ß-Adrenergic Modulation of IKs Gating in the Guinea Pig: What Can Be Learned by Numerical Modelling

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Abstract

Mechanistic interpretation of ion channel function strictly based on experimental data is not always possible due to insurmontable technical difficulties. This is the case of native I_{Ks}, for which important aspects remain unresolved. This work aimed to test whether a structurally realistic scheme of I_{Ks} gating recently proposed by Silva and Rudy could account for I_{Ks} gating under baseline conditions and during β-adrenergic stimulation. To this end, the Silva-Rudy model was optimized on a coherent set of experimental data concerning various aspects of I_{Ks} kinetics in control and during exposure to isoprenaline. The results obtained showed that the scheme proposed by Silva and Rudy is suitable to reproduce the full complexity of I_{Ks} gating both in baseline conditions and during β-adrenergic stimulation. Analysis of model predictions disclosed a novel mechanism potentially accounting for a previously unresolved aspect of I_{Ks} modulation.

1. Introduction

The slowly activating delayed rectifier potassium current, I_{Ks}, contibutes to ventricular repolarization and is strongly upregulated by PKA-mediated phosphorylation. The observation that genetic defects in I_{Ks} (e.g. LQT1 syndrome) are associated with repolarization abnormalities and arrhythmias typically triggered by emotional stress [1], suggests that I_{Ks} has a central role in maintaining repolarization stability during sympathetic activation in man.

As a consequence of its kinetics, I_{Ks} during the action potential is exquisitely sensitive to heart rate. In vivo, sympathetic activation modulates repolarization currents by the concurrence of direct action on adrenergic receptors and heart rate changes. Thus, to understand the role of I_{Ks} these two factors need to be considered simultaneously and their interaction analyzed.

Rate-dependent increase in I_{Ks} has been until recently attributed to its slow diastolic deactivation, leading to accumulation of channels in the open state at high heart rates. This is expected to increase the I_{Ks} component immediately available during fast depolarization and accordingly named "instantaneous". However, we recently reported that high stimulation rates enhance I_{Ks} also by accelerating the onset of its time-dependent component [2] and that this action is the target of concomitant β-adrenergic stimulation [3].

In view of the high difficulties involved in the recording of unitary I_{Ks} currents even under baseline conditions, in silico modelling may be essential in providing clues for the interpretaion of this complex behaviour in terms of channel gating.

Previous modelling studies on I_{Ks} and its adrenergic modulation [4,5] had two significant shortcomings. The first one concerned the failure to include the tetrameric structure of I_{Ks} channels, potentially relevant to the kinetic behavior. The second was the need to hypothesize a biologically implausible increase in the number (or conductance) of functional channel units to account for the adrenergically-induced increase in "maximal" I_{Ks} conductance, which is far too rapid to allow for changes in channel expression.

The former problem was recently addressed by Silva and Rudy, who proposed a structurally accurate markovian I_{Ks} model (Fig. 1), capable of reproducing rate-dependency of I_{Ks} activation [6]. However, whether this model could account for the complex effect of β-adrenergic modulation was not tested.

The purpose of the present work was to test whether the full complexity of I_{Ks} kinetics and of its β-adrenergic modulation could be interpreted within the framework of a structurally accurate kinetic scheme, such as the one proposed by Silva and Rudy. To this aim, I_{Ks} kinetics, its rate-dependency and the effects of β-adrenergic modulation (by isoproterenol) were experimentally characterized in guinea-pig ventricular myocytes and subsequently used as the reference for the identification and validation of a mathematical description of I_{Ks} within the framework proposed by Silva and Rudy model.

2. Methods

Ventricular myocytes from Hartley guinea-pigs were isolated by using a retrograde coronary perfusion method.

The features of I_{Ks} kinetics tested in experimental studies and then used as reference data for model identification were: 1) steady-state activation curves, 2) voltage-dependency of activation rate, 3) voltage-dependency of deactivation rate; 4) pause-dependency of activation rate. These features, which encompass all aspects relevant to I_{Ks} physiology, including rate-dependency, were analyzed under basile conditions and during isoproterenol superfusion (0.1 μM). To allow for direct application of results to modelling under physiological conditions, the reference experimental data were obtained at physiological temperature (36.5 °C).

The Markov model structure (Fig. 1) directly derived by Silva and Rudy [6]. It accounts for 4 independent voltage sensors each undergoing two conformational changes before channel opening. The group of closed states representing channels for which the first transition has been completed by only part of the 4 voltage sensors is called "Zone 2". The group of closed states representing channels in which the first transition has been completed by all 4 voltage sensors is called "Zone 1". As in the original Silva and Rudy formulation, two open states are included, in keeping with previous experimental observations [7]. Single channel currents are translated into whole-cell I_{Ks} by:

$$I_{Ks} = G_{Ks} \cdot P_o \cdot (V - E_{Ks}) \quad where \quad G_{Ks} = \sigma \cdot g_{Ks}$$

in which P_O represents the overall probability of the open states, G_{Ks} is the maximum steady-state I_{Ks} conductance and $(V\text{-}E_{Ks})$ represents the electrical driving force. G_{Ks} is obtained as the product of channel density (σ) and the unitary channel conductance (g_{Ks}). Because the reference experiments were performed during blockade of the major Ca^{2+} influx pathways (I_{CaL} and I_{NCX}), G_{Ks} dependence on changes in cytosolic Ca^{2+} was neglected in the model.

Model optimization was performed by the Nelder-Mead simplex direct algorithm [8], which identifies parameter values by minimizing the sum of squared differences between predicted and observed data values. The values of parameters defining the voltage-dependency of state transitions were first optimized in basal conditions. The optimization process was iterated until a single set of parameters adequate to reproduce the experimental data of all kinetic features (see above) was identified. The parameters identified under baseline conditions were then used as the initial guess for model optimization during adrenergic stimulation.

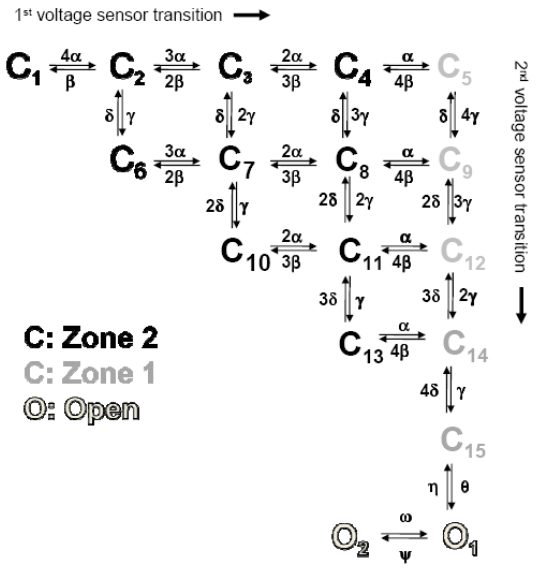

Figure 1. The I_{Ks} model [6] contains 15 closed (C_1 to C_{15}) states to account for 2 transitions of each of the 4 voltage sensors before channel opening. Black closed states represent channels in Zone 2 that have not completed the first transition for all 4 channel subunits. Grey closed states represent channels in Zone 1 that have completed 4 first voltage sensor transitions. The transition to the open state O_1 (rate θ) is voltage independent. There are 2 open states (O_1 to O_2, white) with no inactivation.

Matlab 7 and Simulink (The MathWorks Inc.- Natick, Mass) were used for all the numerical computations.

3. Results and discussion

In myocytes ISO significantly enhanced I_{Ks} amplitude so that the maximum current, at 50 mV, was more than doubled. ISO slowed down the I_{Ks} deactivation with respect to CTRL. When the two-step protocol was applied, an ISO-induced enhancement of I_{Ks} reactivation rate was observed. Moreover, ISO slowed the restitution process with respect to CTRL.

Model optimization led to satisfactory reproduction of all reference I_{Ks} properties determined experimentally both at baseline and during β-adrenergic stimulation. This provided validation of the kinetic scheme under very stringent conditions. Thus, we proceeded to analyze model predictions on the gating changes required to account for β-adrenergic modulation of I_{Ks} properties.

The parameter adjustments required to simulate ISO effects concerned only half of the transitions described in the kinetic scheme of Figure 1, namely the rates of the transitions within Zone 2 (α and β) and of the transition

between Zone 1 and the open state (η and θ). Importantly, changes in the term G_{Ks} of equation 1 were not required to simulate ISO effects. This implies that the "maximal" steady state I_{Ks} conductance observed in control conditions corresponded to the opening of only part of the functional I_{Ks} channels present in the membrane (i.e. limiting $P_O<<1$), with the remaining part achieving the open state only in the presence of ISO (i.e. limiting Po approaching 1). An analysis of voltage-dependent changes in the occupancy of states showed that under control conditions a limiting value of P_O of about 0.4 (representing "maximum conductance") resulted from the achievement, at depolarizations beyond approximately +50 mV, of a balance between voltage-induced gating changes which lead to decrease and increase in P_O, respectively. The mechanism of ISO-induced increase in maximum conductance was therefore an increase of the level of P_O at which the limiting balance was achieved. This finding is of particular interest because it suggests that receptor-induced modulation of maximal channel conductance does not necessarily require a change in the number (or conductance) of channel units, but can result simply from modulation of transitions between channel states, such as the one operated by channel phosphorylation. This novel interpretation might help to explain the puzzling observation that "maximal conductance" is modulated by β-adrenergic receptors within seconds, a time compatible with PKA-mediated phosphorylation, but not with changes in channel protein turnover.

4. Conclusions

The use of a markovian kinetic scheme which keeps into account channel multimeric structure allowed reproducing the full complexity of I_{Ks} kinetic behaviour. Moreover, I_{Ks} modulation by β-adrenegic receptors was reproduced without appealing to biologically implausible mechanisms. Most importantly, analysis of model behaviour disclosed a previously overlooked mechanism potentially accounting for unresolved aspects of I_{Ks} modulation. This highlights the value of *in silico* functional modelling in providing new theoretical frameworks for the interpretation of molecular mechanisms.

Acknowledgements

The present work was partially funded by an institutional grant to A.Zaza (FAR 2007) and by Hospal SpA grant to S.Severi.

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