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MEETING ABSTRACT

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Privileged ER Ca^{2+} refilling in vascular endothelial cells: evidence for a role of the Na^+/Ca^{2+} exchanger (NCX)

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Background: The endoplasmic reticulum (ER) is an organelle involved in the majority of cellular processes such as lipid synthesis, protein synthesis and folding, and post-translation modification. The ER is also the main intracellular Ca^{2+} store. Ample experimental evidence suggests that there is a relation between Ca^{2+} signals and the above-mentioned processes. Under ER stress conditions, misfolded proteins accumulate in the ER; this, in turn, leads to Ca^{2+} leakage from the ER and, in general, to an alteration in the healthy Ca^{2+} transport to and from the ER. Deterioration of the ER function, as happens during ER stress, appears linked to several diseases such as neurodegenerative disorders (Parkinson's, Alzheimer's), bipolar disorders and diabetes. Since changes in Ca^{2+} in the ER can affect the quantity and the efficiency of protein folding, it is important to understand the mechanism of ER Ca^{2+} refilling. Na^+/Ca^{2+} exchangers (NCX), Ca^{2+} ATPases (SERCA), inositol trisphosphate receptors (IP_3R) and ryanodine receptors (RyR) regulate Ca^{2+} movement into and out of the ER, including to and from the extracellular space. We investigate the role of NCX in the transport of Ca^{2+} in endothelial cells under various conditions of cell stimulation and membrane polarization on the heels of previous findings showing that in vascular smooth muscle cells the NCX plays a critical role in the refilling of the SR with extracellular Ca^{2+}.

Methods: We employed Fura-2 AM as a ratiometric cytoplasmic Ca^{2+} indicator and D1ER cameleons as luminal ER Ca^{2+} indicators to image Ca^{2+} signals in our cell system by standard fluorescence microscopy. We also measured the membrane potential by whole-cell patch and micro-electrode methods.

Results: Our findings point to an involvement of the NCX Ca^{2+} influx mode in the refilling of ER. The data suggest a significant contribution of NCX reverse-mode operation in addition to, or in conjunction with, store-operated Ca^{2+} entry via STIM-Orai in a process of privileged refilling of the ER at small or negligible changes in global cytosolic Ca^{2+}. We propose that this process occurs in plasma membrane (PM)–ER junctions. These results are corroborated by a comparison between our own measurements of the membrane potential and calculation of the NCX potential in the experimental condition used during the experiments.

Discussion: Our results provide further elucidation of the mechanism and function of a previously hypothesized subplasmalemmal Ca^{2+} control unit during the refilling of the ER under physiological conditions.

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