1432
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Quantitative Assessment of Cardiac Intercalated Disk Ultrastructure and Molecular Organization by Indirect Correlative Light and Electron Microscopy

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Purpose of Study: During each heartbeat ~12 billion muscle cells (cardiomyocytes) must contract in perfect synchrony. This is achieved through electrical signals, which spread through the heart, triggering cardiomyocytes to contract in sequence. At the cellular scale, cell-to-cell propagation of these signals occurs at the intercalated disk (ID) – a specialized structure that affords electrical (gap junctions) and mechanical (adherens junctions, desmosomes) coupling between cardiomyocytes. Previous research suggests that cardiac sodium channels ($Na_V1.5$), the primary source of excitatory current in the heart, are enriched within ID nanodomains, where they may play a key role in cell-to-cell electrical communication[1,2]. For well over half a century, the ID has been recognized as a complex three dimensional structure comprised of distinct, heterogeneous niches[3]. Yet, current computational models of cardiac conduction do not include the ID or at best, grossly oversimplify it to a uniform flat circle with homogeneous properties. Little experimental data is available to enable realistic *in silico* representation of the ID and its ion channel components. We are using indirect correlative light and electron microscopy (*i*CLEM) to quantitatively assess the ultrastructure (TEM) and molecular organization (STORM) of $Na_V1.5$-rich ID nanodomains under baseline conditions and following acute (60 minutes) structural perturbation using adhesion inhibitor peptides that selectively disrupt different ID nanodomains.

Research Methods: Murine hearts were isolated and stained for ultrastructure (TEM) and molecular organization (STORM). TEM images of the ID, particularly gap junctions and mechanical junctions, were obtained at 6,000x, 10,000x, and 20,000x magnification on a FEI Technai G2 Spirit transmission electron microscope. Morphometric quantification was performed using ImageJ. Images at 6,000x magnification were used to quantify: the total ID cross-sectional length and tortuosity. Images at 10,000x magnification were used to quantify: length of plicate and interplicate regions, amplitude and frequency of plicae, and lengths of gap junctions and mechanical junctions in plicate and interplicate domains of the ID. Images at 20,000x magnification were used to quantify inter-membrane distance inside and outside mechanical junctions and outside gap junctions (within gap junctions, the apposed membranes are only a few angstroms apart). Stochastic optical reconstruction microscopy (STORM), a single molecule localization technique, was used to assess the spatial organization of landmark structural proteins - connexin43 for gap junctions, N-cadherin for adherens junctions, and desmoglein for desmosomes – as well as populations of key electrogenic proteins – specifically, the cardiac sodium channel 1.5 ($Na_V1.5$), the inward-rectifier potassium channel ($K_{ir}2.1$), and the sodium potassium ATPase (NKA) – in relation to the landmark structural proteins. Spatial protein organization was quantified by STORM-based relative localization analysis (STORM-RLA). In addition to studies in unperturbed murine hearts, similar assessments were performed in hearts treated with peptide mimetics of adhesion domains to selectively inhibit adhesion within different ID nanodomains: 1) Nadp1 (target: N-cadherin), 2) dadp1 (target: Desmoglein-2), and 3) βadp1 (target: sodium channel β1 subunit, SCN1b). The effects of each active peptide were compared against a corresponding inactive control peptide (Nadp1-c, dadp1-c, βadp1-scr). The functional consequences of perturbing ID structure were assessed by optical mapping (electrical signal conduction velocity) and electrocardiography (arrhythmia vulnerability).

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Findings: Ultrastructure measurements revealed the following – total ID length: 11.59 µm; length of plicate domains:1.63 µm; length of inter-plicate domains: 1.64 µM; plicae amplitude: 0.17 µm; plicae frequency: 2.65 waves/µm; gap junction length in plicate domains: 0.27 µm and in inter-plicate domains: 0.57 um; mechanical junction length in plicate domains: 0.33 µm and in inter-plicate domains: 0.14 µm; inter-membrane width – outside gap junctions: 14.08 nm, outside mechanical junctions: 19.61 nm, inside mechanical junctions: 16.55 nm. Preliminary STORM-RLA results indicate that ~50% of ID-localized $Na_V1.5$ are located within gap junction-adjacent perinexi with another ~35% being associated with mechanical junctions. Disrupting adhesion within ID nanodomains locally increases intermembrane spacing (electron microscopy), and leads to dynamic (within 30-60 minutes) dispersal of $Na_V1.5$ clusters (STORM). In contrast, the Kir2.1 clusters were evenly distributed between gap junction and mechanical junction -associated ID nanodomains and did not undergo redistribution following adhesion inhibitor treatments. Junctional protein distributions were not affected by the peptide inhibitors. Functionally, these effects correlate with conduction slowing and increased arrhythmia vulnerability. Furthermore, the magnitude of functional impacts are likely determined by the amount of sodium channels contained within the nanodomains disrupted.

Implications: We use these experimentally-derived data to generate realistic finite-element meshes of ID nanodomain structure and probe their functional roles via computational modeling of cardiac electrical signaling. This approach could reveal previously unanticipated mechanisms of electrical signaling at ID nanodomains and uncover local control of the heartbeat at the nanoscale.

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