response enhances mitochondrial metabolism triggered mitochondrial hyperpolarization and enhanced ATP production. Li⁺ a substrate cation of NCX but not of NCX replaces Na⁺ in enhancing the cytosolic and mitochondrial Ca²⁺ responses. Altogether, our results show that combined electrical and ion flux activity of TTX sensitive Na⁺ channels initiates a cytosolic Na⁺ and Ca²⁺ signals propagating by the MCU and NCLX to the mitochondria, thereby shaping cytosolic or mitochondrial Ca²⁺ transients and metabolism of beta cells.

1225-Plat The Potential for Another Calcium Uptake Mode in Cardiac Mitochondria

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Ultrafast Genetically Encoded Calcium Indicators for Visualizing Calcium Signaling Inside Cilia Upon Mechanical Bending

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The contractile force of cardiomyocytes is controlled by Ca²⁺ across-signaling between L-type Ca²⁺ channels and ryanodine receptors (RyR2) across the narrow dyadic cleft. To detect the functional Ca²⁺ signal, we designed a peptide probe (Calstabi-Cam) with calmodulin as its Ca²⁺ sensor, yellow fluorescent protein (EYFP) as reporting fluorophor, and FKBP12.6 (calstabin2) as subunit of RyR2. Effective adenoviral expression in cultured adult rat cardiomyocytes was verified after 48 hours when Calstabi-Cam co-localized with fluorescent RyR antibodies in a sarcomeric z-lines pattern. Dissociation constant (Kd) of Calstabi-Cam for Ca²⁺ measured in permeabilized myocytes was 80 nM. The kinetic of Ca²⁺ signals was measured in voltage-clamped cells with a Leica TIRF microscope which allowed comparison of rapidly interlaced images of cytosolic Ca²⁺ probes (fura-2 or fluo-4) and Calstabi-Cam. Fluo-4 Ca²⁺ sparks were detected superimposed on the sarcomeric fluorescence patterns of Calstabi-Cam. On activation of Ca²⁺ release by caffeine or membrane depolarization, Calstabi-Cam fluorescence signals had slower rise times compared to fura-2, but had much slower decay kinetics. Scans of focal Calstabi-Cam signals at different sites, occurring spontaneously or at the onset of evoked Ca²⁺ releases, appeared to have a significant distribution of magnitudes and latencies. We conclude that Ca²⁺-sensing biological peptides may be targeted to the cleft-space occupied by DHPR/RyR complexes as to make it possible to record the variance of Ca²⁺ signals at different dyadic junctions.

1229-Plat CaMKII-Mediated Amplification is Essential to NAADP Signalling in Cardiac Myocytes

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NAADP is a highly potent endogenous Ca²⁺-mobilising second-messenger forming part of the beta-adrenergic response in cardiac myocytes. Our previous studies using NAADP photorelease in cardiac myocytes revealed that NAADP-elicited Ca²⁺ release is Ca²⁺ dependent, occurring upon or at the onset of evoked Ca²⁺ releases. This observation was supported by the demonstration that NAADP-elicited Ca²⁺ release was abolished in TPC2KO mice. This study aimed to test the hypothesis that NAADP-elicited Ca²⁺ release is dependent on TPC2 channels. To test this hypothesis, we used a genetically encoded calcium indicator targeted to primary cardiac myocytes. This study demonstrated that NAADP-elicited Ca²⁺ release was abolished in TPC2KO mice. This study aimed to test the hypothesis that NAADP-elicited Ca²⁺ release is dependent on TPC2 channels. To test this hypothesis, we used a genetically encoded calcium indicator targeted to primary cardiac myocytes.

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