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STORM and TEM Identify the Cardiac Ephapse: An Intercalated Disk Nanodomain with Previously Unanticipated Functions in Cardiac Conduction

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Electrical coupling between cells across a narrow extracellular cleft, termed ephaptic coupling, is known to occur in the brain and has been hypothesized to occur in the heart as well. Computer models of cardiac conduction suggest that this would be feasible provided sodium channel ($Na_v1.5$) -rich membranes of adjacent myocytes were closely apposed (<30 nm) [1-3]. In recent studies, we have used transmission electron microscopy (TEM) and gSTED super-resolution imaging that the perinexus, an intercalated disk (ID) nanodomain surrounding connexin43 (Cx43) gap junctions (GJ), possesses both characteristics. [4,5] Further, experimental disruption of close apposition between perinexal membranes slowed conduction and precipitated arrhythmias. Therefore, we investigated the localization and functions of $Na_V1.5$ and $Na_V\beta1$ (a sodium channel auxiliary subunit and cell adhesion molecule [6]) within different ID nanodomains.

Super-resolution STochastic Optical Reconstruction Microscopy-based Relative Localization Analysis (STORM-RLA) [7] and immuno-electron microscopy identified two populations of $Na_V1.5$ within the ID in guinea pig ventricles (GPVs): One, localized to the perinexus, accounted for 47% of ID-localized $Na_V1.5$ and the other, which co-distributed with N-Cadherin, accounted for 29% of ID-localized $Na_V1.5$. $Na_V\beta1$ was preferentially localized to the perinexus (48% of ID-localized $Na_V\beta1$) over N-Cadherin-rich plicate regions (8%). βadp1, a novel peptide inhibitor of $Na_V\beta1$ adhesion, inhibited the barrier function in $Na_V\beta1$-overexpressing 1610 cells but not in native 1610 cells in electric cell-substrate impedance spectroscopy studies. However, neither βadp1 nor a scrambled control peptide (βadp1-scr) affected I_{Na} or action potentials in isolated GPV myocytes. βadp1 (100 μM) compromised the diffusion-resistance of the ID as assessed by perfusion of fixable fluorescent dyes in GPVs. βadp1 (48 ± 4 μm) but not βadp1-scr (22 ± 1 μm) increased perinexal intermembrane spacing compared to control GPVs (17±1μm). In optical mapping studies, βadp1 but not βadp1-scr preferentially decreased transverse conduction velocity, increased anisotropy and precipitated spontaneous tachyarrhythmias in a dose-dependent manner.

We provide the first report that ID-localized $Na_V1.5$ may segregate into a Cx43-adjacent perinexal pool and a plicate pool that co-distributes with N-Cadherin. Further, we demonstrate that $Na_V\beta1$ may preferentially co-distribute with the perinexal $Na_V1.5$ pool. Importantly, we demonstrate that the perinexus likely functions as the cardiac ephapse with $Na_V\beta1$-mediated adhesion as a critical and dynamic modulator of close apposition between $Na_V1.5$-rich perinexal membranes and thereby, ephaptic conduction.

References:

[1] Kucera JP, Rohr S, & Rudy Y (2002) Circulation research **91(12)**:1176-1182.
[2] Lin J & Keener JP (2013) IEEE transactions on bio-medical engineering **60(2)**:576-582.
[3] Mori Y, Fishman GI, & Peskin CS (2008) Proceedings of the National Academy of Sciences of the United States of America **105(17)**:6463-6468.
[4] Veeraraghavan R, *et al* (2015) Pflugers Arch. **467(10)**:2093-105.
[5] Veeraraghavan R, *et al* (2016) Pflugers Arch **468(10)**:1651-1661.
[6] Isom LL (2001) Neuroscientist **7(1)**:42-54.
[7] Veeraraghavan R & Gourdie R (2016) Molecular biology of the cell. **27(22)**:3583-3590.

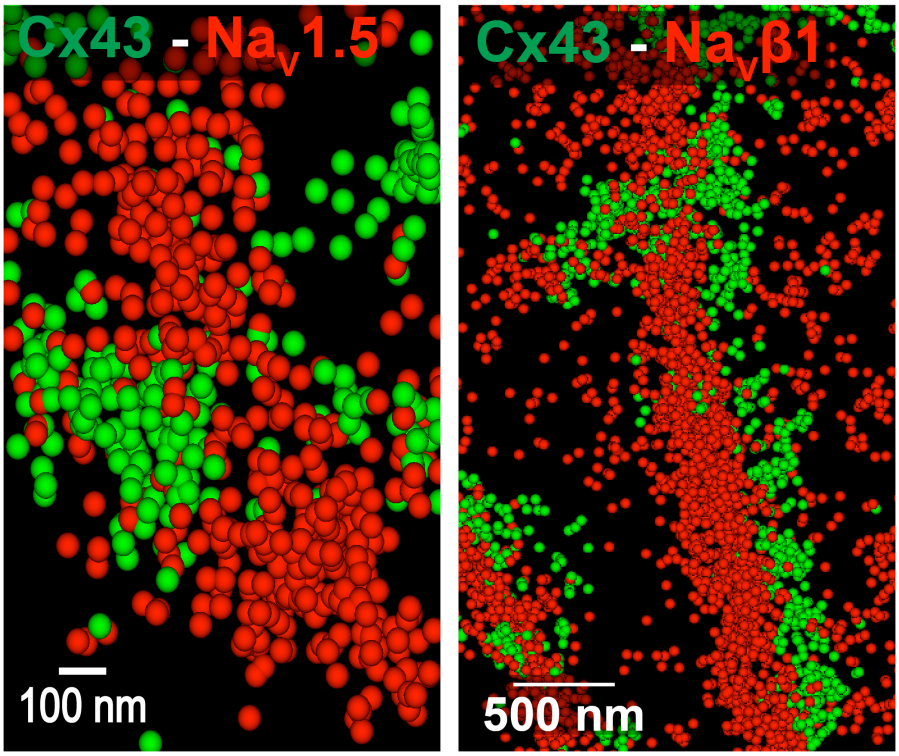

Figure 1. Representative STORM data showing a clusters of fluorophores corresponding to Cx43 (green) in close apposition to $Na_v1.5$ (red; left) and $Na_V\beta1$ (red; right). Each sphere represents an individual fluorophore molecule localized.

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