Long QT Syndrome-associated Mutations in the Voltage Sensor of I_{Ks} Channels

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Key Words

Cardiac action potential • KCNE • KCNQ • LQT • Heart • Arrhythmia

Abstract

The plateau phase of the ventricular action potential is the result of balanced Ca^{2+} influx and K^+ efflux. The action potential is finally terminated by repolarising K^+ currents. Under β -adrenergic stimulation the slowly activating component of the human cardiac delayed rectifier K^+ current I_{Ks} provides the major repolarising component. I_{Ks} channels are heteromeric channels composed of KCNQ1 and KCNE1. Mutations in the voltage sensor S4 of KCNQ1 are associated with long-QT syndrome 1 (LQTS1). Here, we study the effects of the mutations S225L, I235N and L239P located in S4. The respective channels were expressed in *Xenopus* oocytes and analyzed by dual electrode voltage clamp. As a result all mutants shifted the voltage dependence of activation to the right and reduced the voltage dependence of deactivation kinetics. The activation kinetics were differently affected in homomeric mutant channels compared to wild type KCNQ1. All three mutations reduced KCNQ1/KCNE1 channel currents in a dominant-negative manner when the mutants were coexpressed with wt

subunits suggesting reduced I_{Ks} as the molecular basis of LQT1.

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Introduction

The plateau phase of the cardiac ventricular action potential is accomplished by a delicate balance of Ca^{2+} influx and K^+ efflux [1]. Finally, the action potential is terminated by repolarising K^+ currents [2]. The slowly activating component of the human cardiac delayed rectifier K^+ current I_{Ks} is a major contributor to the repolarisation of the cardiac action potential [3]. I_{Ks} channels are formed from heteromeric co-assemblies of α -subunits KCNQ1 (Kv7.1, KvLQT1) and β -subunits KCNE1 (MinK, Isk) [4, 5]. KCNQ1 shares topological homology with other voltage-gated K^+ channels: four voltage sensor transmembrane domains (S1-S4) and a pore region (S5-S6). The β -subunit KCNE1 contains a single transmembrane spanning domain with external N-terminus and intracellular C-terminus [6, 7]. Four KCNQ1 α -subunits seem to assemble with 2 β -subunits to form the functional I_{Ks} channel [8, 9].

The sympathetic nervous system mediates the fast physiological stress response to physical and psychological

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stressors. Stimulation of the sympathetic nervous system includes positive chronotropic effects on the heart rate and positive inotropic effects on the ventricles. The control of cardiac function by the sympathetic nervous system results from binding of catecholamines to G protein-coupled receptors, including the β 1-adrenergic receptor (β 1-AR) [10, 11]. Receptor activation results in activation of heterotrimeric G-proteins and subsequent stimulation of adenylate cyclase leading to increased production of cAMP, which activates the cAMP-dependent protein kinase A (PKA) [12]. Active PKA stimulates downstream effectors, including L-type Ca^{2+} channels and delayed rectifier potassium channels KCNQ1/KCNE1 (I_{Ks}) [13–15]. The influence of the sympathetic nervous system on ventricular delayed rectifier potassium channels is more profound on the slow component (I_{Ks} , KCNQ1/KCNE1) than the fast component (I_{Kr} , HERG) of the repolarising potassium current I_{K} [16]. Compartmentalization of the response to cAMP is achieved by KCNQ1/KCNE1 channel-bound A kinase-anchoring protein yotiao to a leucine zipper motif in the KCNQ1 C-terminal domain [17]. Yotiao binds and therewith recruits PKA and PP1 (protein phosphatase 1) to the KCNQ1/KCNE1 channel complex to form a channel micro-signaling domain to regulate the phosphorylation of KCNQ1-Ser27 [17]. The response to cAMP may also be facilitated by β -AR-KCNQ1/KCNE1 channel colocalization [18]. β -AR signaling is coupled to L-type Ca^{2+} channel modulation [19] structurally and functionally linking L-type Ca^{2+} channels, KCNQ1/KCNE1 channels and β -ARs.

The long QT syndrome 1 (LQTS1) results from loss-of-function mutations in the α -subunit KCNQ1. Several hundred mutations have been reported to be associated with LQTS1 (summarized in <http://www.fsm.it/cardmoc/>). Locations of KCNQ1 mutations include the voltage sensor transmembrane helix S4. Recently, mutagenesis scanning has shown that the functional integrity of this protein region is very sensitive in respect to amino acid substitutions [20, 21]. About half of the S4 residues mutated in LQTS1 were associated with swimming-triggered arrhythmia suggesting heavy phenotypes [22].

In the present study, we introduced disease-associated mutations into human KCNQ1 and studied the effects on homomeric mutant KCNQ1 channels and heteromeric wt-KCNQ1/mutant-KCNQ1/KCNE1 channels closely resembling the situation in the cardiac ventricular muscle. As a result the mutations S225L, I235N and L239P yielded complex kinetic alteration in mutant KCNQ1 channels, leading to right shifted voltage

dependence and destabilization of the second open and inactivated state of the channels. The heteromeric wt-KCNQ1/mutant-KCNQ1/KCNE1 channels all showed more than 50% reduced channel currents compared to wt-channels, suggesting dominant-negative suppression of channel function by mutated subunits.

Materials and Methods

Molecular Biology

The molecular biological procedures were the same as previously described [23]. Human *KCNQ1* (NM_000218.2, variant 1) was subcloned into oocyte expression vector pSGEM. The clone was mutated at the positions mentioned in the text by QuickChange site directed mutagenesis kit (Stratagene). All constructs were confirmed by automated DNA-sequencing. The vectors were digested using *Nhe*I and *in-vitro* synthesis of cRNA was performed with T7 mMessage mMachine kit (Ambion).

Electrophysiology

Xenopus laevis oocytes were harvested according to German law as described previously [23]. Animals were tricaine-anesthetized and ovary lobes were removed from frogs and digested with collagenase (Type II, Worthington, 1 mg/ml in calcium-free Barth's solution) for 120 min. Stage V oocytes were collected and injected with 60 nl of cRNA solution. Oocytes were injected with 5 ng of wt or mutant KCNQ1 cRNA alone or with 2.5 ng wtKCNQ1 cRNA plus 2.5 ng wt/mutant KCNQ1 cRNA plus 1 ng of KCNE1 cRNA. The oocytes were stored for 3–4 days at 17°C in Barth's solution containing (in mM): 88 NaCl, 1.1 KCl, 2.4 NaHCO_3 , 0.3 $\text{Ca}(\text{NO}_3)_2$, 0.3 CaCl_2 , 0.8 MgSO_4 , 15 HEPES-NaOH, penicillin-G (31 mg/l), streptomycin sulfate (20 mg/l), gentamycin (50 mg/l), pH 7.6. Standard TEVC recordings were performed at 22–23°C using a Turbo Tec-10CX (NPI, Germany) amplifier equipped with an ITC-16 interface and pCLAMP 8.0 software (Axon Instruments Inc./Molecular Devices, USA) and analyzed with Clampfit 8.0 and Origin 6.0 (Additive, Germany). Recording pipettes were filled with 3M KCl and had resistances of 0.4–1 M Ω . Channel currents were recorded in ND96 recording solution containing (in mM): 96 NaCl, 4 KCl, 1.8 MgCl_2 , 1.0 CaCl_2 , 5 HEPES; pH 7.6. Reagents where purchased from Sigma unless otherwise noted.

Data analysis

The activation curves were obtained by plotting initial (determined ~5 msec after the end of the test pulse) normalized tail current amplitudes (I_{tail}) versus the respective test potential. These curves were fitted to a Boltzmann equation of the form:

$$I_{\text{tail}} = [A_1 - A_2 / 1 + \exp((V_{1/2} - V) / k)] + A_2 \quad (\text{formula 1})$$

$V_{1/2}$ is the voltage of half-maximal activation; k is the slope factor; V is the voltage of the test pulse; A_1 and A_2 are the maximal and minimal current amplitudes, respectively. The apparent gating valence z_g at ~20°C is given by:

$$z_g = 25,26/k \quad (\text{formula 2})$$

The deactivating phases of wt-KCNQ1 tail currents were fitted with a single exponential function and the fit was extrapolated to the beginning of the repolarisation pulse. Extrapolated tail current amplitudes were used to plot the activation curves. Activating and deactivating current traces were fitted using the simplex algorithm to one (formula 3) or two (formula 4) exponential functions:

$$y = A_0 + A_1 * \exp(-t/\tau) \quad (\text{formula 3})$$

$$y = A_0 + A_1 * \exp(-t/\tau_1) + A_2 * \exp(-t/\tau_2) \quad (\text{formula 4})$$

With the time constant(s) τ , and its amplitude(s) A , and a steady-state current A_0 .

KCNQ1 channels showed a pronounced hook in the tail indicating recovery from inactivation. In order to fit only the deactivation and not the recovery from inactivation only the decaying component was fitted to a single exponential function (formula 3).

Statistical analysis was performed with student's t-test (unpaired). Differences were assumed significant based on $p = 0.05$ and are marked with "*" in the figures.

Results and Discussion

The mutations S225L, I235N and L239P were reported to be associated with LQTS1 [22, 24-26]. The mutations are positioned in the S4 voltage sensor transmembrane domain. The mutated residues are either homologous (lipophilic residue, Leu239) or not conserved (Ser225, Ile235) in other Kv channels (Fig. 1a). During the opening/closing of the channels the conformation of the S4 segment is expected to undergo substantial conformational changes: In the closed/resting state the side chains of the residues are expected to partially interact with the surrounding α -helices and the lipid phase. In the open state Ser225 is expected to be close to the outer membrane border and Ile235 and Leu239 may interact with the pore α -helix S5 (Fig. 1b). A rotational and translational motion may allow the transition between these putative stable channel conformations [27]. Recently, the importance of specific S4 transmembrane helical charges was assayed, showing that the respective mutations exerted diverse phenotypes [21]. However, the location of the residues mutated in LQTS1 did not affect the charged residues tested by Abbott's group. Further, a tryptophan scan in the KCNQ1 S4-segment revealed complex energetic perturbations by the respective mutations, which were modulated by the presence of KCNE1 [20]. These authors did not analyze the LQT1-mutations S225L, I235N and L239P. Thus, the phenotypes of those mutations could not be predicted. In the present study KCNQ1 was mutated to study the functional impact of the disease associated mutant channels. It should be

Fig. 1. Localization of LQTS1-associated mutations in the KCNQ1 S4 transmembrane helix. **a.** Alignment of the voltage sensor domains S4 from Kv channels, Kv7.1 is synonymous with KCNQ1. The yellow boxes mark the positions of the LQTS1-mutations and the shaded boxes indicate positions of the highly conserved arginine residues in Kv channel S4 segments. **b.** We downloaded the published closed/open state partial KCNQ1 three-dimensional coordinates [27]. In these molecular 3D-models the transmembrane domains of the four α -subunits are depicted as ribbons colored by individual colors (red, green, blue yellow). The amino acids at the positions of the LQT1 associated mutations in S4 are shown in space fill and are colored in shades of pink). In these molecular 3D-models all three residues associated with LQT1 undergo substantial transpositions during channel opening/closing. The figure was prepared using DeepView/ Swiss-PdbViewer 4.0 [29].

noted that Bianchi *et al.* studied KCNQ1(S225L) [28]. Here, we investigate S225L in more detail.

Wt and mutant KCNQ1 channels were expressed in *Xenopus laevis* oocytes and all channels were functional. Interestingly, the mutations led to diverse functional effects (Fig. 2a). The I235N channels showed weak voltage dependence and did not close completely at -140 mV, whereas the S225L and L239P channels closed at hyperpolarizing potentials. Notably, all mutant channels showed right shifted activation curves compared to wt channels ($V_{1/2-wt-KCNQ1}$: -19.3 ± 2.6 mV; $V_{1/2-S225L}$: 30.2 ± 1.7 mV; $V_{1/2-I235N}$: 76.1 ± 15.9 mV and $V_{1/2-L239P}$: 68.6 ± 16.7 mV; $n = 5-9$; Fig. 2 a-c). Also voltage dependence of the mutant channels was highly variable. The mutation S225L increased and the mutation I235N

Fig. 2. Mutations in KCNQ1 S4 shift the activation curves to the right. Wt and mutant channels were expressed in *Xenopus laevis* oocytes and channel functions were analyzed with the two-electrode voltage clamp technique. a. Representative current traces of wt and mutant KCNQ1 channel currents. Currents were activated by a pulse protocol like indicated. Arrows indicate where currents were determined. b. The activation curves were obtained by normalization of the initial tail currents to the respective value at 60 mV (n = 5-8, error as +/- SEM). The activation curves were fit to a Boltzmann function (formula 1) and the fits are shown. c. The results of the Boltzmann fits (voltage of half maximal activation ($V_{1/2}$) and slope (k)) are depicted (error bars indicate +/- SEM; *, significantly different, $p < 0.05$).

Fig. 3. Mutations in KCNQ1 S4 modulate activation and deactivation kinetics. a, b. Activating channel currents shown in Fig. 2 were fitted to a two-exponential function and the time constants of fast (τ_{fast}) and slow activation (τ_{slow}) are plotted vs. the depolarising potential. c. The relative amount of the fast component is shown plotted vs. test pulse potentials (error bars indicate +/- SEM, n = 4-8). d. Currents were recorded with a tail current protocol: Channels were activated by a 3 sec prepulse to +40 mV followed by pulses to various potentials. e. Representative normalized currents traces at -140 mV are shown. f. The tail currents (for wt-KCNQ1 only the decaying component was analyzed, see methods) were fitted to single-exponential functions and the time constants of deactivation (τ_{deact}) are depicted vs. the potentials (error bars indicate +/- SEM, n = 4-8; *, significantly different, $p < 0.05$).

decreased the voltage dependence of channels compared to wt-KCNQ1 ($k_{wt-KCNQ1}$: 19.2 ± 3.7 mV, z_g : 1.32; k_{S225L} : 10.1 ± 1 mV, z_g : 2.49; k_{I235N} : 38.8 ± 3.4 mV, z_g : 0.65; k_{L239P} : 11.1 ± 3.3 mV, z_g : 2.28; n = 5-9; Fig. 2 c). The exchange of Ile235 by Asn caused rather weak voltage dependence and a low apparent gating valence (z_g) (Fig. 2).

The mutations resulted in complex effects on channel activation. The activation of all channels could be sufficiently described by a double exponential fit, whereby the relative contribution of the two components was quite different from those of the wt channels (Fig. 3c). In L239P channels τ_{fast} was relatively unaltered, whereas τ_{slow} shows increased voltage dependence compared to wt channels (Fig. 3). Whereas the time constants of activation were similar to those of wt channels, the relative contribution of the fast component was reduced in mutant I235N channels. Moreover, the time constants of activation of mutant S225L channels were slower as

compared to wt channels (Fig. 3). In terms of deactivation, we observed that S225L and L239P channels deactivated faster than wt channels, while deactivation of the I235N channel is faster than deactivation of the wt channel at potentials above -80mV (Fig. 3d, e). Taken together, the right shifted activation curve, the increased voltage dependence, the decreased rate of activation and the increased rate of deactivation of S225L channels suggest that this mutation stabilizes the channels in closed conformations (Fig. 2, 3). Interestingly, none of the mutant channels showed a characteristic hook in the tail, indicative of KCNQ1-type inactivation suggesting that the mutant channels do not reach late open states.

In patients the mutant α -subunits transcribed from one chromosome may associate with wt- α -subunits from the second chromosome and KCNE1 β -subunits to form

Fig. 4. Mutations in KCNQ1 S4 domain modulate heteromeric wt-KCNQ1/mutant-KCNQ1/KCNE1 channel current amplitudes. a. Currents were recorded with the protocol indicated above. Representative currents traces are shown. Peak currents at the end of 7-sec test pulses ($V_{\text{hold}} = -80$ mV) were determined (positions indicated by arrows) and plotted vs. the respective test potentials (error bars indicate $\pm$ SEM, $n = 5-10$; *, significantly different, $p < 0.05$).

the I_{Ks} channels. We mimicked this situation by coexpression of mutant KCNQ1 with wt-KCNQ1 and KCNE1 and compared the respective channel currents with those of oocytes expressing wt-KCNQ1 + KCNE1 (Fig. 4). All oocytes expressing heteromeric mutant channel subunits showed currents decreased by more than 50%, indicating a dominant-negative phenotype. The strongest suppressing effects were found for S225L followed by I235N and L239P (Fig. 4).

Coexpression of KCNQ1 α -subunits with KCNE1 β -subunits resulted in increased current amplitudes at depolarized potentials above ~ 0 mV [4, 5]. In order to test if the decreased current amplitude in heteromeric mutant channels is due to defective amplitude stimulation by KCNE1 subunits we compared currents of heteromeric mutant and wt channels expressed together with or without KCNE1. Indeed, KCNE1 failed to stimulate channels containing mutant S225L subunits, which may contribute to the particularly strong suppression of currents by this mutant under situations similar to the heterozygous inheritance of most LQTS1 (Fig. 5).

The I_{Ks} represents the functional counterpart to the I_{CaL} under stress conditions and I_{Ks} and I_{CaL} may be structurally linked together [18, 19]. This balanced relationship of potassium efflux and calcium influx is key to the well dosed calcium influx preventing calcium accumulation which favors ventricular arrhythmias including *torsade de pointes* arrhythmias. The stronger the reduction in I_{Ks} by the inherited mutation in the I_{Ks} channel is the larger may be the calcium accumulation under β -adrenergic conditions. This phenomenon may be the basis for stress as a trigger of potentially lethal arrhythmias in LQTS1. Further, the effectiveness of β -blockade and left thoracic sympathectomy may be explained by a prevention of situations of dysbalanced I_{Ks} vs I_{CaL} . The three mutants represent strong inhibiting effect on heterologously expressed I_{Ks} channels. All three mutations may render patients particularly sensitive to stress via the sympathetic nervous system due to strong dominant negative suppression of I_{Ks} channels which may explain the swimming-triggered arrhythmia as a result of mutations KCNQ1(S225L) and KCNQ1(I235N) [22].

Fig. 5. Mutations in KCNQ1 S4 modulate KCNE1-mediated current facilitation. a. Heteromeric wt-KCNQ1/mutant-KCNQ1 were expressed alone or coexpressed with KCNE1 and channel currents were recorded with the same protocol used in Fig. 4. Peak currents at the end of the test pulses were analyzed and plotted vs. the potentials (error bars indicate $\pm$ SEM, $n = 5-10$; *, significantly different, $p < 0.05$).

In summary, the three mutations in the voltage sensor domain of KCNQ1 channels dominantly suppress heteromeric KCNQ1/KCNE1 channel function explaining the long QT syndrome reported in people carrying the respective mutation.

Abbreviations

LQTS1 (long QT syndrome 1); wt (wild type);
 β -AR (β -adrenergic receptor).

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