Mutations in the KvLQT1 gene are the cause of the long QT syndrome 1. KvLQT1 gene product is associated with the regulator protein IsK to produce a component of the delayed rectifier K⁺ current in cardiac myocytes. We identified an N-terminal truncated isoform of the KvLQT1 gene product, referred to as isoform 2. In RNase protection assays, isoform 2 represented 28.1 ± 0.6% of the total KvLQT1 expression in the human adult ventricle. COS-7 cells injected intranuclearly with KvLQT1 isoform 1 cDNA exhibited a fast-activating K⁺ current, whereas those injected with a KvLQT1 isoform 1 plus IsK cDNA showed a slow-activating K⁺ current. Cells injected with KvLQT1 isoform 2 plasmid showed no detectable K⁺ current. Those injected with a 1/1 isoform 2/isoform 1 ratio showed no detectable K⁺ current. Those injected with 1/5 and 2/5 ratios showed a K⁺ current with markedly reduced amplitude. Coexpression of the IsK regulator consistently reduced the dominant negative effects of isoform 2. Our results indicate that KvLQT1 isoform 2 exerts a pronounced negative dominance on isoform 1 channels and that the cardiac KvLQT1 K⁺ channel complex is composed of at least three different proteins as follows: isoform 1, isoform 2, and IsK.

Mutations in the KvLQT1 gene are the most frequent cause of the congenital long QT syndrome, a familial disorder characterized by prolonged cardiac repolarization, syncope, and a high incidence of sudden death (1). KvLQT1 is also responsible for the Jervell and Lange-Nielsen cardioauditory syndrome in which prolonged cardiac repolarization is associated with bilateral congenital deafness (2). In a recent study, the KvLQT1 gene was found to encompass chromosomal rearrangements associated with the Beckwith-Wiedemann syndrome which causes prenatal overgrowth and cancer (3). Thus, the same gene at locus 11p15.5 could be implicated in at least three distinct genetic disease entities. The KvLQT1 gene product is a six-transmembrane domain protein which, in association with a membranous regulator termed IsK (previously known as minK), produces a K⁺ current with physiological characteristics similar to those of the major component of the delayed rectifier sustained K⁺ current in cardiac myocytes (4, 5). KvLQT1 expression is not limited to the heart muscle but can also be observed in human pancreas, lung, kidney, inner ear, and placenta (4–6). In autosomal dominant long QT syndrome 1, mutations in KvLQT1 supposedly decrease the amount of K⁺ current available for repolarization, thereby prolonging the cardiac action potential and inducing arrhythmias. In the recessively inherited Jervell and Lange-Nielsen syndrome, bi-allelic mutations in KvLQT1 (2) not only alter cardiac electrophysiology but also inner ear endolymph homeostasis, leading to congenital deafness.

In the present work, we have cloned an N-terminal truncated isoform of the KvLQT1 gene product corresponding to KvLQT1 isoform 2 simultaneously identified by Lee et al. (3) and Yang et al. (7). It was determined that KvLQT1 isoform 2, which is constitutively expressed in adult heart muscle, is a strong dominant negative of KvLQT1 isoform 1. Our results demonstrate that the cardiac K⁺ channel responsible for the long QT syndrome 1 is a complex protein assembly composed of isoform 1 (the channel pore), isoform 2 (a dominant negative), and IsK (the channel regulator). These findings could have important physiopathological implications.

**EXPERIMENTAL PROCEDURES**

Cloning of KvLQT1 Isoform 2—To obtain the 5’ sequence of KvLQT1, a rapid amplification of cDNA ends (RACE)³ experiment was performed using a Marathon cDNA Amplification kit and Marathon ready cDNA from human adult heart (CLONTECH). Two nested primers (LQTN1, 5’ GAT GTA CAG GGT GGT TAT CAG C 3’; and LQTN2, 5’ TGA TGG CGA TGA TGG AAA TGG G 3’) were derived from the previously published partial KvLQT1 sequence (1), and RACE was performed according to the manufacturer’s protocol. The fragment of 320 bp obtained was cloned in pUC 18 using the Sure Clone Ligation kit (Pharmacia Biotech Inc.). Sequencing reactions were subsequently performed on two independent clones using the dye terminator cycle sequencing kit (Applied Biosystems). Sequencing reactions were analyzed on 4.0% polyacrylamide gels using an ABI-377 automated DNA sequencer (Applied Biosystems). The sequence of both clones was identical, revealing a start codon 6 bp upstream from the previously published sequence (1).

Molecular Expression of KvLQT1 Isoforms 1 and 2—Human heart RNA was prepared using left or right ventricular tissues from the explanted heart of a patient with cystic fibrosis. Right atrial appendage biopsies were also obtained during open heart surgery from six patients with coronary heart disease. Tissue samples were quick-frozen in liquid nitrogen, and total RNA was then isolated separately from each sample using the classical guanidinium isothiocyanate method (8). RNA samples were quantified by spectrophotometric analysis. The lack of degradation of RNA samples was monitored by the observation of appropriate 28 S to 18 S ribosomal RNA ratios as determined by ethidium

³ The abbreviations used are: RACE, rapid amplification of cDNA ends; bp, base pair(s); RT-PCR, reverse transcriptase-polymerase chain reactions; HA, hemagglutinin; PBS, phosphate-buffered saline.
bromide staining of the agarose gel. Reverse transcriptase-polymerase chain reactions (RT-PCR) were performed on total RNA. cDNA was synthesized from approximately 1 μg of RNA. RT-PCR reactions contained 5 mM MgCl$_2$, 1 × PCR Buffer II, 1 μM dNTP, 1 unit/μl RNAse inhibitor, 2.5 units/μl murine leukemia virus reverse transcriptase (Perkin-Elmer), and 0.75 μl primers in a final volume of 20 μl. Controls without reverse transcriptase were performed to check that amplification did not proceed from residual genomic DNA. cDNA was used for PCR reactions with 2 μM MgCl$_2$, 1 × PCR Buffer II, 2.5 units/100 μl AmpliTaq DNA polymerase (Perkin-Elmer), and 0.5 μM primers (Genosys) in a final volume of 100 μl. PCR reactions were performed as follows: DNA was denatured (95°C for 4 min), followed by 35 cycles of 92°C for 1 min, 57°C for 1 min, and 72°C for 2 min, and finally extended at 72°C for 7 min. Ten microliters of each PCR product were analyzed by electrophoresis on a 2% agarose gel (Pharmacia) followed by ethidium bromide staining. Sequences of the sense (F1) and antisense (R) oligonucleotide primers for human KvLQT1 isoform 1 are shown in Fig. 1. The sense primer (F2) used for specific detection of human KvLQT1 isoform 2 is also shown in Fig. 1. Sense 5′-TCTC-GTCTACACCAACCAGGG-3′ and antisense 5′-TTCACAGCACTACAGCACGAGC-3′ were used for Isk. Sense 5′-GGCATCGTGGATTGCTCCG-3′ and antisense 5′-GCTGAAAGGTGGCAGAGCGA-3′ were used for human β-actin. The expected fragment lengths calculated from cDNA sequences were 245, 232, and 215 bp for KvLQT1 isoform 1, isoform 2, Isk, and β-actin, respectively.

Three different cDNAs were used as hybridization probes for RNase protection assays. The first was a human 274-bp KvLQT1 isoform 1 cDNA corresponding to the 5′ 37 nucleotides upstream and 237 nucleotides downstream from the start codon (Fig. 1). This cDNA contains 62 nucleotides specific for isoform 1, which in an RNase protection assay provides a 274-nucleotide isoform 1-specific protected fragment and a 212-nucleotide protected fragment that hybridizes non-isotopically 1 transcript. The probe was PCR-cloned from the expression vector pCI-KvLQT1 (see below) using tailing primers between the BamHI and HindIII sites of a pBluescript SK vector (Strategene). The second probe was a human 347-bp KvLQT1 isoform 2 cDNA corresponding to the 25′ 27 nucleotides upstream and the 320 nucleotides downstream from the start codon. This cDNA contains 53 nucleotides specific for isoform 2, which in an RNase protection assay provides a 347-nucleotide isoform 2-specific protected fragment and a 314-nucleotide non-isotopically 2 protected fragment. This probe was subcloned from the expression vector pCE-KvLQT1 between the HindIII and PstI sites of a pBluescript SK vector (Strategene). The third probe was a human 393-bp cDNA corresponding to the human KvLQT1 sequence (clone pCMV/HisK).

Antisense RNA probes were produced using T7, T3, and SP6 polymerases (Ambion) on linearized KvLQT1 isoform 1, KvLQT1 isoform 2, and Isk templates, respectively, in the presence of [α-32P]UTP (NEN Life Science Products). RNase protection assays were carried out as described previously (9) using the RPA II Kit (Ambion). For each tissue source, 15 μg of total RNA were hybridized to 0.5–1 × 10$^8$ cpm of the RNA probe, which was run after denaturing in formamide, 8 μl urea gel. Sense KvLQT1 isoform 1 RNA was also hybridized and was run on the same gel for use as a size marker (not illustrated), as well as undigested RNA probes. Negative controls were run using t-yeast RNA hybridized with the isoform 1 and isoform 2 probes. Gels were exposed for 48 h at −80 °C on x-ray films (Biomax-M, Eastman Kodak Co.) with an intensifying screen. Autoradiograms of RNase protection assays were subjected to densitometric analysis using the ImageQuant program (Molecular Dynamics). The ratios of KvLQT1 isoform 1 and KvLQT1 isoform 2 mRNA levels were determined directly from specific signals since respective protected bands were hybridized with the same probe.

Intracellular Injection of Plasmids—The kidney-derived COS-7 cell line was obtained from the American Type Culture Collection. Cells grown on plastic Petri dishes were microinjected with plasmids at day 1 after plating. Our protocol to microinject cell cultured cells using the Eppendorf ECET microinjector 5246 system has been reported in detail elsewhere (10). Plasmids were diluted at a final concentration of 1–5 μg/ml in a buffer composed (in mM): HEPES 50, NaOH 50, NaCl 40, pH 7.4, supplemented with fluorescein isothiocyanate isomer I (excitation 490 nm, emission 520 nm). Human KCNQ1 isoform 1 (Ref. 26; GenBank accession number AF000571), KvLQT1 isoform 2, as well as Isk cDNAs were subcloned into the mammalian expression vector pCI (Promega, Madison, WI) under the control of a cytomegalovirus enhancer/promoter. The KvLQT1 isoform 2 construct was derived from the isoform 1 pCI plasmid using polymerase chain reaction and tailed primers. A green fluorescence protein pCI plasmid (a kind gift from Dr. Rainer Waldmann, Sophia-Antipolis, France) was used as an inert plasmid to ensure that cells were always injected with a constant 15 μg/ml plasmid concentration.

Patch-clamp Recordings—Whole cell currents were recorded as described previously (10). A Petri dish containing cells was placed on the stage of a Nikon Diaphot inverted microscope (Nikon) with a coverslip (Perkin-Elmer), and 0.75 μM primers (Genosys) were used for priming the standard extracellular solution containing (in mM) NaCl 145, KCl 4, MgCl$_2$ 1, CaCl$_2$ 1, HEPES 5, glucose 5, pH adjusted to 7.4 with NaOH. Patch pipettes with a tip resistance 2.5–5 MΩ were electrically connected to a patch-clamp amplifier (Axon Instruments). The intracellular medium contained (in mM): potassium gluconate 145, HEPES 5, MgCl$_2$ 1, CaCl$_2$ 1, glucose 5, pH 7.2 with NaOH, whereas the extracellular medium used to record pure K$^+$ currents contained (in mM): sodium gluconate 145, potassium gluconate 4, 1/2 calcium gluconate 7 (free Ca$^{2+}$, 1), 1/2 magnesium gluconate 4 (free Mg$^{2+}$, 1), HEPES 5, glucose 5, pH 7.2 with NaOH. Stimulation, data recording, and analysis were performed through an A/D 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 the mean ± S.E. The statistical significance of the observed effects was assessed by means of the standard t test.

Immunocytochemistry and Immunoblotting of Epitope-tagged KvuLQT1 Constructs—The sequence encoding the influenza hemagglutinin (HA) epitope (YPYDVPDYA) was inserted in frame with the KvLQT1 isoform 1 coding sequence after nucleotide position 654 (see GenBank accession number AF000571). This position corresponds to the second external loop of the protein, between serine 218 and lysine 219. pCI-KvLQT1 isoform 1 plasmid was digested with EcoRI and AfII, and the 716-bp fragment so produced was subcloned into pUC 19 and then digested with BamHI and PstI. A sense oligonucleotide containing the tag sequence 5′-TACCCATAGCTTCGAGATACGGT-3′ was annealed with its reverse oligonucleotide and inserted between BamHI and PstI sites. The resulting product was digested with EcoRI and AfII and subcloned into the pCI-KvLQT1 isoform 1 plasmid. To insert the HA tag into KvLQT1 isoform 2 coding sequence, a PCR reaction was performed using the epitope-tagged KvLQT1 isoform 1 plasmid as a template. Epitope-tagged KvLQT1 plasmids were checked by sequencing. Functional expression of the epitope-tagged plasmids was verified by patch-clamp analysis of transfected cells (data not shown).

For immunoblotting experiments, COS-7 cells cultured in 25-cm$^2$ flasks were transfected with tagged pCI-KvLQT1 plasmids using 22-kDa polyethyleneimine as a polycationic transfection vector. Twenty-four hours after transfection, 100 μl were washed twice with cold phosphate-buffered saline (PBS), incubated with 200 μl of SDS gel-loading buffer (50 mM Tris-HCl, pH 6.8, 100 mM dithiothreitol, 2% SDS, 0.1% bromphenol blue, 10% glycerol), and scanned. The samples were placed in a boiling water bath for 10 min, and the chromosomal DNA was sheared by sonication for 2 min. Samples were centrifuged at 10,000 × g for 10 min at room temperature, and the supernatant was used for protein determination. SDS-polyacrylamide gel electrophoresis was performed on 10% slabs and transferred to Immobilon-P polyvinylidene difluoride membrane (Sigma). The blots were probed with a 1:1000 dilution of a biotin-conjugated goat anti-mouse antibody (Sigma). The membrane was washed three times with cold PBS and incubated for 30 min in 1% non-fat dry milk and 0.1% Tween 20 and incubated with the blots for 1 h at room temperature. After primary antibody reaction, the filters were rinsed five times with quench buffer and incubated for 1 h at room temperature in a 1:1000 dilution of a biotin-conjugated goat anti-mouse antibody (Sigma). The blots were then incubated for 30 min with conjugated extravidin peroxidase and finally developed by the enhanced chemiluminescence Western blotting system. Biomax-MR Kodak films were used to detect protein in the blot.

For in situ immunocytochemistry, transfected COS-7 cells seeded on 12-mm collagen-coated coverslips were washed three times with PBS supplemented with 1 mM Ca$^{2+}$ and 0.5 mM Mg$^{2+}$ and incubated with the monoclonal anti-HA antibody dilution 1:20 in PBS, 0.5% ovalbumin in 1 h at 37 °C. Cells were washed three times and incubated for 30 min at room temperature with a fluorescein isothiocyanate conjugated goat anti-mouse antibody (Sigma) diluted at 1:25 in PBS, 0.5% ovalbumin. The cells were subsequently washed and then fixed using a 15-min incubations.
tion with 4% formaldehyde. The glass coverslips were further washed and then mounted in a mounting medium (Citifluor) to prevent photobleaching. Staining was observed using a standard epifluorescence oil-immersion microscope (Axioskop; Carl Zeiss, Oberkochen, Germany).

RESULTS

Cloning—5′-RACE experiments performed on human heart cDNA identified a clone predicted to encode a truncated KvLQT1 protein lacking an intracytoplasmic N-terminal tail. The predicted N-terminal sequence (Fig. 1) was only 2 amino acids (Met and Asp) longer than the previously published partial sequence (1) and 131 amino acids shorter than the cardiac KvLQT1 isoform 1 (6). A 27-bp sequence identified 5′-upstream from the start codon matched the KvLQT1 exon 1b sequence recently published by Lee et al. (3).

RNA Expression in the Heart—When RT-PCR primers specific for each isoform (Fig. 1) were used, both full-length and N-terminal truncated KvLQT1 isoform RNAs were detected in human ventricular tissues from an explanted heart (Fig. 2). Isoform 2 mRNA was also detected in six right atrial appendages sampled during open heart cardiac surgery (Fig. 2). KvLQT1 expression was further quantified in adult human cardiac muscle using an RNase protection assay (Fig. 3). The probe used for isoform 2 mRNA detection spanned 347 bp and covered a portion of isoform 2-specific 5′-coding and non-coding sequences as well as a 314-bp sequence common to isoforms 1 and 2 RNA. Thus, with our isoform 2 probe, two RNA fragments were RNase-protected as follows: a 347-bp sequence corresponding to isoform 2 and a 314-bp sequence corresponding to non-isoform 2 (Fig. 3). For isoform 1, the RNase protection hybridization probe covered a 274-bp segment which comprised a 62-bp fragment of isoform 1-specific 5′-sequence and a 212-bp segment common to isoform 1 and 2 sequences (Fig. 1). With this probe, the following two RNA fragments were RNase-protected: a 274-bp sequence corresponding to isoform 1 and a 212-bp sequence corresponding to non-isoform 1 (Fig. 3). This approach allowed direct comparison of the expression level of the different isoforms without the need for an internal marker. Densitometric analysis was used to compare isoform 1/non-isoform 1 bands and isoform 2/non-isoform 2 bands from cardiac tissues. This analysis revealed that isoform 1 and isoform 2 represented 71.9 ± 0.6 and 28.1 ± 0.6%, respectively, of the...
Functional Expression—We first investigated whether KvLQT1 isoform 2 is fully processed to the cell membrane. To this end, COS-7 cells were transfected with epitope-tagged plasmids encoding KvLQT1 isoform 1 or KvLQT1 isoform 2. In our tagged constructs, the influenza hemagglutinin (HA) flag was placed within the second extracellular loop of the channel protein so that immunodetection of the tag could be performed in non-permeabilized, non-fixed cells. Fig. 4A shows that specific immunostaining appeared as dots situated at the membrane surface of cells transfected with tagged isoform 1 and isoform 2 but not at the surface of cells transfected with an untagged plasmid. Western blot experiments performed in COS-7 cells transfected with HA-tagged plasmids confirmed that KvLQT1 isoform 1 and KvLQT1 isoform 2 encode proteins at the molecular mass (e.g., 75 and 61 kDa, respectively) predicted by their coding sequences. However, as shown in Fig. 4B, two different bands for each isoform were visible on the immunoblot. We hypothesized that the lower band may correspond to the unglycosylated form of the protein, whereas the upper band may correspond to the glycosylated form (1).

Functional expression experiments were conducted using untagged plasmids. In these experiments, we used the intranuclear plasmid injection technique to ensure precise control of the relative expression of the different transgenes. COS-7 cells intranuclearly injected with 50 μg/ml KvLQT1 isoform 2 exhibited no detectable voltage-activated \( \Delta F \) current either in the absence (Fig. 5A) or presence (Fig. 6A) of the IsK regulator. To investigate a possible effect related to isoform 2 expression, isoform 2 was co-injected with isoform 1 in the absence or presence of IsK. In these experiments, a green fluorescence protein plasmid (pGFP) was used as an inert plasmid substitute to ensure that the total plasmid concentration injected was always 15 μg/ml. Cells injected with 5 μg/ml KvLQT1 isoform 1 (plus 10 μg/ml pCI-GFP) exhibited a voltage-activated \( \Delta F \) current characterized by fast activation kinetics and slow deactivation kinetics (Fig. 5A and Table I). Cells co-injected with 5 μg/ml isoform 1 plus 5 μg/ml isoform 2 (plus pCI-GFP, 5 μg/ml) displayed no detectable \( \Delta F \) current (not illustrated). Cells co-injected with 5 μg/ml isoform 1 plus 1 μg/ml isoform 2 (plus pCI-GFP, 9 μg/ml) exhibited a \( \Delta F \) current with similar kinetics (Table I) but with markedly reduced amplitude (Fig. 5, A and B). Fig. 5C shows that the activation curve for the isoform 1-related \( \Delta F \) current was not affected by the presence of isoform 2. In six different experiments during which current tail protocols were applied, the ionic selectivity of the KvLQT1 isoform 1 current was not modified by the presence of isoform 2 (not illustrated). A series of experiments was also performed in the presence of IsK (Fig. 6). Cells co-injected with 5 μg/ml isoform 1 and 5 μg/ml IsK (plus pCI-GFP, 5 μg/ml) exhibited a

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**Fig. 4. Immunodetection and immunoblots of HA-tagged KvLQT1 in transfected COS-7 cells.** A, immunolabeling of non-permeabilized COS-7 cells transfected with HA-tagged KvLQT1 isoform 1 (Iso 1) or isoform 2 (Iso 2) plasmids or with an untagged KvLQT1 isoform 1 plasmid (Cont). B, immunoblots of cell lysates from COS-7 cells transfected with HA-tagged KvLQT1 isoform 1 (Iso 1) or isoform 2 (Iso 2) plasmids or with an untagged KvLQT1 isoform 1 plasmid (Cont). Molecular mass standards indicated are \( \alpha \)-galactosidase (114 kDa) and bovine serum albumin (81 kDa).

**Fig. 5. Whole cell \( \Delta F \) currents in COS-7 cells injected with KvLQT1 cDNA in the absence of IsK.** A, typical current traces obtained in a control COS-7 cell (C), in a cell injected with 50 μg/ml pCI-KvLQT1 isoform 2 plasmid (Iso 2), in a cell injected with 5 μg/ml pCI-KvLQT1 isoform 1 plasmid (Iso 1), and in a cell co-injected with 5 μg/ml pCI-KvLQT1 isoform 1 plus 1 μg/ml pCI-KvLQT1 isoform 2 plasmids (Iso 1 + Iso 2). In these experiments, cells were also co-injected with a pCI-GFP plasmid, so the total plasmid concentration was always 15 μg/ml. Superimposed current traces were obtained by voltage steps, 20-mV increment, applied from -100 to +60 mV. Holding potential: -80 mV. Current scale: 5 pA/pF. Time scale: 200 ms. B, tail current voltage relations for KvLQT1 current. Circles are from a 5 μg/ml pCI-KvLQT1 isoform 1 plasmid-injected cell (same cell as in A). Squares are from a 5 μg/ml pCI-KvLQT1 isoform 1 and 1 μg/ml pCI-KvLQT1 isoform 2 co-injected cell (different cell from A). Triangles are from the 50 μg/ml pCI-KvLQT1 isoform 2-injected cell shown in A. C, averaged KvLQT1 activation curves in the presence (squares, \( n = 5 \)) and absence (circles, \( n = 5 \)) of isoform 2. \( V_{0.5} \) denotes the potential for half-maximal activation.
The N-terminal end of KvLQT1 appears to be essential for the function but not for the mediation of the subunit association to form the tetrameric K⁺ channel complex. This situation differs notably from the classic status for the voltage-dependent K⁺ channel in which a domain designed as “NAB” domain, conserved in the cytoplasmic N terminus, is crucial for subunit-specific multimerization (13). Such a NAB domain is not found as is the case for insulin-like growth factor 2, for example, which has multiple promoters indicative of developmental and tissue dependence (12).

The N-terminal region of the KvLQT1 gene product is constitutively expressed in adult human heart and that this truncated isoform is a strong dominant negative of KvLQT1 isoform 1 K⁺ channels. The isoform 2 coding sequence reported here corresponds to that identified by Yang et al. (7) and is referred to as variant B. In Northern blot analysis, variant B expression was demonstrated in the heart but not in other organs (7). At the genomic level (3), isoform 2 comprises exon 1b, exon 1, and exon 2 but lacks exon 1c of the KvLQT1 gene. Exon-trapping and exon-connection experiments (3) clearly demonstrate that isoforms 1 and 2 are splice variants. The KvLQT1 gene spans a large region of at least 300 kilobase pairs and comprises a minimum of 14 exons (3). Although the KvLQT1 promoter region has not yet been identified, it can be inferred that isoform 1 and isoform 2 use either the same promoter or, alternatively, a different promoter as is the case for insulin-like growth factor 2, for example, which has multiple promoters indicative of developmental and tissue dependence (12).

**Table I**

| Iso 1 | Iso 2 | IsK | n | τₚₑₓ | τₑₓ | τₑₓ | τₑₓ |
|------|------|-----|---|------|-----|-----|-----|
| 5    | 0    | 14  |   | 18.4±2.0 | 254.2±28.5 | 108.8±27.0 |   |
| 5    | 1    | 8   |   | 23.6±5.8  | 178.1±14.3  | 91.9±11.3  |   |
| 5    | 0    | 5   |   | 150.6±22.0 |   | 75.4±8.9 |   |
| 5    | 1    | 7   |   | 242.4±40.8 |   | 73.1±7.2 |   |
| 5    | 2    | 5   |   | 183.9±20.9 |   | 63.7±15.6 |   |

**Fig. 6.** Whole cell KvLQT1 K⁺ currents in COS-7 cells co-injected with 5 μg/ml pCI-Iso plasmid. The same abbreviations and protocols as in Fig. 5 are used. A, current traces obtained in a cell injected with 50 μg/ml pCI-Iso plasmid (Iso 2), in a cell injected with 5 μg/ml pCI-Iso plasmid (Iso 1), and in a cell co-injected with 5 μg/ml pCI-Iso plasmid plus 1 μg/ml pCI-Iso plasmid (Iso 1 + Iso 2). Time scale: 200 ms for Iso 2 and 500 ms for the Iso 1 and Iso 1 + Iso 2. Current scale: 5 pA/pF. B, corresponding current voltage relations in cells injected with isoform 1 (circles), isoform 1 plus isoform 2 (squares), and with isoform 2 (triangles) cDNA. Same cells as in A, averaged KvLQT1 activation curves in the presence (squares, n = 4) and absence (circles, n = 5) of isoform 2. V₀₅ denotes the potential for half-maximal activation.

**Discussion**

Our results demonstrate that an N-terminal truncated isoform of the KvLQT1 gene product is constitutively expressed in adult human heart and that this truncated isoform is a strong voltage-activated K⁺ current with a similar amplitude but slower activation kinetics than cells injected with isoform 1 alone (Table I). Again, co-injection of isoform 2 (1 μg/ml) together with isoform 1 (5 μg/ml) consistently reduced the K⁺ current amplitude in the presence of IsK (Fig. 6, A and B). As shown in Fig. 6C, the presence of isoform 2 did not change the half-activation voltage but shifted the activation threshold by ~20 mV to a more depolarized voltage. Again, in the presence of IsK, isoform 2 did not alter the activation and deactivation kinetics of the isoform 1-related K⁺ current (Table I). As summarized in Fig. 7, isoform 2 reduced average current tail amplitude dose-dependently. In the presence of 1, 2, and 5 μg/ml isoform 2 but without IsK, the current tail amplitude induced by 5 μg/ml isoform 1 was reduced to 12.6, 4.7, and 0%, respectively, of its control value. In the presence of 5 μg/ml IsK, the same concentrations of isoform 2 reduced current tail amplitude to 47, 33, and 1.8% of its control value, respectively. Thus, the current related to isoform 1 expression was more sensitive to the negative dominance of isoform 2 in the absence than in the presence of IsK. Fig. 7 also shows that the dominant negative effects produced by 1 μg/ml isoform 2 were partially reversed by increasing the IsK plasmid concentration in the injected mixture from 5 to 9 μg/ml.
FIG. 7. Effects of KvLQT1 isoform 2 on the averaged current tail density related to isoform 1 expression. The left panel summarizes data obtained in the absence of IsK, and the right panel summarizes data obtained in the presence of IsK. The tail amplitude of the current related to isoform 1 expression was determined for variable isoform 1/isoform 2 cDNA ratios in the injected mixture. Respective concentrations (µg/ml) in the injection mixture for isoform 1 (Iso 1), isoform 2 (Iso 2), and IsK plasmids are indicated on the x axis. Cells were either co-injected with a pCI-GFP plasmid so the total plasmid concentration was always 15 µg/ml. * denotes p < 0.05, and *** denotes p < 0.001 when comparison is made with cells injected with KvLQT1 isoform 1 alone. NS is non-significant. Numbers in parentheses indicate n values.

In our experimental conditions, the amplitude of the current induced by KvLQT1 isoform 1 expression approximated that induced by coexpression of isoform 1 plus IsK. However, previous findings for transfected COS and Chinese hamster ovary cells as well as Xenopus oocytes (4, 5, 15) showed that the channel regulator not only affected activation kinetics (as with our cells) but also markedly increased its density. Differences in experimental procedures may account for this discrepancy, particularly with Xenopus oocytes in which IsK-induced KvLQT1 current is highly temperature-dependent (16). Previous experiments were performed at 20–22 °C, whereas in our study a physiological temperature (37 °C) was chosen. Moreover, we used direct cDNA injection, whereas previous studies chose classical synthetic vector-mediated gene transfer.

In the heart ventricle, we observed that ~30% KvLQT1 mRNA expression is linked to isoform 2, and ~70% is linked to isoform 1. This corresponds to the 5/2 isoform 1/isoform 2 cDNA ratio used in COS-7 expression experiments. In the absence of IsK, this ratio produced only 4.7% of the current detected in the absence of isoform 2. However, in the presence of 5 µg/ml IsK cDNA, injection of the 5/2 ratio produced 33% of the current detected in the absence of isoform 2. Our findings suggest that the characteristics of endogenous K+ current available for cardiac repolarization depend on the relative expression of the following three different proteins: isoform 1, isoform 2, and IsK. In the presence of IsK, isoform 2 expression shifted the KvLQT1 activation voltage toward more depolarized potentials (see Fig. 6C), providing a better representation of the activation curve of the slow component of the endogenous cardiac delayed rectifier K+ current (17). Moreover, the dominant negative effects of isoform 2 were increased in the absence of IsK. This property may contribute to the inner ear defect observed in IsK knock-out mice (18), although isoform 2 expression has not yet been demonstrated in the inner ear during embryonic development or after birth.

Mutations in the following three genes are considered responsible for the dominantly inherited Romano-Ward long QT syndrome: (i) KvLQT1 localized to chromosome band 11p15.5, responsible for the most frequent LQT1 phenotype (19); (ii) HERG, a K+ channel encoding gene at 7q35–36 and responsible for the LQT2 phenotype (20); and (iii) SCN5A, a voltage-dependent Na+ channel encoding gene at 3p21–24 responsible for the LQT3 phenotype (21). A fourth locus (LQT4) has been identified by our laboratory at 4q25–27 (22). The vast majority of the mutations responsible for LQT1 lie within the KvLQT1 transmembrane domains and the pore region (Refs. 1 and 2) and thus supposedly affect both isoform 1 and isoform 2. In the heart of a Romano-Ward LQT1 patient, the delayed rectifier K+ current available for repolarization would result from the association within heterotetrameric channel protein complexes of the following: (i) mutated isoform 1, (ii) wild-type isoform 1, (iii) mutated isoform 2, (iv) wild-type isoform 2, and (v) IsK proteins. This implies that the functional effects of KvLQT1 mutations on isoform 2 negative dominance also need to be evaluated. Indeed, a mutation within isoform 2 could very well reduce or even suppress its dominant negative effect on wild-type isoform 1. Paradoxically, this would increase the K+ current related to KvLQT1 isoform 1 expression from the unaffected allele since less dominant negative isoform 2 transcripts would be available. Therefore, depending on the effects of a familial mutation on isoform 2, the amplitude of the delayed rectifier K+ current available for cardiac repolarization (and hence the phenotype) may vary. Current research programs in our laboratory are exploring the consequences of familial mutations on isoform 2 negative function.

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Addendum—Recently, Jiang et al. (23) reported that KvLQT1 isoform 2 exerts strong dominant negative effects on KvLQT1 isoform 1-related current recorded in Xenopus oocytes but does not affect the current produced by expression of Kv1.4, Kv4.3, or HERG.

REFERENCES
1. Wang, Q., Curran, M. E., Splawski, I., Burn, T. C., Millholland, J. M., Van Raay, T. J., Shen, J., Timothy, K. W., Vincent, G. M., De Jager, T., Schwartz, P. J., Towbin, J. A., Moss, A. J., Atkinson, D. L., Landes, G. M., Conners, T. D., and Keating, M. T. (1996) Nat. Genet. 12, 17–23
2. Neyroud, N., Tesson, F., Derjouy, I., Leibovich, M., Donger, C., Barhanin, J., Fauré, S., Gary, F., Coumel, P., Petit, C., Schwartz, K., and Guicheney, P. (1997) Nat. Genet. 15, 186–189
3. Lee, M. P., Hu, R. J., Johnson, L. A., and Feinberg, A. P. (1997) Nat. Genet. 15, 181–185
4. Barhanin, J., Lesage, F., Guillemaire, E., Fink, M., Lazzouni, M., and Romey, G. (1996) Nature 384, 78–80
5. Sanguinetti, M. C., Curran, M. E., Spector, P. S., Zou, A., Shen, J., Atkinson, D., and Keating, M. T. (1996) Nature 384, 80–83
6. Chouabe, C., Neyroud, N., Guicheney, P., Lazzouni, M., Romey, G., and Barhanin, J. (1997) EMBO J. 17, 5472–5479

P. Guicheney, personal communication.
7. Yang, W. P., Levesque, P. C., Little, W. A., Lee Conder, M., Shalaby, F. Y., and Blanar, M. A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4017–4021
8. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
9. Péront, Y., Navarro, J., Sorrentino, V., Louboutin, J. P., Noireaud, J., and Palade, P. T. (1997) Pfluegers Arch. 433, 221–229
10. Mohammad-Panah, R., Demolombe, S., Riochet, D., Loussouarn, G., Leblais, V., Baró, I., and Escande, D. (1998) Am. J. Physiol., in press
11. Wilson, I. A., Niman, H. L., Houghten, R. A., Cherenson, A. R., Connolly, M. L., and Lerner, R. A. (1984) Cell 37, 767–778
12. Vu, T. H., and Hoffman, A. R. (1994) Nature 371, 714–717
13. Xu, J., Yu, W., Jan, Y. N., Jan, L. Y., and Li, M. (1995) J. Biol. Chem. 270, 24761–24768
14. MacKinnon, R. (1991) Nature 350, 232–235
15. Romey, G., Attali, B., Chouabe, C., Abitbol, I., Guillemare, E., Barhanin, J., and Lazdunski, M. (1997) J. Biol. Chem. 272, 16713–16716
16. Busch, A. E., and Lang, F. (1993) FEBS Lett. 334, 221–224
17. Sanguinetti, M. C., and Jurkiewicz, N. K. (1990) J. Gen. Physiol. 96, 195–215
18. Vetter, D. E., Mann, J. R., Wangemann, P., Liu, J., McLaughlin, K. J., Lesage, F., Marcus, D. C., Lazdunski, M., Heinemann, S. F., and Barhanin, J. (1996) Neuron 17, 1251–1264
19. Roden, D. M., Lazzara, R., Rosen, M., Schwartz, P. J., Towbin, J., and Vincent, G. M. (1996) Circulation 94, 1996–2012
20. Curran, M. E., Saplaki, L., Timothy, K. W., Vincent, G. M., Green, E. D., and Keating, M. T. (1995) Cell 80, 785–803
21. Wang, Q., She, J., Saplaki, L., Atkinson, D., Li, Z., Robinson, J. L., Moss, A. J., Towbin, J. A., and Keating, M. T. (1995) Cell 80, 805–811
22. Schott, J. J., Charpentier, F., Feltier, S., Foley, P., Drouin, E., Bouhour, J. B., Donnelly, P., Vergnaud, G., Rischner, L., Moisan, J. P., Le Marec, H., and Pascal, O. (1995) Am. J. Hum. Genet. 57, 1114–1122
23. Jiang, M., Tseng-Crank, J., and Tseng, G.-N. (1997) J. Biol. Chem. 272, 24109–24112
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