

Redox-mediated PKA-R1 α localisation to the lysosome inhibits myocardial calcium release and robustly reduces myocardial injury

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Background: Kinase oxidation is a critical signalling mechanism through which changes in the intracellular redox state alter cardiac function. In the myocardium, type-1 PKA (PKAR1 α) can be reversibly oxidised, forming interprotein disulfide bonds within the holoenzyme complex. However, the effect of PKAR1 α oxidation on downstream signalling in the heart, particularly under states of oxidative stress, remains unexplored.

Purpose: To determine the direct functional consequences of PKAR1 α oxidation in the heart and investigate their impact on ischaemia/reperfusion (I/R) injury.

Methods & Results: Experiments using the AKAR3ev FRET biosensor in murine left ventricular (LV) myocytes and Fluorescence Recovery After Photobleaching (FRAP) of GFP-tagged WT and mutant R1 α proteins in R1 α -null fibroblasts showed that PKAR1 α oxidation does not increase the kinases's catalytic activity, but enhances its binding to A-kinase anchoring proteins (AKAP; n=30-39/N=3, p<0.01). Super-resolution microscopy revealed localisation of oxidised PKAR1 α to lysosomes in WT myocytes, which was completely absent in "redox dead" Cys17Ser PKAR1 α knock-in mice (KI; *panel A*; n=38-41/N=3, p<0.01) and reduced when AKAP binding was prevented using the RIAD disruptor peptide (30.6 \pm 5.1% reduction; n=35-37/N=3, p<0.01).

Displacement of PKAR1 α from lysosomes resulted in spontaneous sarcoplasmic reticulum Ca²⁺ release and dramatic Ca²⁺ oscillations in KI LV myocytes (*panel B*), which were preventable by ryanodine receptor blockade (1 mM tetracaine; n=14, p<0.01), acute depletion of endolysosomal Ca²⁺ stores (100 nM bafilomycin; n=7; p<0.01), or lysosomal two-pore channel (TPC) inhibition (5 μ M Ned-19; n=9; p<0.05).

I/R (secondary to cardiopulmonary bypass) was found to induce PKAR1 α oxidation in the myocardium of patients undergoing cardiac surgery (*panel C*; n=18, p=0.02).

Absence of this response in KI mouse hearts resulted in 2-fold larger infarcts ($p < 0.01$) and a concomitant reduction in LV contractile recovery (final LVDP of 55.9 ± 8.6 vs 82.5 ± 7.1 mmHg in WT; $n = 7-8$, $p < 0.05$), both which were prevented by addition of Ned-19 at the time of reperfusion (*panel D*; $n = 4$, $p < 0.01$).

Conclusions: Oxidised PKAR α acts as a potent inhibitor of intracellular Ca $^{2+}$ release in the heart through its redox-dependent interaction with the lysosome. In the setting of I/R, where PKA oxidation is induced, this regulatory mechanism is critical for protecting the heart from injury and offers a novel target for the design of cardioprotective therapeutics.

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