Markovian Model for Wild-Type and Mutant (Y1795C and Y1795H) Human Cardiac Na^+ Channel

*S Vecchietti, °I Rivolta, *S Severi, °C Napolitano, °SG Priori, *S Cavalcanti

*Biomedical Engineering Laboratory, DEIS, University of Bologna, Bologna, Italy
°Molecular Cardiology Laboratory, Fondazione Salvatore Maugeri, IRCCS, Pavia, Italy

Abstract

Long QT syndrome (LQTS) and Brugada syndrome (BrS) are inherited syndromes predisposing to ventricular arrhythmias and sudden death. Emerging evidences related LQTS and BrS to dysfunctions of cardiac ion channels. Recently, two novel missense mutations in gene encoding for the cardiac Na channel have been identified (Y1795C for LQTS and Y1795H for BrS). Both mutations alter inactivation, intermediate inactivation, onset of inactivation of Na current and cause a sustained Na current. In this study we present a Markovian model of wild type and mutant Na channels. Model includes three closed states, an open state, and five inactivated states. Transition rates between these states were identified on the basis of electrophysiological experiments. The model is able to reproduce the current alterations observed in mutant channels just by alter the transition rates with respect to wild type assignment.

1. Introduction

Cardiac Na channel is a voltage dependent channel, composed of 3 subunits (α, β1, β2), that allows the fast inward current to cross through the cell membrane during the initial depolarisation phase of the cardiac action potential. Na channel α subunit consists of four homologous domain, each domain is composed of 6 transmembrane segments. The four domains fold together to create a central pore whose conformational state determine the selectivity and conductance properties of the channel [1].

Kinetic models have been proposed to schematise the changes in the channel conformation due to changes in membrane voltage [2]. These models are useful to understand how genetic mutations alter the kinetic properties of channel and to simulate the effects of mutation on the action potential morphology [3-6].

Mutations in the gene encoding the human cardiac Na channel α subunit (SCN5A), have been linked to long QT syndrome (LQTS) and Brugada syndrome (BrS), two inherited cardiac disorder associated with syncope, ventricular tachycardia and sudden death. LQTS is associated to a lengthening of cardiac action potential duration, and thus QT prolongation, due to an imbalance between inward and outward currents during the depolarization or plateau phases [7]. BrS is characterized by ST elevation in the right precordial lead and apparent bundle-branch block due to a potential gradient through the right ventrical [1;7-9].

Recently, two novel mutations located in the C terminus tail of cardiac Na channel were identified. Interestingly, these mutations lead to two different substitutions of the same amino acid: the Y1795C for the LQTS and the Y1795H [9]. In the present preliminary study a nine states Markovian model able to reproduce the Na current measured in both wild-type and mutant channels is presented.

2. Methods

2.1. Voltage-clamp experiments

For a detailed description of molecular screening, mutagenesis, expression of recombinant Na channels and electrophysiology measurements see reference 9. Briefly, Na channels were expressed in human embryonic kidney 293 cells. Transient transfections were carried out with equal amount of Na channel α subunit and with hβ1. Expression of channels was studied using patch clamp procedures 48 h after transfection. Membrane current were measured using conventional whole-cell patch clamp procedures. The holding potential was –100 mV. Data are presented in mean values ± S.D.

2.2. Markov model

The Markov model (Fig.1) is composed of one conducting state (open state O), three closed states (C1,C2,C3) and five inactivated states: two close-inactivated states (IC1, IC2), one fast inactivation state (IF) and two intermediate inactivation states (IM1,IM2) [4]. A complete list of the transition rates indicated in Fig. 1 can be found in references 4. Only the expression of transition rate a2 (see Tab. 1) was changed respect to [4] to obtain an accurate fitting of the experimental data.

For the sake of brevity only the expression of

transition rates discussed in the present paper are listed in Tab.1 and the values of related parameters are reported in Tab. 2. A complete list of parameter values can be requested to the authors.

Associated to each state there is the probability of channel to be in that state. The state probability is a function of the membrane potential. Time changes in the state probabilities due to membrane potential changes are described by a set of nine linear first order differential equations:

$$\frac{dP(t)}{dt} = Q(V)P(t) \tag{1}$$

where P is the vector (n=9) of state probability, Q is the transition rate matrix (n=9x9) and V the membrane voltage.

In conventional voltage-clamp experiments, voltage steps from a holding potential (V_O) to a test potential (V_∞) are applied to the cell membrane. Under the hypothesis of voltage step stimulation the system equation (1) admit an analytical solution. Brefly, transition rate matrixes $Q(V_O)$, $Q(V_\infty)$ can be decomposed in

$$Q = MLN \tag{2}$$

where M is the matrix of eigenvector of Q, L the matrix of eigenvalue and $N=M^{-1}$. Thus, the analytical solution can be expressed as

$$P(t) = P(0) \cdot e^{Q(V_\infty) \cdot t} \tag{3}$$

where P(0) is the initial probability state vector calculated as

$$P(0) = N(Vo)_{ji} \tag{4}$$

where i is the position in L(Vo) of the zero eigenvalue and j=1,2…,9.

The expression of the Na current (I_{Na}) is

$$I_{Na} = G_{\max} P_o (V - E_{Na}) \tag{5}$$

where *Gmax* is the maximal Na channel conductance, *Po* is probability to be in the conducting open state and E_{Na} is the sodium Nernst potential.

2.3. Parameters identification

The parameters in the expression of transition rates were estimated by fitting the prediction of the Markovian model on the curves obtained by all the voltage-clamp experiments [9]. For each experimental curve a sensitivity analysis was preliminarily performed in order to establish the transition rates that maximally influence the curve predicted by the model. After establishing the transition rates to be changed, the relative parameters were identify by a minimization procedure best-fitting simulation prediction on experimental data (solid line in all figures).

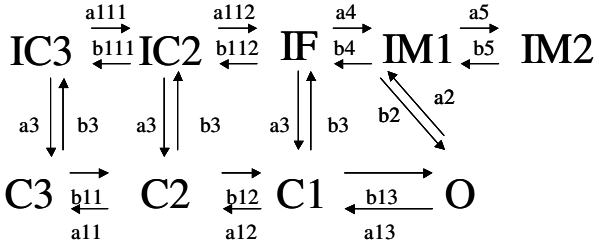

Figure.1 The nine state Markov model

Transition rate
a11=(P1a11/(P2a11*exp(-v/17)+P3a11*exp(-v/150)))
a12=(P1a11/(P2a11*exp(-v/15)+P1a12*exp(-v/150)))
a13=(P1a11/(P2a11*exp(-v/12)+P1a13*exp(-v/150)))
a3=P1a3*exp(-v/ P2a3)
a2=P1a2/(P2a2*exp(-v/16.5)+P3a2*exp(-v/200)
a4=P1a4*a2/P2a4
b4=P1b4*a3/10
a5=P1a5*a2/9.5e4
b5=P1b5*exp(-v/P2b5)/P3b5

Table 1. Transition rate expression

3. Results

Inactivation curve (see Fig. 2) was obtained considering the peak current elicited by a fixed depolarisation step starting from different initial holding potentials (Vo ranging from –130 to –20 mV) to V_∞= -10 mV. Inactivation curve represents the percentage of channel that are inactivated as a function of the membrane potential, i.e. the percentage of channel that cannot conduct until membrane is again repolarized or hyperpolarized. It can be noticed that channel linked to LQTS (Y1795C) exhibits a shift towards negative potential of inactivation. This effect is more evident in channel linked to BrS (Y1795H). To reproduce the differences in the inactivation curves we identified different values for the parameters of transition rate a3 for the wild-type and mutant channels (see Tab. 2). With the different parameter assignment the model is able to

distinguish between the three channel with a good fitting of experimental points (see Fig. 2).

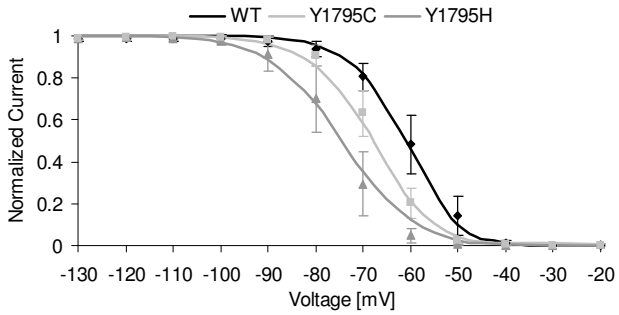

Figure 2. Inactivation curve.

Time to half inactivation curve (see Fig.3) is obtained as the time to decay to half of peak current and it characterizes the onset of inactivation kinetic. Y1795H mutation speeds inactivation especially at negative potential, while Y1795C mutation slows inactivation at voltages greater than –20 mV. To fit the kinetic model on the experimental points (see Fig. 3) the parameters in a11, a12, a13 transition rates are assigned differently for the wild-type and mutant channels (see Tab.2).

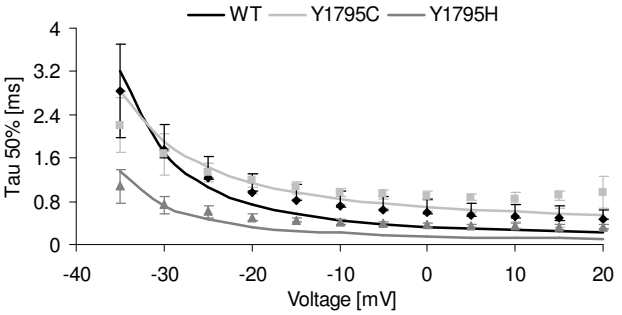

Figure 3. Time to half inactivation.

Sustained current (Fig. 4) is the current assessed at the end of an impulse lasting 150 ms from the holding potential of -100 mV to a test potential of -10 mV. Y1795C mutation induces a sustained current, surprisingly also Y1795H mutation linked to BrS induced a light maintained current. The model is able to reproduce quite well the sustained current and permit to differentiate the three channels. To reproduce the sustained current it was necessary to change parameters in the a4 rate.

The activation curve (Fig.5) describes the percentage of channels that are able to open for depolarization at different potentials. The voltage dependence of activation is obtained by normalizing with respect to driving force the current assessed during pulses with Vo=–100 mV as holding potential and test potential (V∞) ranging from –80 to 50 mV (10-mV increments). There are not significant differences in activation curve between the three channels and the model was able to reproduce the data for all the 3 channels considered.

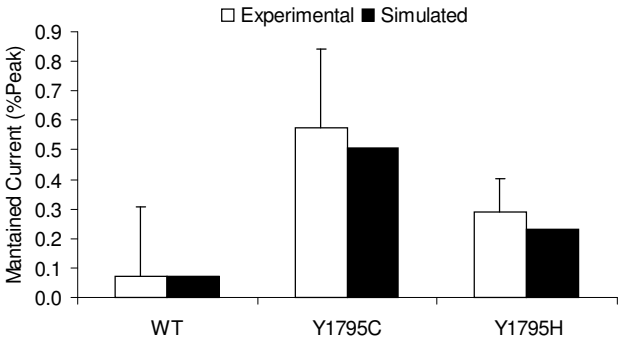

Figure 4. Sustained current

Parameters	WT	Y1795H	Y1795C
P1a11	3.1869	8.5545	1.44704931
P2a11	0.12054	0.12054	0.0112054
P3a11	2.6	0.26	0.34
P1a12	0.299	0.299	0.391
P1a13	0.325	0.325	0.425
P1a3	3.7933e-7	11.3799e-7	4e-7
P2a3	6.1839	7.60291839	7.1839
P1a2	5.04	6	3.73333
P2a2	0.08	0.08	0.00001518
P3a2	0.364	0.364	0.476
P1a4	0.22	0.36	0.075
P2a4	100	250	1000
P1b4	1	7.2	0.75
P1a5	0.8775	4.95	0.015
P1b5	1.7070e-7	20.48382e-7	0.0126443333-7
P2b5	7.7	7.7	7
P3b5	20	50	50

Table 2. WT, Y1705H, Y1795C parameters identified

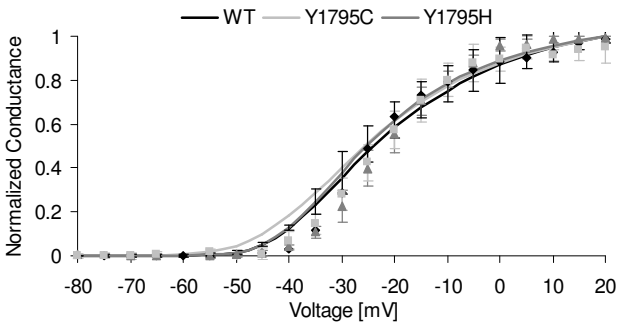

Figure 5. Activation

4. Discussion

The two mutations considered were characterized by a shift towards negative potential in the inactivation curve, a different onset of inactivation kinetic and a sustained current to respect to wild-type channel. Assigning different values to model parameters is possible to reproduce both wild-type and mutant currents with good accordance to experimental data.

The Markov model chosen to describe the wild-type and the mutant channels was proposed by Clancy and Rudy in 2002 [4]for only the wild-type channel. Clancy and Kass [3] used a different structure, in which a "burst mode" was included, for Y1795C mutant channel. This "burst mode" should account for the different gating mode in which sodium channels enter when a sustained current is present. The small percentage of channels in the "burst mode" should fail to inactivate creating the sustained current [2-4]. As shown in the present study is not necessary to include the "burst mode" to reproduce the sustained current in mutant channels. In particular, it is sufficient to act on the a4 rate that regulates the transition between fast inactivation and intermediate inactivation states. Thus, a4 parameter determine the probability to leave the open state.

The Markov model presented can be introduced in comprehensive model of ventricular cell to estimate the effects of mutation on the action potential [6].

5. Conclusions

The Markov model with different assignment of model parameters is able to reproduce all the features that characterized WT cardiac Na current and Y1795C and Y1795H mutations. In particular, the model structure with 9 states is sufficient to reproduce the sustained current that usually is a distinctive mark of LQT3 mutation.

References

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Address for correspondence.

Vecchietti Stefania.
Viale Risorgimento 2
Biomedical Engineering Laboratory,
DEIS, University of Bologna,
40137 Bologna, Italy.
svecchietti@deis.unibo.it