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Journal of Molecular and Cellular Cardiology 44 (2008) 571–581
Journal of Molecular and Cellular Cardiology
www.elsevier.com/locate/yjmcc

Original article

Cardiac potassium channel dysfunction in sudden infant death syndrome

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Received 27 August 2007; received in revised form 26 November 2007; accepted 26 November 2007
Available online 28 January 2008

Abstract

Life-threatening arrhythmias have been suspected as one cause of the sudden infant death syndrome (SIDS), and this hypothesis is supported by the observation that mutations in arrhythmia susceptibility genes occur in 5–10% of cases. However, the functional consequences of cardiac potassium channel gene mutations associated with SIDS and how these alleles might mechanistically predispose to sudden death are unknown. To address these questions, we studied four missense *KCNH2* (encoding HERG) variants, one compound *KCNH2* genotype, and a missense *KCNQ1* mutation all previously identified in Norwegian SIDS cases. Three of the six variants exhibited functional impairments while three were biophysically similar to wild-type channels (*KCNH2* variants V279M, R885C, and S1040G). When co-expressed with WT-HERG, R273Q and K897T/R954C generated currents resembling the rapid component of the cardiac delayed rectifier current (I_{Kr}) but with significantly diminished amplitude. Action potential modeling demonstrated that this level of functional impairment was sufficient to evoke increased action potential duration and pause-dependent early afterdepolarizations. By contrast, *KCNQ1*-I274V causes a gain-of-function in I_{Ks} characterized by increased current density, faster activation, and slower deactivation leading to accumulation of instantaneous current upon repeated stimulation. Action potential simulations using a Markov model of heterozygous I274V-I_{Ks} incorporated into the Luo–Rudy (LRd) ventricular cell model demonstrated marked rate-dependent shortening of action potential duration predicting a short QT phenotype. Our results indicate that certain potassium channel mutations associated with SIDS confer overt functional defects consistent with either LQTS or SQTS, and further emphasize the role of congenital arrhythmia susceptibility in this syndrome.

Keywords: Sudden death; Genetics; Ion channels; Long QT syndrome; Short QT syndrome

1. Introduction

Life-threatening arrhythmias including the congenital long QT syndrome (LQTS) have been proposed to contribute to some cases of the sudden infant death syndrome (SIDS), the leading cause of death among infants 1 month to 1 year of age [1–3]. Supporting this theory are recent observations that mutations in genes responsible for LQTS are found in approximately 5–10% of SIDS cases [4–6]. Congenital LQTS is an inherited disorder associated with mutations in genes predominantly encoding subunits of cardiac ion channels [7]. The disorder is caused by impaired ventricular repolarization leading to prolongation of the QT interval and an increased risk for life-threatening ventricular arrhythmias. Other mutations in some of the same genes (*KCNH2*, *KCNQ1*) can enhance repolarization and cause shortening of the QT interval [8–10]. In the short QT syndrome (SQTS), there is also increased risk for cardiac arrhythmias and sudden death [11,12].

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0022-2828/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.yjmcc.2007.11.015

Data linking cardiac potassium channel mutations with SIDS include anecdotal reports and population surveys. In 2001, Schwartz and colleagues reported discovery of a *de novo KCNQ1* mutation (P117L) in a SIDS victim [13]. The same mutation was also observed to segregate with autosomal dominant LQTS at reduced penetrance in an unrelated Italian family [13]. Another missense *KCNQ1* variant (H105L) without functional consequences was discovered among 41 German SIDS cases [14]. Two novel *KCNH2* mutations (K101E, P1157L) have also been reported in single SIDS probands [5,15], and in one case this finding led to discovery of undiagnosed LQTS in family members [15]. But, there have been no studies demonstrating that any of these mutant channels are dysfunctional.

Ackerman et al. reported a systematic survey of autopsied SIDS cases for mutations in the LQTS genes, an effort that originally identified two *SCN5A* mutations in 93 subjects [4]. The study later reported novel mutations in cardiac potassium channel genes (*KCNH2*-G294V, *KCNQ1*-T600M) occurring in two SIDS victims [5]. Three other potassium channel variants were identified, but these alleles were also observed in ethnically matched population controls, albeit rarely. Most recently, the largest series of cases screened for mutations in arrhythmia-susceptibility genes revealed novel missense *KCNH2* and *KCNQ1* mutations in Norwegian SIDS victims [6]. Importantly, the electrophysiological consequences of these novel SIDS-associated potassium channel mutations were previously unknown.

We report here the functional characterization of novel *KCNH2* and *KCNQ1* mutations identified in the Norwegian SIDS study. Our findings demonstrated a diversity of functional phenotypes that revealed plausible explanations for sudden death in this clinical setting.

2. Materials and methods

2.1. Mutagenesis and heterologous expression

Four single *KCNH2* variants (V279M, R273Q, R885C, S1040G), one compound genotype (K897T/R954C) and a *KCNQ1* mutation (I274V) were constructed in HERG or KCNQ1 cDNA vectors, respectively, using recombinant PCR mutagenesis (primer sequences available upon request). The final constructs were assembled in bicistronic mammalian expression plasmids (pIRES2-EGFP or pIRES2-DsRed, BD Biosciences-Clontech, Palo Alto, CA) to enable monitoring of transfection success by co-expression of fluorescent proteins. All constructs were sequenced to verify the mutation and to exclude polymerase errors. Recombinant human KCNE1 cDNA was subcloned into pIRES2-DsRed for use in I_{Ks} expression experiments. In co-expression experiments, the WT channel (either KCNQ1 or HERG) was coupled to DsRed while mutants were expressed from vectors encoding EGFP.

Chinese hamster ovary cells (CHO-K1, ATCC) cells were grown as previously described [16,17]. For HERG experiments, cells were transiently co-transfected with 3 μg of both WT and mutant plasmid DNA using Fugene-6 (Roche Diagnostics, Indianapolis, IN). For KCNQ1 experiments, cells were transiently transfected with 1 μg KCNQ1 plasmid DNA, whereas to study I_{Ks}, cells were co-transfected with 1 μg KCNQ1 and 1 μg KCNE1 plasmids. In some experiments, we performed transient transfection of WT or mutant KCNQ1 in a stable I_{Ks} cell line [17]. For these experiments, we initially determined by plasmid DNA titration that 1 ng of WT-KCNQ1 was sufficient to boost current amplitude in stable I_{Ks} cells by 2- to 3-fold and thus used this amount in transfections to achieve approximately equal levels of stably and transiently expressed KCNQ1 alleles. Following transfection (48–72 h), fluorescent cells were selected by epifluorescence microscopy (green for single transfections, yellow for co-transfections) for use in whole-cell patch clamp recording experiments. Non-transfected cells grown under these conditions did not exhibit measurable endogenous potassium currents.

2.2. Electrophysiology

Whole-cell currents were measured in the whole-cell configuration of the patch clamp technique [18] using an Axopatch 200B amplifier (Molecular Devices, Corp., Sunnyvale, CA, USA) as previously described [16,17]. Patch pipettes were pulled from thick-wall borosilicate glass (World Precision Instruments, Inc., Sarasota, FL, USA) with a multistage P-97 Flaming-Brown micropipette puller (Sutter Instruments Co., San Rafael, CA, USA) and fire-polished to a pipette resistance of 2–4 MΩ. A 1–2% agar-bridge composed of bath solution was utilized as a reference electrode. For HERG recording, the bath solution consisted of (in mM): NaCl 145, KCl 4, $MgCl_2$ 1, $CaCl_2$ 1.8, glucose 10, HEPES 10, adjusted to pH 7.35 with NaOH, ~275 mosM/kg. The pipette solution consisted of (in mM): KCl 110, ATP (dipotassium salt) 5, $MgCl_2$ 2, EDTA (ethylenediaminetetraacetic acid) 10, HEPES 10, adjusted to pH 7.2 with KOH, ~265 mosM/kg. For KCNQ1 and I_{Ks} recording, the bath solution consisted of (in mM): NaCl 132, KCl 4.8, $MgCl_2$ 1.2, $CaCl_2$ 2, glucose 5, HEPES 10, pH 7.4, ~275 mosM/kg. The pipette solution consisted of (in mM): K-aspartate 110, ATP (dipotassium salt) 5, $MgCl_2$ 1, EGTA 11, HEPES 10, $MgCl_2$ 1, pH 7.3, ~265 mosM/kg. Pipette solutions were diluted with distilled water (to ~250 mosM/kg) to prevent activation of swelling-activated currents. Chemicals were purchased from Sigma (St. Louis, MO, USA).

For HERG experiments, the holding potential was −80 mV and whole-cell currents were measured from −80 to +70 mV (in 10 mV steps) 1990 ms (activation) and 2200 ms (tail currents) after the start of the voltage pulse. For KCNQ1 and I_{Ks} experiments, the holding potential was −80 mV and whole-cell currents were measured from −80 to +60 mV (in 10 mV steps) 1990 ms after the start of the voltage pulse. Whole-cell current traces were filtered at 5 kHz and acquired at 2 kHz. The access resistance and apparent membrane capacitance were estimated as described by Lindau and Neher [19]. Pulse generation, data collection and analyses were done with Clampex 8.1 (Molecular Devices, Corp.). Specific voltage pulse protocols are described in the figures and legends. The apparent voltage dependence of activation for I_{Ks} was obtained by fitting data with the Boltzmann function: $I = 1/(1+\exp(V - V_{1/2})/k)$. To quantify the time course of deactivation, tail currents were fit with a single