

## Redox-mediated PKA-R1 $\alpha$ localisation to the lysosome inhibits myocardial calcium release and robustly reduces myocardial injury

Jillian N. Simon<sup>1</sup>, Besarte Vrellaku<sup>1</sup>, Stefania Monterisi<sup>2</sup>, Sandy Chu<sup>1</sup>, Nadiia Rawlings<sup>1</sup>, Oliver Lomas<sup>1</sup>, Gerard A. Marchal<sup>1</sup>, Dominic Waithe<sup>3</sup>, Parag Gajendragadkar<sup>1</sup>, Raja Jayaram<sup>1</sup>, Keith Channon<sup>1</sup>, Pawel Swietach<sup>2</sup>, Manuela Zaccolo<sup>2</sup>, Phil Eaton<sup>4</sup>, Barbara Casadei<sup>1</sup>

<sup>1</sup>Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, UK

<sup>2</sup>Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK

<sup>3</sup>Wolfson Imaging Centre, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, UK

<sup>4</sup>Cardiovascular Division, The Rayne Institute, King's College London, London, UK

**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 $\alpha$ ) can be reversibly oxidised, forming interprotein disulfide bonds within the holoenzyme complex. However, the effect of PKAR1 $\alpha$  oxidation on downstream signalling in the heart, particularly under states of oxidative stress, remains unexplored.

**Purpose:** To determine the direct functional consequences of PKAR1 $\alpha$  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 $\alpha$  proteins in R1 $\alpha$ -null fibroblasts showed that PKAR1 $\alpha$  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 $\alpha$  to lysosomes in WT myocytes, which was completely absent in "redox dead" Cys17Ser PKAR1 $\alpha$  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 ( $30.6\pm5.1\%$  reduction;  $n=35-37/N=3$ ,  $p<0.01$ ).

Displacement of PKAR1 $\alpha$  from lysosomes resulted in spontaneous sarcoplasmic reticulum Ca<sup>2+</sup> release and dramatic Ca<sup>2+</sup> 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 Ca<sup>2+</sup> stores (100 nM bafilomycin;  $n=7$ ;  $p<0.01$ ), or lysosomal two-pore channel (TPC) inhibition (5  $\mu$ M Ned-19;  $n=9$ ;  $p<0.05$ ).

I/R (secondary to cardiopulmonary bypass) was found to induce PKAR1 $\alpha$  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  $55.9 \pm 8.6$  vs  $82.5 \pm 7.1$  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 $\alpha$  acts as a potent inhibitor of intracellular  $\text{Ca}^{2+}$  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.

### Funding Acknowledgements:

British Heart Foundation grants CH/12/3/29609 (to J.N.S., B.C.), RG/16/12/32451 (to J.N.S., B.V., B.C.), FS/17/17/32438 (to P.G.), RG/17/6/32944 (M.Z., S.M.), PG/15/5/31110 (to M.Z.), RG/15/9/31534 (to P.S.), and PG/17/44/33064 (to P.E.); Medical Research Council grants MR/P023150/1 (to P.E) and MR/S005382/1 and MRC/BBSRC/EPSRC MR/K01577X/1 (to D.W.); Garfield-Weston Foundation MPS/IVIMS-11/12-4032 (to N.R., B.C.); Wellcome Trust Fellowship 0998981Z/12/Z (to O.L.)

**Figure:**

