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PRE-CLINICAL RESEARCH

A Molecular Mechanism for Adrenergic-Induced Long QT Syndrome

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Objectives This study sought to explore molecular mechanisms underlying the adrenergic-induced QT prolongation associated with *KCNQ1* mutations.

Background The most frequent type of congenital long QT syndrome is LQT1, which is caused by mutations in the gene (*KCNQ1*) that encodes the alpha subunit of the slow component of delayed rectifier K^+ current (I_{Ks}) channel. We identified 11 patients from 4 unrelated families that are heterozygous for *KCNQ1*-G269S. Most patients remained asymptomatic, and their resting corrected QT intervals ranged from normal to borderline but were prolonged significantly during exercise.

Methods Wild-type (WT) *KCNQ1* and/or *KCNQ1*-G269S (G269S) were expressed in mammalian cells with *KCNE1*. I_{Ks}-like currents were measured in control conditions or after isoproterenol or protein kinase A (PKA) stimulation using the patch-clamp technique. Additionally, experiments that incorporated the phosphomimetic *KCNQ1* substitution, S27D, in WT or *KCNQ1*-G269S were also performed.

Results The coexpression of WT-*KCNQ1* with varying amounts of G269S decreased I_{Ks}, shifted the current-voltage *I-V* relation of I_{Ks} to more positive potentials, and accelerated the I_{Ks} deactivation rates in a concentration-dependent manner. In addition, the coexpression of G269S and WT blunted the activation of I_{Ks} in response to isoproterenol or PKA stimulation. Lastly, a phosphomimetic substitution in G269S did not show an increased I_{Ks}.

Conclusions G269S modestly affected I_{Ks} in control conditions, but it almost completely blunted I_{Ks} responsiveness in conditions that simulate or mimic PKA phosphorylation of *KCNQ1*. This insensitivity to PKA stimulation may explain why patients with G269S mutation showed an excessive prolongation of QT intervals on exercise. (J Am Coll Cardiol 2014;63:819–27) © 2014 by the American College of Cardiology Foundation

Congenital long QT syndrome (LQTS) is characterized by an abnormal QT interval prolongation on the electrocardiogram (ECG), syncope due to a polymorphic ventricular tachycardia called "torsade de pointes," and ventricular fibrillation (1,2). At least 13 genes are responsible for different subtypes of the syndrome (LQT1 to LQT13), with LQT1 being the most common and accounting for approximately 40% to 50% of genotyped patients (3,4). LQT1 is caused by mutations in *KCNQ1*, the alpha subunit of the slow component of delayed rectifier K^+ current (I_{Ks}), which is a major repolarizing current during the plateau phase of cardiac action potentials (5).

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The impaired expression or dysfunction of I_{Ks} channels (6,7) can lead to a prolongation in the cardiac action potential and the QT interval on an ECG (7,8). A major role for I_{Ks} is to maintain the ventricular action potential duration by offsetting the increase in L-type Ca^{2+} current ($I_{Ca,L}$) after adrenergic stimulation. Adrenergic stimulation activates protein kinase A (PKA), which directly increases I_{Ks} by phosphorylating the *KCNQ1* alpha subunit at S27 (8). Not surprisingly, most

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Abbreviations and Acronyms

cDNA = complementary deoxyribonucleic acid
CHO = Chinese hamster ovary
DNA = deoxyribonucleic acid
ECG = electrocardiogram
FK = forskolin
HEK293 = human embryonic kidney 293
IBMX = 3-isobutyl-l-methyl-xanthine
$I_{Ca,L}$ = L-type Ca^{2+} current
I_{Ks} = the slow component of delayed rectifier K^+ current
I-V = current-voltage
LQTS = long QT syndrome
QTc = corrected QT
PBS = phosphate-buffered saline
PKA = protein kinase A
WT = wild type

LQT1 patients experience triggered cardiac events during adrenergic stimulation (i.e., while exercising) (9,10).

More recently, we identified a heterozygous missense *KCNQ1* mutation, G269S, in 11 patients from 4 unrelated families. Similar to previous clinical reports (10–12), most of our patients have normal to borderline corrected QT (QTc) intervals at rest, but their QTc intervals are significantly prolonged after exercise. We characterize the functional consequences of the I_{Ks} channel reconstituted with G269S in mammalian cells and provide important insight into molecular mechanisms underlying the adrenergic-induced LQTS. Specifically, we found G269S modestly affected I_{Ks} but severely blunted the increase in I_{Ks} with isoproterenol, pharmacological activators of PKA, and with the PKA phosphomimetic mutation *KCNQ1*-S27D. These findings may explain why patients with G269S mutation showed an excessive prolongation of QT intervals on exercise and suggest a potential benefit of beta-blocker therapy.

Methods

Clinical investigation and genetic testing. The clinical diagnosis of LQTS was referred to the criteria of Schwartz et al. (2). The protocol for genetic analysis was approved by the institutional ethics committee and performed under its guidelines. Written informed consent was obtained from every subject before the analysis. Genomic deoxyribonucleic acid (DNA) used for genetic evaluation was isolated from venous blood lymphocytes. In addition to *KCNQ1*, genetic screening for mutations in other LQTS-related genes including *SCN5A*, *KCNH2*, *KCNE1*, *KCNE2*, and *KCNJ2* was conducted by denaturing high-performance liquid chromatography (WAVE system, Transgenomic Inc., Omaha, Nebraska). For abnormal screening patterns, sequencing was performed with an automated sequencer (ABI PRISM 3100x, Applied Biosystems, Foster City, California).

Heterologous expression of cDNA in CHO and HEK293 cells. Full-length complementary deoxyribonucleic acid (cDNA) encoding human wild-type (WT) *KCNQ1* (GenBank AF000571, Institut de Pharmacologie Moleculaire et Cellulaire, CNRS, Valbonne, France) was subcloned into a pIRES2-EGFP expression vector. *KCNQ1*-G269S, *KCNQ1*-S27D, and *KCNQ1*-(S27D-G269S) mutants were constructed using a Quick Change II XL site-directed mutagenesis kit (Stratagene, La Jolla, California), and they were also subcloned into the pIRES2-EGFP expression vector. Full-length cDNA encoding human *KCNE1* (GenBank M26685) subcloned into the pCDNA3.1 expression vector was obtained by polymerase chain reaction from human heart cDNA library (Clontech, Mountain View, California). Full-length cDNA encoding human A-kinase-anchoring protein 9 (Yotiao or AKAP9) was subcloned into pCDNA3.1 expression vector (Department of Bio-Informational Pharmacology, Tokyo Medical and Dental University, Japan). *KCNQ1*-WT and/or its mutants, *KCNE1* and Yotiao cDNA were transiently transfected into Chinese hamster ovary (CHO) or human embryonic kidney 293 (HEK293) cells using Lipofectamine (Invitrogen Life Technologies Inc., Carlsbad, California) according to the manufacturer's instructions.

Solutions and chemicals. The pipette solution contained (in mmol/l) 70 potassium aspartate, 40 KCl, 10 KH_2PO_4, 1 $MgSO_4$, 3 Na_2 adenosine triphosphate (Sigma, St. Louis, Missouri), 0.1 Li_2 Guanosine-5'-triphosphate (Roche Diagnostics GmbH, Mannheim, Germany), 5 ethylene glycol tetraacetic acid, and 5 *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid; and the pH was adjusted to 7.2 with KOH. The extracellular solution contained (in mmol/l) 140 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 0.5 $MgCl_2$, 0.33 NaH_2PO_4, 5.5 glucose, and 5.0 *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid; the pH was adjusted to 7.4 with NaOH. Isoproterenol (Sigma) was dissolved in distilled water (containing 1 mmol/l ascorbic acid) to yield 10 mmol/l stock solution and kept in the dark at 4°C. Forskolin (FK, Sigma) and 3-isobutyl-l-methyl-xanthine (IBMX, Sigma) were respectively dissolved in dimethyl sulfoxide (Sigma) to yield stock solutions of 5 mmol/l and 15 mmol/l, respectively.

Electrophysiological recordings and data analysis. Forty-eight hours after transfection, cells attached to a glass coverslip were transferred to a 0.5-ml bath chamber perfused with extracellular solution and maintained at 25°C. Patch-clamp experiments were conducted on green fluorescent protein-positive cells. Whole-cell membrane currents were recorded with an EPC-8 patch-clamp amplifier (HEKA, Lambrecht, Germany). I_{Ks} were evoked by depolarizing voltage-clamp steps given from a holding potential of −80 mV to various test potentials. I_{Ks} amplitude was determined by measuring the amplitude of tail current elicited on repolarization to −50 mV following 2-s depolarization to 30 mV every 10 s, and currents were normalized to the cell membrane capacitance to obtain current densities (pA/pF). Voltage-dependence of I_{Ks} activation was evaluated by fitting the *I-V* relation of the tail currents to a Boltzmann equation: $I_{K,tail} = 1/[1+\exp([V_h - V_m]/k)]$, where $I_{K,tail}$ is the tail current amplitude density, V_h is the voltage at half-maximal activation, V_m is the test potential, and k is the slope factor. The deactivation kinetics of I_{Ks} after depolarization was determined by a single exponential fit of tail current trace.

Immunocytochemistry. Forty-eight hours after transfection, CHO cells were fixed with 3.7% formaldehyde in