Synthesis of cADPR and NAADP by intracellular CD38 in heart: role in inotropic and arrhythmogenic effects of beta-adrenoceptor signaling

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Background: Nicotinic acid adenine dinucleotide phosphate (NAADP) and cyclic ADP-ribose (cADPR) are calcium mobilising messengers that are upregulated during the activation of beta-adrenoceptors. They are positive inotropic agents that have roles in modulating excitation-contraction coupling and disease development of the heart. The enzyme responsible for producing these molecules is a potential therapeutic target, but its identity remains unclear. CD38 is an ADP-ribose cyclase that catalyses synthesis of both NAADP and cADPR in various tissues, although this is frequently found on the extracellular surface of the plasmalemma.

Methods: Synthesis of NAADP was detected and quantified using sea urchin egg homogenates, and synthesis of cADPR from NAD was gauged from the conversion of its analogue (NGD) to a fluorescent product (cGDPR). Calcium transients were measured using Fluo-5F and spinning disk confocal microscope and sarcomere shortening was measured using an IonOptix system. A whole heart arrhythmia study was carried out with programmed electrical stimulation and monophasic action potential measurement.

Results: Membrane preparations as well as single cells from CD38⁻/⁻ mouse hearts failed to synthesise NAADP and cADPR. Isolated superfused ventricular myocytes from CD38⁻/⁻ mice showed smaller peak calcium transients and contractions than myocytes from WT mice, both under basal conditions (F/F₀: WT, 3.73 ± 0.45 vs KO, 2.65±0.57; contractions (µm): WT, 0.07 ± 0.01 vs KO, 0.04 ± 0.02) and when stimulated by the 5 nM isoproterenol (F/F₀: WT, 8.11 ± 1.55 vs KO, 5.23 ± 0.35; contractions (µm): WT, 0.17 ± 0.02 vs KO, 0.14 ± 0.05). In isolated WT cardiac myocytes, membrane permeabilisation with Triton X-100 increased NAADP synthesis, suggesting there might be additional intracellular CD38. This is further supported by the observation that permeabilised WT myocytes showed a striated immunostaining pattern (consistent with location of CD38 on sarcoplasmic reticulum) for CD38 while CD38⁻/⁻ myocytes and non-permeabilised WT myocytes showed little or no staining.

Our experiments showed that sarcoplasmic reticulum enriched membrane preparations from sheep hearts showed ability to synthesise NAADP and cADPR, with enzyme properties consistent with a CD38 identity. This synthesis of NAADP and cADPR was blocked by a novel drug, SAN4825. Blind docking experiments showed that SAN4825 binds to the active site of CD38 with possible pi-pi interactions with the Trp 125 and Trp 189. Whole hearts isolated from CD38⁻/⁻ mice as well as hearts perfused with SAN4825, a CD38 inhibitor, showed a reduced tendency to arrhythmias in the presence of isoproterenol (300 nM).

Conclusion: The above observations support the hypothesis that intracellular CD38 generates NAADP and cADPR, thereby enhancing excitation-contraction coupling under basal conditions and contributing towards effects of β-adrenoceptor stimulation (positive inotropy and arrhythmogenecity).