Involvement of IsK-Associated $K^+$ Channel in Heart Rate Control of Repolarization in a Murine Engineered Model of Jervell and Lange-Nielsen Syndrome

Milou-Daniel Drici, Isabelle Arrighi, Christophe Chouabe, Jeffrey R. Mann, Michel Lazdunski, Georges Romey, Jacques Barhanin

Abstract—The Jervell and Lange-Nielsen (JLN) syndrome affects the human cardioauditory system, associating a profound bilateral deafness with an abnormally long QT interval on the ECG. It results from mutations in $KVLQT1$ and IsK genes that encode the 2 subunits forming the $K^+$ channel responsible for the cardiac and inner ear slowly activating component of the delayed rectifier $K^+$ current ($I_k$). A JLN mouse model that presents typical inner ear defects has been created by knocking out the isk gene ($isk^{−/−}$). This study specifically reports on the cardiac phenotype counterpart, determined in the whole animal and at mRNAs and cellular levels. Surface ECG recordings of $isk^{−/−}$ mice showed a longer QT interval at slow heart rates, a paradoxical shorter QT interval at fast heart rates, and an overall exacerbated QT–heart rate adaptation compared with wild-type (WT) mice. A 300-ms increase in the heart rate cycle length induces a $0.309±0.21\%$ increase in the QT duration of the WT mice versus a $500±50\%$ in $isk^{−/−}$ mice ($P<0.001$). It is concluded that the $isk$ gene product and/or $I_k$, when present, blunts the QT adaptation to heart rate variations and that steeper QT-RR relationships reflect a greater susceptibility to arrhythmias in patients lacking $I_k$. (Circ Res. 1998;83:95-102.)

Key Words: long-QT syndrome ■ $KCNE1$ ■ MinK ■ electrocardiography ■ sex difference

Congenital LQTS and acquired LQTS are characterized by an abnormally prolonged ventricular repolarization, responsible for a polymorphic type of ventricular arrhythmia (known as torsades de pointes) that may lead to syncope and sudden death. Two forms of congenital LQTS, RW and JLN, can be distinguished on the basis of the mode of transmission and specific symptoms. In the case of RW syndrome, the mode of transmission is dominant, with few clinical features, but cardiac. In the JLN syndrome, the disease is recessively transmitted and includes a profound bilateral deafness in the whole animal and at cellular levels. Recent information on the identity of the genes involved in both syndromes has permitted us to comprehend some of the complex gene interactions and mechanisms underlying the congenital LQTS. All the genes responsible for these syndromes identified so far are ion channel genes, including the voltage-sensitive $Na^+$ channel gene $SCN5A$, and the $K^+$ channel genes $HERG$, $KVLQT1$, and $ISK$ (also called $KCNE1$). These latter have been shown to encode subunits of the same channel protein complex that is responsible for the slow component, $I_k$, of the cardiac delayed outward rectifier current. Moreover, mutations in $SCN5A$ or $HERG$ are associated with RW syndrome only, whereas $KVLQT1$ and $ISK$ can be implicated in both JLN and RW syndromes, depending on the mutation they carry. Expression studies have shown that mutations found in RW syndrome abolish channel function but also display a dominant-negative effect by partially inactivating the normal channel subunits encoded by the WT allele in heterozygous patients. By contrast, mutations responsible for JLN syndrome have no pronounced dominant-negative effect but abolish the current in the homozygous state.

Transgenic and gene-targeted mice have gained great importance as models for cardiovascular congenital affections. In order to analyze the in vivo function of the IsK protein (also referred as minK), null mutant mice with a targeted disruption of the $isk$ gene have been engineered. At the homozygous state, these mice present the genotypic characteristics of the $isk$ gene–associated form of the human cardioauditory JLN syndrome. Notably, they suffer from inner ear defects strikingly similar to those observed in JLN syndrome. As in JLN patients, the mice bear a profound bilateral deafness from birth that is shown to be due to the absence of $K^+$ secretion in the endolymph. However, the cardiac phenotype is still unexplored. The goal of the present study was to determine the cardiac role of IsK in this mouse model and to evaluate its putative influence in the different cardiac parameters (ie, QT duration, QT-RR adaptation, and T-wave alternans) classically associated with LQTS. The patient’s outcome is also known to be influenced by factors such as sex or bradycardia, which are explored in this model.
Materials and Methods

Animals

Knockout isk mice were generated by the gene-targeting methodology as previously described. The mutation has been maintained on the 129/Sv genetic background, and all the animals used in this study, isk−/− and WT, are inbred 129/Sv. Mice were maintained on sterile regular rodent chow (R03, Usine d’Alimentation Rationnelle [France]) and allowed free access to food and water in a facility (at 21 ± 1°C with 12-hour light/dark cycles) monitored by the Institut de Pharmacologie Moléculaire et Cellulaire staff in full compliance with the French Government animal welfare policy.

Northern Blot Analysis

Total brain and heart RNAs were isolated from 3 to 6 days, 4 weeks, and 8 weeks in 129 Sv/j WT and isk−/− mice. PolyA+ RNA (2 µg) was separated by electrophoresis on 1% agarose gel and transferred onto nylon membranes (Hybond N, Amersham). Blots were probed with 32P-labeled specific cDNA fragments of the different cardiac delayed rectifier K+ channel subunits in Express Hyb solution (Clontech) at 60°C for 16 hours and washed stepwise with 1°C with 12-hour light/dark cycles) monitored by the Institut de Pharmacologie Moléculaire et Cellulaire staff in full compliance with the French Government animal welfare policy.

Electrocardiography

Animal Preparation

Twelve 3-week-old (9 to 11 g each) and thirty-one 12-week-old (23 to 29 g each) male and female mice were studied. For each experiment, a mouse was anesthetized with sodium pentobarbital (10 mg/kg IP for the male adults, 45 mg/kg IP for the female adults, and 8 weeks in 129 Sv/J WT and isk−/− mice were generated by the gene-targeting methodology as previously described. The mutation has been maintained on the 129/Sv genetic background, and all the animals used in this study, isk−/− and WT, are inbred 129/Sv. Mice were maintained on sterile regular rodent chow (R03, Usine d’Alimentation Rationnelle [France]) and allowed free access to food and water in a facility (at 21 ± 1°C with 12-hour light/dark cycles) monitored by the Institut de Pharmacologie Moléculaire et Cellulaire staff in full compliance with the French Government animal welfare policy.

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Electrophysiological Recordings

Primary cultures of ventricular cardiomyocytes from WT and isk−/− mice were prepared as previously described with some modifications. Ventricles from 1- to 4-day-old mouse pups were dissected at 4°C and dissociated at room temperature for 15 minutes in 1.25 mg/mL trypsin in Joklik’s MEM (M0518, Sigma) with gentle agitation. Ventricles were then digested for 10 minutes with 0.5 mg/mL collagenase (type CLSII, Worthington) under gentle agitation. This was followed by mechanical dissociation using a Pasteur pipette. Cells released in the medium were centrifuged (1000 rpm for 5 minutes), collected, and washed in Joklik’s MEM. Cells obtained

![Northern blot analysis of the expression of major cardiac outward rectifier K+ channel subunits. Equal amounts (2 µg) of heart or brain polyA+ RNA from 1-, 4-, and 8-week-old animals were loaded in each lane. The expression of Isk and KvLQT1 was not detected in brain. Except for Isk, none of the intensities of the different bands in both tissues were influenced by the knockout.](image-url)
Comparison of the QT-RR Adaptation Slopes in WT and Knockout Mice

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Results are shown as mean±SEM. Continuous variables, such as slope of adaptation, QT values, and their increase from baseline, were analyzed by ANOVA (Statview 4.5 and SuperAnova 1.11, Abacus Corp) or a Mann-Whitney-Wilcoxon rank sum test, when applicable. The Bonferroni/Dunn correction was used to adjust for multiple comparisons. A value of P<0.05 was considered statistically significant.

Northern Blot Analysis

In order to check for an eventual compensation of the isk gene knockout by a modification of the expression of other K+ channel subunits, the level of mRNA corresponding to the major cardiac IsK was analyzed at different developmental stages (Figure 1). As expected from previous work,28 the level of IsK mRNA was high in neonatal hearts and decreased with age in WT hearts. At 8 weeks, the heart IsK message reached a low but still detectable level that did not change with aging (not shown). The IsK message was totally absent in null mutants of the isk gene (Figure 1). Conversely, the amount of mRNA for the other delayed rectifier subunits, including the IsK partner KvLQT1, Kv1.5 (encoding the sustained K+ current29), and merg, the mouse counterpart of HERG, were not influenced by the absence of IsK at any age. It is particularly noteworthy that only IsK presented a strong developmental regulation. KvLQT1 expression was totally independent of that of its IsK partner, both in WT and knockout mice.

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ECG Characteristics

Forty-three animals allotted as indicated in the Table were analyzed. Three bipolar lead ECGs (DI, DII, and DIII) were recorded under anesthesia, with a good stability of the signal and data acquisition and analysis were performed using PCLAMP software. The pipette solution contained (mmol/L) KCl 140, MgCl2 4, EGTA 1, and Na2ATP 3. This solution was buffered at pH 7.3 with NaCl 30, trimethyl ammonium chloride 110, CaCl2 1, KCl 5, MgCl2 1, and glucose 2. This solution was buffered at pH 7.4 with 10 mmol/L HEPES/NaOH.

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Progressively lengthened their RR intervals to an average of 247±11 ms (range, 134 to 445 ms) over a 45-minute period. PR and QT intervals also increased with a similar pattern (Figure 2A and 2B). The average QT-interval duration at the end of the experiment was 160±9 ms (range, 63 to 303 ms). In our model, a linear relationship, QT (ms) or PR (ms)=A×RR (ms)+B, fitted best the QT-RR and the PR-RR interval relationships in 40 of 43 mice (average r²=0.94±0.06, Figure 2C and 2D), with A being the slope of the QT-RR regression and B being the QT or PR intercepts for a theoretical RR value of 0.

**Gene-Related Modifications of the QT-RR Relationship**

The lengthening of the QT subsequent to bradycardia was different according to the genotype of the mouse. The isk−/− mice had a greater adaptation of their QT interval to the lengthening of the RR interval than did the WT mice. The slope of the QT-RR adaptation was 0.637±0.06 in the WT mice (n=22) versus 0.755±0.027 in females versus 0.632±0.043 in males, P<0.05 in both cases). The resulting QT interval over a 300-ms range in cycle length is 309±21% in WT mice and 500±50% in isk−/− mice. *P<0.05, **P<0.001.

**Absence of Gene-Related Modifications of the PR Interval**

The PR interval lengthened progressively according to the increase in the cycle length (Figure 2). The relationship was best fitted with a linear regression. No statistical difference was seen in the RR-induced adaptation of the PR interval according to the presence or absence of the isk gene (PR=0.15±0.01RR+17±3 in isk−/− mice [n=6] and PR=0.16±0.03RR+16±4 in WT mice [n=8], P=0.8). At

An average measured cycle length of 249±3 ms, where the RR-QT relationships are diverging, the average PR interval was 55±1 and 54±2 ms in the isk−/− and WT mice, respectively.

**Influence of Age on Gene-Related Differences in the QT-RR Relationship**

The adaptation of the QT to RR intervals differed in WT and isk−/− mice. Since the level of IsK mRNA drastically decreases during development, it was important to analyze the influence of age of the animals on these parameters. The QT intervals differed with aging in WT mice (Figure 4A and 4B). Although the slopes of the QT-RR relationships were only moderately changed from young to adult stages (0.571±0.06 [n=5] versus 0.657±0.03 [n=17], P<0.05; Table), the absolute QT values were significantly shorter in young mice in the whole range of heart rates (Figure 4A). The influence of age observed in WT mice was almost abolished in the isk−/− mice, with overlapping QT-RR relationships in young and adult mice (Figure 4B; QT=0.812±0.08RR−34±12 and QT=0.786±0.035RR−25±5, respectively). This resulted in accentuated differences in the gene-dependent QT-RR adaptation in young compared with adult animals.

**Sex Differences**

The QT-RR relationship presented a higher slope in females than in males, regardless of the presence or absence of the isk gene (0.755±0.027 in females versus 0.632±0.043 in males,
noninactivating time-dependent current at membrane potential.

Cultured Cardiomyocytes

I component of IsK that was present in both WT and isk/− mutant cells. This difference is related to the sexual maturity of the animals, since the slope of the QT-RR relationship was sex independent before puberty (0.723±0.065 in males versus 0.703±0.098 in females, P=0.88; Figure 4C, right). There was no statistically significant interaction between gene and sex (P=0.80), whereas both factors significantly influenced adaptability (gene, P<0.01; sex, P=0.02).

Isoproterenol Challenge

In order to increase their heart rate, isk/− and WT mice were injected intraperitoneally with increasing doses of isoproterenol (20 and 200 nmol, n=5). The shortest RR intervals attained in sinus rhythm were 95±3 ms (range, 88 to 101 ms). The QT interval decreased accordingly to an average value of 54±2 ms (range, 44 to 57 ms), with no noticeable difference between groups. In both groups the T wave increased significantly in amplitude (Figure 5), and at the highest dose, a T-wave alternans phenomenon developed regardless of the gene status of the mice (2 of 3 WT mice and 1 of 2 isk/− mice, Figure 5).

K+ Current Recordings in Cultured Cardiomyocytes

In order to apprehend which cellular events could be involved in the ECG changes observed in isk/− mice, K+ currents in cultured ventricular myocytes from both WT and isk/− mutant mice were analyzed. Under voltage-clamp conditions, IsK were present in both types of cells. Figure 6 (panels A and B, upper traces) shows representative K+ currents in response to depolarizing voltage pulses from a holding potential of −80 mV. Typical slow tail currents were elicited on repolarizations to −40 mV. IsK was the dominant component of IK that was present in both WT and isk/− mutant cells. This current was identified by its sensitivity to the specific blocker E-4031 (Figure 6, panels A and B, lower traces) and by its bell-shaped current-voltage relationship (Figure 6C). The ISK component of IK could be detected after ISK blockade by E-4031, essentially by its remaining slow tail current and its nonactivating time-dependent current at membrane potential biased toward 0 mV (Figure 6C). However, even if the cells analyzed originated from neonates, ISK could only be recorded in a mere 10% of the WT cells (7 of 60). Conversely, none of the mutant cells (0 of 55) exhibited this current. The E-4031–sensitive current was not significantly different according to the gene status. Because of numerous studies that implicate a contributing role for IsK to ISK, and IK, the present study was limited to these currents.

Discussion

The cardiac phenotype of the mice has been thoroughly investigated. In contrast to what has been previously stated,33,37 we found that the QT duration in mice not only varies with heart rate but that it does so with a strict linearity over a wide range of heart rates. Such a linearity has been reported in humans when the heart rate tends to a steady state,35 contrasting with the usual nonlinear QT-RR relationship observed in humans, rabbits, and guinea pigs under non–steady-state conditions.23,33 This is the case in the present study, with a steady anesthesia-induced lengthening of the RR interval and a beat-to-beat variability rarely exceeding a few milliseconds (not shown). Therefore, no correction of the QT values for RR intervals was necessary, avoiding correction bias.25,36

The most important result is that when IsK is present, the QT adaptation to heart rate variations is blunted in WT mice compared with isk/− mice. The knockout mice showed a larger lengthening of their cardiac repolarization on the decrease of the heart beat frequency. In fact, compared with WT mice, isk/− mice have a longer QT interval in bradicardic conditions (by 31 ms at 150 bpm, P<0.05) and a
shorter QT interval at fast heart rates (Figure 2). Such results raise several hypotheses. In bradycardic conditions, it is likely that $I_{Ko}$ slowly develops during the time course of the action potential in WT mice. At slow heart rates, the action potential gets longer, allowing $I_{Ko}$ to reach a higher level, thus limiting the increase of the APD. As in patients suffering from LQTS resulting from $ISK$ mutation, the absence of $I_{Ko}$ in $isk^{-/-}$ mice may result in a longer QT interval at slow heart rates. At fast heart rates, the shorter QT intervals observed in $isk^{-/-}$ mice are more intriguing. The classic role attributed to $I_{Ko}$ in shortening the APD (due to its open state accumulation at fast rates) does not seem to hold in our model. This finding may be relevant to the following: (1) The gene invalidated in the present mouse model is $isk$, which encodes the regulatory subunit, and not $kvlqt1$, which is responsible for the pore-forming subunit of the channel complex. When these conditions are reproduced in COS cells transfected with KvLQT1 alone, a rapidly activating small-amplitude K$^+$ current is obtained. The presence of such a current in the $isk^{-/-}$ mice could shorten the APD at fast heart rates. The fact that no KvLQT1 current was detected in either cultivated cardiomyocytes (Figure 6) or in the inner ear stria vascularis epithelium still does not invalidate such a hypothesis. The membrane resistance during the plateau phase of the action potential is rather high, and it is conceivable that a very small outward current (not detectable under our experimental conditions) could have a marked effect on the APD. Creation of mice with a knockout of the $kvlqt1$ gene instead of $isk$ could help to verify this hypothesis. However, the human JLN syndrome was recently shown to result from mutations in either the $ISK$ or the $KvLQT1$ gene, with no distinguishable clinical difference so far. (2) A modification of other currents involved in cardiac repolarization, such as $I_{Kr}$, the rapid sustained outward current, $I_{Ks}$, the transient outward current, or $I_{Ko}$, some of which having been previously linked to the $isk$ gene, could occur. However, according to this hypothesis, the lack of IsK would diminish $I_{Ko}$ even further or any other current that has a possible positive interaction with IsK, therefore tending to a longer QT at fast heart rates. (3) Compensation by overexpression of rapidly activating channels like $I_{Kr}$ secondary to the $isk$ gene knockout could contribute to a shortening of the QT at fast heart rates. (4) A dysregulation of the autonomic nervous system leading to an excessive QT shortening cannot be eliminated, given the beneficial effects of β-blockers or left stelllectomy in human patients with LQTS.

When cardiac parameters are compared at different developmental stages, it is found that young WT mice have shorter QT intervals than do the adults. This makes sense, considering that the amplitude of $I_{Ko}$ is related to the amount of IsK and that IsK is more heavily expressed in young hearts (Figure 1). The lack of difference between the 2 ages observed in $isk^{-/-}$ mice is in good agreement with this interpretation. In a way, young $isk^{-/-}$ hearts look like adult ones with regard to the QT-RR relationship.

A sex difference affects the outcome of both acquired and congenital LQTS, with more cardiac events in women than in men, especially after puberty. In fact, females are known to have longer QT interval values than males in several mammalian species. The mouse complies with this rule. An obvious sex difference has been observed in adult mice in the present study (Figure 4C). Moreover, this difference is lacking in sexually immature young mice. It was of interest to investigate the inference of the $isk$ gene on the sex difference. Although sex difference has been attributed to differences in K$^+$ currents through genomic and nongenomic effects of sex steroid hormones, no significant interaction between gene and sex could be supported by the present study.

Among several abnormalities in membrane ion currents accounting for the T-wave alternans phenomenon, $I_{Ko}$ was a relevant candidate at fast heart rates, because of its peculiar slow deactivation. The fact that T-wave alternans occurs regardless of the gene status renders the involvement of the KvLQT1/IsK current unlikely.

The present study clearly shows that the invalidation of the $isk$ gene does cause alterations of the functional properties of the heart. In this study, $I_{Ko}$ could be recorded only in cells originating from WT mice and in a small proportion of the cells analyzed. Conversely, the E-4031–sensitive current was consistently recorded in all cells, regardless of the $isk$ gene status. This first study was limited to $I_{Ko}$ and $I_{Ks}$, since too extensive an analysis would be required to assess changes in other currents or at other developmental stages, possibly accounting for the ECG changes. However, no compensatory process resulting from the $isk$ gene invalidation could be assessed by Northern blot analysis of heart transcripts of major K$^+$ channel subunits.

Which lessons do we gain from this mouse model? Although one must remain cautious, it appears that the change in QT-RR adaptability, which has drawn much less attention than the QT duration itself, is cardinal to the disease. Torsades de pointes ventricular arrhythmias are favored by a slow heart rate in humans. The proposed underlying mechanism is the triggering of oscillations known as early afterdepolarizations that interrupt the normal repolarizing time course of the APD, especially at slow heart rates. The lack of $I_{Ko}$ may facilitate the occurrence of early afterdepolarizations in 2 ways: (1) by delaying the repolarization phase and lengthening the action potential first, enabling inward currents to reactivate, and (2) by opposing weakened outward conductances on the emergence of such depolarizations. Furthermore, the onset of torsades de pointes is constantly preceded by a sudden increase in the RR interval with an abnormally prolonged QT interval. Therefore, it is likely that LQTS patients are prone to the occurrence of such arrhythmias through an instantaneous greater adaptability of their QT interval to their heart rate. Indeed, LQTS patients have previously been shown to have a greater adaptability of both monophasic APD and QT intervals to their heart rate, at rest and during exercise. The $isk^{-/-}$ mouse clearly is a relevant model for the JLN syndrome. The enhanced adaptability of the QT interval to the heart rate appears therefore to be a valuable criterion identifying patients at risk in an otherwise asymptomatic population of mutation carriers among relatives in RW families.

Acknowledgments
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