DEFECTS OF T-TUBULAR ELECTRICAL ACTIVITY UNDERLIE LOCAL ALTERATIONS OF Ca\(^{2+}\) RELEASE IN HEART FAILURE

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ABSTRACT

Action potentials (APs), via the transverse axial tubular system (TATS), synchronously trigger uniform Ca\(^{2+}\) release throughout the cardiomyocyte. In heart failure (HF), TATS structural remodeling occurs, leading to asynchronous Ca\(^{2+}\) release across the myocyte and contributing to contractile dysfunction. In cardiomyocytes from failing rat hearts, we previously documented the presence of TATS elements which failed to propagate AP and displayed spontaneous electrical activity; the consequence for Ca\(^{2+}\) release remained, however, unsolved. Here, we develop an imaging method to simultaneously assess TATS electrical activity and local Ca\(^{2+}\) release. In HF cardiomyocytes, where T-tubules fail to conduct AP, show a slower and reduced local Ca\(^{2+}\) transient compared with regions with electrically coupled elements. It is concluded that TATS electrical remodeling is a major determinant of altered kinetics, amplitude, and homogeneity of Ca\(^{2+}\) release in HF. Moreover, spontaneous depolarization events occurring in failing T-tubules can trigger local Ca\(^{2+}\) release, resulting in Ca\(^{2+}\) sparks. The occurrence of tubulodepolarization and Ca\(^{2+}\) sparks may contribute to the arrhythmic burden in heart failure.

THE RANDOM ACCESS MICROSCOPE

A high power femtosecond pulsed fiber laser at 1064 nm provides the excitation light (TP1560-24, Florida). ~300-nm pulse width, repetition rate 80 MHz, wavelength 1050 nm. The laser beam is focused for optimal linear polarization via a half-wave (A/2) plate polarized after the AOM to optimize the diffraction efficiency of the two orthogonally mounted AOMs (AOD-x and AOD-y). The light is focused onto the specimen by the objective lens. The isotropic two-photon fluorescence (TPF) signal is collected in forward direction by an immersion objective equipped with band pass filters for each channel. The TPF signal is discriminated by using dichroic mirror beamsplitters at 560 nm, whereas the voltage-sensitive dye (light grey) together with the corresponding membrane potential of 47 aligned and averaged CTRL Ca\(^{2+}\) cytes. The blue arrows highlight spontaneous Ca\(^{2+}\) sparks (green) recorded at the sites indicated in panel (A). Voltage-associated polarization events (black arrowheads). Voltage in magenta and [Ca\(^{2+}\)]\textsubscript{i} in green. The grey dashed line indicates the Ca\(^{2+}\) release time-to-peak measured nearby SS. (C) Graph showing mean value for Ca\(^{2+}\) transient time-to-peak (TTP) and 50% of Ca\(^{2+}\) decay (CaT50) nearby SS and TT. The falling TT\(_{AP}\) (A) have been distanced from the electrophysiological spontaneous sparks (B, C). White lines represent the Ca\(^{2+}\) kinetics features measured nearby SS and TT (solid) or SS (dashed). Student’s t-test p=0.001. Ochre asterisks refer to the comparison with CTRL values. Data from SS/TL 24 AP+ TT, and 23 HF+ TT (n=51 cells).

STOCHASTIC NATURE OF Ca\(^{2+}\) RELEASE

DELAY OF Ca\(^{2+}\) RELEASE IN AP FAILING TT OF ACUTE DETUBULATED CELLS

DELAY OF Ca\(^{2+}\) RELEASE IN AP FAILING TT OF HEART FAILURE FAILURE

VOLTAGE-ASSOCIATED Ca\(^{2+}\) SPARKS (V-SPARKS)

(A) Fluorescence traces (CIF\(_{2}\)) from failing TT of HF cardiomyocytes and isoproterenol-treated HF cells (HF+ISD) displaying spontaneous electrical activity. Electrical trigger at 200 ms (black arrowheads). Voltage in magenta and [Ca\(^{2+}\)]\textsubscript{i} in green. (B) Frequency of spontaneous depolarization events that are associated with a correspondent local Ca\(^{2+}\) spark (V-sparks). Bars mean ± standard error (SE). (C) Percentage of spontaneous depolarization events that are associated with a correspondent local Ca\(^{2+}\) spark (V-sparks). Bars mean ± standard error (SE). (D) Representative fluorescence traces (CIF\(_{2}\)) from failing TT of HF cardiomyocytes and isoproterenol-treated HF cells (HF+ISD) displaying spontaneous electrical activity. Electrical trigger at 200 ms (black arrowheads). Voltage in magenta and [Ca\(^{2+}\)]\textsubscript{i} in green. (A) Time traces of Ca\(^{2+}\) transient (TTP) and 50% of Ca\(^{2+}\) decay (CaT50) nearby SS and TT. The falling TT\(_{AP}\) (A) have been distanced from the electrophysiological spontaneous sparks (B, C). White lines represent the Ca\(^{2+}\) kinetics features measured nearby SS and TT (solid) or SS (dashed). Student’s t-test p=0.001. Ochre asterisks refer to the comparison with CTRL values. Data from SS/TL 24 AP+ TT, and 23 HF+ TT (n=51 cells).

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