The functional role of cardiac T-tubules explored in a model of rat ventricular myocytes

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The morphology of the cardiac transverse-axial tubular system (TATS) has been known for decades, but its function has received little attention. To explore the possible role of this system in the physiological modulation of electrical and contractile activity, we have developed a mathematical model of rat ventricular cardiomyocytes in which the TATS is described as a single compartment. The geometrical characteristics of the TATS, the biophysical characteristics of ion transporters and their distribution between surface and tubular membranes were based on available experimental data. Biophysically realistic values of mean access resistance to the tubular lumen and time constants for ion exchange with the bulk extracellular solution were included. The fraction of membrane in the TATS was set to 56%. The action potentials initiated in current-clamp mode are accompanied by transient K⁺ accumulation and transient Ca²⁺ depletion in the TATS lumen. The amplitude of these changes relative to external ion concentrations was studied at steady-state stimulation frequencies of 1–5 Hz. Ca²⁺ depletion increased from 7 to 13.1% with stimulation frequency, while K⁺ accumulation decreased from 4.1 to 2.7%. These ionic changes (particularly Ca²⁺ depletion) implicated significant decrease of intracellular Ca²⁺ load at frequencies natural for rat heart.

Keywords: accumulation–depletion of ions; transverse tubule; heart; mathematical model; cardiomyocyte; rat

1. Introduction

The transverse-axial tubular system (TATS) of cardiac cells is a structure that allows rapid propagation of excitation into the cell interior. As suggested in recent experimental works (Shepherd & McDonough 1998; Christé 1999; Brette & Orchard 2003), the electrical activity of the cell might be significantly affected by accumulation and/or depletion of ions in the restricted space of TATS. Our previous modelling work (Pásek et al. 2003) indicated that such phenomena

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could be studied quantitatively. However, the description of membrane transport systems in that model merged formulations suited to ventricular cells of different species. The natural next step in the modelling work was to design models complying with the species-related differences, namely in the morphology of the TATS (Forbes & van Neil 1988; Amsellem et al. 1995; Soeller & Cannell 1999), in distribution of ionic currents between peripheral and tubular membrane and in diffusion rate within TATS (Yao et al. 1997; Shepherd & McDonough 1998; Brette & Orchard 2003). Thus, we developed a specific model based on available data related to ventricular cells of rat, particularly on recent microscopic analysis of rat ventricular cell ultrastructure (Satoh et al. 1996; Soeller & Cannell 1999) and on data from measurements of sarcolemmal ionic currents before and after formamide-induced detubulation (Brette et al. 2002; Yang et al. 2002). Using this model, we investigated the extent of ion concentration changes in the tubular space and their dependence on stimulation frequency. Part of this work was communicated at the Biocomputation and Modelling in Physiology Workshop at Oxford, 1–3 October 2004 (Pásek et al. 2004; Christé et al. 2004).

2. Development of model

Generally, the model is based on a quantitative description of electrical activity of the rat ventricular myocyte proposed by Pandit et al. (2001). Included are ionic transporting systems in surface and tubular membranes, description of the uptake and release compartment of sarcoplasmic reticulum (NSR and JSR), subsarcolemmal compartment (subspace with free calcium concentration $[Ca^{2+}]_{ss}$) and intracellular $Ca^{2+}$ buffers. A schematic of the model is shown in figure 1. It was assumed that the properties of ionic transfer mechanisms did not differ between the peripheral and the tubular membrane. Despite the report of an immunological difference between peripheral and tubular Na$^+$–K$^+$ pump molecules (McDonough et al. 1996), their electrical properties do not differ (Despa et al. 2003). Regarding other currents in the model, we found no report of such regional differences in properties.

The formulation of the TATS as well as the modifications we have introduced to the quantitative description of Pandit et al. (2001) are summarized in the following sections.

(a) Myocyte ultrastructure

The geometric parameters of the TATS were set to comply with the results of microscopic analysis in rat ventricular myocytes (Satoh et al. 1996; Soeller & Cannell 1999). The published data include fractional volume of TATS (approx. 3.6%), ratio TATS-area per cellular volume (0.44 $\mu m^2$ $\mu m^{-3}$), ratio total membrane area per cellular volume (0.68–0.89 $\mu m^2$ $\mu m^{-3}$), tubular diameter (0.2–0.3 $\mu m$) and spacing of tubular mouth in peripheral membrane (approx. 1.8 $\mu m$). Applying these parameters and ratios in the model with total membrane area 10 000 $\mu m^2$, the peripheral membrane represents 4395 $\mu m^2$ (43.95 pF), the tubular membrane 5605 $\mu m^2$ (56.05 pF), the cellular volume 12 739 $\mu m^3$ and the tubular volume 420.38 $\mu m^3$. For density of tubule mouths in the surface membrane 0.3 tubules $\mu m^{-2}$ and diameter of the tubules ($d_t$) 0.3 $\mu m$, their mean

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length \( (l) \) was determined to be 4.5 \( \mu \text{m} \). The fractional volumes of intracellular compartments (NSR, JSR and subspace) were set according to Pandit et al. (2001).

(b) Modification of membrane transport systems

The description of \( I_{\text{Na}} \)-channel gating was restricted to three activation (\( m^3 \)) and one inactivation (\( h \)) gates. The rate constants for activation (\( \alpha_m, \beta_m \)) and inactivation (\( \alpha_h, \beta_h \)) were formulated according to experimental data of Brown et al. (1981) and data from our experiments (M. Bebárova, P. Matejovic, M. Pásek, M. Šimurdova & J. Šimurda 2003, unpublished work). The maximum \( \text{Na}^+ \) conductance \( (g_{\text{Na}, \text{max}}) \) was adjusted to generate the maximum upstroke velocity \( (dV/dt)_{\text{max}} = 160 \text{ V s}^{-1} \). The reversal voltage \( (E_{\text{Na}}) \) was decreased by introducing a 12\% fractional permeability for \( \text{K}^+ \) ions to fall into the range of experimentally observed values (Brown et al. 1981; Zhang et al. 2003).

Figure 1. Schematic of a ventricular myocyte. The description of electrical activity of surface (s) and tubular (t) membrane comprises formulation of ion transporters that are labelled in the scheme. The intracellular space contains the subspace, the \( \text{Ca}^{2+} \)-uptake and \( \text{Ca}^{2+} \)-release compartments of sarcoplasmic reticulum (NSR, JSR) and the \( \text{Ca}^{2+} \) buffers calmodulin (B\text{cm}), troponin (B\text{trpn}, B\text{ltrpn}) and calsequestrin (B\text{cs}). The small filled rectangles in JSR membrane represent ryanodine receptors. The small bi-directional arrows denote \( \text{Ca}^{2+} \) diffusion. The dashed arrow represents ionic diffusion between the tubular and the bulk space. The currents included in the model were: \( I_{\text{Ca}} \), calcium inward current; \( I_{\text{Kt}} \), time-dependent transient outward potassium current; \( I_{\text{Kss}} \), steady-state outward potassium current; \( I_{\text{Kf}} \), hyperpolarization-activated current; \( I_{\text{K1}} \), time-independent inward rectifier potassium current; \( I_{\text{Nab}} \), background sodium current; \( I_{\text{Cab}} \), background calcium current; \( I_{\text{Kb}} \), background potassium current; \( I_{\text{NaCa}} \), \( \text{Na}^+ - \text{Ca}^{2+} \)-exchanger current; \( I_{\text{NaK}} \), electrogenic \( \text{Na}^+-\text{K}^+ \)-ATPase pump current; \( I_{\text{pCa}} \), sarcolemmal \( \text{Ca} \)-pump current.
The resulting formulae expressing voltage dependence of rate constants are:

\[
\alpha_m = 117.26(V_m + 59.3)/(1 - \exp(-0.55(V_m + 59.3))), \\
\beta_m = 3800 \exp(-0.072(V_m + 61)), \\
\alpha_h = 284.4/(1 + \exp(0.0812(V_m + 115.9))), \\
\beta_h = 18.77 \times (V_m + 64.4)/(1 - \exp(-0.22(V_m + 64.4))).
\]

The Ohm relation in the formulation of \(I_{Ca}\) was replaced by the equation based on constant field theory that accounts for the permeation properties of the L-type channels (Sun et al. 2000) and better describes dependence of \(I_{Ca}\) on external \(Ca^{2+}\). Hence,

\[
I_{Ca,\text{max}} = P_{Ca}A(V_m F^2/(RT)) \times ([Ca^{2+}]_{ss} \exp(2V_m F/(RT)) - 0.341[Ca^{2+}]_e)/(\exp(2V_m F/(RT)) - 1).
\]

To get a better fit with experimental data published by Katsube et al. (1998), the constant term (0.017 s) in the description of time constants of fast and slow \(I_{Ca}\) inactivation (\(\tau_{f1}, \tau_{f2};\) Pandit et al. 2001) was omitted.

The description of \(I_{K1}\) was corrected to be consistent with experimental data from rat myocytes (shown in Pandit et al. 2001). The corrected formulation is

\[
I_{K1} = g_{K1}(V_m - E_K - 1.7)/(1 + \exp(1.613F/(RT)(V_m - E_K - 1.7)) \times (1.0 + \exp(-(K_e - 0.9988)/0.124))).
\]

The stimulus current (5.2 nA, 1 ms) was incorporated into the equation controlling the intracellular potassium concentration to comply with the charge conservation principle (Hund et al. 2001).

To ensure realistic levels of diastolic and systolic [\(Ca^{2+}\)] at stimulation frequencies up to 5 Hz (for lower stimulation frequencies see Frampton et al. 1991b) and proportions of sarcolemmal transport systems in extrusion of systolic [\(Ca^{2+}\)] (Negretti et al. 1993; Varro et al. 1993), some changes in setting of properties of membrane channels had to be done. They comprise: increase of scaling factor of \(Na^+–Ca^{2+}\) exchange (\(k_{NaCa}\)) to 0.18 nA cm\(^{-2}\) mM\(^{-4}\), increase of maximum current of sarcolemmal \(Ca^{2+}\) pump (\(I_{pCa,max}\)) to 0.85 \(\mu\)A cm\(^{-2}\), increase of maximum conductivity of the background \(Ca^{2+}\) current (\(g_{Ca,b}\)) to 0.648 \(\mu\)S cm\(^{-2}\) and increase of maximum \(Na^+–K^+\) pump current (\(I_{NaK,max}\)) to 1 \(\mu\)A cm\(^{-2}\).

The maximum specific conductivity and current density of individual ion transfer mechanisms in surface membrane (\(g_{X,max,s}, I_{X,max,s}\)) and in tubular membrane (\(g_{X,max,t}, I_{X,max,t}\)) were computed according to the relations:

\[
g_{X,max,s} = g_{Xmax}(1 - f_{X,t})(S_{m,s} + S_{m,t})/S_{m,s}, \quad g_{Xmax,t} = g_{Xmax}f_{X,t}(S_{m,s} + S_{m,t})/S_{m,t}, \quad I_{X,max,s} = I_{Xmax}(1 - f_{X,t})(S_{m,s} + S_{m,t})/S_{m,s}, \quad I_{Xmax,t} = I_{Xmax}f_{X,t}(S_{m,s} + S_{m,t})/S_{m,t},
\]

where \(g_{X,max}\) and \(I_{X,max}\) denote the maximum specific conductivity and current density related to total membrane and \(f_{X,t}\) denote the fractions of individual currents flowing through tubular membrane (see table 1). Detubulation data were used to set the values of \(f_{Ca,t}\) (Kawai et al. 1999), \(f_{Kss,t}\) (Komukai et al. 2002), \(f_{NaCa,t}\) (Yang et al. 2002; Despa et al. 2003) and \(f_{NaK,t}\) (Despa et al. 2003).
S_{m,s} and S_{m,t} stand for the areas of peripheral and tubular membrane, respectively.

(c) Electrical interaction between surface and tubular membrane

The TATS was described as a single compartment separated from the surface membrane by the mean resistance of the tubular system (R_{st}) connected to the bulk pericellular solution. The contribution of one tubule to R_{st} was expressed as the resistance of a cylindrical conductor, whose length, radius and specific resistivity corresponded to one-half of the tubular effective length (l_{t}/2), its average radius (r_{t}) and the specific resistivity of the extracellular solution (\rho_{ext}), respectively. (For the Tyrode solution, \rho_{ext} \approx 83.33 \Omega \text{ cm}.)

Taking into account that, from the electric point of view, the TATS represents a parallel combination of all (n_{t}) tubules in the model cell, the mean resistance of the tubular system (R_{st} = 20 k\Omega) can be calculated from the relation

\[ R_{st} = \rho_{ext} l_{t} / (2\pi r_{t}^{2} n_{t}). \]

As follows from the electrical equivalent scheme of the model in figure 2, the stimulating current I_{m} equals the sum of the sarcolemmal current I_{ms} and the current through the TATS membrane I_{mt}:

\[ I_{m} = I_{ms} + I_{mt}. \]

In current-clamp conditions, I_{m} = 0 throughout, except for the duration of the short (1 ms) suprathreshold stimuli. It follows that a common current,

\[ I_{circ} = I_{ms} = -I_{mt} = (V_{ms} - V_{mt})/R_{st}, \]

circulates through both membrane systems.

Table 1. Electrical properties of membrane transport systems in the model. (The values of the parameter f_{X,t} denote fractions of membrane currents flowing through tubular membrane. The values marked by asterisks were obtained by the group of Clive Orchard (Leeds) and others were determined under assumption of uniform density of corresponding transporters in peripheral and tubular membrane. g_{X,max} and I_{X,max} represent maximum specific conductivity and current density related to 1 cm\(^2\) of total membrane set in the model, P_{X} is permeability.)

<table>
<thead>
<tr>
<th>f_{X,t} (%)</th>
<th>g_{X,max}, I_{X,max}, P_{X}</th>
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<tbody>
<tr>
<td>I_{Na}</td>
<td>56</td>
</tr>
<tr>
<td>I_{Ca}</td>
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<tr>
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<td>59*</td>
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<tr>
<td>I_{pCa}</td>
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Intracellular Ca\textsuperscript{2+}-handling

In their model, Pandit et al. (2001) adopted a description of intracellular Ca\textsuperscript{2+}-handling from a quantitative model of canine midmyocardial ventricular cell (Winslow et al. 1999) that exhibits significant differences from the behaviour of rat ventricular cell, in particular when higher stimulation rates are applied. To simulate realistic dynamic changes of intracellular Ca\textsuperscript{2+} and to prevent reactivation of SR Ca\textsuperscript{2+}-release channels at a stimulation rate characteristic for rat (5 Hz), some model parameters related to intracellular Ca\textsuperscript{2+} translocation had to be changed: the forward rate parameter of SR Ca\textsuperscript{2+}-ATPase was increased to 0.4 mM s\textsuperscript{-1}, the time constant of Ca\textsuperscript{2+}-transfer from NSR to JSR was increased to 20 ms and the time constant of Ca\textsuperscript{2+}-transfer between subspace and cytoplasm was decreased to 8 ms.

Ionic diffusion between tubular and extracellular space

The time constants of ionic diffusion between the TATS and the extracellular space were set to 500 ms for divalent ions (Ca\textsuperscript{2+}) and to 150 ms for monovalent ions (K\textsuperscript{+} and Na\textsuperscript{+}) to fit the experimental data by Yao et al. (1997) (see §3 for more details).

Numerical integration technique

The model was implemented in the program system MATLAB v. 6.5 (developed by MathWorks, Inc.) and the numerical computation of the system of 59 nonlinear differential equations was performed using the solver for stiff systems ODE-15s. The model equations were simultaneously solved using a time-step adjusted to keep the estimated relative error of inner variables below a threshold value of 0.001. After every change in model equations or parameter values, the

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Figure 2. Electrical interaction between the surface and tubular membrane. $V_{ms}$, $I_{is}$, $I_{Cs}$, $C_s$ and $V_{mt}$, $I_{it}$, $I_{Ct}$, $C_t$ are, respectively, membrane voltage, total ionic current, capacitive current and membrane capacity of the surface and tubular membranes. $R_{st}$ is the mean luminal resistance of the TATS and $R_{el}$ mimics the series resistance of patch-clamp pipette. $I_m$ is the stimulating current.
model was run for 20 min of equivalent cell lifetime to ensure that steady state was reached. The values of all variables at this time were assigned as starting values before running model trials.

The basic units in which the equations were solved were: mV for membrane voltage, μA for membrane currents, mM for ionic concentrations, s for time and ml for volumes.

3. Results

(a) Comparison of the model with a real cell

(i) Action potentials

The comparison of action potentials produced by the model at different stimulation rates with experimental records obtained from rat ventricular myocytes (M. Bebárova, P. Matejovic, M. Pásek, M. Šimurdova & J. Šimurda 2003, unpublished data) is depicted in figure 3. The shape of simulated action potentials is similar to that obtained by experimental measurements and corresponds to epicardial myocytes (Shimoni et al. 1995; Volk et al. 1999). The experimentally observed frequency-induced lengthening of action potential duration (Shimoni et al. 1995; Fauconnier et al. 2003) is also well reproduced in our model.

(ii) Kinetics of tubular K\(^+\) diffusion

To test the validity of our formulation describing the K\(^+\) and Ca\(^{2+}\) exchange between tubular lumen and bulk solution (single compartment), we reproduced the experiments performed by Yao et al. (1997) on a single rat ventricular myocyte (their fig. 1 and 6, rat traces).

Their experimental data was digitized from image scans and superimposed with model output. In current-clamping mode, after a period of rest at 4.4 mM

\[ \text{(a)} \]

\[ \text{(b)} \]
The external medium was switched for 860 ms to a solution with 6.6 mM $\text{K}^+$ ions, and then switched back to 4.4 mM. The resting membrane voltage was continuously recorded (Figure 4a). The experimental and computed responses could be well approximated with a sum of two exponential functions. The faster exponential can be hypothetically ascribed to the response of peripheral membrane, while the slower one relates to the response of the TATS. In line with this conception, the fast component is much faster in the model than in the experiment. In the model (continuous line in Figure 4a), the change of external $\text{K}^+$ was assumed instantaneous. Thus, the fast initial phase corresponds to the time constant of charging of the peripheral membrane ($C_m \times R_m$). In the real situation (dotted line), restricted diffusion due to own complexity of the peripheral membrane and buffering effect come in addition to slow down the time course of change in $\text{K}^+$ seen by the peripheral membrane (as discussed by Yao et al. 1997). On the other hand, the time constant of the slower exponential component (assessed from the downward step of $[\text{K}^+]_e$) appeared to be equal (147 ms) in experimental and simulated responses and corresponds well with single-exponential decrease of $[\text{K}^+]_t$ (time constant 144 ms) that is available as a model output (Figure 4a, lower panel).

Figure 4. Investigation of the changes in tubular concentrations of $\text{K}^+$ ions (left) and of $\text{Ca}^{2+}$ ions (right). The results of model simulations are presented as full lines, experimental results as dotted lines. The experiments of Yao et al. (1997), as depicted in their fig. 1 and 6, respectively, were reproduced in the model. Yao et al.’s numerical data were extracted from image scans of their figures. (a) The changes in $V_m$ induced by temporarily switching from 4.4 to 6.6 mM $[\text{K}^+]_e$ are plotted in the upper panel in (a). The time course of changes in tubular $[\text{K}^+]$ as output from the model is plotted in the lower panel in (a). (b) The changes in peak $I_{\text{Ca,L}}$ current at various times after switching to a $\text{Ca}^{2+}$-free external solution are plotted as empty squares connected by a dotted line (upper panel in (b)). The underlying changes in tubular $[\text{Ca}^{2+}]$ were computed by the model (lower panel in (b)). Note that the initial value was 0.9 versus 1.08 mM in the external control perfusion, owing to steady tubular depletion during prepulse at $-40 \text{ mV}$. 

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(iii) **Kinetics of Ca\(^{2+}\) washout from transverse-axial tubular system**

The time course of tubular Ca\(^{2+}\) washout from TATS was explored using \(I_{\text{CaL}}\) measurement at various times after switch to zero external \([\text{Ca}^{2+}]_e\) (figure 4b). The model responded to step-wise removal of Ca\(^{2+}\) with an immediate decrease of \(I_{\text{CaL}}\) by 13% corresponding to the fraction of Ca-channels in peripheral membrane (table 1). The slow phase of exponential decline of \(I_{\text{CaL}}\) amplitude (time constant 405 ms) correlates with the time constant (410 ms) of the decrease of \([\text{Ca}^{2+}]_t\) as computed in the model (figure 4b, lower panel). Experimental results (Yao *et al.* 1997) did not include measurements within the first 100 ms after the step of \([\text{Ca}^{2+}]_e\), and the course between 100 and 500 ms departs considerably from single exponential decline. Detailed simulations in a distributed model (Šimurda *et al.* 2004a,b) are needed to reveal whether this divergence may be related to spatial distribution of Ca\(^{2+}\) concentration within TATS (see §4).

(b) **Activity-related changes in the rat cell model with transverse-axial tubular system**

To explore activity-related changes in currents and concentrations at physiological heart rate, the model was driven at 5 Hz stimulation frequency at steady state in current-clamping mode. This stimulation frequency corresponds to the resting heart rate of rat. Figure 5 shows the dynamic changes of membrane potential, ionic currents and concentrations in one full cycle. The traces related to surface membrane are drawn as full lines, those related to TATS as dotted lines.

(i) **Tubular and surface action potentials**

Action potentials at surface and tubular membrane are almost identical (figure 5a), which indicates that propagation of the depolarization to the tubular membrane occurred with negligible delay. As discussed by Pásek *et al.* (2003), this results from the large space constant of the TATS, i.e. approximately 240 μm, as compared to the tubular length. This is also consistent with experimental data showing that the capacitance transient during voltage clamp pulses decays mono-exponentially.

(ii) **Ionic currents**

The differences between ionic currents across the surface and tubular membrane (figure 5a) are mainly due to differences in densities of channels and different membrane areas. However, the tubular currents are modulated to a certain extent by variations of the tubular concentrations. This effect, indistinguishable in figure 5a, could be evaluated from model simulations (see §3c for further details).

(iii) **Na\(^+\) concentrations**

Intracellular [Na\(^+\)] (see panel [Na\(^+\)]\(_i\) in figure 5b) is significantly elevated at 5 Hz (slightly above 19 versus 4.52 mM at rest), which is a consequence of the higher intake of Na\(^+\) into the cell at higher rate of stimulation (Frampton *et al.* 1995).
Figure 5. Basic behaviour of rat model at 5 Hz stimulation (resting rat heart rate). (a) Action potentials and membrane currents. Continuous lines refer to peripheral membrane and dotted lines to tubular membrane. Horizontal lines show the zero level (0) for voltage and the membrane currents. (b) Intracellular and tubular ion concentrations. Horizontal solid lines show the zero level for \([\text{Ca}^{2+}]_i\) and the bulk extracellular concentration for \([\text{Ca}^{2+}]_t\), \([\text{K}^+]_t\), and \([\text{Na}^+]_t\). In addition, horizontal dotted lines show the rested state values for \([\text{Ca}^{2+}]_i\), \([\text{Ca}^{2+}]_t\), and \([\text{K}^+]_t\).
1991a). Tubular $[Na^+]$ is negligibly different from bulk extracellular concentration (140 mM, see continuous line in panel $[Na^+]_t$ of figure 5b). None of these concentrations show significant variations during a cycle.

(iv) $K^+$ concentrations and fluxes

While intracellular $[K^+]$ shows negligible fluctuations, tubular $K^+$ ions undergo transient accumulation (figure 5b). The voltage-dependent increase in the three outward $K^+$ currents $I_{K_{tor}}$, $I_{K_{ss}}$ and $I_{K1}$ (figure 5a) contribute to the rise of tubular $[K^+]$ that peaks at the end of the action potential (see panel $V_m$ in figure 5a). Both $I_{K_{tor}}$ and $I_{K_{ss}}$ return to zero, while $I_{K1}$ later decays towards an inward current value of $-28$ pA at the end of diastole (figure 5a). Inversion of $I_{K1}$ in the late part of the cycle is caused by the membrane voltage ($-80.4$ mV) becoming negative to the reversal voltage for $K^+$ ions ($-78.3$ and $-78.1$ mV at tubular and surface membrane, respectively). $I_{NaK}$ is permanently outward and is transiently doubled during depolarization. The persistent activation of the Na$^+$–K$^+$ pump, due to elevated $[Na^+]_i$, permanently depletes $K^+$ ions from the tubules, upon which the outflow of $K^+$ ions due to transient activation of $K^+$ channels causes transient tubular $K^+$ accumulation. The slow decay phase that ensues is altogether due to permanent uptake of $K^+$ ions by the Na$^+$–K$^+$ pump and to exchange with the extracellular bulk solution by diffusion.

(v) $Ca^{2+}$ concentrations and fluxes

$[Ca^{2+}]_i$ is transiently increased and relaxes towards an end-diastolic value higher than the rested state value of 0.024 μM (horizontal dotted line in panel $[Ca^{2+}]_i$ of figure 5b). Meanwhile, concentrations in both compartments of the SR ($[Ca^{2+}]_{JSR}$ and $[Ca^{2+}]_{NSR}$) vary around values far from the rested state value of 0.14 mM (figure 5b), illustrating frequency-induced $Ca^{2+}$ load. Tubular $[Ca^{2+}]$ is reduced rapidly at the beginning of the action potential (figure 5b), owing to the large $I_{Ca}$ and partly to the $I_{NaCa}$ that is transiently outward (Egan et al. 1989; figure 5a). The latter reverses direction and becomes inward, carrying $Ca^{2+}$ ions outward for the rest of the cycle.

(vi) Effect of frequency on the dynamic changes of tubular concentrations

To explore the effect of frequency on concentration changes in the TATS, we drove the model at steady state at frequencies from 1 to 5 Hz. We analysed the minimum value of tubular $[Ca^{2+}]$ (light grey) and its end-diastolic value (dark grey), and for tubular $[K^+]$ the maximal value (light grey) and end-diastolic value (dark grey). Figure 6 shows these quantities expressed in percentage of change referred to bulk extracellular concentrations. Accumulation above bulk extracellular concentration is plotted as an upward column and depletion is a downward column.

The maximal depletion of tubular $Ca^{2+}$ during a cycle at 1 Hz was 7%, whereas a moderate accumulation (2.3%) was present at the end of diastole. With increased frequency, the peak depletion of tubular $Ca^{2+}$ was progressively increased to reach 13.1% at 5 Hz, whereas maximal $Ca^{2+}$ accumulation decreased to reach almost zero at 4 Hz. At 5 Hz, end-diastolic $Ca^{2+}$ was
depleted by 1%. Thus, tubular Ca\(^{2+}\) oscillates between depletion and accumulation at 1–4 Hz, whereas it is depleted throughout the cycle at 5 Hz.

At 1 Hz, tubular accumulation of K\(^{+}\) peaked at 4.1% of bulk [K\(^{+}\)] (set at 5.4 mM) and reversed into slight depletion (0.7%) at the end of diastole. The maximal accumulation decreased with frequency to be 2.7% at 5 Hz, whereas depletion increased up to 3 Hz (1.3%) and decreased at higher frequencies (1% at 5 Hz). Thus, tubular K\(^{+}\) exhibits accumulation as well as slight depletion.

\((c)\) Consequences of ionic concentration changes in transverse-axial tubular system

Activity-related concentration changes in TATS may influence the magnitude of ionic currents through the TATS membrane. To quantify this effect, we compared electrical charges transferred within one cycle by individual membrane currents with those transferred under condition of constant tubular concentrations suddenly set to the levels of extracellular concentrations (table 2\(a\)). The relative alterations of transferred charge are introduced in table 2\(b\). As follows from table 2\(b\), the presence of TATS tends to moderate most explored ionic currents. A consequence of this immediate effect of invalidation of TATS is a prolongation of the action potential, both at 1 and 5 Hz (figure 7\(a\)). Thus, the presence of TATS tends to shorten action potential duration.

Altered concentrations within TATS also induce changes in intracellular ionic concentrations. Figure 7b shows the gradual increase of free calcium concentration in the SR uptake compartment ([Ca\(^{2+}\)]\(_{NSR}\)) and in cytoplasm ([Ca\(^{2+}\)]\(_i\)) resulting from the sudden fixation of tubular concentrations at the extracellular levels. Dotted traces indicate results obtained at steady state from the non-modified model. The changes at 1 Hz are negligible. However, at 5 Hz, both concentrations in the modified model increase markedly. As follows from the data in Table 2, during the first cycle after switching to the mode of constant concentrations in the TATS, the amount of Ca\(^{2+}\) inside the cell is increased by 17.4 amol. The increments gradually decline and vanish as soon as the new steady state is achieved. However, the Ca\(^{2+}\) concentrations in both compartments remain significantly enhanced (figure 8). At 1 Hz, there is a slight increase of free calcium concentration in the SR uptake compartment ([Ca\(^{2+}\)]\(_{NSR}\)) but no visible change in the [Ca\(^{2+}\)]\(_i\) transient. In contrast, at 5 Hz, there is a prominent difference between the two models. The diastolic level of [Ca\(^{2+}\)]\(_{NSR}\) and the peak value of [Ca\(^{2+}\)]\(_i\) transient increase by 0.28 mM (18.34%) and by 0.28 µM (24.5%), respectively, when concentration changes in TATS are disabled. As shown in the lowest panels of figure 8, the tubular Ca\(^{2+}\) in the model cell with variable TATS concentrations, at 1 Hz, is transiently depleted and returns to a level above bulk external [Ca\(^{2+}\)] near to the level attained at steady state at rest (1.21 mM). At 5 Hz, maximal tubular Ca\(^{2+}\) depletion is twice as large and [Ca\(^{2+}\)]\(_i\) still remains below bulk [Ca\(^{2+}\)] at the end of diastole.

The accumulation and depletion of tubular ions in the model also affected the levels of intracellular Na\(^+\) and K\(^+\) concentrations. At steady state, elimination of concentration changes in TATS led to the enhancement of intracellular Na\(^+\) load by 1.43 mM (7.4%) and to reduction of the intracellular K\(^+\) load by 1.55 mM (1.24%).

Table 2. Effects of concentration changes in TATS on ion fluxes. (a) Ionic charges transferred by membrane transport systems during one cycle at 5 Hz. Upper line shows steady-state values from the model with concentration changes in TATS. Lower line shows values obtained during the first cycle after the concentrations in TATS were suddenly set constant equal to external values. (b) Relative changes of the transferred charges. The reference values are from the model with concentration changes in TATS.

<table>
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<tr>
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<th>(Q_{Ca}) [pC]</th>
<th>(Q_{Kto}) [pC]</th>
<th>(Q_{Ksi}) [pC]</th>
<th>(Q_{K1}) [pC]</th>
<th>(Q_{NaCa}) [pC]</th>
<th>(Q_{NaK}) [pC]</th>
<th>(Q_{PCa}) [pC]</th>
<th>(Q_{CP,b}) [pC]</th>
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<tr>
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<td>-3.504</td>
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<tr>
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The functional role of cardiac T-tubules

Phil. Trans. R. Soc. A (2006)
Figure 7. Effects of sudden fixation of the concentrations in TATS at the external bulk values in the model driven at 1 and 5 Hz. Results of simulations obtained from the model before (dotted lines) and immediately after fixation of tubular concentrations (solid lines). (a) Action potentials. (b) Ca$^{2+}$ concentrations in intracellular compartments ([Ca$^{2+}$]$_{NSR}$ and [Ca$^{2+}$]$_{i}$).

Figure 8. Comparison of a model cell with and without concentration changes in TATS, at steady state. [Ca$^{2+}$] in the uptake compartment of the sarcoplasmic reticulum ([Ca$^{2+}$]$_{NSR}$, top row), cell cytoplasm ([Ca$^{2+}$]$_{i}$, middle row) and TATS lumen ([Ca$^{2+}$]$_{t}$, bottom row) are plotted versus time, in the presence (dotted line) and absence (solid line) of concentration changes in TATS, during stimulation at (a) 1 Hz and (b) 5 Hz at a bulk extracellular [Ca$^{2+}$] of 1.2 mM (indicated by the horizontal solid line in the lower panels).

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4. Discussion

A variety of mathematical models have been formulated in the last decades describing electrical phenomena and excitation–contraction (E–C) coupling in cardiac cells from different species and different regions of the heart. None of them, however, has explored the possible role of the cardiac TATS in the physiological modulation of electrical activity and intracellular calcium homeostasis. For so doing we have developed a mathematical model of rat ventricular cardiomyocytes, in which the TATS is described as a single compartment. The simulations at steady state under regular stimulation in current-clamp mode revealed significant activity-dependent $\mathrm{Ca}^{2+}$ depletion and $\mathrm{K}^+$ accumulation in the tubular system. One consequence which is examined in the present work is that the SR calcium load in the model is permanently moderated at physiological frequencies.

(a) Limitations of the model

In the present model, the tubular system is represented as a single compartment, which neglects the fact that equilibration of concentrations through diffusion depends on the depth in the TATS (Soeller & Cannell 1999), on the inhomogeneities of distribution of channels along the TATS (Scriven et al. 2000) and on the ion-buffering properties of the TATS luminal content (Kostin et al. 1998). Such features influence the spatial pattern of changes in tubular concentrations as detected in adult ventricular myocytes by Blatter & Niggli (1998). Temporal and spatial distribution of ionic concentrations was investigated using partial differential equations in a continuous model of unbranched T-tubules closed at the inner end (Simurda et al. 2004a,b). Under uniform density of ion transfer mechanisms over the depth of T-tubules, the computer simulations have shown that the profile of activity-induced changes in tubular ion concentrations increases in a roughly exponential manner with depth along the T-tubule, with a steepness that is maximal at the mouth and reaches zero at the inner end. With local regions of higher channel density, undulations appeared in the dynamic concentration profile, which were considerably smoothed when ion binding within the T-tubules was included. The present lumped model satisfactorily describes concentration changes averaged over the length of tubular axis, but fails to reproduce details, such as the wave-like propagation of $[\mathrm{Ca}^{2+}]$ at low velocity along the tubular system observed by Blatter & Niggli (1998).

The change in tubular concentrations as estimated by the change of ionic currents magnitude measured in ventricular cells in response to step changes of extracellular ion concentrations was well approximated by a single exponential function in rabbit and guinea-pig myocytes (Yao et al. 1997; Shepherd & McDonough 1998; Levi et al. 1998). This phase is well accounted for by our model. However, a fast initial phase with time constant around 20 ms was detected in real ventricular cells (Shepherd & McDonough 1998) and attributed to a peripheral juxtamembrane compartment with restricted diffusion, which is neglected in our model. Among these neglected features of real cells, only the buffering of ions in TATS lumen is likely to dampen concentration changes, whereas axial gradients, inhomogeneities in the density of ion transfer mechanisms in TATS and peripheral compartment of restricted diffusion ought to enhance activity-related changes in tubular concentrations.
Another limitation of the present model relates to the description of E–C coupling. To avoid oversized computational complexity, the calcium-induced calcium release process is assumed to be controlled by $[\text{Ca}^{2+}]$ in a single compartment represented by restricted subspace. Such ‘common-pool models’ fail to depict some experimental results, namely smoothly graded Ca-release with $I_{\text{CaL}}$ at high gain of E–C coupling. To account for these observations, interactions at the molecular level between Ca$^{2+}$ ions and calcium channels within functional release units must be considered (for a review see Soeller & Cannell 2004). On the other hand, the common pool models proved able to satisfactorily describe many aspects of E–C coupling. As the aim of the present modelling work was to roughly estimate the effect of TATS on the whole cell behaviour, the simplification introduced into our model seems acceptable. However, re-examination of the simulated results with a local-control model of the Ca-release process will be necessary in the future.

In our present model, the formulation of the reuptake of calcium into the SR does not produce the shortening of the intracellular Ca$^{2+}$ transient with increasing frequency as observed in rat ventricular myocytes (Frampton et al. 1991a; Layland & Kentish 1999). Although the mechanism of this phenomenon is still unclear, a future version of the model ought to account for this effect.

The model construction has been complicated by some controversial experimental results related to E–C coupling in rat ventricular cells. For example, experimental studies of the rate dependence of SR Ca$^{2+}$ content, intracellular Ca$^{2+}$ transients and twitch force resulted in positive (Frampton et al. 1991b; Layland & Kentish 1999; Taylor et al. 2004) as well as negative (Bers 1991, 2000; Maier et al. 2000) relationships. The results appeared to be very sensitive to experimental conditions, and different behaviour was observed even in cells from the same experimental series (Frampton et al. 1991a). In our model, the SR Ca$^{2+}$ content (0.14 mM at rest) becomes progressively loaded with increasing stimulation frequency (see figure 7), causing gradual increase in intracellular Ca$^{2+}$ transients.

(b) Properties of transverse-axial tubular system

The fraction of the cell membrane present in TATS was estimated to be 32% from the cell capacitance that was lost after detubulation of rat ventricular myocytes (Despa et al. 2003). We introduced a value of 56% that was derived to comply with morphological features measured by Soeller & Cannell (1999) and with surface-to-volume ratio evaluated by Satoh et al. (1996). These could not be respected in the model when the fractional area of TATS was lowered to 32%, which implicated too small mean length of tubules (1.66 μm) and fractional volume of TATS (1.88%). As a consequence, ion concentration changes in TATS were increased: tubular Ca$^{2+}$ depletion was 20.7% and K$^+$ accumulation was 4.4% at 5 Hz stimulation, expressed versus extracellular concentrations. Estimating the fractional area of TATS from changes of membrane capacity (or the converse) is done using the implicit assumption of equal specific capacitance of peripheral and tubular membranes (1 μF cm$^{-2}$ in our model). It can be speculated as to whether a lower value of specific capacitance of TATS membrane could explain, at least partly, the low estimate of tubular membrane.
area published by Despa et al. (2003). This would allow our model to account for the results of experimental detubulation while respecting morphological features.

The time constants of monovalent (150 ms) and divalent (500 ms) ion diffusion between external space and TATS were set for the model to reproduce experimental data from rat myocytes published by Yao et al. (1997). This difference in diffusion rate conforms with the ratio of diffusion constants \( D_K/D_{Ca^2+} \approx 2.5 \) in aqueous solution (Kirsch et al. 1977; Hille 1992) and with slow \( Ca^{2+} \) diffusion in TATS observed by Blatter & Niggli (1998). However, in experiments on guinea-pig ventricular cells, Shepherd & McDonough (1998) came even to reverse ratio of the time constants of \( Ca^{2+} \) and \( Na^+ \) exchange (\( \tau_{Ca} = 195 \text{ ms} \) and \( \tau_{Na} = 230 \text{ ms} \)). To assess the impact of diffusion rates on the model cell, we repeated the simulations illustrated in figure 5 with shorter time constants of \( Ca^{2+} \) diffusion. Surprisingly, with time constants of 350 and 150 ms, the maximum calcium depletion decreased only to 11.9 and 10.1%, respectively, in comparison with 13.13% obtained with the time constant of 500 ms. Thus, changing the time constant of diffusion between 150 and 500 ms did not considerably affect \( Ca^{2+} \) depletion in TATS.

(c) Cumulative tubular \( Ca^{2+} \) depletion and moderation of calcium load at physiological stimulation rate

The present results support the concepts of transient accumulation of \( K^+ \) ions (Attwell et al. 1979) and transient depletion of \( Ca^{2+} \) ions (Bers 1983) in cardiac TATS (figure 5). Increasing the rate of stimulation from 1 to 5 Hz, in the model explored at steady state, increased transient \( Ca^{2+} \) depletion (from 7 to 13.1%) and decreased \( K^+ \) accumulation (from 4.1 to 2.7%) versus bulk extracellular concentrations (figure 6). It is worth noting that the largest relative change at all frequencies is \( Ca^{2+} \) depletion. In our model driven at 5 Hz, tubular \( Ca^{2+} \) depletion led to a reduction of the unidirectional \( Ca^{2+} \) influx during a cycle, suggesting a moderation of cellular calcium load. When we switched to a modified model, in which TATS concentrations were set constant and equal to extracellular levels, the intracellular \( Ca^{2+} \) transients and the \( Ca^{2+} \) content in SR increased substantially (by 24.5 and 18.34%, respectively; figure 8). Thus, the presence of TATS exerted a significant effect on the degree of intracellular \( Ca^{2+} \) load. This effect is likely to be still more significant at frequencies above 5 Hz as under effort or stress. Thus, TATS may play a significant part in the mechanisms controlling the homeostasis of calcium in ventricular cardiomyocytes of rat.

Editors’ note

Please see also related communications in this focussed issue by Crampin et al. (2006) and Iribe et al. (2006).

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