Compound Mutations in Long QT Syndrome Assessed by a Computer Model

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Abstract

Long QT syndrome (LQTS) is an electrical disorder that predisposes affected individuals to sudden death from cardiac arrhythmias. Recently, it has been shown that compound mutations in LQTS are more common than expected and cause a severe phenotype. We used a mathematical model of rabbit ventricular myocyte (LabHEART) to investigate the simultaneous effects of three compound mutations reported in LQTS patients. Our results show that the mutations prolong the action potential (AP), being the impact of compound mutations stronger than the additive effects of single ones. The user-friendly characteristic of LabHEART allows combining easily different levels of current alterations to evaluate their outcome. This feature makes it an invaluable tool for researchers who want to explore the effects of channel mutations on the AP waveform.

1. Introduction

Long QT syndrome (LQTS) is a cardiac disease characterized by prolongation of the QT interval on the ECG. It is caused by mutations in genes coding for cardiac ion channel subunits involved in the cardiac AP. Up to now 8 types of LQTS have been described, each of them corresponding to a particular gene [1]. There is considerable variation in the clinical presentation of LQTS, ranging from no symptoms to cardiac arrest, even among members of the same family. Recently, molecular screenings showed that, within LQTS families, some individuals may carry more than a single mutation and the presence of compound mutations is more frequent than expected [2;3]. These studies showed interesting genotype-phenotype correlations, being striking the differences in the clinical manifestations of the carriers of compound mutations and their family members carriers of a single mutation only. Symptoms as syncope and cardiac arrest are observed almost in all carriers of compound mutations but in only a small percentage of patients with only one mutation.

Computational modeling was used in the last years to provide mechanistic insights into the LQTS-associated arrhythmogenesis. So far, only the effects of single mutations were taken into account. The use of mathematical models helps to overcome the uncertainties in predicting the combined effects of multiple mutations on the action potential (AP), calcium transient and force generation due to the synergistic interaction of the different mechanisms involved in the excitation-contraction coupling. In the present study, a model of the rabbit ventricular myocyte was used to simulate the effects of three compound mutations already reported in LQTS patients (Tab. 1), by reproducing the currents alterations experimentally assessed in Xenopus oocytes heterologous expression systems [3-5]. Our model confirms the severity of compound mutations in the LQTS carriers.

Table 1. The compound mutations considered in this study alter the slowly and rapidly activating delayed-rectifier potassium currents (I_{Ks} and I_{Kr}) and the fast sodium current (I_{Na}).

Protein (Gene)	Mutation	Protein (Gene)	Mutation
I_{Ks} α subunit (KvLQT1)	G168R[3]	I_{Ks} β subunit (KCNE1)	D85N[3]
I_{Ks} α subunit (KvLQT1)	D611Y[4]	I_{Kr} α subunit (HERG)	D609G[4]
I_{Na} α subunit (SCN5A)	R1623Q[5]	I_{Ks} β subunit (KCNE1)	D85N[3]

2. Methods

The general approach of using LabHEART as a model of the rabbit ventricular cell has been described previously [6]. An upgraded version (LabHEART 4.9.6) was implemented for this study [7].

The equations describing the sodium and potassium currents were modified in order to reproduce the features of the mutants. The formulations of I_{Ks}, I_{Kr} and I_{Na}, which are listed below, were based on experimental data from heterologous expression in heterozygous or homozygous conditions. Mutations were then incorporated into the model to reproduce the heterozygous condition by considering 50% of wild type (WT) and 50% of mutant

channels.

G168R and D85N reduced I_{Ks}, whereas only minor effects were noticed on the kinetics of the current [3]. Accordingly, the maximal conductance of I_{Ks} was decreased.

D611Y did not significantly affected current amplitudes with respect to WT, however the mutation slowed down the I_{Ks} activation process [4]. We simulated the effects of the mutation by increasing the slow and fast time constant of I_{Ks} activation.

D609G strongly suppressed the peak and tail I_{Kr} currents [4]. The effect of the mutation was reproduced by reducing the maximal conductance of I_{Kr}.

R1623Q slowed the rate of I_{Na} decay. Time from peak current to 50% decay (T_{50}) was prolonged threefold in the mutant with respect to WT [5]. This feature was reproduced by altering the parameters describing the I_{Na} inactivation process.

I_{Ks} (WT)

$$I_{Ks} = \overline{G_{Ks}} X_{s1} X_{s2} (V - E_{Ks})$$

$$\overline{G_{Ks}} = 0.0057 + \frac{0.019}{1 + e^{\frac{-7.2 - \log([Ca^{2+}]_i) + 3}{0.6}}}$$

$$X_{s1\infty} = X_{s2\infty} = \frac{1}{1 + e^{-\frac{V+6}{14.5}}}$$

$$\tau_{Xs1} = \frac{1}{\frac{3.53 \cdot 10^{-5}(V+10)}{1 - e^{-0.4476(V+10)}} - \frac{9.035 \cdot 10^{-7}(V+10)}{e^{-4.921 \cdot 10^{-4}(V+10)} - 1}}$$

$$\tau_{Xs2} = 4\tau_{Xs1}$$

I_{Ks}(G168R, heterozygous)

$$\overline{G_{Ks}} = 0.55 \cdot \overline{G_{Ks}}$$

I_{Ks} (D85N, heterozygous)

$$\overline{G_{Ks}} = 0.7 \cdot \overline{G_{Ks}}$$

I_{Ks} (G168R+D85N, heterozygous)

$$\overline{G_{Ks}} = 0.2 \cdot \overline{G_{Ks}}$$

I_{Ks}(D611Y, heterozygous)

$$\tau_{Xs1} = \frac{1}{\frac{3.6044 \cdot 10^{-5}(V+10)}{1 - e^{-3.3544(V+10)}} + \frac{5.7625 \cdot 10^{-5}(V+10)}{e^{0.0385(V+10)} - 1}}$$

I_{Kr} (D609G, heterozygous)

$$\overline{G_{Kr}} = 0.2 \cdot \overline{G_{Kr}}$$

I_{Na} (R1623Q, homozygous)

$$I_{Na} = \overline{G_{Na}} m^3 hj (V - E_{Na})$$

If V≥-40mV,

$$\alpha_h = \alpha_j = 0;$$

$$\beta_h = \frac{1}{0.4333\left(1 + e^{-\frac{V+10.66}{11.1}}\right)};$$

$$\beta_j = \frac{0.03 e^{-2.535 \cdot 10^{-7} V}}{1 + e^{-0.1(V+32)}}.$$

If V≤-40mV,

$$\alpha_h = 0.135 e^{-\frac{80+V}{6.8}};$$

$$\beta_h = 1.78 e^{0.079V} + 1.55 \cdot 10^5 e^{0.35V};$$

$$\alpha_j = \frac{\left(-1.2714 \cdot 10^5 e^{0.2444V} - 3.474 \cdot 10^{-5} e^{-0.04391V}\right)(V + 37.78)}{1 + e^{0.311(V+79.23)}};$$

$$\beta_j = \frac{0.01212 e^{-0.01052V}}{1 + e^{-0.1378(V+40.14)}}.$$

For the entire range of V,

$$\alpha_m = \frac{0.32(V + 47.13)}{1 - e^{0.1(V+47.13)}};$$

$$\beta_m = 0.08 e^{-\frac{V}{11}}.$$

Field stimulation of the cardiomyocyte was simulated at different frequencies (0.25, 0.5, 1, 2, 3, 4, 5 Hz). Action potential duration was assessed at 90% of repolarization (APD_{90}).

3. Results

The agreement between the main experimental and simulated functional effects of single mutations on potassium and sodium currents is shown in Figures 1 and 2. Figure 1 shows the experimental findings of Yamaguchi et al. [4] and the fitting of our model for the

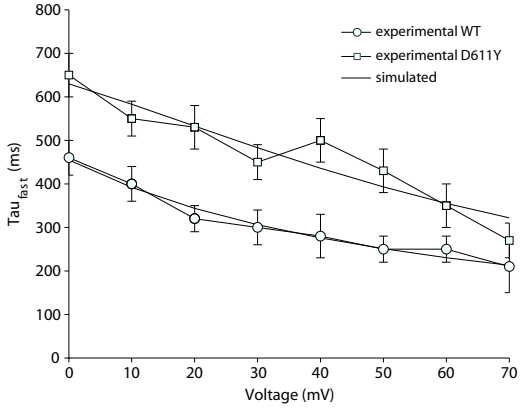

Figure 1. WT and mutant (D611Y) time constants of I_{Ks} activation.

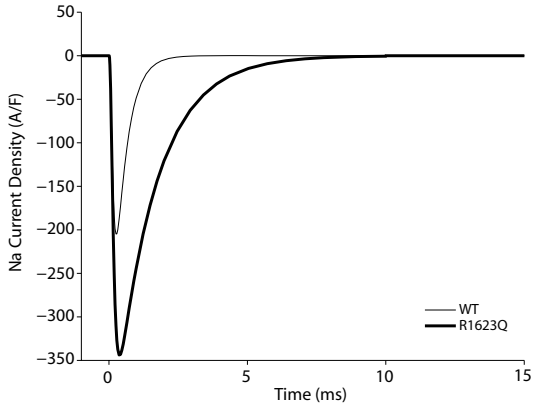

Figure 2. Simulated WT and mutant (R1623Q) I_{Na}.

fast time constant of WT and D611Y I_{Ks} activation. The simulated R1623Q I_{Na} current showed a slowed rate of decay with respect to WT (T_{50} = 0.3 ms in WT vs. 0.9 ms in mutant), in addition to a higher peak amplitude (Fig.2), according with [5].

Next we determined the consequences of single or double mutations on the AP. The reduction of I_{Ks} maximal conductance, which is the only functional impact of D85N and G168R, produced only negligible effects on the simulated action potential of the rabbit ventricular myocyte (see Fig. 3). Both the mutations caused an increased of APD_{90} (at 1 Hz, WT: 175 ms; D85N: 177 ms; G168R: 179 ms), as well as the compound mutation (at 1 Hz, D85N+G168R: 181 ms).

The increase in the time constant of I_{Ks} activation due to D611Y only slightly affected the AP with respect to the WT (at 1 Hz, APD_{90}=181 ms), whereas the reduction in the I_{Kr} maximal conductance (D609G) produced a more significant lengthening of the AP (at 1 Hz, APD_{90}=258 ms). The combination of the two mutant currents (D611Y+D609G) had a stronger impact on the AP leading to a further increase in its duration (APD_{90}=285 ms, see Fig. 4).

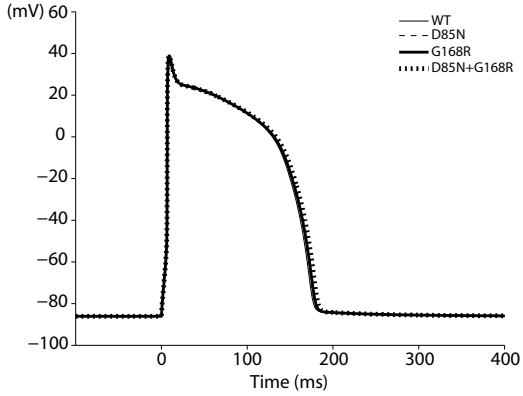

Figure 3. Simulated APs (at 1 Hz) for WT (solid thin line), G168R (dashed line), D85N (solid thick line), and compound mutation (dotted line).

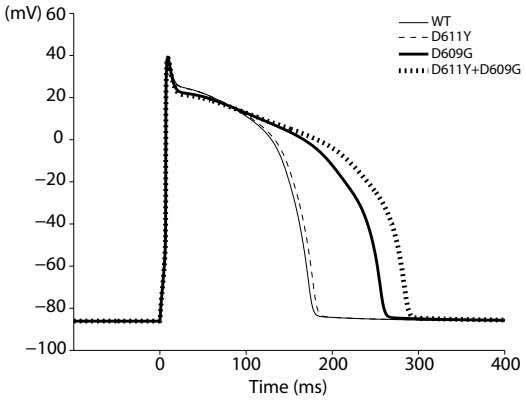

Figure 4. Simulated APs (at 1 Hz) for WT (solid thin line), D611Y (dashed line), D609G (solid thick line), and compound mutation (dotted line).

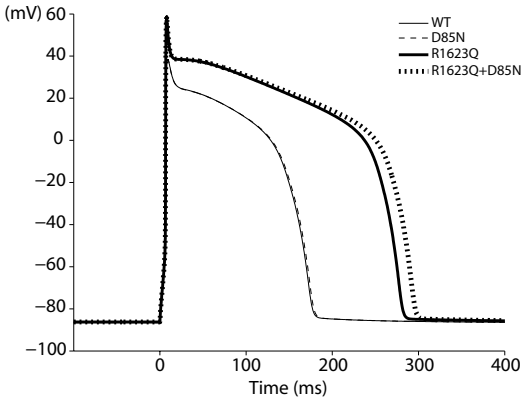

Figure 5. Simulated APs (at 1 Hz) for WT (solid thin line), D85N (dashed line), R1623Q (solid thick line), and compound mutation (dotted line).

Figure 5 shows the effects on the AP of the third couple of mutations. As already shown, D85N only slightly affected the AP, while R1623Q had a marked impact, by increasing the upstroke and delaying the repolarization process (APD_{90}=278 ms). The combination of both the mutant currents had a more pronounced effect on the rabbit cardiomyocyte, prolonging the APD_{90} up to 293 ms.

Finally, Figure 6 shows the APD_{90} as a function of the stimulation frequency for WT and compound mutations.

4. Discussion and conclusions

In the present study, the effects of single and compound LQTS mutations on the ventricular AP were assessed by using LabHEART. The phenotypic consequences of compound mutations are more severe

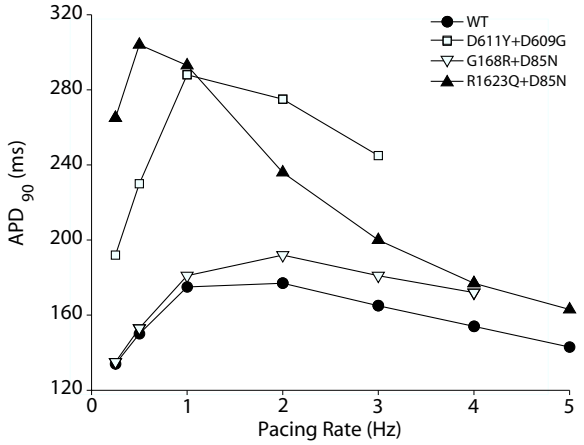

Figure 6. APD_{90} vs. pacing rate for WT and compound mutations (0.25, 0.5, 1, 2, 3, 4, 5 Hz)

than the effects due to one mutation alone and even than the algebraic addition of the effects of single ones, leading to a more marked increase of the APD_{90}. This is in accordance with clinical and experimental studies showing that LQTS-associated compound mutations cause a severe phenotype and are associated with increased arrhythmic risk [2;3].

Syncope events in LQTS patients are often associated to a sudden increase in the sympathetic activity, in example during emotional stress (fear or anger) or exercise (swimming in particular), or the combination of both. This sort of trigger is frequent in patients with LQT1 (KvLQT1). A sudden awakening is almost a specific trigger for LQT2 patients (HERG). Accordingly, beta-blockers are widely used to prevent the lethal cardiac events associated with the LQTS, especially in KvLQT1-related LQTS patients [1]. In our simulations, the mutations associated with LQT1 and LQT2 (D611Y+D609G and G168R+D85N) are susceptible to the increase in the pacing rate and they fail to follow the high frequencies (see Fig. 6).

On the other hand, LQTS families have been reported in which cardiac arrest happened at rest, most frequently during sleep. Cardiac events at rest are rare in LQT1, whereas they are frequent in patients with LQT3 [1]. Accordingly, the simulated R1623Q+D85N compound mutation showed a worse effect on the action potential at low pacing frequencies (Fig. 6).

In this study, we used experimental data from heterologous expression in Xenopus oocytes. We are aware the limitation of this approach, however in each case the mutant currents were compared to the WT currents. Such comparison also reduces the limits due to the use of a model of the rabbit ventricular myocyte, instead of a human model.

From the modelling point of view LabHEART provides the unique feature of alterating not only the conductance of the channels but the equations as well without the need of recompiling the program. This characteristic makes our model a versatile tool to explore compound mutations on the cardiac myocytes.

References

[1] Ching CK, Tan Ec. Congenital long QT syndromes: clinical features, molecular genetics and genetic testing. Expert Review of Molecular Diagnostics 2006;6(3):365-74.

[2] Schwartz PJ, Priori SG, Napolitano C. How Really Rare Are Rare Diseases?:. The Intriguing Case of Independent Compound Mutations in the Long QT Syndrome. Journal of Cardiovascular Electrophysiology 2003;14(10):1120-1.

[3] Westenskow P, Splawski I, Timothy KW, Keating MT, Sanguinetti MC. Compound Mutations: A Common Cause of Severe Long-QT Syndrome. Circulation 2004 Apr 20;109(15):1834-41.

[4] Yamaguchi M, Shimizu M, Ino H, Terai H, Hayashi K, Kaneda T, et al. Compound heterozygosity for mutations Asp611-->Tyr in KCNQ1 and Asp609-->Gly in KCNH2 associated with severe long QT syndrome. Clin Sci (Lond). 108[2], 143-150. 2005.

[5] Kambouris NG, Nuss HB, Johns DC, Tomaselli GF, Marban E, Balser JR. Phenotypic Characterization of a Novel Long-QT Syndrome Mutation (R1623Q) in the Cardiac Sodium Channel. Circulation 1998 Feb 24;97(7):640-4.

[6] Puglisi JL, Bers DM. LabHEART: an interactive computer model of rabbit ventricular myocyte ion channels and Ca transport. Am J Physiol Cell Physiol 2001 Dec 1;281(6):C2049-C2060.

[7] LabHEART The Official Homepage 2006 Available from: URL: http://www.labheart.org

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