Propagation Malfunctions due to Gap Junction Dysregulation

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Abstract

Gap junctions are membrane channels that connect the cytoplasm of adjacent cells allowing the cell-to-cell electrical coupling necessary for action potential propagation. Pathological conditions, such as malformations in connexins, mutations affecting phosphorylation of regulatory sites of connexins, alterations in gap junction organization, and type and quantity of connexin expression, can impede the normal electrical propagation. All these malfunctions can produce a dispersion of repolarization, implicated in ventricular arrhythmias. In fact, ventricular tachycardia and spontaneous ventricular arrhythmia occurred in more than twice as many connexin deficient hearts than wild-type hearts.

We perform numerical simulations of a human ventricular model in order to mimic some of these pathological conditions. In particular, we consider a diminished Cx43 connexin expression, as well as altered connexin conductance dynamics, i.e., modified maximum and minimum conductances g_{max} and g_{min}, half-inactivation voltage $V_{1/2}$ and decay kinetics. Physiologically these modifications can appear due to mutations or to different connexin configurations, i.e., forming heteromeric channels. Under these conditions we study the change in action potential duration (APD) and CV-restitution properties. We observe that, although CV diminishes with decreased connexin expression, the APD remains almost constant up to the point of conduction block. Also, propagation differs for constant or time-dependent voltage conductance, conduction block occurring earlier for the former. While mutations resulting in a stronger dependence of the delay time produced an appreciable change intercellular conductances, this effect was not so important when the mutations affected the overall delay time. Thus, our results suggest that a correct description of gap junctional conductance is of big importance for understanding action potential propagation under pathological conditions.

1. Introduction

A proper heart contraction requires a sequential propagation of the electrical stimulus from one cell to another through the cardiac tissue. Gap junctions (GJ) are membrane channels that connect the cytoplasm of adjacent cells and allows the cell to cell current transfer. Each gap junction channel contains specific proteins, named connexins, distributed in two hexameric structures referred as hemichannels or connexons, one from each neighboring cell. Each connexon can be constituted by one type of connexin (homomeric connexon) or more than one type of connexin (heteromeric connexon). Furthermore, an intercellular channel can be composed by two identical homomeric connexons (homotypic junction) or two connexons of different heteromeric or homomeric composition (heterotypic junction) [1]. Characteristics and type of connexon affects the electrical coupling between adjacents cells and then the properties of Action Potential (AP) propagation. As in most tissues and organs, multiple connexin types with different expresion or combination are found in the heart, depending on the specific animal and the specialized cardiomyocyte. In this way, three main connexins are found in mammalian cardiac cells: connexin40 (Cx40), connexin43 (Cx43) and connexin45 (Cx45), with different amounts and combinations in different heart tissues. Cx43 is the far the most abundant in cardiomyocytes. Moreover, the gap junctions of atria of most mammalians species contain abundant connexin Cx40 coexpresed with Cx43. Connexin45 (Cx45) is found in atrial and ventricular myocytes but in less quantities [2].

In the last years a considerable atention has been centered in the role of the gap junctions and conexins in arrhythmias. In fact mutations and alterations of gap junction organization and connexin expression are related with human heart disease with arrhythmic tendency. In this sense a reduction of expresion of Cx43 in ventricles has been observed in patients with ischemic cardiomyopathy and other chronic myocardial disease states. In failling hearts one of the consequences of the observed downregulation of Cx43 expression is the conduction slowing [3]. Hypertrophic and dilated cardiomyopathy produces a rearrangament of connexin Cx43, normally located in intercalated disc and with low density in lateral membranes, originating an heterogeneous connexin redistribution and down-regulation

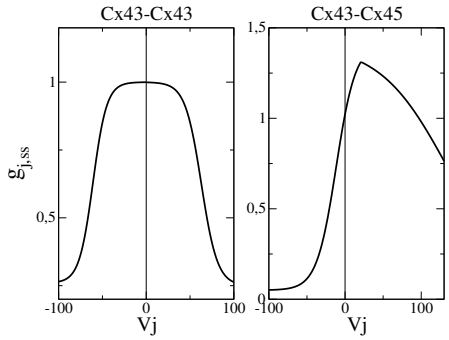

Figure 1. Steady state conductance for homomeric-homotypic (Cx43) and homomeric-heterotypic (Cx43-Cx45) channels.

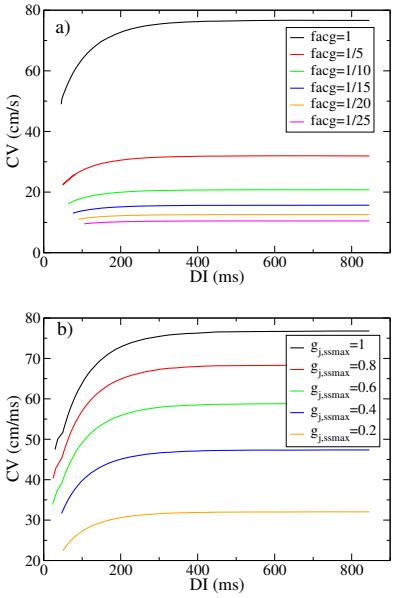

Figure 2. Curves of velocity restitution corresponding to a fiber with homotypic-homomeric connexin Cx43 gap junctions for different a) $g_{j,ss}$ multiplying factors and b) maximum conductivity $g_{j,max}$ values.

expression resulting in conduction defects [4] . Mutations that originate changes in phosphorylation of hemichannels affect, not only to process of degradation, expression and distribution of connexins, but also to the funcionality of channels and the conductance of gap junctions [5]. Furthemore a mutation in a connexin protein is implicated in the pathogenesis of sudden infant death syndrome [6] Consequently, pathological remodeling of connexins contributes to arrhythmogenic substrates and development of reentrant arrhythmias.

In this work we use a simplified ventricular model to analyze the effect of several pathological circustances in order to mimic some of the above mentioned pathological condictions. We observe the strong behaviour of connexins, being necessary big changes to observe appreciable reduction in velocity conductivity.

2. Model and approach

We use the simplified human model of Peñaranda et al [7] to provide the action potential dynamics with the parameters corresponding to a human ventricular cardiomyocyte. This model is applied to a cardiac fiber, where we solve the discrete cable equation assuming that cardiomyocytes are isopotential

$$\frac{\partial V}{\partial t} = \frac{D}{dx^2}\left[g_{j+1}(V_{j+1}-V_j) - g_j(V_j - V_{j-1})\right] - \frac{I_{ion}-I_{stim}}{C_m}\ , \quad (1)$$

where V_j and g_j are, respectively, the voltage and conductance differences between the j-th cell and its neighbor.

Simulations have been performed using a simple Euler forward algorithm with a time discretization dt = 0.01 ms. Spatial discretization dx = 0.01 cm is used. Dynamics of gap junctions are introduced following the model of Vogel/Harris [8]/[9] in a way similar to the works of Lin and Desplantez [10], [11]:

$$\frac{dg_j}{dt} = \frac{g_{j,ss}-g_j}{\tau_g} \quad (2)$$

with steady state conductance:

$$g_{j,ss} = \frac{g_{j,max}-g_{j,min}}{1+e^{[A(V_j-V_{1/2})]}} + g_{j,min} \quad (3)$$

and conductance time constant $\tau_g = A_\tau e^{BV_j}$. Here $g_{j,max}/g_{j,min}$ are the maximum/minimun connexin conductances and A, A_τ, B and $V_{1/2}$ correspond to parameters fitted to experimental data from Lin and Desplantez [10], [11].

We use the values of parameters obtained by these authors for the different homomeric-homotypic and homomeric-heterotypic channels as original parameters (see Fig. 1). In the latter case we measure the conduction delay between two cells presenting Cx43 and Cx45 connexins. Changes in conductance dynamics are analyzed to mimic malfunctions of gap-junctions. We analyze the changes produced in restitution properties when expresion of connexins is diminished and/or when conductivity of channels is reduced, reproducing the effect of some mutations.

3. Results

In Fig. 2 we show the effect of either an overall reduction of the conductance or a reduction of the maximal conductance $g_{j,max}$ in a tissue with Cx43-Cx43 channels.

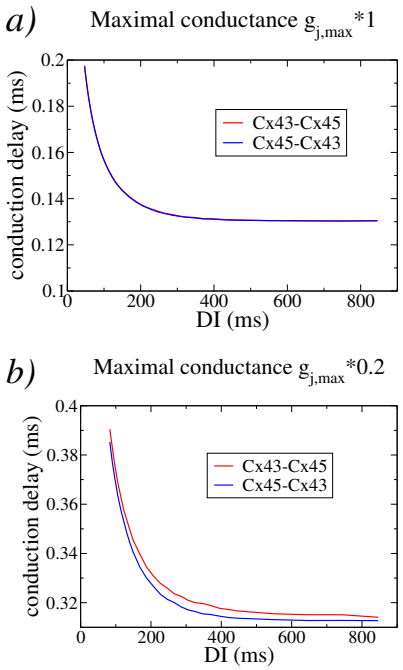

Figure 3. Conduction delay vs diastolic interval (DI) curves for either homomeric-heterotypic channels Cx45-Cx43 (red) or Cx43-Cx45 (blue) for a) reference conductance and b) 20% of maximal conductance $g_{j,max}$.

This results in an overall reduction of the conduction velocity, according to the scaling $CV \sim \sqrt{g}$. The difference from a continuum description is not observed until very small velocities, corresponding to conductances of the order of 1/50 times the standard value. This case has been very well studied [12]. Interestingly, even for very low conductances, the action potential resitution curves remain unaltered.

A more interesting situation is the case of homomeric-heterotypic channels. In this case the steady-state conductance is asymmetrical and the conduction delay time could depend on the direction in which the channels are transversed. We consider the case of Cx43-Cx45 gap junction and in Fig. 3 plot the conduction delay vs diastolic interval (DI) curves for propagation in either direction. For standard conditions the difference is negligible. This was to be expected. At standard delay times of $\sim$ 0.15 ms between cells and for an upstroke of $\sim$ 3-5 ms duration and $\sim$ 100 mV amplitude, this corresponds to a difference of $2.5 - 4$ mV between contiguous cells. For those values of V_j the conductance is always maximal. Lowering the maximal conductance (Fig. 3b) the conduction delays increase and the asymmetry effect begins to be appreciable (for the parameters in Fig. 3b, $V_j \sim 6 - 10$ mV).

We have investigated the effect of changing the time constant τ_g, either its overall factor A_τ or its voltage dependence B. The effect is very different. Starting from a value of g_j equal to that of the maximal conductance $g_{j,max}$, the conductance decreases with time until it reaches a steady value. Typically the time constant of g_j (10-100 s) is much larger than the duration of a stimulation. This means that it cannot follow the changes in voltage polarity during an action potential and its changes during an action potential are typically negligible. However, due to the voltage dependence of the time constant, the dynamics of the conductance is faster during the upstroke than during the plateau phase. This means that, even if in the time of an stimulation the conductance does not have enough time to reach a low value, it can decrease as the continued effect of many stimulations, since the conductance can hardly increase, due to the very slow time scales at $V_j \sim 0$. This is the effect observed in Fig. 4. While an overall change of the conductance time scale τ_g does not change the final value of the conductance and only how fast it approaches it, a change in its voltage dependence does indeed change the final average conductance. This accentuates the difference already observed in Fig. 3 between the two directions of propagation. In fact, a feedback mechanism can be produced, in which a decrease in conductance increases the conduction delays that in turn increase the intercellular potential difference, resulting in a further decrease of conductance. We have observed that this can result in a branch of low conductance action potentials.

4. Discussion and Conclusions

We have performed simulations considering discrete connexions between cells and studied the effect of reducing the maximal conductance and the time scales for its dynamics. Unless very low values of the conductance are achieved, discrete effects are negligible for homotypic connexins. A more interesting situation happens when heterotypic channels are considered. In this case, a reduction of the maximal conductance results in a difference in conduction delays between two cells presenting Cx45 and Cx43 connexins, depending on the direction of propagation. This effect is accentuated when the voltage dependence of the conductance time scale is enhanced. This may have proarrythmic effects in the atria or at the junction of ventricular muscle and Purkinje fibres where a change from Cx43 to Cx45 connexins has been observed [10], [11].

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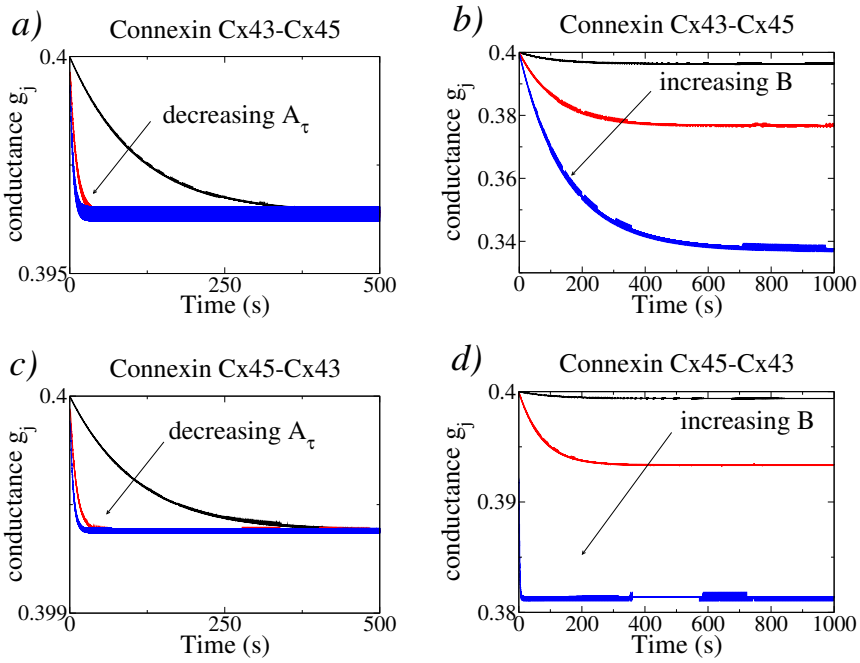

Figure 4. Instantaneous value of the conductance as a function of time for homomeric-heterotypic channels Cx45-Cx43 (a) and (b) and Cx43-Cx45 (c) and (d) with 40% of maximal conductance $g_{j,max}$. In panels (a) and (c) we show the result of modifying the parameter A_τ in the conductance time constant, while in panels (b) and (d) we show the results of modifying the parameter B that takes into account the voltage dependence of the conductance time constant.

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