In-silico Evaluation of β-adrenergic Effects on the Long-QT Syndrome

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Abstract

Patients suffering from the congenital Long-QT syndrome have been reported to react highly sensitive to the presence of β-adrenergic agents that are produced by the sympathetic nervous system. In this work we used an anisotropic and electrophysiologically heterogeneous in-silico model to reproduce wedge experiments in which the Long-QT syndrome was induced pharmacologically. The integration of an intracellular signaling cascade allowed the prediction of the effects of adrenergic agents on the different subtypes of the Long-QT syndrome. For LQT1 the in-silico model predicted a QT prolongation in the transmural pseudo ECG without an increase in transmural dispersion of repolarization. For LQT2 and LQT3 the QT prolongation was accompanied by an increased transmural dispersion of repolarization. β-adrenergic tonus shortened the QT interval and increased transmural dispersion of repolarization. These findings were consistent with the experimental reports.

1. Introduction

The congenital Long-QT syndrome (LQTS) is caused by mutations in specific ion channel genes. Clinically it manifests as QT prolongation and changes in T Wave morphology that can lead to polymorphic ventricular tachycardia from the Torsade de Pointes (TdP) type. Especially LQT1 patients have been reported to react highly sensitive to the presence of β-adrenergic agents that are produced by the sympathetic branch of the autonomous nervous system during episodes of physical activity.

Antzelevitch et al. have conducted canine wedge experiments in which they pharmacologically induced different LQTS subtypes and investigated the effects of various drugs and β-blockers while trying to provoke episodes of TdP [1–3]. Although different research groups have created in-silico models of the LQTS, not all features of the pharmacological experiments could be reproduced. E.g. in case of LQT1 most computational models predict a reduced T Wave width and amplitude as the mutation affects the heterogeneously distributed channel I_{Ks} [4, 5]. The mutation-induced loss of function homogenizes the repolarization thereby reducing the transmural dispersion of repolarization (TDR) and thus T Wave width and amplitude. However, previous in-silico models did not consider the effects of β-adrenergic regulation which is known to shorten action potential duration (APD), increase TDR and elevate both inotropy and chronotropy. Recently, Saucerman et al. presented a model for the intracellular β-adrenergic signaling cascade [6] which we integrated into the model of human ventricular electrophysiology from ten Tusscher et al. [7].

In this work we used an extended version of this model to mimic the wedge experiments from Antzelevitch's group with pharmacologically induced LQT1, LQT2 and LQT3. We focused especially on changes in TDR that were introduced by the different LQTS subtypes both in the presence and absence of β-adrenergic stimulation.

2. Methods

The in-silico model of the ventricular wedge preparation was composed of 120 x 20 x 20 cubic elements with a side length of 0.1 mm. This resulted in a wedge-length of 12 mm which was comparable to the experimental preparations [1, 3]. The anisotropic electrical conduction of the myocard was considered by assigning fiber orientation using a rule-based method which was parameterized with measurement data from Streeter et al. [8].

The electrophysiological properties were described using the second version of the ten Tusscher model for human ventricular myocytes [9] in which we integrated the adrenergic signaling cascade from Saucerman et al. [7]. We recently expanded the model by adding adrenergic effects on the Na/K-ATPase (NKA). If adrenergic influences on NKA are not considered, this can lead to spontaneous Ca transients from the sarcoplasmic reticulum into the cytosol as shown in knockout mice experiments [10]. Transmural electrophysiological heterogenity was modelled using three distinct tissue layers: 20% endocardium, 30% midmyocardium and 50% epicardium. The maximum conductance of the slowly activating delayed rectifier current g_{Ks} was adapted for each of the three cell types to create a similar ratio of $APD_{90,Epi}/APD_{90,M}/APD_{90,Endo}$ as in the wedge experiments [2] ($g_{Ks,Endo} = 0.45$, $g_{Ks,M} =$

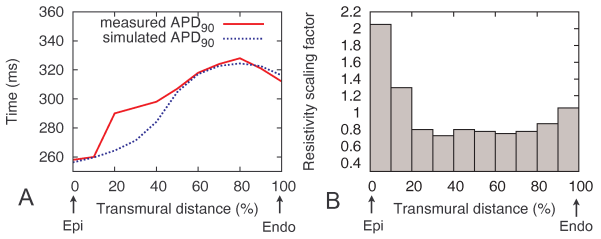

Figure 1. Wedge setup: A: Simulated and measured [2] transmural APD_{90} distribution for the wild-type case. A constant offset of 35 ms was added to the measured data to allow a better comparison of the shorter APDs of the canine measurements and the simulations which used a model of human electrophysiology. B: Extracted resistivity scaling factors from the wedge experiments [2] that were used to adapt the transmural conductivities.

0.08, $g_{Ks,Epi} = 0.75$). In addition to that, changes in tissue resistivity throughout the wall of the wedge preparations have been reported [2]. Resistivity scaling factors were derived from these measurements and used to transmurally adapt the conductivities accordingly. Fig. 2 shows the transmural distribution of the APD_{90} and the tissue resistivities between epicardium and endocardium.

Electrical excitation of the wedge was initiated by applying intracellular current at the endocardial front surface. The bidomain model was used to describe the current flow through gap junctions as well as through intra- and extracellular space. A transmural pseudo ECG (tECG) was calculated by submerging the wedge in blood ($\sigma_{blood} = 0.7$ S/m), placing a ground electrode near the endocardium and recording the extracellular potentials at an electrode in 1 mm distance from the epicardial front surface.

LQT1 and LQT2 were modelled by reducing the maximum conductance of g_{Ks} and g_{Kr} to 50%, respectively. To investigate the effects of LQT3, we used a Markov model of I_{Na} which describes a mutation in the C terminus of the channel (1795insD) [11]. β-adrenergic effects were triggered by applying $1\mu M$ of the adrenergic agonist isoproterenol (ISO). Prior to the simulation of the excitation spread in the wedge, all parameter configurations of the electrophysiological model were pre-calculated in an uncoupled environment for 120 s with a basic cycle length of 2000 ms to adjust gating variables and ionic concentrations.

3. Results

After stimulating the endocardial front surface of the computational wedge, the excitation propagated towards the epicardium. Due to the chosen APD_{90} distribution (see Fig. 2A) the repolarization started in the epicardial regions and ended in the M cells. The adopted transmural

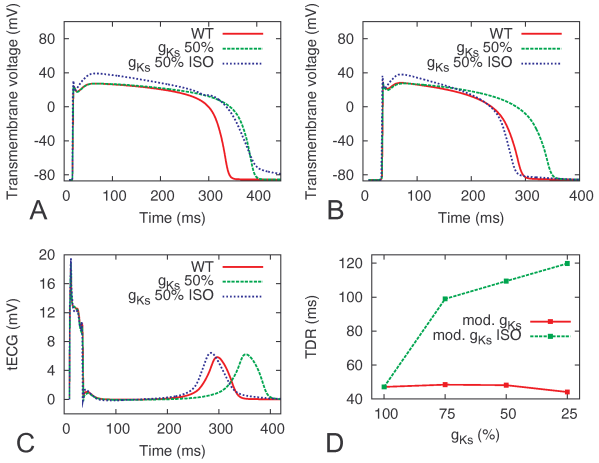

Figure 2. AP course of M (A) and epicardial cells (B) are shown for WT, LQT1 (50% g_{Ks}) and LQT1 with ISO. C: tECGs. D: TDR as a function of different $_{Ks}$ reduction levels with (mod. g_{Ks} ISO) and without ISO (mod. g_{Ks}).

conductivity arrangement (see Fig. 2B) resulted in a conduction time of 26 ms which translated into a propagation velocity of 46 cm/s. This velocity was close to experimental recordings [12].

The AP plots for all the LQT mutations were extracted from the tissue simulation. The reported APD_{90}-changes which are stated in the following are relative changes compared to the wild-type (WT) setup. Fig. 2 shows the effects of a loss of function of I_{Ks} which is associated with LQT1. In case of a $g_{Ks,max}$ reduction of 50% the APD_{90} of M and epicardial cells were both prolonged ($APD_{90,M}$ +16%, $APD_{90,EPI}$ +21%). After the application of ISO the APD_{90} of the M cells was slightly longer than in LQT1 without ISO ($APD_{90,M}$ +21%) whereas the APD_{90} of epicardial cells was abbreviated by 4%. The resulting tECG showed a QT prolongation for LQT1 which was compensated by the effects of ISO. The TDR was not significantly changed for different levels of I_{Ks}-block. However, if ISO was applied in addition to a $g_{Ks,max}$-reduction the TDR increased significantly.

The reduced maximal g_{Kr} conductivity that was used to model LQT2 led to a slight APD_{90} prolongation in both M and epicardial cells ($APD_{90,M}$ +7%, $APD_{90,EPI}$ +6%, see Fig.3). The administration of ISO lead to a dramatic shortening of epicardial APD_{90} (-21%) whereas M cells were not affected (+7%). The QT time of the tECG was only slightly prolonged for LQT2 but severly shortened in case of ISO. With increasing reduction of $g_{Kr,max}$ the TDR was increased resulting in a widening of the T Wave in the tECG. The application of ISO further increased the TDR, whereas no additional widening effects on the T Wave were visible.

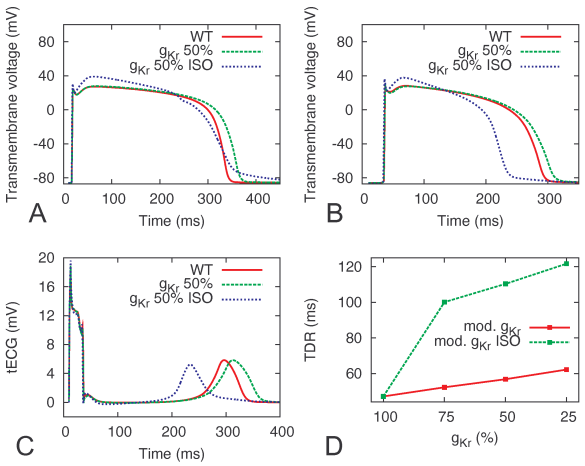

Figure 3. AP course of M (A) and epicardial cells (B) are shown for WT, LQT2 (50% g_{Kr}) and LQT2 with ISO. C: tECGs. D: TDR as a function of different g_{Kr} reduction levels with (mod. g_{Kr} ISO) and without ISO (mod. g_{Kr}).

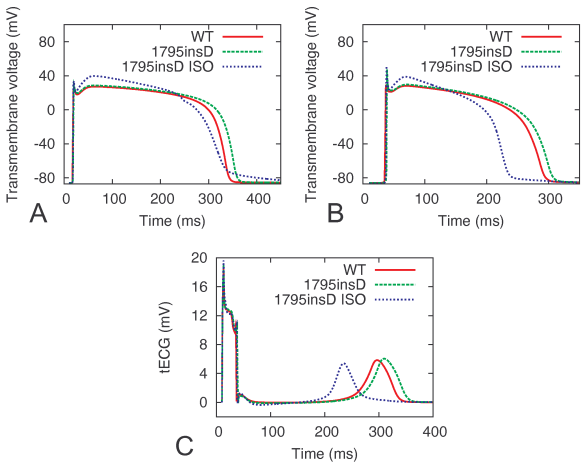

Figure 4. AP course of M (A) and epicardial cells (B) are shown for WT, LQT3 (1795insD) and LQT3 with ISO. C: tECGs.

When we included the mutated I_{Na} to model LQT3, the APD_{90} of M and epicardial cells were marginally prolonged (+5% and +3% respectively) as seen in Fig. 4. The addition of ISO again preferentially shortened the APD_{90} of epicardial cells ($APD_{90,M}$ +2%, $APD_{90,EPI}$ -23%) thus increasing the TDR ($TDR_{WT} = 47ms$, $TDR_{LQT3} = 53ms$, $TDR_{LQT3,ISO} = 96ms$). Similarly as in the case of LQT2, the mutation prolonged the QT time only slightly whereas ISO shortened the QT time to smaller values than in the WT case.

4. Discussion and conclusions

In this work, we reproduced the LQTS wedge experiments from Antzelevitch et al. with an in-silico model. Although previous studies had similar aims [4, 13], they were not able to investigate the effects of β-adrenergic regulation as no model for the corresponding intracellular signaling processes was available at that time.

Great care was taken in trying to reproduce the original experimental setup as close as possible. We therefore adapted endocardial, midmyocardial and epicardial g_{Ks} in order to mimic the transmural APD distribution that was measured in the wedge (see Fig. 2). Unfortunately, Antzelevitch et al. did not explicitly state the position and thickness of the endo, M and epi-layers. With the assumed 20%:30%:50% we were not able to exactly match the transmural APD course even though we added a layer of high resistivity that was reported to decouple the epicardium from the M cells [2] (see Fig. 2B). Antezelevitch et al. attributed the existence of such a layer to a "region of sharp transition of cell orientation" [2]. However, this abrupt change in fiber orientation contradicts the generally accepted idea of almost parallel fibers with respect to the endo- and epicardial borders [8].

The results of the computational wedge simulations were in good agreement with the measurements. In case of LQT1 the reduction of maximal g_{Ks} induced a homogeneous prolongation of the APD of the three cell types with no significant reduction in TDR [3]. Only a large reduction to 25% of the baseline g_{Ks} value caused a small reduction in TDR. This is different from previous studies [4,5] where a reduction in g_{Ks} always led to a reduced TDR and thus to narrow or even negative T Waves. The main reason for the almost constant TDR at moderate reduction rates of g_{Ks} was the initial elevation of baseline g_{Ks} values in order to reproduce the transmural APD course in Fig. 2A. If the heterogenously distributed channel I_{Ks} is dominant over the homogeneously distributed I_{Kr} due to elevated g_{Ks}, a moderate reduction of g_{Ks} does not significantly reduce the dispersion of repolarization. This can also be seen in the work from Gima et al. where extremely high densities of I_{Ks}/I_{Kr} were chosen (11:1 endocardial, 4:1 midmyocardial and 35:1 epicardial) [13]. With this setup they were also able to generate upright T Waves with similar width than in the WT case. However, they claim to be able to generate positive T Waves even if I_{Ks} was set to 0%. This directly contradicts their own findings as they reported that a wedge with a homogeneously distributed I_{Ks} will produce negative T Waves.

In case of LQT2 a reduction of the homogeneously distributed I_{Kr} amplified the existing I_{Ks}-based heterogenity. This gave rise to an increase in TDR and a widening of the T Waves in the tECG. A similar effect was seen for LQT3 where the mutation-induced late component of I_{Na} preferentially prolonged the APD of the M cells thus aug-

menting the TDR. The smaller QT prolongation of LQT2 and LQT3 compared to LQT1 was due to the elevated levels of I_{Ks} which partly compensated the reduction in I_{Kr} or the increased influx due to the late component of I_{Na}, respectivley.

The application of ISO always shortened the APD_{90} of epicardial cells. In case of LQT1 and LQT2 ISO prolonged the APD_{90} of the M cells while there was almost no ISO-induced change of M cell APD_{90} in LQT3. Furthermore ISO increased the TDR in all three cases. Except for the increase in TDR under adrenergic influence in case of LQT3, the results of the in-silico model are consistent with the experimental reports [1,3]. However, unlike in the wedge experiments, the ISO-induced increase in TDR did not lead to a significant widening of the associated T Waves. If the ISO effects over time are considered, it is interesting to note, that our computational results match with the effects that Antzelevitch et al. observed 2 minutes after the application of ISO. However, our precalculations ensured that the ISO-effects were maximal in the in-silico model which should in theory correspond to the effects after 10 minutes in Antzelevitch's experiments.

Although this computational study was able to reproduce the main features of the pharmacologically induced LQT syndromes, there are still some unanswered questions when it comes to the interpretation of clincial ECG recordings of patients with LQT1 and LQT2. While the wedge experiments predict no significant changes in TDR and T Wave width for LQT1, patients with this disease show broad-based T Waves [14] (even at rest without adrenergic stimulation). This is difficult to explain as the mutation is associated with a loss of function of the heterogeneously expressed I_{Ks}. A reduction of I_{Ks} cannot cause an increase in TDR which would explain broad-based T Waves. On the other hand the tECGs from the wedge experiments show an increase in TDR for LQT2 which is difficult to connect to the low-amplitude T Waves which are seen in patients suffering from LQT2 [14]. A possible explanation for patients with LQT1 that show a broad-based T Wave could be that they suffer from a recently reported mutation that does not cause a simple loss of function of I_{Ks}, but rather separates I_{Ks} from adrenergic regulation. Due to enhancing effects of ISO on I_{CaL} and other currents the APD and thus QT time is prolonged without a reduction of the intrinsic I_{Ks}-based heterogeneous properties.

Acknowledgements

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