species. Quantitative analysis of Western blots provides important complementary information, allowing for relative quantification of protein expression across species when the antigenic epitope is identical in different species. A limitation of Western blot analyses is that because of potential antibody affinity differences, meaningful quantitative comparisons are not possible for the expression of different subunits within the same species or for expression of the same subunit with epitope sequence differences across species. Our mRNA analysis provides precise information about the relative expression of the various \( I_{Ks} \) subunits in different species as well as about the relative expression of transcripts encoding different molecular species with one another. \( \text{minK} \) and KvLQT1 epitopes are the same among the species we studied, allowing quantitative comparisons for their protein expression across species. However, the relative protein expression of \( \text{minK} \) versus KvLQT1 cannot be compared, and we therefore cannot comment on the relative protein concentrations within each species. Because of epitope differences, we were not able to compare quantitatively ERG1 protein expression across species.

Only normal human tissue was used for this study. Because of the rarity of such samples, we were not able to control for the gender of the samples, but the number of male and female subjects from whom tissues were obtained was at least equal. The guinea pig and rabbit samples were similarly mixed for consistency. Human tissue samples were available from the basal region of the left ventricular free wall. Guinea pig and rabbit samples comprised both basal and lateral left ventricular free wall regions. If the distribution of various cell types (e.g., epicardial, endocardial, and midmyocardial or M cells) were different among the tissue samples obtained from the various species studied, this could have influenced the results. We are not aware of studies comparing quantitatively the distribution of various transmural cell types across the species we studied, and such an analysis was beyond the scope of the present study.

The human tissue was kept in cardioplegic solution at 4°C after surgical excision during the time required for transport to the laboratory and initial processing. In contrast, guinea pig and rabbit tissues were snap frozen immediately on excision. This difference could theoretically have resulted in contamination of the results by RNA or protein degradation of human tissues. We routinely performed RNA gels on all RNA samples to detect degradation. Degradation was minimal and no differences were observed among human, rabbit, and guinea pig samples. We have also compared \( K^+ \) channel subunit protein densities in cardiac tissues that were snap frozen with tissues preserved in cold cardioplegic solution for 4 h before being frozen and observed no differences.

In conclusion, there are quantitative differences in cardiac \( K^+ \) channel subunit expression among guinea pigs, rabbits, and humans, which shed light on the molecular basis of species-specific repolarization prop-
erties and arrhythmia susceptibility. The present ob-
servations provide potentially useful insights into the  
relationships between repolarizing currents and the  
expression of underlying K\(^+\) channel subunits in  
commonly used experimental animals compared with those  
in man. These findings are important for the under-
standing of the molecular control of repolarization and  
for the interpretation of electrophysiological studies in  
various animal models.

The authors thank Evelyn Landry for technical assistance, France  
Thériault for secretarial help with the manuscript, and Dr. Miklos  
Opincario of the Department of Cardiac Surgery, University of  
Szeged, for help and tissue procurement.

DISCLOSURES

This study was funded by the Canadian Institutes of Health  
Research, the Quebec Heart and Stroke Foundation, and by Hun-
garian National Research Foundation Grant OTKA-T037520.

REFERENCES

1. Abbott GW, Sesti F, Splawski I, Buck ME, Lehmann MH,  
Timothy KW, Keating MT, and Goldstein SA. MiRP1 forms  
IKr potassium channels with HERG and is associated with  
2. Barhanin J, Lesage F, Guillemare E, Fink M, Lazdunski  
M, and Romety G. K\((V)\)LQT1 and I\(\text{Ks}\) (minK) proteins  
associate to form the I\((K_{\text{Ca}})\) cardiac potassium current.  
3. Beuckelmann DJ, Nabauer M, and Erdmann E. Alterations  
of K\(^+\) currents in isolated human ventricular myocytes  
4. Biliczki P, Virag L, Iost N, Papp JG, and Varro A. Interac-
tion of different potassium channels in cardiac repolarization  
in dog ventricular preparations: role of repolarization reserve.  
5. Carlson L, Almgren O, and Duker G. QTU-prolongation  
and torsades de pointes induced by putative class III antiarrhythmic  
agents in the rabbit: etiology and interventions. J Cardiovasc  
RS, Cohen IS, and McKinnon D. Role of the Kv4.3 K\(^+\) channel  
in ventricular muscle. A molecular correlate for the transient  
7. Escande D and Coraboeuf E. Two types of transient outward  
currents in adult human atrial cells. Am J Physiol Heart Circ  
8. Findlay I. Is there an A-type K\(^+\) current in guinea pig ventri-
9. Giles WR and Van Ginneken AC. A transient outward current  
in isolated cells from the crista terminalis of rabbit heart.  
10. Han W, Chartier D, Li D, and Nattel S. Ionic remodeling of  
cardiac Purkinje cells by congestive heart failure. Circulation  
MR, Antzelevitch C, Escande D, Franz M, Malik M, Moss A,  
and Shah R. The potential for QT prolongation and pro-ar-
rythmia by non-anti-arrhythmic drugs: clinical and regulatory  
implications. Report on a Policy Conference of the European  
12. Inoue M and Imanaga I. Masking of A-type K\(^+\) channel  
in guinea pig cardiac cells by extracellular Ca\(^{2+}\). Am J Physiol  
13. Lathrop DA, Nanasi PG, Schwartz A, and Varro A. Ionic  
basis for OPC-8212-induced increase in action potential duration  
in isolated rabbit, guinea-pig and human ventricular myocytes.  
14. Li GR, Yang B, Sun H, and Baumgarten CM. Existence of a  
transient outward K\(^+\) current in guinea pig cardiac myocytes.  
15. Lu HR, Marien R, Saels A, and Do Clerck F. Species plays an  
important role in drug-induced prolongation of action potential  
duration and early afterdepolarizations in isolated Purkinje fi-
and kinetics of I\(\text{Ks}\) and I\(\text{Kc}\) in guinea-pig and rabbit ventricular  
myocytes explain different efficacy of I\(\text{Kc}\) blockade at high  
heart rate in guinea-pig and rabbit: implications for arrhythmogenesis  
17. Nattel S. The molecular and ionic specificity of antiarrhythmic  
18. Roden DM, Balser JR, George AL Jr, and Anderson ME.  
19. Sanguinetti MC, Curran ME, Zou A, Shen J, Spector PS,  
Atkinson DL, and Keating MT. Coassembly of K\((V)\)LQT1 and  
minK (I\(\text{Ks}\)) proteins to form cardiac I\(\text{Ks}\) potassium channel.  
20. Sanguinetti MC, Jiang C, Curran ME, and Keating MT. A  
mechanistic link between an inherited and an acquired cardiac  
arrhythmia: HERG encodes the IKr potassium channel. Cell 21:  
Ionic currents and action potentials in rabbit, rat, and guinea-
22. Virag L, Iost N, Opincariu M, Szolnoky J, Szecsi J, Bogats  
G, Zsenohradszky P, Varro A, and Papp JG. The slow  
component of the delayed rectifier potassium current in undis-
S. Potential molecular basis of different physiological properties  
of the transient outward K\(^+\) current in rabbit and human atrial  
24. Wang Z, Ferrini B, and Nattel S. Repolarization differences  
between guinea pig atrial endocardium and epicardium: evi-
dence for a role of I\(\text{Kc}\). Am J Physiol Heart Circ Physiol 280:  
25. Wang Z, Yue L, White M, Pelletier G, and Nattel S. Differe-
tial distribution of inward rectifier potassium channel tran-
26. Weerapura M, Nattel S, Chartier D, Caballero R, and  
Hebert TE. A comparison of currents carried by HERG, with  
and without coexpression of MiRP1, and the native rapid de-
layed rectifier current. Is MiRP1 the missing link? J Physiol 540:  
27. Yue L, Feng J, Li GR, and Nattel S. Transient outward and  
delayed rectifier currents in canine atrium: properties and role  
H2168, 1996.
mechanisms underlying ionic remodeling in a dog model of atrial  
29. Zeng J, Laurita KR, Rosenbaum DS, and Rudy Y. Two  
components of the delayed rectifier K\(^+\) current in ventricular  
myocytes of the guinea-pig type. Theoretical formulation and  