Properties of KvLQT1 K⁺ channel mutations in Romano–Ward and Jervell and Lange-Nielsen inherited cardiac arrhythmias

Christophe Chouabe, Nathalie Neyroud¹,
Pascale Guicheney¹, Michel Lazdunski²,
Georges Romey and Jacques Barhanin

Institut de Pharmacologie Moléculaire et Cellulaire, CNRS, 660 route des Lucioles, Sophia Antipolis, 06560 Valbonne and ¹INSERM U153, Groupe Hospitalier Pitié-Salpêtrière, Institut de Myologie, 47 boulevard de l'Hôpital, 75651 Paris Cedex, France

²Corresponding author

Mutations in the delayed rectifier K⁺ channel subunit KvLQT1 have been identified as responsible for both Romano–Ward (RW) and Jervell and Lange-Nielsen (JLN) inherited long QT syndromes. We report the molecular cloning of a human KvLQT1 isoform that is expressed in several human tissues including heart. Expression studies revealed that the association of KvLQT1 with another subunit, IsK, reconstitutes a channel responsible for the *I_{Ks}* current involved in ventricular myocyte repolarization. Six RW and two JLN mutated KvLQT1 subunits were produced and co-expressed with IsK in COS cells. All the mutants, except R555C, fail to produce functional homomeric channels and reduce the K⁺ current when co-expressed with the wild-type subunit. Thus, in both syndromes, the main effect of the mutations is a dominant-negative suppression of KvLQT1 function. The JLN mutations have a smaller dominant-negative effect, in agreement with the fact that the disease is recessive. The R555C subunit forms a functional channel when expressed with IsK, but with altered gating properties. The voltage dependence of the activation is strongly shifted to more positive values, and deactivation kinetics are accelerated. This finding indicates the functional importance of a small positively charged cytoplasmic region of the KvLQT structure where two RW and one JLN mutations have been found to take place.

Keywords: delayed-rectifier/dominant-negative/*I_{Ks}*/IsK/long QT/MinK

Introduction

Long QT (LQT) syndromes are inherited cardiac disorders characterized by prolonged QT interval on the ECG associated with syncopal attacks, and high risk of sudden death due to ventricular tachyarrhythmia (Schwartz, 1985). These LQT syndromes include the autosomal dominant Romano–Ward (RW) syndrome (Romano, 1965; Ward, 1964) and the autosomal recessive Jervell and Lange-Nielsen (JLN) syndrome (Jervell and Lange-Nielsen, 1957) as well as sporadic forms. In the case of the JLN syndrome, the patient suffers from a severe bilateral deafness in addition to the cardiac disorder. Although congenital LQTs

are not a frequent diagnosis, acquired forms of abnormal repolarization and susceptibility to arrhythmia are very common. Thus inherited LQT syndromes provide an opportunity to study these life-threatening cardiac disorders at the molecular level.

A recent flurry of publications described multiple mutations within two K⁺ channel genes that cause the RW and JLN syndromes. Mutations in the *HERG* gene are responsible for the chromosome 7-linked RW syndrome (LQT2) (Curran *et al.*, 1995), while different mutations in the single *KVLQT1* gene can cause either a chromosome 11-linked RW (LQT1) (Wang *et al.*, 1996) or the JLN (Neyroud *et al.*, 1997) syndrome. It has also been reported that the *KVLQT1* gene is imprinted in most tissues, with the exception of the heart, and it has been suggested that it could be involved in the development of the overgrowth malformation disorder called Beckwith–Wiedemann syndrome (Lee *et al.*, 1997).

The *KVLQT1* gene encodes the pore-forming subunit of a K⁺ channel, but this subunit has to co-assemble with another transmembrane protein, IsK, to produce the cardiac slow delayed rectifier current known as *I_{Ks}* (Sanguinetti and Jurkiewicz, 1990; Attali, 1996; Barhanin *et al.*, 1996; Sanguinetti *et al.*, 1996). No mutation in the IsK gene has yet been reported to be responsible for a human cardiac dysfunction, but mice with a null mutation of this gene display inner ear defects very similar to those of JLN patients (Vetter *et al.*, 1996).

The manifestations of the LQT disease are variable, with a high incidence of sudden death in some affected families but not in others (Roden *et al.*, 1996). This observation suggests that different mutations could account for different phenotypes in affected families. In this study, we have cloned a full-length KvLQT1 cDNA from human kidney and we demonstrate that this cDNA is also the major form found in cardiac tissue. It was then used as a template to produce eight mutated subunits found in ten RW and four JLN families (C.Donger, I.Denjoy, M.Berthet, N.Neyroud, C.Cruaud, M.Bennaceur, G.Chivoret, K.Schwartz, P.Coumel and P.Guicheney, in preparation) for a functional expression study in transfected COS cells.

Results

Molecular cloning of human KvLQT1

The full sequence of the recently cloned mouse isoform of the *KVLQT1* gene was used to screen a human cDNA kidney library and four independent clones were isolated. The 3–3.2 kb inserts of these clones all bear the same ORF and differ only in the length of their 5' non-coding sequences. The ORF is 2028 nucleotides long, corresponding to a protein of 676 amino acids. This KvLQT1 sequence is aligned in Figure 1 with the previously published mouse heart and *Xenopus* oocyte

Fig. 1. Primary sequence alignment of the KvLQT1 cloned in this study with published [human pancreatic and *Xenopus* (Sanguinetti *et al.*, 1996); mouse (Barhanin *et al.*, 1996)] and *C.elegans* sequences. Putative transmembrane segment S1–S6, as well as the pore-associated P domain, are underlined. Amino acid mutations in RW (circles) and JLN (square or boxed) syndromes are indicated over the sequence, and the mutations analysed in this work are shown in black type on white. C25B8 (EMBL/DBJ/GenBank accession number U14556) is a *C.elegans* sequence issued from the Genome Sequencing Project (Wilson *et al.*, 1994). A sequence of 22 residues has been substituted with '22 aa' in the C25B8 sequence to aid in alignment. The nucleotide KvLQT1 kidney sequence is deposited in the EMBL/DBJ/GenBank database under the accession number AF000571).

sequences, and with the *Caenorhabditis elegans* sequence identified by a BLAST search in sequence databases. The kidney KvLQT1 protein is identical to that reported by Sanguinetti *et al.* (1996) in its C-terminus, up to the Gln107, but is very different in its N-terminal part

(Figure 1). We failed to detect the mRNA corresponding to this latter isoform in human heart following specific PCR amplification of the cDNA (not shown). Therefore, all the ensuing mutagenesis work has been performed on the kidney isoform.

Fig. 2. Northern blot analysis of the distribution of the KvLQT1 and IsK mRNA in human tissues. Each lane contains 2 μ g of poly(A)⁺. The KvLQT1 common and 5' probes correspond to the fragments 130–431 and 75–98 of the kidney amino acid sequence. For IsK, only the 3.4 kb transcript is shown. The small 0.8 kb transcript is also detected in the same tissues, but at a lower abundance. Autoradiograms exposed for 5, 7, 9 days and 6 h for the KvLQT1 common and 5', IsK and β -actin probes, respectively.

Tissue distribution

A DNA probe specific for the kidney sequence (corresponding to amino acids 75–98) was used to hybridize human multiple tissue Northern blots (Clontech). The tissue distribution of the 3.2 kb transcript was found to be very similar to the distribution revealed with a probe located in the core domain, being most abundant in heart, pancreas, prostate, kidney, small intestine and peripheral blood leukocytes, and less represented in placenta, lung, spleen, colon, thymus, testis and ovaries (Figure 2). Hybridization of the same blots with an IsK probe shows that some tissues such as heart, lung, kidney, testis, ovaries, small intestine or peripheral blood leukocytes have both KvLQT1 and IsK transcripts, with the level of IsK being generally lower than the level of KvLQT1 mRNA. Testis and ovaries are exceptions with a higher IsK mRNA level than that of KvLQT1. Conversely, pancreas, spleen, prostate and colon tissues have relatively high levels of KvLQT1 and no detectable IsK transcripts. It is unknown at this time whether KvLQT1 and IsK can co-assemble with other partners. However, it is likely that the ratio KvLQT1/IsK is variable in a given tissue depending upon regulating factors, such as hormones (Pragnell *et al.*, 1990; Drici *et al.*, 1996).

Functional expression of KvLQT1

The new human KvLQT1 cDNA coding sequence has been inserted in the pCI (Promega) vector for expression in COS cells. The KvLQT1 cDNA produced a functional K⁺ outward current when transfected alone, but co-expression with IsK drastically modified its characteristics. In Figure 3, the functional properties of the KvLQT1 and KvLQT1 + IsK currents are compared. As previously described for the mouse counterpart, the salient features are: (i) a 4-fold increase in the current density [10.2 ± 1.3 pA/pF at +30 mV ($n = 29$) for KvLQT1 and 43.1 ± 4.1 pA/pF ($n = 31$) for KvLQT1 + IsK, Figure 3C], (ii) a shift towards positive voltage values of the activation curve [$V_{0.5} = -23.6 \pm 1.6$ mV, slope factor $k = 11.1 \pm 0.9$ mV ($n = 8$) for KvLQT1 to $V_{0.5} = 19.6 \pm 2.0$ mV, $k = 24.1 \pm 0.6$ mV ($n = 24$) for KvLQT1 + IsK,

Fig. 3. Transient expression of KvLQT1 and KvLQT1 + IsK in transfected COS cells. (A and B) superimposed K⁺ currents in response to voltage pulses from -80 to $+60$ mV in 20-mV steps from a holding potential of -80 mV. Tail currents were elicited during repolarizations to -40 mV. (A) KvLQT1 alone. (B) KvLQT1 + IsK. (C–F) (○) KvLQT1 and (●) KvLQT1 + IsK. (C) K⁺ currents measured at the end of the test pulses normalized to cell capacitance (current density) and plotted against test potentials (mean $\pm$ SEM, $n = 22$). (D) Relative activation curves determined at the beginning of the deactivating tail currents after return to -40 mV (mean $\pm$ SEM, $n = 7$). Data points were fitted to a Boltzmann distribution. (E) Time constant of activation versus membrane potential obtained by fitting current traces with a single exponential (mean $\pm$ SEM, $n = 4$). (F) Time constant of deactivation versus membrane potential. Decay was fitted using a single exponential (mean $\pm$ SEM, $n = 5$). Inset: voltage protocol.

Figure 3D], (iii) a large decrease in the activation kinetic (Figure 3E) and (iv) only minor changes in the slow deactivation process [time constant at -40 mV of 621.4 ± 27.0 ms ($n = 10$), and 691.1 ± 21.1 ms ($n = 24$) for KvLQT1 and KvLQT1 + IsK, respectively, Figure 3F].

Localization of the mutations in the KvLQT1 channel protein

Mutations in *KVLQT1* have been reported to cause the 11-linked LQT1 RW syndrome (Russell *et al.*, 1996; Wang *et al.*, 1996; Tanaka *et al.*, 1997). We report 13 new naturally occurring mutations in the same K⁺ channel protein that are also associated with the LQT1 syndrome (Figure 1, Table I and C.Donger, I.Denjoy, M.Berthet, N.Neyroud, C.Cruaud, M.Bennaceur, G.Chivoret, K.Schwartz, P.Coumel and P.Guicheney, in preparation).

Table I. Properties of KvLQT1 (WT and mutant) currents in the presence of IsK

KvLQT1 channel type	Syndrome	Current density at +30 mV (pA/pF)	Activation curve		Time constant of deactivation (ms)	E_{rev} (mV)
			$V_{0.5}$ (mV)	Slope (mV)		
WT (2 μ g/ μ l)		43.1 $\pm$ 4.1 (31)	19.6 $\pm$ 2.0 (24)	24.1 $\pm$ 0.6 (24)	691.1 $\pm$ 21.1 (24)	-82.3 $\pm$ 0.8 (10)
WT (4 μ g/ μ l)		40.2 $\pm$ 4.2 (22)	18.5 $\pm$ 2.5 (16)	25.6 $\pm$ 0.8 (16)	657.8 $\pm$ 25.0 (16)	-82.7 $\pm$ 0.7 (7)
WT + R174C <i>R45C</i>	RW	24.7 $\pm$ 5.2 (9)*	22.7 $\pm$ 2.5 (6)	23.9 $\pm$ 0.5 (6)	634.2 $\pm$ 20.6 (8)	-80.7 $\pm$ 1.2 (3)
WT + G269D <i>G140D</i>	RW	25.5 $\pm$ 5.6 (11)*	21.2 $\pm$ 3.2 (7)	23.4 $\pm$ 1.0 (7)	613.2 $\pm$ 35.4 (9)	-83.3 $\pm$ 1.1 (6)
WT + W305S <i>W176S</i>	JLN	32.2 $\pm$ 6.1 (10)	18.5 $\pm$ 1.9 (9)	23.8 $\pm$ 0.7 (9)	603.4 $\pm$ 28.7 (9)	-83.2 $\pm$ 1.0 (5)
WT + G314S <i>G185S</i>	RW	16.4 $\pm$ 5.8 (6)*	21.9 $\pm$ 3.7 (6)	23.3 $\pm$ 0.5 (6)	643.3 $\pm$ 38.3 (7)	-82.5 $\pm$ 1.0 (6)
WT + Y315S <i>Y186S</i> ^a	RW	22.7 $\pm$ 6.0 (7)*	18.3 $\pm$ 3.2 (6)	23.6 $\pm$ 1.7 (6)	610.8 $\pm$ 41.7 (7)	-81.8 $\pm$ 1.2 (5)
WT + L342F <i>L213F</i>	RW	23.4 $\pm$ 7.6 (10)*	18.0 $\pm$ 2.5 (8)	24.0 $\pm$ 0.8 (8)	591.3 $\pm$ 30.1 (9)	-82.8 $\pm$ 0.9 (5)
WT + Δ 544 <i>I244, -7 +8</i>	JLN	32.4 $\pm$ 5.7 (10)	18.2 $\pm$ 1.8 (8)	24.3 $\pm$ 0.8 (8)	608.9 $\pm$ 22.3 (8)	-81.0 $\pm$ 0.4 (4)
WT + R555C <i>R426C</i>	RW	18.1 $\pm$ 4.3 (10)*	50.2 $\pm$ 8.3 (9)*	32.0 $\pm$ 1.4 (9)*	628.3 $\pm$ 42.5 (11)	-82.0 $\pm$ 1.2 (7)
R555C (2 μ g/ μ l)		10.9 $\pm$ 2.0 (10)*	69.3 $\pm$ 4.6 (10)*	21.7 $\pm$ 2.4 (10)	337.5 $\pm$ 41.4 (10)*	-82.7 $\pm$ 1.4 (10)

The positions of the mutations are given according to the sequence of Figure 1 in regular letters and according to the sequence of Wang *et al.* (1996) in italic letters. Data are expressed as mean $\pm$ SEM (n = number of cells). E_{rev} : reversal potential of the tail current.

^aFor WT/Y315S ratios of 10:1 and 1:10, the current densities were 40.4 $\pm$ 6.2 pA/pF (n = 10) and below the detection level (n = 13), respectively.

*Statistically significant difference (P < 0.05) compared with WT (2 μ g/ μ l) and (4 μ g/ μ l), for homomeric and heteromeric associations, respectively.

This brings the number of reported *KVLQT1* mutations associated with LQT1 to a total of 29. It is clearly apparent that all these mutations are located in five domains of the protein including the cytoplasmic loops S2–S3 and S4–S5, the P-domain, the hydrophobic segment S6 and a short positively charged region in the COOH tail. This finding underlines the functional significance of these domains, a prominence that was expected for the P-, S4–S5 and S6 domains, but was less evident for the others. For instance, except for the P-domain, the mutation locations are quite different in HERG, the other LQT associated K⁺ channel protein in which many natural mutations have been described (Curran *et al.*, 1995; Dausse *et al.*, 1996). Interestingly, the two known *KVLQT1* mutations associated with the JLN syndrome (Neyroud *et al.*, 1997; C.Donger, I.Denjoy, M.Berthet, N.Neyroud, C.Cruaud, M.Bennaceur, G.Chivoret, K.Schwartz, P.Coumel and P.Guicheney, in preparation) also fall in ‘RW-related domains’, namely the P-domain and a small region in the cytoplasmic COOH sequence (Figure 1 and Table I). It is interesting to note that one of the JLN mutations is a missense W305S mutation, while the other one (called herein Δ 544) is a deletion-insertion leading to a modification of the following 107 amino acid sequence and a premature stop codon. Representative RW mutations

in the five domains and the two JLN mutations have been individually introduced by site-directed mutagenesis into the human KvLQT1 cDNA, and the resulting subunits expressed in COS cells.

Functional expression of the mutated KvLQT1 channels

The positions of the mutations that have been studied are shown in the schematic representation of Table I. All mutants were first transfected in the absence of IsK. None of them produced K⁺ currents under these conditions. The same result was obtained for all mutants co-expressed with the IsK subunit (not shown), with the exception of R555C. This C-terminal mutant is able to form functional channels with IsK, and representative currents produced are shown in Figure 4. Beside the reduction in current density at +30 mV [10.9 $\pm$ 2.0 pA/pF (n = 10) versus 43.1 $\pm$ 4.0 pA/pF (n = 31) for the wild type (WT) KvLQT1], the main difference from the WT current is a +50 mV shift of the voltage dependence of activation [$V_{0.5}$ = 69.3 $\pm$ 4.6 mV (n = 10) instead of 19.6 $\pm$ 2.0 mV (n = 24) for the WT, Figure 4D]. Furthermore, Figure 4F shows clearly that R555C channels deactivate more rapidly than WT channels [337.5 $\pm$ 41.4 ms (n = 10) instead of 691.1 $\pm$ 21.1 ms (n = 24) for WT]. In

Fig. 4. Expression and biophysical properties of the mutant R555C. Same voltage protocols as in Figure 3. Expression of R555C + IsK (A) or with the wild type KvLQT1 (B). (C–F) (●), KvLQT1 + IsK; (■), R555C + IsK; (○), R555C + KvLQT1 + IsK. (C) Current densities plotted against membrane potential (mean $\pm$ SEM, $n = 10$). (D) Relative activation curves (mean $\pm$ SEM, $n = 9$). (E) Time constants of activation versus membrane potential (mean $\pm$ SEM, $n = 3$). (F) Time constants of deactivation (mean $\pm$ SEM, $n = 5$).

contrast, the time course of activation remains unchanged (Figure 4E).

When co-assembled with WT, all the KvLQT1 mutants affect channel function. In Table I, the main parameters of the KvLQT1 + IsK currents are compared with those obtained after co-expression with the mutated subunits. Whatever the location of the mutation, the general rule is that the only discernable effect is a decrease of the current density, corresponding to a dominant-negative suppression of KvLQT1 function. The activation parameters, the time constant of deactivation and the reversal potential of the tail current are not significantly changed (Table I). Again, the R555C mutant is an exception, since the $V_{0.5}$ for its activation is shifted towards positive values. The $V_{0.5}$ value in the case of the WT/R555C heteromeric association is in between the values found for the two homomeric channels (WT and R555C) (Table I and Figure 4). However, the time constant of deactivation of the WT-R555C current remains close to the value found for the WT form. Hence, it seems likely that a new current with specific properties has been created by the association of WT with the R555C mutated subunit.

The two JLN mutations also produce a dominant-negative effect, but the extent of the inhibition is lower than the one seen with the six RW mutations. Although

the differences do not attain statistical significance, it remains remarkable that the presence of the JLN mutated subunits slightly alters the current density.

Discussion

A human cDNA encoding a KvLQT1 channel protein has been cloned by conventional screening of a kidney library. Only one isoform was identified, which contains the known exon 1A (Lee *et al.*, 1997) as well as the 5' upstream sequence that was not identified before. Analysis of the very large *KVLQT1* gene by Lee *et al.* demonstrated that at least four alternative splice variants can exist that differ in the use of exons located 5' to the so-called exon 1 (position 130–159 in the kidney sequence, Figure 1) or the addition of an exon 2a not found in kidney transcripts. It is likely that all the isoforms contain the exons 1–14, defining a common core for all these putative K^+ channel subunits. The cDNA cloned in this study probably corresponds to the full-length isoform 1. This isoform is not specific for the kidney, since it is also found in all tissues expressing the common core, especially in the heart.

The functional expression study shows, as in previous studies with other KvLQT1 clones (Barhanin *et al.*, 1996; Sanguinetti *et al.*, 1996), that KvLQT1 alone expresses a fast current of small amplitude. Co-expression with IsK increases the current amplitude, slows down the activation rate and shifts the $V_{0.5}$ of activation to more positive potentials, giving rise to currents very similar, if not identical, to the slow cardiac delayed rectifier I_{Ks} (Barhanin *et al.*, 1996; Sanguinetti *et al.*, 1996). Therefore, analysis of the properties of the mutated forms of KvLQT1 was performed in the presence of IsK in order to reproduce, as far as possible, the I_{Ks} current in patients.

Expression of KvLQT1 subunits from individuals affected by the RW syndrome demonstrates that the point mutations genetically linked to the disease produce large alterations in the function of the resulting K^+ channels. Except for the R555C, none of the RW subunits forms functional homomeric channels in the presence as well as in the absence of IsK. Indeed, their co-expression with the WT KvLQT1 leads to dominant-negative effects, i.e. a decrease in the current density, without much alteration in the kinetic properties of the remaining current. Although other explanations cannot be excluded, our results are consistent with the idea that mutated subunits assemble with WT subunits to form inactive channels. Since I_{Ks} current in the heart is essential to adapt action potential duration to high frequency stimulations (Jurkiewicz and Sanguinetti, 1993; Romey *et al.*, 1997), the reduction of I_{Ks} current due to mutations in KvLQT1 is the probable reason for the high susceptibility of RW and JLN patients to tachycardias and early post-depolarizations.

The two JLN mutations, W305S and Δ 544, have globally the same effects as the RW mutations, but with lower dominant-negative effects. Neither of them produce functional channels in our experimental protocol, and they hardly change the expression of the WT subunits. This is consistent with the clinical observation that heterozygous JLN carriers only show slight cardiac dysfunctions (Fraser *et al.*, 1964; Neyroud *et al.*, 1997). The single WT allele would produce a sufficient level of K^+ channel protein to ensure I_{Ks} function. The complete or almost complete

absence of the KvLQT1 protein in the homozygous state would lead to a severe disease characterized by a very high susceptibility to ventricular tachyarrhythmias and to deafness due to the total loss of K^+ transport into the ear endolymph (Vetter *et al.*, 1996; Neyroud *et al.*, 1997).

The exact mechanisms by which each of the mutations examined in this study, with the exception of R555C, cause the suppression of channel function has yet to be determined. Concerning mutations in the P domain and the S6 segment, it is very probable that they modify the pore function, making the channel non-conducting (Heginbotham *et al.*, 1994; Lopez *et al.*, 1994; Taglialatela *et al.*, 1994). All the amino acids involved in these mutations are very conserved, not only in the KvLQT1 channel family, but also in the whole family of voltage-dependent K^+ channels. The case of the G314S mutation deserves a special comment. This glycine residue belongs to the GYG motif, which is the most conserved motif in all K^+ selective channels, including outward, inward and non-rectifying channels (Durell and Guy, 1996). A naturally occurring mutation of the first glycine of the GYG sequence in a G-protein-activated K^+ channel subunit (GIRK2) has been recently described in the neurological mutant mouse *weaver* (Patil *et al.*, 1995). *Weaver* GIRK2 homopolymers display a higher Na^+ versus K^+ permeability upon expression in *Xenopus* oocyte (Kofuji *et al.*, 1996; Slesinger *et al.*, 1996). However, it is not clear whether this ionic selectivity change also takes place in neurons and if the neurodegenerative phenotype of the *weaver* mice is due to gain-of-function effects such as loss of K^+ selectivity, or to a loss-of-function effect due to the drastic decrease associated, or not, with dominant-negative effects on K^+ channel activity (Surmeier *et al.*, 1996; Lauritzen *et al.*, 1997; Signorini *et al.*, 1997). In the case of the KvLQT1 G314S mutation, the homopolymer is totally inactive and no change of ionic selectivity is observed in cells coexpressing the mutated and wild type subunits since the reversal potential of the tail current is not modified. The same conclusion applies to the Y315S mutation. In this case, a change in ionic selectivity could have been expected from the study of the role of this amino acid in the pore of *Shaker* K^+ channels (Heginbotham *et al.*, 1994), but the only detectable effect is a dominant loss of function.

The S4–S5 loop and the S6 segment are known to be important for voltage-dependent K^+ channel function in lining the internal part of the pore (Isacoff *et al.*, 1991; Slesinger *et al.*, 1993; Lopez *et al.*, 1994). Therefore, it is not surprising that RW mutations are found in these domains and that the mutants G269D and L342F that we analyzed in this work were totally inactive.

We report two mutations in the cytoplasmic S2–S3 loop (Figure 1 and Table I) and three other mutations in the same region have previously been reported (Wang *et al.*, 1996; Tanaka *et al.*, 1997). This is not a conserved region between the diverse families of K^+ channels. However, it appears that most of the mutated positions, including the R174C studied in this work, are conserved, not only in the mouse and *Xenopus* orthologs of KvLQT1, but also in the *C.elegans* more distant KvLQT1-like sequence C25B8. This conservation may underline a crucial role for these amino acids in this particular family of K^+ channels, as indicated by the total lack of function of

the R174C mutant. Two RW mutations occur in a small region situated in the cytoplasmic C-terminal domain. Again, the conservation of this small part of the large C-terminal end that characterizes the KvLQT1-related subunits, is relatively high. In particular, the arginine in position 555 of the KvLQT1 form presently being studied, is conserved in all KvLQT1-related sequences, including C25B8. This is the only mutation that leads to a functional homomeric channel. Interestingly, all the major biophysical properties of the mutated channel are modified. The removal of a single positive charge in this highly positively charged domain (Figure 1), surprisingly produces a large shift in the $V_{0.5}$ of activation, by nearly 50 mV, and a decrease in the time constant of deactivation. These two modifications of the biophysical properties create a channel that is probably deficient in action potential duration adaptation to the heartbeat frequency. This conclusion is consistent with the known particularly high susceptibility to antiarrhythmic drugs of patients carrying this mutation.

Functional analysis of RW and JLN alleles show that a reduction of I_{Ks} is the molecular mechanism of LQT syndromes linked to chromosome 11. Depending upon the severity of the dominant-negative effect of the different mutations, the disease is either dominant or recessive. In the latter case, the reduction of the current normally carried by the KvLQT1 subunit is so high that the defect becomes apparent in other tissues expressing this protein, the inner ear being the most evident one. Due to the very wide distribution of KvLQT1 transcripts (Figure 2), it is likely that other defects exist in JLN patients that have not yet been clearly diagnosed.

Concerning the mutations found in heterozygous RW patients, there is no clear correlation between the severity of the disease as estimated by the frequency of the symptoms and the strength of the dominant-negative effect on the current. All the mutations studied in this paper are classified as 'severe', except the R555C. The same reduction in the current density was observed for co-expression of this mutant with the WT as for co-expression of the WT with other RW mutants (Table I). However, this mutant is the only one that is functional in the homomeric form and it is also the only one for which the decrease in the K^+ current carried by the heteromeric WT/R555C channels results mainly from a shift of the activation-potential relationships. It is likely that other factors that remain to be discerned are important either to aggravate or to diminish the consequences of the I_{Ks} channel dysfunction on heartbeat control.

Materials and methods

Isolation of cDNA clone encoding the human KvLQT1

An oligo(dT)-primed cDNA library (a generous gift of the Dr Rainer Waldmann) derived from poly(A)⁺ RNA isolated from human adult kidney was screened with the ³²P-labeled mouse KvLQT1 cDNA coding sequence. Filters were hybridized in 50% formamide, 5× SSC, 4× Denhardt's solution, 0.1% SDS and 100 µg denatured salmon sperm DNA at 50°C for 18 h and washed to a final stringency of 0.1× SSC, 0.3% SDS at 55°C. Ten positive clones were isolated from ~5×10⁵ clones and four of them were further analyzed. The λZAPII phages containing the cDNA inserts were converted to cDNA plasmids by rescue excision (Stratagene). The cDNA inserts were characterized by restriction enzyme analysis and by partial or complete DNA sequencing on both strands by the dideoxy nucleotide method using an automatic sequencer (Applied

Biosystems model 373A). The coding sequence was amplified using a low error rate DNA polymerase (Pwo DNA pol, Boehringer) with appropriate restriction sites to be subcloned into pCI plasmid (Promega) to give pCI-KvLQT1 for functional expression studies.

Northern blot

For RNA analysis, two human multiple tissues Northern blots (Clontech, catalog numbers 7759-1 and 7760-1) were probed with ³²P-labeled probes in ExpressHyb™ hybridization solution (Clontech) according manufacturer's instructions. The common and 5' specific probes (nucleotides 498–1403 and 286–408, respectively from DDBJ/EMBL/GenBank accession number AF000571) were obtained by PCR amplification and labeled using the Klenow DNA polymerase and random (common probe) or specific (5' specific probe) primers. The full coding sequence of the human IsK cDNA (Attali *et al.*, 1992) was used as IsK probe.

Site-directed mutagenesis

Long QT KvLQT1 mutated subunits were prepared by mutagenesis on the double-stranded plasmid using the Transformer™ site-directed mutagenesis kit (Clontech). Nucleotide sequence analysis was performed on each mutant prior to expression studies.

Electrophysiological measurements in transfected COS cells

COS cells were seeded at a density of 7×10^5 cells per 35 mm Petri dish 24 h prior to transfection. They were transfected by the calcium phosphate precipitation method using 2 µg or 4 µg (in the co-expression experiments) of pCI-KvLQT1 (WT or mutant) and 2 µg of pCMV-hIsK (Lesage *et al.*, 1993) per dish. A CD8-expressing plasmid (1 µg) was added in all transfection experiments to visualize transfected cells using anti-CD8 antibody-coated beads (Jurman *et al.*, 1994). Cells were tested in electrophysiology 48 h after transfection using the whole-cell configuration of the patch-clamp technique (Hamill *et al.*, 1981). The internal solution contained 150 mM KCl, 2 mM MgCl₂, 5 mM EGTA and 10 mM HEPES-KOH at pH 7.2 and the external solution contained 150 mM NaCl, 5 mM KCl, 1 mM CaCl₂, 2 mM MgCl₂ and 10 mM HEPES-NaOH at pH 7.4.

Acknowledgements

We are very grateful to Dr R.Waldmann for the gift of the human kidney cDNA library and Dr A.Patel for reading the manuscript. We thank M.M.Larroque, M.Jodar and N.Leroudier for expert technical assistance, and D.Doume for secretarial assistance. We thank the Association Française contre les Myopathies (AFM), the Ministère de l'Enseignement Supérieur et de la Recherche (Contract MESR ACC SV9 No. 9509113), the Clinical Research Network No. 494012, the Direction de la Recherche Clinique des Hôpitaux de Paris (PHRC P-920308), the European Community specific RTD program No. BMH4-CT96-0028. This work was supported by the Centre National de la Recherche scientifique (CNRS) and the Institut National de la Santé et de la Recherche Médicale (INSERM). C.C. is recipient of a grant from the Association Française contre les Myopathies.

References

- Attali,B. (1996) Ion channels - a new wave for heart rhythms. *Nature*, **384**, 24–25.
- Attali,B., Romey,G., Honoré,E., Schmid-Alliana,A., Mattéi,M.G., Lesage,F., Ricard,P., Barhanin,J. and Lazdunski,M. (1992) Cloning, functional expression and regulation of two K⁺ channels in human T lymphocytes. *J. Biol. Chem.*, **267**, 8650–8657.
- Barhanin,J., Lesage,F., Guillemare,E., Fink,M., Lazdunski,M. and Romey,G. (1996) K(v)LQT1 and IsK (minK) proteins associate to form the I-Ks cardiac potassium current. *Nature*, **384**, 78–80.
- Curran,M.E., Splawski,I., Timothy,K.W., Vincent,G.M., Green,E.D. and Keating,M.T. (1995) A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. *Cell*, **80**, 795–803.
- Dausse,E., Berthet,M., Denjoy,I., André-Fouet,X., Cruaud,C., Bennaceur,M., Fauré,S., Coumel,P., Schwartz,K. and Guicheney,P. (1996) A mutation in HERG associated with notched T waves in long QT syndrome. *J. Mol. Cell. Cardiol.*, **28**, 1609–1615.
- Drici,M.D., Burklow,T.R., Haridas,V., Glazer,R.I. and Woosley,R.L. (1996) Sex hormones prolong the QT interval and downregulate potassium channel expression in the rabbit heart. *Circulation*, **94**, 1471–1474.
- Durell,S.R. and Guy,H.R. (1996) Structural model of the outer vestibule and selectivity filter of the Shaker voltage-gated K⁺ channel. *Neuropharmacology*, **35**, 761–773.
- Fraser,G.R., Froggatt,P. and Murphy,T. (1964) Genetical aspects of the cardio-auditory syndrome of Jervell and Lange-Nielsen (congenital deafness and electrocardiographic abnormalities). *Ann. Hum. Genet.*, **28**, 133–157.
- Hamill,O.P., Marty,A., Neher,E., Sakmann,B. and Sigworth,F.J. (1981) Improved patch-clamp techniques for high resolution current recording from cells and cell-free membranes patches. *Pflügers Arch.*, **391**, 85–100.
- Heginbotham,L., Lu,Z., Abramson,T. and Mackinnon,R. (1994) Mutations in the K⁺ channel signature sequence. *Biophys. J.*, **66**, 1061–1067.
- Isacoff,E.Y., Jan,Y.N. and Jan,L.Y. (1991) Putative receptor for the cytoplasmic inactivation gate in the Shaker K⁺ channel. *Nature*, **353**, 86–90.
- Jervell,A. and Lange-Nielsen,F. (1957) Congenital deaf-mutism, functional heart disease with prolongation of Q-T interval and sudden death. *Am. Heart J.*, **54**, 59–68.
- Jurkiewicz,N.K. and Sanguinetti,M.C. (1993) Rate-dependent prolongation of cardiac action potentials by a methanesulfonanilide class-III antiarrhythmic agent - specific block of rapidly activating delayed rectifier K⁺-current by dofetilide. *Circ. Res.*, **72**, 75–83.
- Jurman,M.E., Boland,L.M. and Yellen,G. (1994) Visual identification of individual transfected cells for electrophysiology using antibody-coated beads. *BioTechniques*, **17**, 876–881.
- Kofuji,P., Hofer,M., Millen,K.J., Millonig,J.H., Davidson,N., Lester,H.A. and Hatten,M.E. (1996) Functional analysis of the weaver mutant GIRK2 K⁺ channel and rescue of weaver granule cells. *Neuron*, **16**, 941–952.
- Lauritzen,I., De Weille,J., Adelbrecht,C., Lesage,F., Murer,G., Raisman-Vozari,R. and Lazdunski,M. (1997) Comparative expression of the inward rectifier K⁺ channel GIRK2 in the cerebellum of normal and weaver mutant mice. *Brain Res.*, **753**, 8–17.
- Lee,M.P., Hu,R., Johnson,L.A. and Feinberg,A.P. (1997) Human KvLQT1 gene shows tissue-specific imprinting and encompasses Beckwith-Wiedemann syndrome chromosomal rearrangements. *Nature Genet.*, **15**, 181–185.
- Lesage,F., Attali,B., Lakey,J., Honoré,E., Romey,G., Faurobert,E., Lazdunski,M. and Barhanin,J. (1993) Are *Xenopus* oocytes unique in displaying functional IsK channel heterologous expression? *Receptors and Channels*, **1**, 143–152.
- Lopez,G.A., Jan,Y.N. and Jan,L.Y. (1994) Evidence that the S6 segment of the Shaker voltage-gated K⁺ channel comprises part of the pore. *Nature*, **367**, 179–182.
- Neyroud,N., Tesson,F., Denjoy,I., Leibovici,M., Donger,C., Barhanin,J., Faure,S., Gary,F., Coumel,P., Petit,C., Schwartz,K. and Guicheney,P. (1997) A novel mutation in the potassium channel gene KVLQT1 causes the Jervell and Lange-Nielsen cardioauditory syndrome. *Nature Genet.*, **15**, 186–189.
- Patil,N., Cox,D.R., Bhat,D., Faham,M., Myers,R.M. and Peterson,A.S. (1995) A potassium channel mutation in *weaver* mice implicates membrane excitability in granule cell differentiation. *Nature Genet.*, **11**, 126–129.
- Pagnell,M., Snay,K.J., Trimmer,J.S., Macluskay,N.J., Naftolin,F., Kaczmarek,L.K. and Boyle,M.B. (1990) Estrogen induction of a small, putative K⁺ channel mRNA in rat uterus. *Neuron*, **4**, 807–812.
- Roden,D.M., Lazzara,R., Rosen,M., Schwartz,P.J., Towbin,J. and Vincent,G.M. (1996) Multiple mechanisms in the long-QT syndrome - current knowledge, gaps, and future directions. *Circulation*, **94**, 1996–2012.
- Romano,C. (1965) Congenital cardiac arrhythmia (Letter). *Lancet*, **1**, 658–659.
- Romey,G., Attali,B., Chouabe,C., Abitbol,I., Guillemare,E., Barhanin,J. and Lazdunski,M. (1997) Molecular mechanism and functional significance of the Mink control of the KvLQT1 channel activity. *J. Biol. Chem.*, **272**, 16713–16716.
- Russell,M.W., Dyck,M., II, Collins,F. and Brody,L.C. (1996) KVLQT1 mutations in three families with familial or sporadic long QT syndrome. *Hum. Mol. Genet.*, **5**, 1319–1324.
- Sanguinetti,M.C. and Jurkiewicz,N.K. (1990) Two components of cardiac delayed rectifier K⁺ current - Differential sensitivity to block by class-III antiarrhythmic agents. *J. Gen. Physiol.*, **96**, 195–215.
- Sanguinetti,M.C., Curran,M.E., Zou,A., Shen,J., Spector,P.S., Atkinson,D.L. and Keating,M.T. (1996) Coassembly of K(v)LQT1 and MinK (IsK) proteins to form cardiac I-Ks potassium channel. *Nature*, **384**, 80–83.

- Schwartz,P.J. (1985) Idiopathic long QT syndrome: progress and questions. *Am. Heart. J.*, **109**, 399–411.
- Signorini,S., Liao,Y.J., Duncan,S.A., Jan,L.Y. and Stoffel,M. (1997) Normal cerebellar development but susceptibility to seizures in mice lacking G protein-coupled, inwardly rectifying K⁺ channel GIRK2. *Proc. Natl Acad. Sci. USA*, **94**, 923–927.
- Slesinger,P.A., Jan,Y.N. and Jan,L.Y. (1993) The S4-S5 loop contributes to the Ion-Selective pore of potassium channels. *Neuron*, **11**, 739–749.
- Slesinger,P.A., Patil,N., Liao,Y.J., Jan,Y.N., Jan,L.Y. and Cox,D.R. (1996) Functional effects of the mouse weaver mutation on g protein-gated inwardly rectifying K⁺ channels. *Neuron*, **16**, 321–331.
- Surmeier,D.J., Mermelstein,P.G. and Goldowitz,D. (1996) The *weaver* mutation of GIRK2 results in a loss of inwardly rectifying K⁺ current in cerebellar granule cells. *Proc. Natl Acad. Sci. USA*, **93**, 11191–11195.
- Taglialatela,M., Champagne,M.S., Drewe,J.A. and Brown,A.M. (1994) Comparison of h-5, s-6, and H-5-S-6 exchanges on pore properties of voltage-dependent K⁺ channels. *J. Biol. Chem.*, **269**, 13867–13873.
- Tanaka,T., Nagai,R., Tomoike,H., Takata,S., Yano,K., Yabuta,K., Hanada,N., Nakano,O., Shibata,A., Sawayama,T., Kasai,H., Yazaki,Y. and Nakamura,Y. (1997) Four novel KvLQT1 and four novel HERG mutations in familial long-QT syndrome. *Circulation*, **95**, 565–567.
- Vetter,D.E., Mann,J.R., Wangemann,P., Liu,J.Z., McLaughlin,K.J., Lesage,F., Marcus,D.C., Lazdunski,M., Heinemann,S.F. and Barhanin,J. (1996) Inner ear defects induced by null mutation of the *IsK* gene. *Neuron*, **17**, 1251–1264.
- Wang,Q., Curran,M.E., Splawski,I., Burn,T.C., Millholland,J.M., Vanraay,T.J., Shen,J., Timothy,K.W., Vincent,G.M., DeJager,T., Schwartz,P.J., Towbin,J.A., Moss,A.J., Atkinson,D.L., Landes,G.M., Connors,T.D. and Keating,M.T. (1996) Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. *Nature Genet.*, **12**, 17–23.
- Ward,O.C. (1964) A new familial cardiac syndrome in children. *J. Irish Med. Assoc.*, **54**, 103–106.
- Wilson,R. *et al.* (1994) 2.2 Mb of contiguous nucleotide sequence from chromosome III of *C.elegans*. *Nature*, **368**, 32–38.

Received on April 29, 1997; revised on June 2, 1997