Effects of the Reggae Mutation on Sinus Node Function: A Simulation Study

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Abstract

The sinus node is the primary pacemaker of the heart. A complex interplay of heterogeneities is assumed to be the basic mechanism that the sinus node can drive the heart. This interplay can be disturbed by e.g. diseases, drugs or mutations. In this work the effect of a mutation on the sinus node function were investigated. Therefore, measurement data of wild-type and mutant I_{Kr} channels were integrated with aid of optimization procedures into the heterogeneous sinus node model of Zhang et al. The measurement data shows a shift of the steady-state inactivation to more positive potentials. Simulated central sinus node cells lose their ability to depolarize spontaneously. Peripheral cell are also effected by the mutation. The main changes are the shortening of the action potential duration from $108\,ms$ to $84\,ms$ and the increase of auto-rhythmic frequency from $6.37\,Hz$ to $7.62\,Hz$ due to an increased mean I_{Kr} current. In a future study the bradycardial effect of this mutation will be shown in a tissue model.

1. Introduction

The autonomous activity of the heart is caused by a small, but complex part of the right atrium, the sinus node [1]. Here, specialized cells permanently initiate an electrical excitation of the surrounding tissue. From this primary pacemaker the action potentials propagate over the atria and via the atrioventricular node to the ventricles leading to a well balanced mechanical contraction.

The sinus node is a heterogeneous structure composed of two types of cells with different morphology and electrophysiological properties [1]. One type is distributed more densely in the periphery the other in the center. Varying gap junction types and densities exist leading also to a heterogeneity in conduction. It is supposed that this complex interplay of heterogeneities is the basic mechanism that the small sinus node is able to electrically drive the surrounding atrial muscle [2]. If this interplay is disturbed, the function of the sinus node can be effected massively.

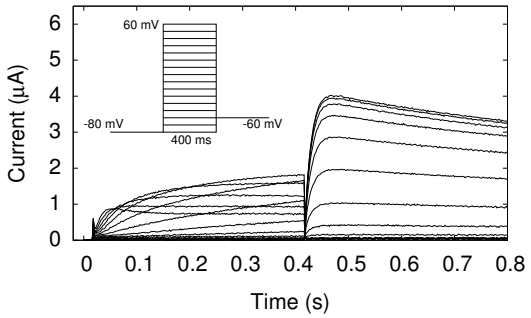
Mutations of ion channels are known to alter the channel kinetics. This can lead to a loss- or a gain-of-channel function. Since these changes lead to an unphysiological variation of ionic currents, patients suffering from channel mutations have normally higher risk of life-threatening cardiac fibrillation. One diagnostic criterion for the Long and Short QT syndrome in especially young patients is their bradycardia i.e. slower heart rate. This was shown e.g. for hERG mutations changing the rapid delayed rectifier potassium I_{Kr} current [3], KCNQ1 mutations altering the slow delayed rectifier potassium I_{Ks} current [4], HCN4 mutations influencing the pacemaker current I_f [5] and many others. The bradycardia could be generated either by an atrioventricular block due to atrial fibrillation or changed electrophysiological properties in the atrioventricular node or due to a slowed sinus node activity.

In this simulation study the effects of the L532P mutation in hERG called reggae on sinus node electrophysiology were demonstrated. Therefore, voltage clamp measurement data with a specific step protocol from wild-type (physiological) and mutant channels expressed in oocytes were used [6]. These data was integrated into the Zhang et al. cell model with aid of optimization methods. The electrophysiological changes were than analyzed.

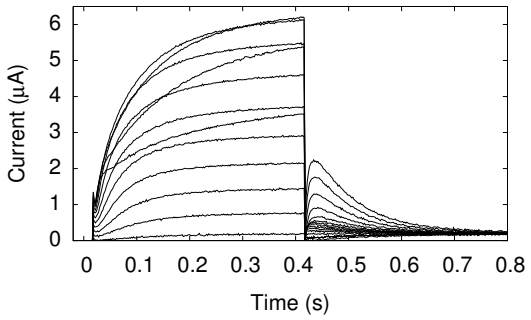
2. Methods

The so called reggae mutation shows similar clinical features as the human Short QT syndrome namely accelerated repolarization of the myocardium which might lead to life-threatening cardiac fibrillation. This L532P mutation is located in the voltage sensor segment of the hERG protein forming the α-subunit of I_{Kr} channels in human myocardial cells.

In order to characterize the function changes, wild-type and mutant hERG channels were expressed in xenopus oocytes and the channel properties were measured with voltage-clamp technique [6]. The cells were measured at $22°C$ and an extracellular potassium concentration of $5\,mM$. The voltage protocol consisted of a $400\,ms$ prepulse at $-80\,mV$ followed by a varying depolarization



(a) Wild-type current traces



(b) Reggae mutant current traces

Figure 1. Current traces of wild-type (a) and reggae mutant (b) hERG channels achieved with the voltage clamp protocol shown in inset of (a).

voltage step between $-80\,mV$ and $60\,mV$ in $10\,mV$ steps for $400\,ms$ (see fig. 1 inset). After the depolarization steps a repolarization step to $-60\,mV$ was applied for $400\,ms$.

The data showed mainly a shift of the steady-state inactivation to more positive potentials. This leads to an increase of the ionic current during the depolarized phase (compare fig. 1 a and b) since the channels stay in the open state for higher voltages. Additionally, the tail current during to the repolarization step is much smaller since more channels are in the open state and can directly go back into the closed state.

The heterogeneous rabbit sinus node model of Zhang et al. [7] (see fig. 2) was used in order to describe the effects of this mutation on single cell sinus node function. The model is describing the properties of central and peripheral cells with a set of non-linear, coupled ordinary differential equations. The model is based on rabbit sinus node experimental data and is constructed of 14 Hodgkin-Huxley-like currents. Some of the maximum conductances of the model were differing to describe the cells from central and peripheral regions. In this model I_{Kr} which is mainly equivalent to the hERG current is defined as

$$I_{Kr} = g_{Kr} p_a p_i (V_m - E_K) \tag{1}$$

with the maximum conductance g_{Kr}, the activation gating variable p_a, the inactivation gating variable p_i, the transmembrane voltage V_m and the Nernst potential of potassium E_K. As the L532P mutation alters the inactivation process p_i is affected. It is described by

$$\frac{dp_i}{dt} = \frac{p_{i_\infty} - p_i}{\tau_{p_i}} \tag{2}$$

$$p_{i_\infty} = \frac{1}{1 + e^{(V_m + 19.6)/10.1}} \tag{3}$$

$$\tau_{p_i} = 0.002 \tag{4}$$

The measurement data (fig. 1) was integrated in the model of I_{Kr} by adapting the parameters of the p_i inactivation variable with aid of optimization methods [8] using the same stimulation protocol as in the measurements. For the optimization the Powell method was used and the optimization value was the root mean square error (E_{RMS}) between the measured and the simulated current traces. For the optimization procedure, the temperature was set in the model to $22°C$ and the extracellular potassium concentration to $5\,mM$.

First, the physiological measurements were used to identify the maximum conductance g_{Kr} and time constant τ_{p_i} of the inactivation process. g_{Kr} needed to be adjusted since the density of I_{Kr} might be different between the model and the measurement. The change of τ_{p_i} is describing the slowing of the protein movements due to the lower temperature. In the next step g_{Kr} was not variable and the parameters of the inactivation gating variable p_i were adjusted to the mutant measurement data. By re-adjusting the variables g_{Kr} and τ_{p_i} this mutant I_{Kr} current was able to work properly in the Zhang et al. sinus node model.

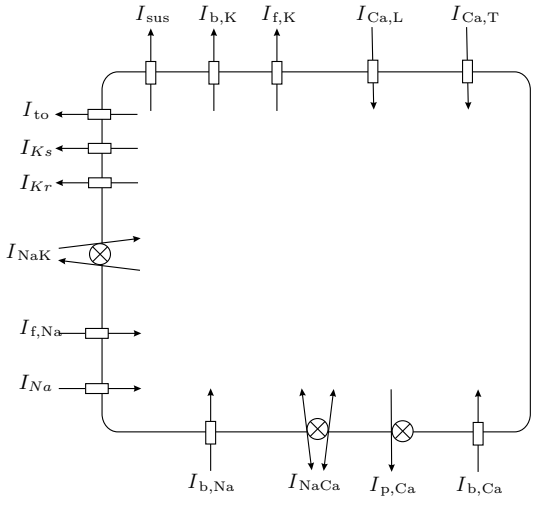


Figure 2. Schematic representation of the Zhang et al. model of sinus node cells. This model does not calculate intracellular ion concentrations.

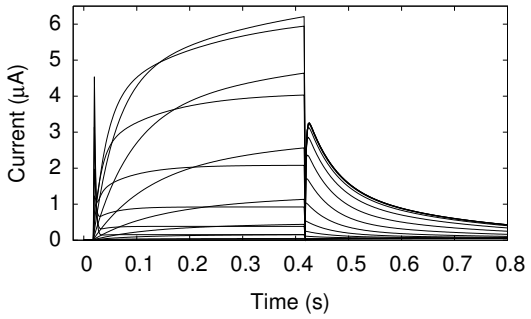


Figure 3. Current traces of simulated reggae mutant hERG channels achieved with the voltage clamp protocol shown in inset of fig. 1 (a). These simulated curves show good agreement to the measured one displayed in fig. 1 (b).

3. Results

The most sensitive parameter of the optimization process was the shift voltage of the steady-state inactivation p_{i_∞} from $-19.6\,mV$ in the physiological case to $10.1\,mV$ in the mutant model. For the final simulation only this change was inserted in the model due to the small effects of the other parameters. The simulated current traces of the mutant I_{Kr} current are displayed in fig. 3. For this simulation the same stimulation protocol as for the measurement was used. The measured and simulated current traces for the mutant case show good agreement.

When inserting this mutant I_{Kr} current in the central sinus node model of Zhang et al., the ability of the central cells to depolarize spontaneously was eliminated (see fig. 4 (a)). Peripheral cell still beat but are affected by the mutation (Fig. 4 (b)). The slope of the pre-potential and the upstroke velocity were not changed. The maximum diastolic potential was increased by $2\,mV$ and the maximum systolic potential decreased by $1.5\,mV$. The diastolic interval was shortened slightly by $3\,ms$. The main effect was a reduction of the action potential duration (APD) from $108\,ms$ in the physiological case to $84\,ms$ in the reggae mutant case leading to a frequency increase from $6.37\,Hz$ to $7.62\,Hz$. The average repolarizing I_{Kr} current increases from $105\,pA$ to $253\,pA$ due to the mutation.

4. Discussion and conclusions

Aim of this simulation study was to investigate the effects of a hERG mutation on single cell sinus node function. Therefore, measurement data was used to adapt a biophysically detailed model of I_{Kr} that was taken from a sinus node model. The simulated increase of the shift voltage is in good agreement with the measured changes.

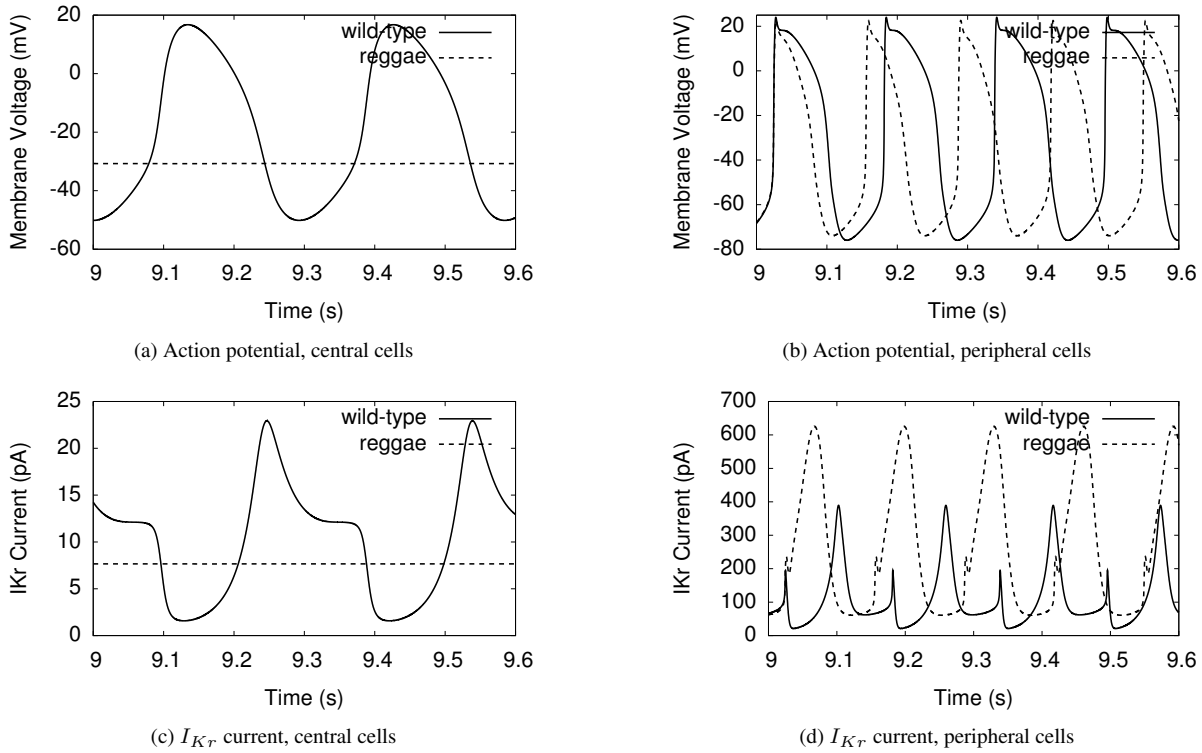
When inserting this mutant current into the sinus node model, central cells stop beating and get to a steady membrane voltage of $-31.1\,mV$. As the mutant I_{Kr} is already opening during the depolarization phase and depolarization in sinus node cells is driven slowly by calcium currents there is a balance between inward and outward currents leading to this quiescence behavior.

The main effect of this mutation on peripheral cells is the shortening of the APD that also increases the autorhythmic frequency. This shortening is due to the larger mean I_{Kr} current that is increased during the depolarization cycle (see fig. 4 (d)). This behavior reflects the shift of the inactivation to more positive potentials since more channels stay in open state during activation than passing into the inactive state for the physiological case.

Especially the loss of auto-rhythmicity in the central sinus node cells is expected to change the overall sinus node activity. Normally, the excitation is initiated in the central cells although single peripheral cells beat faster than central ones. This effect can be explained by both the heterogeneity in gap junction density that decouples more the central cells as well as by the the strong coupling of peripheral cells to atrial working myocardium which has a more negative resting membrane voltage and no self-depolarizing properties. This interaction was shown in a previous computational study [2]. Although peripheral cells beat faster being effected by the reggae mutation, we expect a bradycardial function of the complete sinus node because of electrotonic interactions with the silent central sinus node cells and the low resting membrane voltage of surrounding atrial muscle cells.

Limitations of this study are that the measurements were taken from oocytes and only from the hERG channel without the β-subunit of I_{Kr}. Additionally, the mutation was only expressed homozygously leading to an overestimation of the mutation effect. Also the temperature of $22°C$ might lead to inaccurate description of the mutation effect. Further limitations are that the ion concentrations are invariant in the model so that the balance between inward and outward currents for the central cell need not necessarily to be stable. Also, remodeling effects due to the mutation are neglected.

In a further study we want to verify the suggestion of bradycardial function of the complete mutant sinus node in a realistic, anisotropic and electrophysiologically heterogeneous model of the three-dimensional sinus node [2] including gap junction heterogeneity. Additionally, effects of further mutations of the hERG channel like in the previous simulation study of the Short QT syndrome [9] should be investigated. The generated results have to be validated against experimental findings of whole sinus node measurements including the mutations. By this we hope to understand better the physiological and pathological behavior of the cardiac pacemaker and to support the development of sinus node specific therapies like ablation or drugs.



(a) Action potential, central cells

(b) Action potential, peripheral cells

(c) I_{Kr} current, central cells

(d) I_{Kr} current, peripheral cells

Figure 4. Action potential (top) and I_{Kr} current (bottom) for central (left) and peripheral (right) sinus node cell for both wild-type (solid) and reggae mutant (dashed) case. Central mutant cells lose there auto-rhythmicity. Therefore, the membrane voltage and the I_{Kr} current are constant. Peripheral cells beat faster because the action potential duration is shorter due to the larger repolarizing I_{Kr} current.

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