Is “Post-Rest Potentiation” (PRP) a SERCA or NCX function index?

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Abstract: Post-rest potentiation (PRP) is a phenomenon whose magnitude depends on the function of the cellular machinery involved in cardiac excitation-contraction coupling. A steady stimulation is followed by a pause (rest) after which stimulation is resumed; Ca^{2+} transients (CaT) are recorded. We define PRP as the post-rest/pre-rest ratio of CaT features. CaT depends on SR Ca^{2+} load and, during the rest, several proteins could come into play to set it; these mainly include Sarco-endoplasmic reticulum (SR) calcium ATPase (SERCA), Ryanodine Receptor (RyR) channels and the Na^{+}/Ca^{2+} exchanger (NCX). SERCA sits in the SR membrane and sequesters Ca^{2+} into this compartment; RyRs release Ca^{2+} from the SR, NCX is a sarcolemmal protein that extrudes Ca^{2+} to the extracellular space in exchange for Na^{+}.

Our aim is to study the relative roles of SERCA and NCX in PRP; this with the purpose of developing PRP into a reporter of SERCA function usable in drug testing. To this end, we plan to study PRP in two different but complementary cellular models: mature rat ventricular cardiomyocytes and cardiomyocytes derived from human-induced pluripotent stem cells (hiPSC-CMs). Intracellular Ca^{2+} will be optically measured by dynamyc recording of the light emitted by a Ca^{2+}-sensitive fluorophore (Fluo4-AM). CaTs will be recorded during electrical stimulation according to the PRP protocol. Several CaT parameters will be evaluated, such as diastolic Ca^{2+} (CaD, mM), CaT amplitude (mM), the maximal velocity of Ca^{2+} rise (dCa/dt, mM/ms), the time to CaT peak (ms), the time constant of Ca^{2+} decay (ms). Measurements will be repeated under conditions suitable to isolate the contributions of SERCA, NCX and RyRs respectively (Thapsigargin will be used as a SERCA antagonists and PST3093 as a SERCA agonist; RyRs will be blocked by Tetracaine, NCX will be inhibited by SEA-0400). PRP of CaT parameters under the various conditions will be compared to assess their sensitivity and specificity in reporting SERCA function. Preliminary experiments indicate that PRP is significantly affected by Thapsigargin.