Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 354 (2007) 776–782
BBRC
www.elsevier.com/locate/ybbrc

Mutations in the Kv1.5 channel gene *KCNA5* in cardiac arrest patients

Nathalie H. Nielsen [a,b,1], Bo G. Winkel [a,c,d,1], Jørgen K. Kanters [a,b,e,f], Nicole Schmitt [a,b], Jacob Hofman-Bang [a,c], Henrik S. Jensen [a,b], Bo H. Bentzen [a,b], Bjarne Sigurd [f], Lars A. Larsen [g], Paal S. Andersen [a,c], Stig Haunsø [a,d], Keld Kjeldsen [d], Morten Grunnet [a,b,h], Michael Christiansen [a,c], Søren-Peter Olesen [a,b,h,*]

[a] *Copenhagen Heart Arrhythmia Research Centre, Panum Institute, University of Copenhagen, Denmark*
[b] *Department of Medical Physiology, Panum Institute, University of Copenhagen, Denmark*
[c] *Department of Clinical Biochemistry, Statens Serum Institute, Copenhagen, Denmark*
[d] *Laboratory of Molecular Cardiology & Medical Department B, The Heart Centre, Rigshospitalet, Copenhagen, Denmark*
[e] *Department of Medicine P, Gentofte University Hospital and the Department of Internal Medicine, Elsinore Hospital, Denmark*
[f] *Department of Cardiology Y, Medical Center Bispebjerg Hospital, Denmark*
[g] *Wilhelm Johannsen Centre for Functional Genome Research, Institute of Medical Biochemistry and Genetics, University of Copenhagen, Denmark*
[h] *NeuroSearch A/S, Ballerup, Denmark*

Received 5 January 2007
Available online 18 January 2007

Abstract

Mutations in one of the ion channels shaping the cardiac action potential can lead to action potential prolongation. However, only in a minority of cardiac arrest cases mutations in the known arrhythmia-related genes can be identified. In two patients with arrhythmia and cardiac arrest, we identified the point mutations P91L and E33V in the *KCNA5* gene encoding the Kv1.5 potassium channel that has not previously been associated with arrhythmia. We functionally characterized the mutations in HEK293 cells. The mutated channels behaved similarly to the wild-type with respect to biophysical characteristics and drug sensitivity. Both patients also carried a D85N polymorphism in KCNE1, which was neither found to influence the Kv1.5 nor the Kv7.1 channel activity. We conclude that although the two N-terminal Kv1.5 mutations did not show any apparent electrophysiological phenotype, it is possible that they may influence other cellular mechanisms responsible for proper electrical behaviour of native cardiomyocytes.
© 2007 Elsevier Inc. All rights reserved.

Keywords: Cardiac arrest; Kv1.5 channel; KCNE1

Cardiac arrhythmia is a prominent cause of sudden cardiac death (SCD) [1]. The cardiac action potential is the result of a well-orchestrated opening and closing of a multitude of ion channels conducting Na^+-, K^+- and Ca^{2+}-currents. Arrhythmias in patients with Long-QT Syndrome (LQTS) may result from mutations in one of the cardiac ion channels [2]. Since 1995 systematic mutation screening of ion channel genes has revealed the molecular background of several arrhythmias and recently a role of genetic ion channelopathies in sudden adult and infant death [3] syndromes has been established. However, mutations in the presently known LQTS-genes are not found in many cases of familiar arrhythmia, cardiac arrest or sudden cardiac death. In these patients, it is interesting to address whether mutations in genes coding for other key cardiac ion channels could play a role in the disease development.

In two patients with cardiac arrest, we found two individual mutations in the gene *KCNA5* encoding the cardiac potassium channel Kv1.5, which is the molecular correlate of the ultrarapid component of the repolarizing current in the heart (I_{Kur}). The two mutated *KCNA5* genes were

* Corresponding author. Fax: +4535327555.
E-mail address: spo@mfi.ku.dk (S.-P. Olesen).
[1] Joint first authorship.

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.01.048

expressed functionally for electrophysiological characterization.

Methods

Molecular screening. Genomic DNA was extracted from whole blood using a QIAamp reagent set (Qiagen GmbH, Hilden, Germany). The probands were screened for mutations in the coding region of *KCNA5*. In addition control screening was performed in both probands for the LQTS-associated genes (*KCNQ1*, *KCNH2*, *KCNE1*, *KCNE2*, *KCNJ2*, and *SCN5A*) and in other genes known to be involved in shaping the cardiac action potential (*KCNE3*, *KCNE4*, *KCNE5*, *KCND2*, *KCND3*, *KCNJ4*, *KCNJ12*, *SCN1B*, and *CASQ2*). The proband with the E33V mutation was additionally screened for mutations in *CX40*, *CX43*, *CX45*, *SLN*, *KCNC4*, *HCN2*, *HCN4*, and *AQP1*. Mutation-screening was performed by single-strand conformation polymorphism and hetero-duplex analysis using intronic primers and conditions described elsewhere. Aberrant conformers were purified and subjected to automated "Dye terminator" cycle sequencing using an ABI373 DNA sequencer (Perkin-Elmer/Applied Biosystems, Foster City, CA, USA).

Molecular biology. The mutations P91L (CTG for CCG) and E33V (GTG for GAG) were introduced into human *KCNA5* cDNA (NM_002234) by site-directed mutagenesis. For expression in mammalian cells the PCR-fragments were cloned into the pXOOM [4] or pcDNA3 vector (Invitrogen, Taastrup, Denmark) and sequenced using an ABI 377 DNA sequencer (Perkin-Elmer/Applied Biosystems, Foster City, CA, USA). The KCNE1 polymorphism D85N (AAC for GAT) was introduced into human *KCNE1* cDNA (NM_000219) and cloned into the pcDNA3 vector.

Cell culture and transfection. CHO-K1 and HEK293 cells were cultured in DMEM supplemented with 10% fetal calf serum at 37 °C. CHO-K1 cells or HEK293 were transiently transfected with 0.5 μg of the respective WT or mutant cDNAs using Lipofectamine. Fourty eight hours post-transfection, the cells were trypsinized and transferred to cover slips (3.5 mm) for experiments.

Electrophysiology. All experiments were performed in the patch-clamp whole-cell configuration. Data were sampled with Pulse (HEKA electronics, Germany) and analysed with IGOR software (WaveMetrics, Lake Oswego, USA). The pipettes had a resistance of 1.5–2.5 MΩ when filled with the intracellular solution. The series resistances (R_S) recorded in the whole-cell configuration were 2–6 MΩ. Currents were recorded by application of voltage-step protocols detailed in the figures legends. The extracellular solution contained (in mM): NaCl 140, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1 and Hepes 10, pH 7.4. The intracellular solution (pH 7.2) used in the pipettes contained 100 nM free calcium with (in mM): KCl 110, Hepes 10, EGTA 10, $CaCl_2$ 5.17, $MgCl_2$ 1.42. The free calcium concentration was calculated by Eqcal Software (Biosoftware, Cambridge, UK). The pH of all solutions was adjusted before use.

Fitting procedures. The potential where half of the channels (hKv1.5 WT or mutants) are activated or inactivated ($V_{1/2}$) were determined by fitting the current curves with a Boltzmann equation of the type:

$$\frac{I}{I_{\max}} = \frac{1}{1+\exp(\frac{V-V_{1/2}}{k})}$$

where I is the current recorded at an applied potential; $I_{\max}$ is the maximal current, V is the applied potential; $V_{1/2}$ is the potential where half of the channels are activated or inactivated, and k is the slope factor.

IC_{50} values for quinidine were calculated from cumulative equilibrium dose–response experiments. Normalized data were fitted to a Hill equation:

$$\frac{I_c}{I_0} = 1 - \frac{C^n}{C^n + (IC_{50})^n}$$

where I_0 is the percentage of remaining current, I_c is the stationary current level at the blocker concentration C, IC_{50} is the concentration giving 50% block, and n is the Hill-coefficient.

The constants of deactivation for Kv7.1 co-expressed with KCNE1 (WT or D85N) were obtained by fitting the current curves with a one-phase exponential curve of the type:

$$y = y_0 + A^* e^{(-\frac{1}{\tau}*x)}$$

where y is the current, y_0 is the current at time 0, A is the maximum current, τ is the constant of deactivation and x the time. Quinidine was purchased from SIGMA.

Statistics. Data are presented as means ± SEM of n experiments and where appropriate have been analyzed using Student's t-test.

Results

Clinical data

Case 1: a Danish male patient diagnosed with classical LQTS at the age of seven received β-adrenergic blocker treatment (atenolol 75 mg daily) and was asymptomatic, with no episodes of syncope, for 18 years. During a coffee break at work, he suddenly collapsed. His ECG showed a 480 ms QTc interval with an atypical T-wave pattern showing relatively peaked T-waves and a biphasic T-wave in lead V2 (Fig. 1D). This pattern is different from the classical T-wave patterns seen with mutations in *KCNH2*, *KCNQ1* or *SCN5A* [5]. The parents were asymptomatic and had normal QTc intervals (<440 ms). On the mother's side a distant cousin died suddenly at the age of 18.

Case 2: the second patient was a 62-year-old woman diagnosed with intermittent LQTS (with 464 ms QTc) and paroxysmal atrial fibrillation following resuscitation from sudden clinical cardiac arrest in the emergency room, when admitted to hospital for an infection treatment. There was a history of palpitations and during the days following admission the ECG exhibited periods with atrial fibrillation. No relatives were available for analysis but there was no family history of sudden cardiac death or syncope.

Molecular screening

In the genomic DNA of the case 1 patient, the substitution of a nucleotide (T for C) at position 272 was found in the coding sequence of *KCNA5*. The mutation leads to an amino-acid substitution at position 91 from proline to leucine (P91L). The patient displayed no disease-causing mutations in the coding regions of 15 other genes associated with arrhythmia. Molecular screening of the genomic DNA from family members showed that the *KCNA5* missense mutation P91L was inherited from the mother (Fig. 1A–C). The mother was asymptomatic and had normal QTc interval (<440 ms). This suggests reduced penetrance of the mutation. Further, the patient had a D85N (G253A) polymorphism in KCNE1 which was inherited from the asymptomatic father.

Molecular screening of the case 2 patient identified the mutation GAG to GTG at position 98 resulting in an E33V amino acid change. The patient displayed no

Download English Version:

https://daneshyari.com/en/article/1939303

Download Persian Version:

https://daneshyari.com/article/1939303

Daneshyari.com