Modelling depolarization delay, sodium currents, and electrical potentials in cardiac transverse tubules

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ABSTRACT

T-tubules are invaginations of the lateral membrane of striated muscle cells that provide a large surface for ion channels and signaling proteins, thereby supporting excitation-contraction coupling. T-tubules are often remodeled in heart failure. To better understand electrical behavior of T-tubules of cardiac cells in health and disease, this study addresses two largely unanswered questions regarding their electrical properties: (1) the delay of T-tubular membrane depolarization and (2) the effects of T-tubular sodium current on T-tubular potentials.

Here, we present a simple computational model to determine the delay in depolarization of deep T-tubular membrane segments as the narrow T-tubular lumen provides resistance against the extracellular current. We compare healthy and diseased tubules from mouse and human, and conclude that diseased T-tubules depolarize even faster than healthy ones due to tubule widening. We moreover model the effect of a large T-tubular sodium current on T-tubular potentials. We observe that extracellular potentials are slightly negative and, therefore, the sodium current is self-attenuating.

Despite the simplicity of this model, these results show that (1) the excitation-contraction coupling defects seen in diseased cells cannot be explained by T-tubular remodeling alone; and (2) the self-attenuation of the sodium current resembles ephaptic effects that have been described for regions of the intercalated discs where the opposing membranes are very close together.

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This article was submitted to BioRXiv.org on 16 April 2019

INTRODUCTION

Transverse (T-)tubules are deep invaginations of the lateral membrane of skeletal and cardiac muscle cells. In mammalian ventricular cardiomyocytes, T-tubules form a complex network throughout the cell, especially in species with high heart rates such as mice [7, 8], and carry many ion channels and regulatory proteins (reviewed in [10-12]). Consequently, T-tubules function as a platform for excitation-contraction coupling and signaling, which is essential for the initiation and regulation of muscle contraction [10]. Importantly, T-tubular remodeling has been reported for several cardiac diseases [14, 15]. Understanding the electrical properties of T-tubules in health and disease is therefore paramount to understanding cardiac physiology and pathophysiology. Several questions regarding the electrical properties of T-tubules however remain largely open. Firstly, the delay by which deep segments of T-tubules depolarize has hardly been assessed. In the past, T-tubules were modeled as bulk rather than as segments, and the delay due to the charging of the capacitor has been assumed to be negligible [16, 17]. We therefore present an *in silico* model of a simple T-tubule and quantify the depolarization delay of deep T-tubular segments compared to cell surface due to the capacitive current. Thus, the threshold of voltage-gated channels deep in the cell will be reached

slightly later than near the surface. Secondly, we extended this model with a T-tubular sodium current, because the interactions between the sodium current and the extracellular potentials have scarcely been investigated. Please note however that the presence of a T-tubular sodium current is still subject of debate, since the presence of a T-tubular sodium current has only been deducted indirectly by comparing whole-cell sodium current density from control and detubulated cardiomyocytes [18]. This method is however flawed, since current density is determined by dividing the whole-cell peak current by the cell capacitance as a measure for cell surface area. Cell capacitance however significantly underestimates cell surface area in cardiomyocytes [19], which leads to a *de facto* overestimation of T-tubular current.

The effects of a large T-tubular sodium current have been already simulated by Hatano *et al.* using an elaborate model [20]. The extracellular potential transiently becomes slightly negative; as a result the sodium current self-attenuates. We confirm this using our simple model and discuss the physiological implications of this finding.

METHODS

Our model approximated one T-tubule as a cylinder and divided it in 100 segments (for a mouse T-tubule) or 20 segments (for a human T-tubule), separating 101 or 21 nodes, respectively (**Figure 1A, Table 1**). The mouth of the T-tubule (first node) was voltage-clamped perfectly, and intracellular resistivity was assumed negligible. T-tubular radius, length, extracellular resistivity, membrane capacitance, and membrane conductance were set as in **Table 1**.

In the same T-tubular model, sodium current in the different nodes was modeled according to Luo & Rudy [1] with modifications by Livshitz [2] (**Figure 2A**). A voltage pulse from -95 to -20 mV was applied at the mouth of the tubule. The value of -20 mV was chosen to elicit a large sodium current. The sodium current was set to 23 mS/µF, the same value as used in whole-cell simulations [1]. Note that this value is probably on the high side, as the intercalated discs contain a relatively high density of voltage-gated sodium channels and carries a significant proportion of the whole-cell sodium current [21]. Calcium channels were not included in the model as these channels open only when the majority of sodium channels have already inactivated.

Membrane potential and the gating variables of the sodium current were integrated using the forward Euler method using a constant time step of 0.25 ns. Simulations were implemented and run in MATLAB (version 2015a, The MathWorks, Natick, MA, USA). The source code used to generate the data shown in the Figures is provided as **Supplemental material**.

RESULTS

DELAY OF T-TUBULAR MEMBRANE DEPOLARIZATION

First, we set out to answer the question how long it takes to charge the membrane as a capacitor and depolarize the T-tubules. In other words: what is the delay between depolarization at the plasma membrane and deep in the T-tubules?

Table 1. Parameters for T-tubular model of healthy and post-myocardial (MI) T-tubules of human and mouse. Radius (r) and length (l) are based on previously published values. Numbers between brackets correspond to the following publications: (1) [3], (2) [4], (3) [5], (4) [6]. The resistivity of the extracellular space (ρ_e) is set at 100 Ω·cm. Conductance of resting membrane (g_m) is set at 0.143 mS/cm^2 [9], which is attributable to a small I_{K1} and leak currents. Capacitance (C_m) was set at 2 µF/cm^2 in healthy cells to simulate microfolds, and at 1 µF/cm^2 to simulate the loss of microfolds in disease [4, 13].

Species	*r* (nm)	*l* (µm)	ρ_e (Ω·cm)	C_m (µF/cm^2)	g_m (mS/cm^2)	Nodes
Healthy mouse	98.5 (1)	9 (2)	100	2	0.143	101
Post-MI mouse	108 (1)	9 (2)	100	1	0.143	101
Healthy human	147 (3)	2 (4)	100	2	0.143	21
Post-MI human	218 (3)	2 (4)	100	1	0.143	21

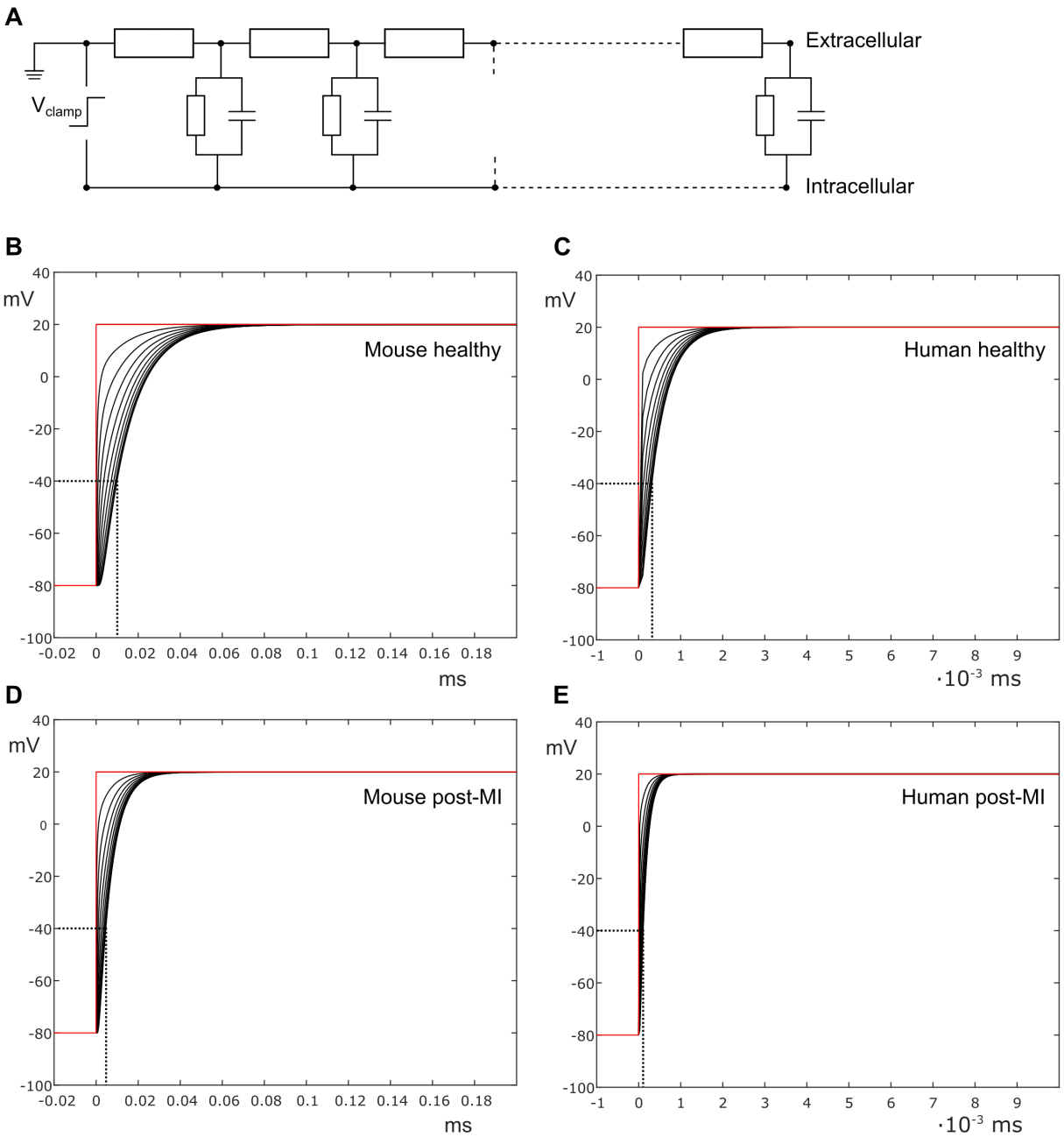

Figure 1. Modeling T-tubule depolarization. **(A)** Schematic representation of the model. The membrane potential at the mouth of the T-tubule is assumed to be perfectly clamped. T-tubule is subdivided in approximately 100-nm-long segments, each consisting of intraluminal resistance, membrane capacitance and membrane resistance. Cytosolic resistance is assumed negligible. Panels **(B-E)** depict simulations of different T-tubules: healthy mouse **(B)**, mouse post-myocardial infarction (MI) **(C)**, healthy human **(D)**, and human post-MI **(E)** T-tubules. Red lines indicate the perfectly clamped mouth of the T-tubule. Note the difference in time scale between mouse and human. See **Table 1** for parameters. The membrane potential of every tenth (**B,D**) and second (**C,E**) node is depicted. Dotted lines indicate the opening threshold for voltage-gated calcium channels (around -40 mV).

When we consider an electrophysiological experiment in which a cardiomyocyte is voltage-clamped, a voltage step at the pipette site will first induce a capacitive current into the cell membrane, quickly followed by depolarization. The capacitive current will travel through the cytoplasm into the T-tubular membrane, but the narrow T-tubules will provide an extracellular "exit resistance" against the capacitive current as it leaves the T-tubule again. The deeper and narrower the T-tubule, the higher the exit resistance, the longer it takes for the T-tubular membrane to depolarize. This delay in membrane depolarization in deep T-tubules directly affects the ion channels in the T-tubule: the deeper in the T-tubule, the later they can open. According to our

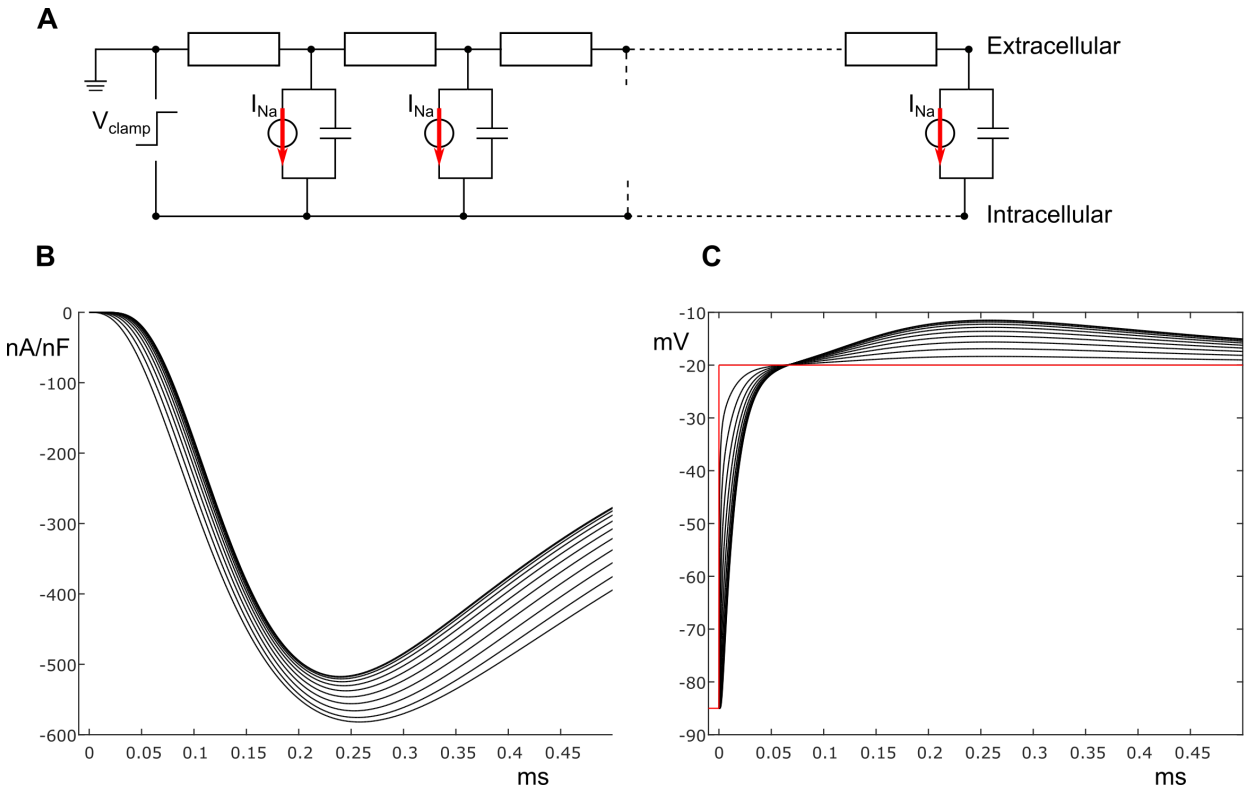

Figure 2. The effect of sodium current on T-tubular potential. **(A)** Schematic representation of the model, based on figure 1A. Here, a voltage-gated sodium current (formulated according to [1, 2]) with a conductance of 23 mS/µF [1] was added to each T-tubular membrane segment, in parallel to the membrane resistance. **(B)** Simulated sodium current upon a voltage clamp step of the tubule mouth from -85 to -20 mV. Note the slightly delayed activation and the reduction of sodium current in the core segments compared to the surface. **(C)** T-tubular membrane potential in the corresponding nodes. Red line indicates the perfectly clamped mouth of the T-tubule (first node). Segments at increasingly deep locations show a larger delay in depolarization and an overshoot of the clamp potential by a few mV due to the positive extracellular potential resulting from the large current flowing along the T-tubule. In panel **(B)** and **(C)**, data for every tenth node are shown.

model, for a typical mouse T-tubule, the threshold of voltage-gated calcium channels (~ -40 mV) in the innermost node of the T-tubule will be reached after ~ 10 µs (**Figure 1B**). For a human T-tubule [6], the membrane depolarization would reach the threshold of the innermost calcium channels much faster than for a murine T-tubule, after ~ 0.3 µs (**Figure 1C**). On a side note, in cardiac disease, T-tubules generally widen [16]. Myocardial infarction (MI) induces an increase in T-tubular diameter of 9% in mice [3], and as much as 33% in humans [5]. Therefore, "exit resistance" of T-tubules decreases and calcium channels deep in the T-tubule open quicker. In a T-tubule from a mouse post-myocardial infarction, the $Ca_v1.2$ opening delay is ~ 7 µs (**Figure 1D**), and in a T-tubule from human post-myocardial infarction, ~ 0.1 µs (**Figure 1E**).

This model also shows that, under the conditions specified in **Table 1**, the voltage drop from the mouth to the end of a healthy murine T-tubule is -4.72 mV for an applied voltage step of 100 mV according to equations derived from classic cable theory [22], starting with

$$\lambda = \sqrt{\frac{r}{2 g_m \rho_e}} \quad \text{(Eq. 1)}$$

where λ is the length constant, r is the T-tubular radius, g_m the conductance of the membrane per unit area, and ρ_e the resistivity of extracellular space. It follows that the length constant for the healthy mouse T-tubule is 186 µm. The second equation to determine the voltage drop for an infinite cable is

$$\Delta V_{inf}(x) = \Delta V_0 \, e^{-\frac{x}{\lambda}}, \quad \text{(Eq. 2)}$$

where ΔV_0 = 100 mV is the voltage applied at the tubule mouth and x is the distance from the tubule mouth. Please note that **Figure 1B** gives a voltage drop much smaller than -4.72 mV, because Eq. 2 applies to infinite cables, whereas our model simulates a sealed cable. For sealed-end cables, by applying the reflection and superposition principle,

a hyperbolic cosine function must be used instead of the exponential [22, 23]:

$$\Delta V_{sealed}(x) = \Delta V_0 \frac{\cosh\left(\frac{L-x}{\lambda}\right)}{\cosh\left(L/\lambda\right)}, \quad \text{(Eq. 3)}$$

It follows that under these conditions the voltage drop is only -0.117 mV. This corresponds with the negligible voltage drop depicted in **Figure 1**. Although the other conditions we modeled show different length constants ($\lambda_{\text{mouse post-MI}}$ = 194 µm; $\lambda_{\text{human healthy}}$ = 227 µm; $\lambda_{\text{human post-MI}}$ = 276 µm), the voltage drops are negligible in all cases (mouse post-MI: ΔV_{inf} = 0.108 mV, ΔV_{sealed} = 0.045 mV; human healthy: ΔV_{inf} = 0.877 mV, ΔV_{sealed} = 0.0039 mV; human post-MI ΔV_{inf} = 0.722 mV, ΔV_{sealed} = 0.0026 mV).

IMPLICATIONS OF T-TUBULAR SODIUM CURRENT

As a next step, we investigated the effect of putative voltage-gated sodium (Na_v) channels on tubular depolarization. **Figure 2B** depicts the sodium current in the different nodes of the healthy mouse T-tubule (see **Table 1** for parameters), and shows that sodium current is smaller deep inside the T-tubule than at the mouth. This smaller current is due to the appearance of a negative extracellular potential in the T-tubule, which results from the flow of current along the narrow tubule. This negative extracellular potential contributes to depolarize the membrane a few mV beyond -20 mV, as shown in **Figure 2C**. The extracellular potential becomes a few mV negative at deep T-tubular nodes, where it brings the transmembrane potential closer to the Nernst potential of sodium, leading to diminished sodium current. Thus, the sodium current is slightly self-attenuating.

DISCUSSION

The results of our computational model show that the delay of membrane depolarization of deep T-tubular segments is small (~ 10 µs for a murine T-tubule), and the sodium current in these deep segments self-attenuate due to negative extracellular potentials.

Consequently, on a cellular level, calcium channels in the deepest part of a T-tubule attain the activation threshold within a very short time after the cell surface is excited when the "exit resistance" of capacitive current is taken into account. The delay of depolarization of mouse and human T-tubules is sufficiently short to ensure excitation-contraction coupling, considering that conduction time along one 100-µm-long cardiomyocyte would be 100 µs [24]. Human T-tubules depolarize considerably quickly because they are relatively wide and short. On a cross-section of a cardiomyocyte, human T- tubules look like spokes on a wheel, and do not form intricate networks like in murine cells [5]. The depolarization delay in human T-tubules is therefore negligible in this model. When adapting our model to simulate remodeled T-tubules post-myocardial infarction, the activation threshold of calcium channels is reached even faster due to the loss of microfolds [2] and increase of luminal diameter [1, 3]. The disturbed calcium cycling associated with disease should therefore have other causes, such as dyad uncoupling [25]. The T-tubular widening might however reduce the relative depletion of calcium and accumulation of potassium in the restricted T-tubular lumen, affecting the driving force of their respective ion channels [8].

It must be noted however that this model does not include constrictions and dilatations of the T-tubular lumen [7, 18], which probably delay calcium channel opening further. The lack of dilatations and constrictions also explains why our model gives a negligible voltage drop of $-3.6 \cdot 10^{-5}$% from mouth to deep T-tubular node. This comparable to previously reported results: for a rat T-tubule of 6.84 µm [26], Scardigli *et al.* calculated a length constant of 290 ± 90 µm and a voltage drop from the surface sarcolemma to the core of ~ -4 mV [27]. Scardigli *et al.* however apply an equation for an infinite cable; applying Eq. 3 for a sealed cable gives a voltage drop of -0.028 mV. A slightly larger but still minuscule value is found for the considerably smaller length constant of 68 µm defined by Uchida *et al.* in a complicated model of branched T-tubules with constrictions [7]. When assuming a murine T-tubule of 9 µm, the voltage drop would be 13 mV for an infinite cable and 0.870 mV for a sealed-end cable and is therefore still negligible. However, care must be taken when interpreting these values, since classic linear cable theory cannot be applied straightforwardly to morphologically heterogeneous T-tubules.

When inserting sodium channels in our computational model, we found that the sodium current would self-attenuate deep in the T-tubules, and the extracellular potential would be a few mV negative (**Figure 2**). This can be explained by the very small T-tubule diameter. At the cardiac

intercalated disc, where two opposing membranes are also very close together, a similar effect has been predicted [28-30]. Importantly, sodium depletion in the T-tubule caused by sodium entering the cell may accelerate the self-attenuation of the sodium current because this depletion will decrease the Nernst potential of sodium. This self-attenuating effect of the sodium current will however not affect calcium current as the effect dies out before the calcium channels open.

In summary, this study presents a relatively simple yet valuable model to assess electrical properties of T-tubules.

ACKNOWLEDGMENTS

The authors express their gratitude to Dr. Jean-Sébastien Rougier for thorough feedback on the manuscript.

AUTHOR CONTRIBUTIONS

Sarah Vermij: conceptualization, methodology, visualization, writing- original draft preparation, reviewing and editing.

Hugues Abriel: supervision, writing- reviewing and editing.

Jan Kucera: supervision, methodology, software, formal analysis, visualization, writing- reviewing and editing.

FINANCIAL STATEMENT

This work was supported by the Swiss National Science Foundation [grant no. 310030_165741; HA group].

SUPPLEMENTARY MATERIAL

The source codes used to generate the data in this manuscript can be found on the BioRXiv.org page for this manuscript under **Supplemental material**.

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