Oxidation of PKA-RIα protects against ischemia-reperfusion injury by inhibiting lysosomal-triggered calcium release

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Introduction: Kinase oxidation is a critical signalling mechanism through which changes in the intracellular redox state alter cardiac function. In the myocardium, type-1 PKA (PKARIα) can be reversibly oxidised, forming interprotein disulfide bonds within the holoenzyme complex. However, the impact of disulfide formation on kinase function, and its influence on PKA signaling in the context of heart disease remains largely unknown.

Methods & Results: Myocardial ischemia-reperfusion (I/R) was found to be a potent inducer of PKARIα disulfide formation *in vivo*, both in mice and in humans. Using imaging modalities with high spatial and temporal resolution, we found that this conformation does not increase intrinsic PKA catalytic activity, but rather facilitated enhanced AKAP-dependent compartmentation of PKARIα in the adult mouse left ventricular (LV) myocyte, with preferential localization to the lysosome under oxidized conditions (n=38-41 myocytes, N=3 biological replicates, $p<0.01$). Investigations in isolated LV myocytes revealed disulfide-modified PKARIα to be a significant regulator of lysosomal two pore channel (TPC)-dependent calcium-induced calcium release, with myocytes from 'redox dead' PKARIα mice (Cys17Ser) displaying spontaneous sarcoplasmic reticulum calcium release events and pronounced intracellular calcium oscillations. These events were prevented by ryanodine receptor blockade (1 mM tetracaine; n=14, $p<0.01$), acute depletion of lysosomal Ca^{2+} stores (100 nM bafilomycin; n=7; $p<0.01$), or TPC inhibition (5 µM Ned-19; n=9; $p<0.05$).

Absence of I/R-induced disulfide formation in "redox dead" PKARIα mouse hearts resulted in larger infarcts (2-fold increase, $p<0.001$) and a concomitant reduction in LV contractile recovery (1.6-fold, $p<0.001$), which could be fully prevented by administering the TPC inhibitor, Ned-19, at the time of reperfusion.

Conclusions: Oxidised PKARIα acts as a potent inhibitor of intracellular calcium 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.