Uncovering an electrically heterogeneous cardiomyocyte by FRAP-quantified diffusion in the T-tubules

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A recent elegant study in PNAS by Scardigli et al. (1) provides a subcellular look at the electrical properties within the cardiomyocyte transverse T-tubular system [transverse-axial tubular system (TATS)] by extrapolation from FRAP-based diffusion measurements of fluorescent dextran and geometric considerations. It was shown that the disarray of the T-tubules in heart failure decreases the electrical conductivity of the extracellular space within the TATS by a factor of 2 and can lead to a spatially heterogeneous action potential response within a cell.

Current understanding and computational modeling of cardiac tissue, in general, preclude spatially heterogeneous action potential responses within a myocyte (e.g., failure to produce an action potential only in some parts of the cell) due to the high "diffusion of voltage" constant, (D_v = 0.3–1 $cm^2 \cdot s^{-1}$) as per cable theory (2, 3). In contrast, calcium dynamics can be quite complex within a cell due to the five–order-of-magnitude lower diffusion of calcium, D_{Ca}^{2+} (4). Therefore, this new study raises fundamental questions about the mechanism of cardiac excitation.

Using the results for diffusion of 3-kDa dextran in the TATS (1) (6e-8 $cm^2 \cdot s^{-1}$) and considering the size of the hydrated sodium and calcium ions in conjunction with the Stokes molecular radius relationship, one obtains diffusion of sodium (D_{Na}^{+} = 2.2e-7 $cm^2 \cdot s^{-1}$) and D_{Ca}^{2+} = 1.5e-7 $cm^2 \cdot s^{-1}$ in the TATS. These FRAP-derived diffusion values are almost three orders of magnitude lower than reported averaged macroscale diffusion of relevant ions within muscle (5); about two orders of magnitude lower than gapFRAP-extracted values for diffusion between myocytes across gap junctions, which involves crossing the plasma membrane; and about fourfold lower than diffusion of calcium in another geometrically complex structure, the sarcoplasmic reticulum (D_{Ca}^{2+} = 6e-7 $cm^2 \cdot s^{-1}$) (6). Such low-diffusivity extracellular space compartments within the cardiomyocytes, as found in this new study, will indeed yield spatial heterogeneity within a single cell. However, the T-tubules, unlike the nucleus, were not implicated as low-diffusivity barriers to intracellular calcium waves (7), perhaps because of the interplay of diffusion and richness of calcium release sites.

Using reciprocal relationships of molecular diffusion coefficients and the TATS geometry, Scardigli et al. (1) estimate the TATS electrical conductivity as 5.3e-4 S/cm. It is interesting to point out that this is an order of magnitude lower value than what is currently assumed for extracellular conductivity in mathematical models of cardiac tissue (2–3e-3 S/cm). In fact, the reported extracellular TATS conductivity is on par with currently used intracellular conductivity measurements for cardiac tissue by Clerc (8) (0.2–17e-4 S/cm). Such results, indicating a more resistive extracellular space (in TATS) than intracellular space (which includes the gap junctions), are surprising in terms of current flow during cardiomyocyte excitation as per the bidomain model of cardiac tissue, and suggest a diffusion-limited operation in the TATS. Alternatively, it is possible that at this subcellular scale, the limits of optical measurements (diffusion of the voltage-sensitive dye) confounded the results. Either way, this is a thought-provoking study, with significant implications for cardiac arrhythmia mechanisms through spatial heterogeneity and for the basic response of cardiac tissue to electric fields (e.g., pacing, defibrillation), namely, suggesting new sources of "virtual electrodes" within each myocyte (3, 9).

1 Scardigli M, et al. (2017) Quantitative assessment of passive electrical properties of the cardiac T-tubular system by FRAP microscopy. *Proc Natl Acad Sci USA* 114:5737–5742.

2 Winfree A (1997) Heart muscle as a reaction-diffusion medium: The roles of electric potential diffusion, activation front curvature, and anisotropy. *Int J Bifurcat Chaos* 7:487–526.

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3 Roth BJ (1994) Mechanisms for electrical stimulation of excitable tissue. *Crit Rev Biomed Eng* 22:253–305.
4 Fabiato A (1992) Two kinds of calcium-induced release of calcium from the sarcoplasmic reticulum of skinned cardiac cells. *Adv Exp Med Biol* 311:245–262.
5 Kushmerick MJ, Podolsky RJ (1969) Ionic mobility in muscle cells. *Science* 166:1297–1298.
6 Wu X, Bers DM (2006) Sarcoplasmic reticulum and nuclear envelope are one highly interconnected Ca2+ store throughout cardiac myocyte. *Circ Res* 99:283–291.
7 Ishida H, Genka C, Hirota Y, Nakazawa H, Barry WH (1999) Formation of planar and spiral Ca2+ waves in isolated cardiac myocytes. *Biophys J* 77:2114–2122.
8 Clerc L (1976) Directional differences of impulse spread in trabecular muscle from mammalian heart. *J Physiol* 255:335–346.
9 Sobie EA, Susil RC, Tung L (1997) A generalized activating function for predicting virtual electrodes in cardiac tissue. *Biophys J* 73:1410–1423.