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USE OF QUANTITATIVE MODEL TO ASSESS THE FRACTIONAL AREA OF T-TUBULAR MEMBRANE IN VENTRICULAR CARDIOMYOCYTE

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Summary: *The transverse (T-) tubules of cardiac ventricular myocytes create a complex network of membrane invaginations. Many of the key proteins involved in excitation-contraction coupling appear to be located predominantly at the T-tubule membrane. Despite their importance, the fraction of cell membrane within the T-tubules remains unclear: for example, measurement of cell capacitance following detubulation in rat cardiomyocytes suggests ~32%, whereas optical measurements suggest up to ~65%. We have therefore investigated the factors that may account for this discrepancy. Calculation of the combinations of T-tubule radius, length and density that produce T-tubular membrane fractions of 32% or 56% suggest that the true fraction is at the upper end of this range.*

1. Introduction

Recent work using rat ventricular myocytes has shown that uncoupling the T-tubules physically and functionally from the surface membrane (detubulation) results in ~32% loss of cell capacitance (Despa et al., 2003), which suggest that ~32% of membrane is present within T-tubules. However, microscopic examinations of rat T-system structure (Soeller & Cannell, 1999) combined with measurement of capacity/volume ratio (Satoh et al., 1996) suggest ~56% of membrane to be present within T-tubules. To clarify this discrepancy and to provide a more reliable estimate of this percentage we explored possible combinations of tubular membrane parameters in our model of rat ventricular myocytes (Pásek et al., 2006).

2. Materials and methods

We determined the combinations of T-tubule radius, length and density that produce fraction of tubular membrane (F_t) of 32% and 56%. Than, we determined which of these two fractions gave combinations of above variables that were closest to those observed experimentally. To do this, the T-tubule length (l_t) was calculated as:

$$l_t = F_t / ((100-F_t)\cdot 2\cdot\pi\cdot r_t\cdot dens_t) \qquad (1)$$

where r_t is T-tubule radius, and $dens_t$ is T-tubule density, expressed as the number of T-tubule openings per unit area of surface membrane.

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3. Results

The coloured graphs in Fig. 1 show the results of the calculation of l_t over the range of T-tubule radii and densities reported experimentally. While the surface corresponding to fractional area of tubular membrane F_t = 56% (A) contains tubular lengths that comply with published data (~6.9 µm; Soeller and Cannell, 1999), the fractional area of 32% (B) produces lengths <4 µm, which is less than half the diameter of a cardiac myocyte and shorter than observed experimentally. These results suggest that, in rat ventricular cardiomyocytes, a fractional area of 56% is closer to the actual value than 32%.

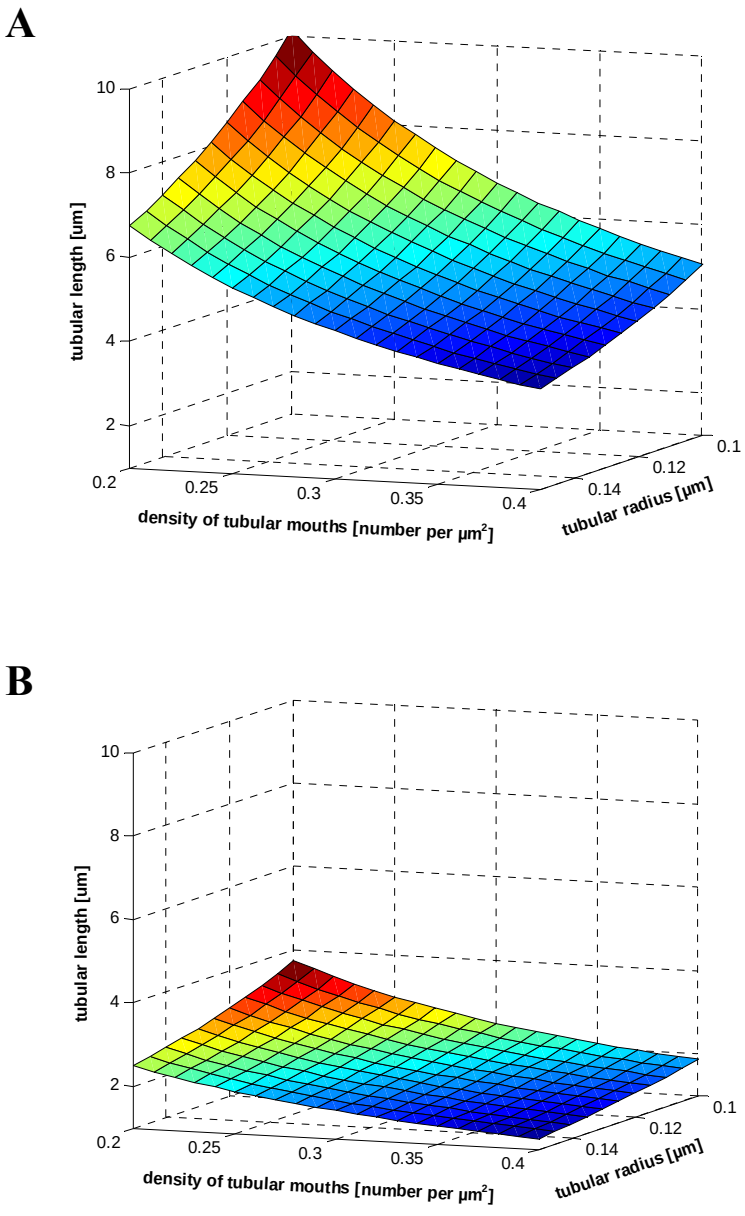

Figure 1. Relationships among T-tubule length, radius and density of tubular openings, calculated from equation (1) for F_t = 56% (A) and F_t = 32% (B). The blue, green and red colours at the surfaces indicate, respectively, the lowest, middle and highest values of tubular lengths.

4. Discussion

The results presented in Fig. 1 indicate that a grater fraction of cardiac cell membrane may be located in tubular system. This raises a question of why the estimates of this parameter are different using the two techniques. One possibility is that the optical dye-filling technique overestimates the fraction of membrane in the T-tubules, for example because it does not take into account folding of the surface membrane. Alternatively, detubulation may underestimate the fraction of cell membrane within the T-tubules, either because some T-tubules remain coupled to the surface membrane or because the specific capacitance of the T-tubule membrane is lower than that of the surface membrane, so that a greater fraction of membrane is lost than capacitance.

To explore this suggestions quantitatively, we used our rat model (Pásek *et al.*, 2006) and determined the loss of membrane capacitance that would be observed upon detubulation if 8% of the T-tubules remained functionally coupled to the surface membrane (found in experiments by Clive Orchard's group in Bristol). As shown in Fig. 2, reducing specific capacitance of tubular membrane ($C_{sp,t}$) from 1 to 0.56 µF/cm^2 (a maximum reduction observed on increasing cholesterol content in artificial membrane) still results in a greater loss of total membrane capacitance (~38%, blue line) than that observed experimentally (32%). However, if the percentage of membrane in the T-tubules is reduced to F_t = 49% (red line) then 32% of membrane capacitance is lost following detubulation. This suggests that real value of rat F_t is close to 49% and thus that optical measurements overestimate the percentage of the cell membrane in the T-tubules while the capacitance measurements underestimate it.

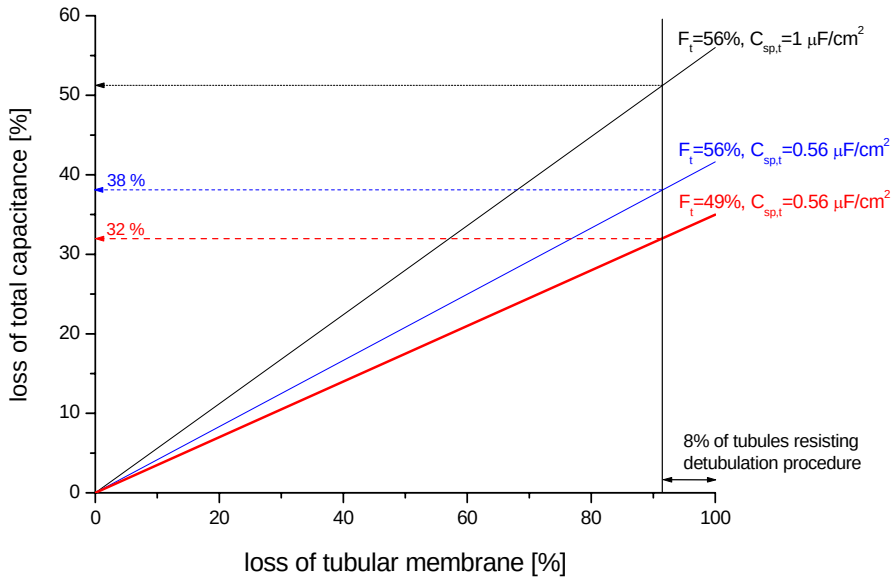

Figure 2. Relation between loss of tubular membrane and loss of total capacitance in the model with F_t = 56% (black and blue full lines) and F_t = 49% (red full line). The values of F_t and $C_{sp,t}$ are indicated in the upper end of the lines.

5. Acknowledgements

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6. References

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