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ROLE OF SUBMEMBRANE SPACES IN THE CONTROL OF TRANSMEMBRANE ION FLUX AND CELLULAR INOTROPIC STATE IN A MODEL OF HUMAN VENTRICULAR CARDIOMYOCYTE

Pásek M.*, Bébarová M., Christé G.*****

Abstract: *We have recently developed a model of human ventricular cardiomyocyte incorporating the t-tubular and surface submembrane spaces and restricted ion exchange between these spaces and cytosol. After incorporating the experimental finding that majority of Na^+-Ca^{2+} exchanger proteins are located at the t-tubular membrane of human ventricular cardiomyocytes, we explored the consequences of ion concentration changes in the submembrane spaces on the electrophysiological activity of these cells. Consistently with the experimental and modelling studies published so far, our model predicts an increased Ca^{2+} extrusion during the action potential. However, our model also predicts a significant reduction of Ca^{2+} extrusion throughout the diastole, which can ultimately lead to an increase of cellular inotropy.*

Keywords: Human ventricular cardiomyocyte, t-tubules, submembrane spaces, submembrane Ca^{2+} gradient, Na^+-Ca^{2+} exchanger, mathematical model.

1. Introduction

In our previous modelling studies, we showed that activity-induced ion concentration changes in t-tubules and extracellular clefts of ventricular cardiomyocytes may be large enough to significantly modulate membrane ionic currents, cellular electrical activity, and cellular inotropic state (Pásek et al., 2003, 2006, 2008, 2012; Hrabcová et al., 2013). However, the magnitude and functional consequences of ion concentration changes that occur in submembrane spaces at the inner side of ventricular cardiomyocytes are still unclear. In 2002, Weber et al. found out that, upon excitation of ventricular cardiomyocyte, the transient increase of Ca^{2+} concentration (so-called Ca^{2+} transient) in intracellular submembrane spaces is substantially higher (>3.2 μM in peak value) than that in bulk cytosol (~1.2 μM). In their novel model of rabbit ventricular cardiomyocytes, Shannon et al. (2004) formulated a new submembrane compartment allowing proteins on the inner side of the membrane to sense ion concentrations that differ from those in the bulk cytosol. They showed that the elevated Ca^{2+} concentration in the submembrane spaces may substantially promote the Ca^{2+} extrusion from the myocyte via Na^+-Ca^{2+} exchanger. Nevertheless, a deeper analysis of the impact of ion concentration changes in the submembrane spaces on action potential (AP) and cytosolic Ca^{2+} transient (CaT_c) has not been done yet.

To fill this gap and translate the impact observed in the animal model to human cardiac electrophysiology, we used our recently published model of human ventricular cardiomyocyte (Synková et al., 2021). Our simulations revealed that the alterations of ion concentrations in the submembrane spaces may play a role in modulating the cellular electrical activity and inotropic state.

* Assoc. Prof. Michal Pásek, PhD.: Institute of Thermomechanics, Czech Academy of Science, Dolejškova 5; 182 00, Prague; CZ and Department of Physiology, Faculty of Medicine, Masaryk University, Kamenice 5; 62500; Brno; CZ, pasek.avcr@centrum.cz

** Assoc. Prof. Markéta Bébarová, PhD.: Department of Physiology, Faculty of Medicine, Masaryk University, Kamenice 5; 625 00; Brno; CZ, mbebar@med.muni.cz

*** Dr. Georges Christé.: Laboratoire de Neurocardiologie, EA4612, Université Lyon 1, F-69003; Lyon; F, christe.georges@laposte.net

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2. Methods

The schematic diagram of our model is illustrated in Fig. 1. The t-tubular fractions of ion transporters are the same as in the original published model (Synková et al., 2021) except for the t-tubular fraction of Na^+-Ca^{2+} exchanger ($f_{NaCa,t}$) that was increased from 0.56 to either 0.8 or 0.9 to respect the experimental finding by Hong et al. (2012) indicating that a great majority of Na^+-Ca^{2+} exchange proteins (roughly 80–90 %) are located at the t-tubular membrane in human ventricular cells. Our 2021 model already included the surface and t-tubular submembrane spaces (see Fig. 1) with time constants controlling the ion fluxes between them and the cytosol being $\tau_{ssc} = 2.8$ ms and $\tau_{stc} = 2.7$ ms, respectively. To reveal the physiological role of these spaces, we compared the simulations on the model with its modified version, in which their function was disabled by multiplying the time constants τ_{ssc} and τ_{stc} by a factor 10^{-6}.

The simulations were performed using the computational system MATLAB 7.2 (MathWorks, Natick, MA, USA) and the solver for stiff systems ODE-15s. To achieve a dynamic steady-state, the model was paced for 600 s of equivalent cell lifetime under all conditions. The standard ion concentrations in the extracellular bulk space $[Na^+]_b$, $[K^+]_b$, and $[Ca^{2+}]_b$ were set to 140, 5.4, and 2 mM, respectively. The basic units in which the equations were solved were mV for the membrane potential, mA for membrane currents, mM for ion concentrations, ml for volumes, and s for the time. The Matlab code of the model is available at https://www.it.cas.cz/en/d3/l033/.

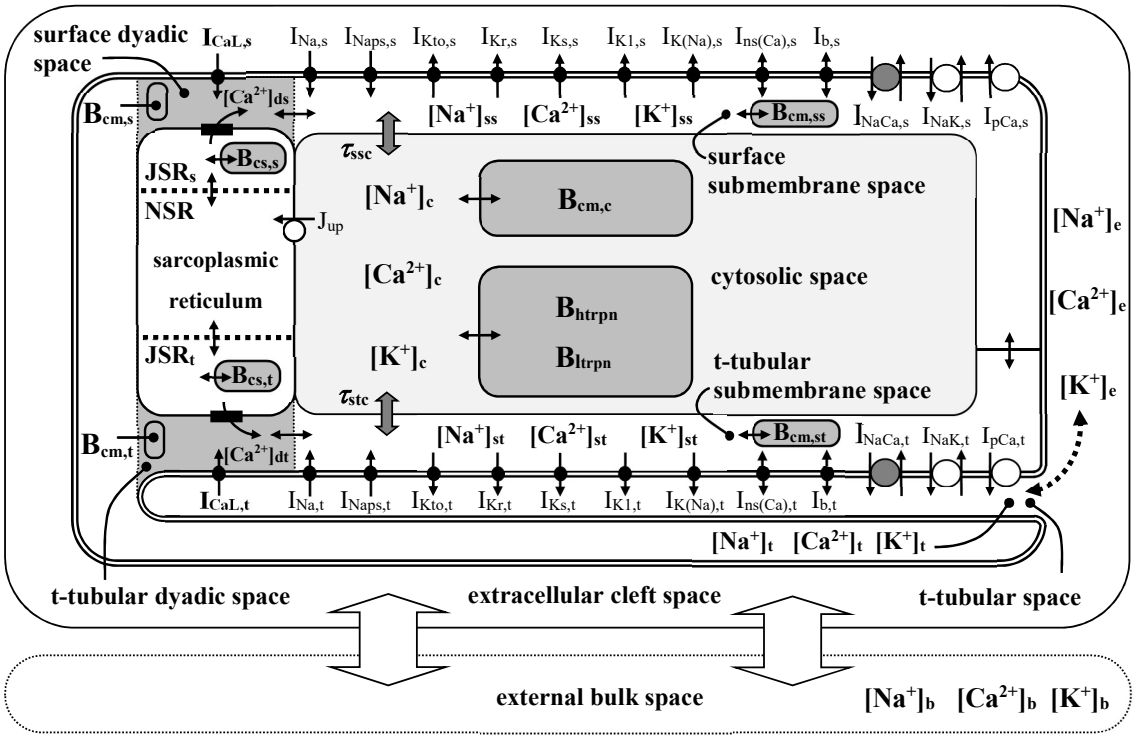

Fig. 1: Schematic diagram of the model of human ventricular cell.
Description of electrical activity of the surface (s) and t-tubular (t) membranes comprises formulations of the following ion currents: fast sodium current (I_{Na}), persistent sodium current (I_{Naps}), L-type calcium current (I_{CaL}), transient outward potassium current (I_{Kto}), rapid and slow components of delayed rectifier potassium current (I_{Kr} and I_{Ks}), inward rectifying potassium current (I_{K1}), background currents (I_b), sodium-activated potassium current ($I_{K(Na)}$), calcium-activated non-specific current ($I_{ns(Ca)}$), sodium-calcium exchange current (I_{NaCa}), sodium-potassium pump current (I_{NaK}), and calcium pump current (I_{pCa}). The intracellular space contains the cytosolic space (c), surface and t-tubular submembrane spaces (ss, st), surface and t-tubular dyadic spaces (ds, dt), and network and junctional compartments of sarcoplasmic reticulum (NSR, JSR_s, JSR_t). J_{up} represents Ca^{2+} flow via SR Ca^{2+} pump and the small filled rectangles in JSR membrane ryanodine receptors. The small black and grey bi-directional arrows denote intracellular ion diffusion. Ion diffusion between the t-tubular and cleft spaces is represented by the dashed arrow, and between the cleft and external bulk spaces by the thick white arrows.

3. Results

Fig. 2 shows the simulated time course of APs, I_{Ca}, I_{NaCa}, $I_{ns(Ca)}$ and Ca^{2+} concentrations in NSR, surface and t-tubular submembrane spaces and cytosol in the original model versus the model with disabled function of both submembrane spaces. As follows from the figure, the presence of functional submembrane spaces led to a substantial increase of Ca^{2+} transients in these spaces (see the increase of amplitude in $[Ca^{2+}]_{ss}$ and $[Ca^{2+}]_{st}$) and, consequently, to the activation of inward I_{NaCa} and of $I_{ns(Ca)}$ at the beginning of AP. The activation of $I_{ns(Ca)}$ resulted in AP shortening (by 5.3 % at 90 % of repolarisation) and, consequently, to a slight decrease of Ca^{2+} intake via I_{Ca} (by 3.1 %). Despite that, the steady-state SR Ca^{2+} load at the end of the cycle and the peak value of CaT_c increased (by 11–16 % and 5.3–8.7 %, respectively, see the graphs of $[Ca^{2+}]_{NSR}$ and $[Ca^{2+}]_c$), which was the consequence of a lower inward I_{NaCa} during the diastolic phase of the stimulation cycle (see the less negative values of I_{NaCa} after the termination of AP). Hence, the performed simulations show that the limited Ca^{2+} diffusion between the submembrane spaces and cytosol in human ventricular cardiomyocytes underlies the substantially higher transient changes in $[Ca^{2+}]_{st}$ and $[Ca^{2+}]_{ss}$ versus those in $[Ca^{2+}]_c$, which promote the higher I_{NaCa}-mediated Ca^{2+} extrusion at the beginning of AP. On the other hand, after the termination of AP, the limited submembrane Ca^{2+} diffusion coupled with a high $f_{NaCa,t}$ (0.8 or 0.9) reduces the level of $[Ca^{2+}]_{st}$

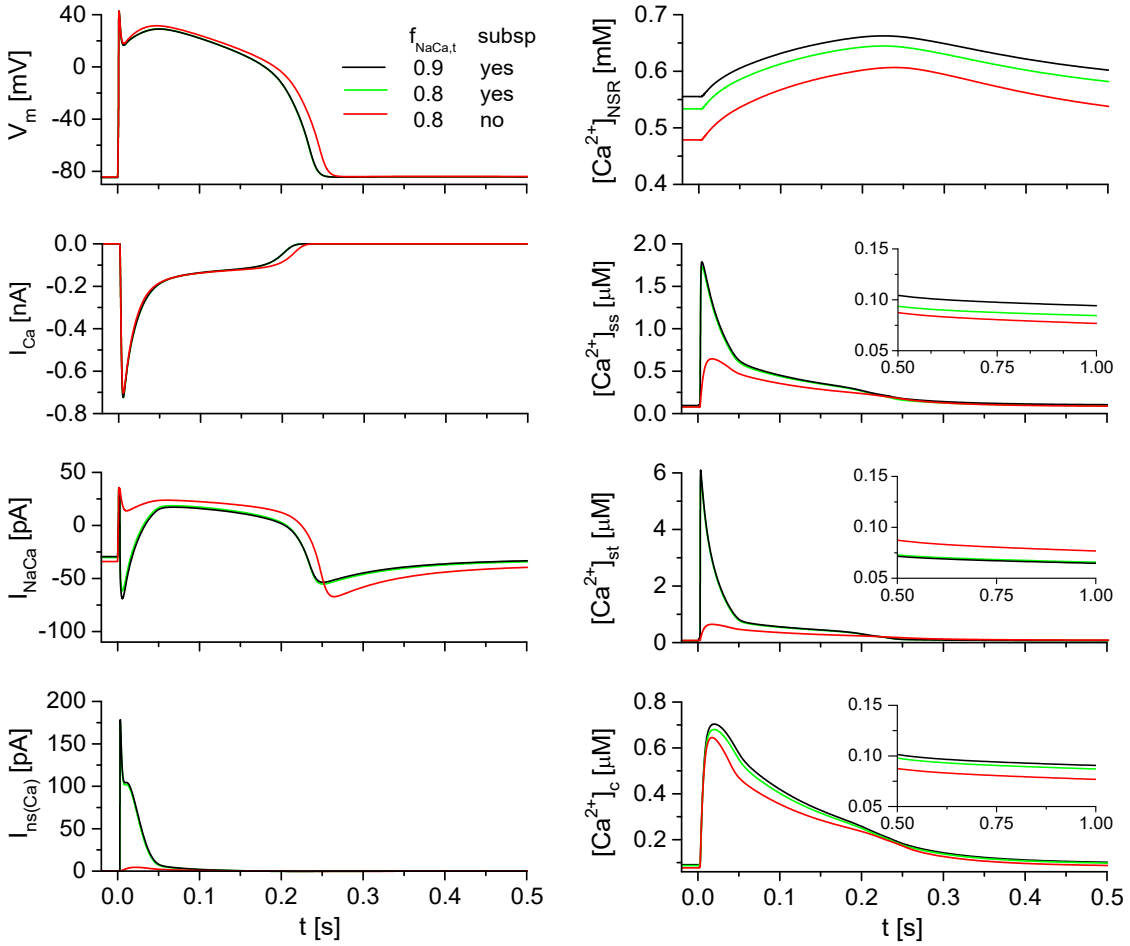

Fig. 2: Simulations of the action potential, I_{Ca}, I_{NaCa}, $I_{ns(Ca)}$, and of Ca^{2+} concentration changes in the network compartment of SR ($[Ca^{2+}]_{NSR}$), submembrane spaces (surface: $[Ca^{2+}]_{ss}$, t-tubular: $[Ca^{2+}]_{st}$), and in the cytosol ($[Ca^{2+}]_c$) during 0.5 s of 1 Hz steady-state stimulation cycle at $f_{NaCa,t}$ of 0.8 (green line) and 0.9 (black line) and at disabled function of the t-tubular and surface submembrane spaces (red line).The disablement of the function of both submembrane spaces was done by multiplying the corresponding time constants controlling the Ca^{2+} fluxes between them and cytosol, τ_{ssc} and τ_{stc}, by 10^{-6}. The insets represent the intracellular Ca^{2+} concentrations $[Ca^{2+}]_{ss}$, $[Ca^{2+}]_{st}$, and $[Ca^{2+}]_c$ at the end of the stimulation cycle.

(see the inset to $[Ca^{2+}]_{st}$ in Fig. 2) and, thus, the Ca^{2+} extrusion via $I_{NaCa,t}$. As the reduction of Ca^{2+} extrusion during the diastolic phase prevailed over its increase at the beginning of AP, the final effect of the changes in $[Ca^{2+}]_{st}$, as predicted by the model, was the increase of SR Ca^{2+} load and of the peak value of CaT_c (by 5–9 %) and, thus, the increase of cellular inotropy. Unlike for $[Ca^{2+}]$, the differences between submembrane and cytosolic $[Na^+]$ and $[K^+]$ during the whole cycle were small (< 2 % and 0.1 %, respectively, not shown) and appeared to have a negligible effect on cellular electrophysiology.

4. Discussion

The results of this study indicate that the ion concentration changes in the limited submembrane spaces of human ventricular cardiomyocytes induce a shortening of AP and an increase of CaT_c. While the shortening of AP was mainly caused by the activation of $I_{ns(Ca)}$ due to the higher submembrane Ca^{2+} transients, the mechanism of the increase of CaT_c was more complex. First, an increased Ca^{2+} extrusion via the Na^+-Ca^{2+} exchanger was apparent in our model in agreement with the published data from rabbit cardiomyocytes (Weber et al., 2002, Shannon et al., 2004). This was due to strong submembrane Ca^{2+} concentration gradients induced by Ca^{2+} induced Ca^{2+} release from the junctional SR at the beginning of the simulated AP. Second, after the termination of AP, the lower concentration of Ca^{2+} in the t-tubular submembrane space caused a reduction of Ca^{2+} extrusion via the Na^+-Ca^{2+} exchanger. This opposite effect during the diastolic phase of the stimulation cycle ultimately led to an increase of SR Ca^{2+} load and CaT_c. Thus, according to the model, the combination of limited Ca^{2+} diffusion between the t-tubular submembrane space and cytosol with predominant localisation of the Na^+-Ca^{2+} exchanger at the t-tubules underlies the formation of submembrane Ca^{2+} concentration gradient that increase the inotropic state of human ventricular cardiomyocytes.

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