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A NEW QUANTITATIVE DESCRIPTION OF INTRACELLULAR Ca^{2+} DYNAMICS IN THE MODEL OF RAT VENTRICULAR MYOCYTE

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Abstract: *In this paper, a new description of intracellular Ca^{2+} dynamics in the model of rat ventricular cardiomyocyte is presented. The principal modifications based on the recently published data comprise: formulation of the function of peripheral dyads, incorporation of peripheral and tubular intracellular subspaces, reformulation of inactivation properties of surface of tubular I_{Ca} and description of the function of exogenous Ca^{2+} buffer in intracellular space. The modified model will be used to explore the activity induced ion-concentration changes in rat transverse-axial tubular system in a more detail and to investigate their effects on excitation – contraction coupling in ventricular cardiomyocytes.*

Keywords: ***cardiac cell, intracellular Ca^{2+}dynamics, quantitative modelling***

1. Introduction

In our previous work we developed a mathematical model of rat ventricular cell electrical activity (Pásek et al., 2006) that firstly included a quantitative description of membrane transverse-axial tubular system (TATS). The model was used to explore the extent of activity induced ion concentration changes in rat TATS and their role in electromechanical activity of rat ventricular myocytes. The experimental data that have been published in the recent years show, however, that some cellular events related to intracellular Ca^{2+} dynamics are more complex than formulated in the model. In this work, we describe a modified model of rat ventricular cardiomyocyte that includes a novel description of intracellular Ca^{2+} handling respecting the recent findings. The principal modifications of the model include: (i) partition of originally single dyadic space into two compartments, one adjacent to surface membrane and another one adjacent to tubular membrane; (ii) incorporation of peripheral and tubular intracellular subspaces; (iii) reformulation of calcium current (I_{Ca}) inactivation including its differentiation between surface and tubular membrane; (iv) incorporation of quantitative description of the function of exogenous Ca^{2+} buffers in intracellular space.

2. Modification of the model

2.1 *Model structure*

The structure of the modified model is based on the previous model (Pásek et al., 2006) and is illustrated in Fig. 1. The presence of peripheral dyads (Brette et al., 2004) and ion gradients under the membrane (Shannon et al., 2004) is taken into account by incorporation of the following new compartments: dyadic space adjacent to surface membrane (surface dyadic space); junctional compartment of sarcoplasmic reticulum adjacent to surface dyadic space (JSR_s); subsarcolemmal spaces adjacent to surface and tubular membranes (surface subsarcolemmal space and tubular subsarcolemmal space). The volumes of all intracellular compartments are specified in Tab. 1.

Readjustment of parameters related to cellular membrane was performed according to the results published in Pásek et al., 2008; the fractional area of tubular membrane was lowered to 49% and the specific capacitances of tubular and surface membrane were set to 1.275 μF/cm^2 and to

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0.714 μF/cm^2, respectively. This adjustment is consistent with commonly used value of total membrane specific capacitance of 1 μF/cm^2.

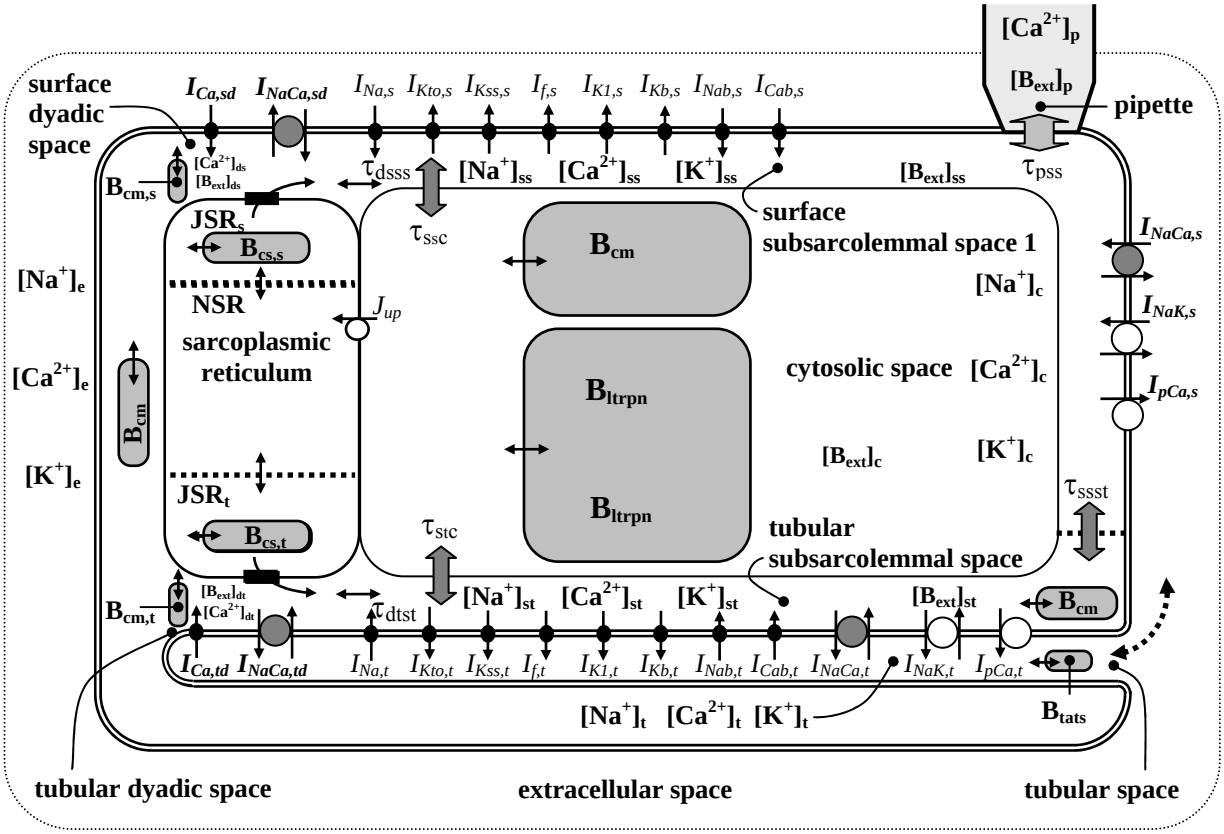

Fig. 1: Schematic diagram of the modified rat ventricular cell model. The description of electrical activity of surface (s, sd) and tubular (t, td) membrane comprises formulations of the following ion currents: fast sodium current (I_{Na}), calcium currents through L-type channels (I_{Ca}), transient outward potassium current (I_{to}), steady-state outward potassium current (I_{Kss}), hyperpolarization-activated potassium current (I_f); inward rectifying potassium current (I_{K1}), background currents (I_{Kb}, I_{Nab}, I_{Cab}), 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, surface and tubular subsarcolemmal subspaces, surface and tubular dyadic spaces, the network and junctional compartments of sarcoplasmic reticulum (NSR, JSR_s, JSR_t), the endogenous Ca^{2+} buffers (calmodulin (B_{cm}), troponin (B_{htrpn}, B_{ltrpn}), calsequestrin (B_{cs})) and the exogenous Ca^{2+} buffer (e.g. BAPTA or EGTA (B_{ext})). B_{tats} denotes the non-specific Ca^{2+} buffer associated with luminal part of tubular membrane. The small filled rectangles in JSR membrane represent ryanodine receptors. The small bi-directional arrows denote Ca^{2+} diffusion. Ionic diffusion between the tubular and the extracellular space is represented by the dashed arrow.

Tab. 1: Volumes of intracellular compartments.

symbol	specification	value [pl]
V_c	cytosolic space	11.137
V_{ss}	surface subsarcolemmal subspace	0.1440
V_{st}	tubular subsarcolemmal subspace	0.0775
V_{ds}	surface dyadic space	0.0001671
V_{dt}	tubular dyadic space	0.0006682
V_{JSRs}	surface junctional compartment of sarcoplasmic reticulum	0.0078
V_{JSRt}	tubular junctional compartment of sarcoplasmic reticulum	0.0312
V_{NSR}	network compartment of sarcoplasmic reticulum	0.3508

2.2 *Membrane transport system*

Voltage dependent inactivation (VDI) and Ca^{2+} dependent inactivation (CDI) of I_{Ca} was newly formulated on the basis of experimental results of Brette et al. (2004). All parameters of CDI are regarded as dependent on the level of calmodulin saturation with Ca^{2+} ($B_{cm,Casat}$, Shannon et al., 2004). The steady state levels and time constants of VDI (ss_{VDI}, τ_{VDI}) and CDI (ss_{CDI}, $\tau_{CDI,s}$, $\tau_{CDI,s}$) are described by the following equations:

$$ss_{VDI,x}=1/(1+\exp((V_{m,x}+26.7)/5.4)),$$

$$\tau_{VDI,x}=1.15\cdot(0.041\cdot\exp(-((V_{m,x}+47)/12)^2)+0.08/(1+\exp(-(V_{m,x}+55)/5))+0.015/(1+\exp((V_{m,x}+75)/25))),$$

$$ss_{CDI,x}=1/(1+0.244\cdot(B_{cm,Casat,x}^4+0.318^4)/B_{cm,Casat,x}^4),$$

$$\tau_{CDI,s}=1/(43.827\cdot B_{cm,Casat,s}^4/(B_{cm,Casat,s}^4+0.976^4)+25.006),$$

$$\tau_{CDI,t}=1/(1160\cdot B_{cm,Casat,t}^8/(B_{cm,Casat,t}^8+1.14^8)+16.66).$$

While the formulations of ss_{VDI}, τ_{VDI} and ss_{CDI} in the description of $I_{Ca,s}$ and $I_{Ca,t}$ are identical (the suffix x in the equations stands for s (surface) or t (tubular)), the τ_{CDI} of these two currents is formulated differently (see $\tau_{CDI,s}$ and $\tau_{CDI,t}$). This takes into account the observed different modulation of surface and tubular I_{Ca} by Ca^{2+} released from SR (Brette et al., 2004).

The conductivity of I_{to} was increased by 20% for action potentials of the model to exhibit physiological duration.

The fractions of membrane currents in the tubular membrane were set to meet the results published in Pásek et al. (2008) except for the values of $f_{ICa,t}$ and $f_{IpCa,t}$ that were set to 80% (Brette et al., 2004) and 95% (Chase & Orchard, 2011), respectively.

2.3 *Intracellular Ca^{2+}- handling*

The formulation of the function of ryanodine receptors in JSR (RyR) was adopted from Shannon et al. (2004). The constants k_s and k_{oCa} were increased from 25 ms^{-1} to 250 ms^{-1} and from 10 mM^{-2} ms^{-1} to 50 mM^{-2} ms^{-1}, respectively, for Ca^{2+} transients in the dyadic space to reach magnitude close to 100 μM at the level of free [Ca^{2+}] in NSR of 0.5 mM (Shannon et al., 2004). Description of SR Ca^{2+} pump (J_{up}) was modified to be consistent with data of Shannon and Bers (1997). The constants used are: V_{max} = 286 μM/s, K_{mf} = 168 nM, K_{mr} = 1.176 mM, h_f = 1.2 and h_r = 1.287. The dissociation constant (K_d) of calsequestrin in JSR was decreased from original value 0.8 μM to 0.65 μM (Shannon et al., 2004). Ca^{2+} buffering by calmodulin was described by differential equations with k_{on} =100000 mM^{-1} s^{-1} and k_{of} = 238 s^{-1}.

The model was supplemented by the description of exogenous Ca^{2+} buffer diffusion (BAPTA or EGTA) among the pipette, subsarcolemmal spaces, dyadic spaces and cytosol. All time constants controlling the rate of exogenous Ca^{2+} buffer and Ca^{2+} diffusion between individual cellular compartments are specified in Tab. 2 (the time constants of intracellular Na^+ and K^+ diffusion were set to the same values as in the case of Ca^{2+} diffusion).

2.4 *Ion diffusion between tubular and extracellular space*

The time constants of ion exchange between the TATS lumen and the extracellular solution ($\tau_{Ca,TATS}$, $\tau_{K,TATS}$, $\tau_{Na,TATS}$) were readjusted for the model to better reproduce the changes in I_{Ca} and resting voltage following rapid decrease or increase of external ion concentrations at 37 °C (Yao et al., 1997). To reconstruct the biphasic time course of I_{Ca}-decrease after rapid exposure of myocytes to Ca^{2+}-free external solution, the model was supplemented by a formulation of Ca^{2+} buffer in TATS (B_{tats}). Using the same pulse and solution change protocols the reconstructions led to the following values of buffer parameters and time constants: k_{on}= 2.2 s^{-1} mM^{-1}, k_{off}= 2.398 s^{-1}, B_{tats} = 2.6 mM, $\tau_{Ca,TATS}$ = 155 ms and $\tau_{Na,TATS}$ = $\tau_{K,TATS}$ = 150 ms. Finally, the time constants were corrected for the lower temperature of the model cell (22 °C, Q_{10} = 1.3) and their final values were: $\tau_{Ca,TATS}$ = $\tau_{Na,TATS}$ = $\tau_{K,TATS}$ = 220 ms.

Tab. 2: Time constants related to intracellular transport of Ca^{2+} and Ca^{2+}-buffers.

symbol	specification	value [s]
$\tau_{pss,buffer\text{-}free}$	controls buffer diffusion from the pipette into the surface subspace	1.36
$\tau_{pss,buffer\text{-}Ca}$	controls buffer-Ca^{2+} diffusion from the pipette into the cytosolic space	1.36
$\tau_{pss,Ca}$	controls Ca^{2+} diffusion from the pipette into the cytosolic space	1.36
$\tau_{dsss,buffer\text{-}free}$	controls buffer diffusion from the surface dyadic space into surface subspace	$0.34E^{-3}$
$\tau_{dsss,buffer\text{-}Ca}$	controls buffer-Ca^{2+} diffusion from surface dyadic space into surface subspace	$0.34E^{-3}$
$\tau_{dsss,Ca}$	controls Ca^{2+} diffusion from the surface dyadic space into the surface subspace	$0.34E^{-3}$
$\tau_{dtst,buffer\text{-}free}$	controls buffer diffusion from the tubular dyadic space into tubular subspace	$0.34E^{-3}$
$\tau_{dtst,buffer\text{-}Ca}$	controls buffer-Ca^{2+} diffusion from the tubular dyadic space into tubular subspace	$0.34E^{-3}$
$\tau_{dtst,Ca}$	controls Ca^{2+} diffusion from the tubular dyadic space into tubular subspace	$0.34E^{-3}$
$\tau_{ssc,buffer\text{-}free}$	controls buffer diffusion from the surface subspace into cytosolic space	0.004
$\tau_{ssc,buffer\text{-}Ca}$	controls buffer-Ca^{2+} diffusion from the surface subspace into cytosolic space	0.004
$\tau_{ssc,Ca}$	controls Ca^{2+} diffusion from the surface subspace into cytosolic space	0.004
$\tau_{stc,buffer\text{-}free}$	controls buffer diffusion from the tubular subspace into cytosolic space	0.001
$\tau_{stc,buffer\text{-}Ca}$	controls buffer-Ca^{2+} diffusion from the tubular subspace into cytosolic space	0.001
$\tau_{stc,Ca}$	controls Ca^{2+} diffusion from the tubular subspace into cytosolic space	0.001
$\tau_{ssst,buffer\text{-}free}$	controls buffer diffusion from the surface subspace into tubular subspace	0.1
$\tau_{ssst,buffer\text{-}Ca}$	controls buffer-Ca^{2+} diffusion from the surface subspace into tubular subspace	0.1
$\tau_{ssst,Ca}$	controls Ca^{2+} diffusion from the surface subspace into tubular subspace	0.1

3. Conclusions

The present novel description of intracellular Ca^{2+} dynamics in the model of rat ventricular myocytes is an important step toward understanding of specific details of excitation-contraction coupling in cardiac ventricular myocytes. The principal modifications based on the recently published data comprise: formulation of the function of peripheral dyads, incorporation of peripheral and tubular intracellular subspaces, reformulation of inactivation properties of surface of tubular I_{Ca} and description of the function of exogenous Ca^{2+} buffer in the intracellular space. The modified model will be used to further investigate the effects of activity induced ion-concentration changes in TATS on electrical and mechanical activity of ventricular cardiomyocytes.

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