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EDITORIAL

What You Did Not Know About Cardiac Ca^{2+} Handling

Lysosomes and Oxidized PKA

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Although the treatment of myocardial infarction (MI) has improved markedly in recent years, MI-associated morbidity and mortality remain substantial. One of the remaining challenges in the treatment of MI is that myocardial reperfusion, although essential to salvage myocardial tissue, also causes damage to the myocardium through a process called ischemia/reperfusion (I/R) injury.[1] Despite decades of intense research and numerous randomized, controlled trials, there are no therapies that prevent I/R injury. Reactive oxygen species (ROS) are major determinants of tissue damage during I/R and, given its central role, efforts to ameliorate I/R injury are often centered around balancing ROS activity. ROS balance, however, is complex, and antioxidants themselves can paradoxically increase the degree of myocardial I/R injury.[2] The mechanism responsible for this partial protective effect of ROS during I/R is thought to reflect the fact that ROS can serve as second messengers.[3]

PKA (protein kinase A) is a critical signaling molecule in cardiomyocytes, involved in calcium (Ca^{2+}) handling, gene transcription, and metabolism.[4] PKA is a heterotetrameric protein composed of 2 catalytic subunits and a dimeric regulatory subunit, the latter of which exists as an RI or RII isoform. PKA is activated by binding of cAMP to the regulatory subunits, triggering a conformational change that releases the catalytic subunits and activates downstream substrate phosphorylation. The spatiotemporal regulation of PKA within the cell is governed by AKAPs (A-kinase anchoring proteins) that restrict PKA into a specific microdomain in close proximity to its activators and downstream targets.[4,5] Recently, the RIα isoform of PKA (PKARIα) was found to be sensitive to oxidation at 2 cysteine (Cys) residues (Cys17 and Cys37), resulting in a disulfide bond between 2 RIα subunits.[4,6] The conformational change in PKARIα promotes AKAP binding and is thought to activate PKA in a cAMP-independent fashion.[1–6] It has been suggested that PKA promotes adaptive angiogenesis and increases cardiac contractility, yet the exact role in I/R injury remains to be established.[6,7]

In this issue of *Circulation,* Simon et al[8] present compelling new evidence that oxidation of PKARIα affords endogenous cardioprotection during myocardial I/R injury that may be amenable to therapeutic interventions. First, the authors set out to determine whether PKARIα oxidation occurs during I/R. Using human atrial tissue obtained before and after cardioplegic arrest, as well as murine heart samples after I/R, the authors discovered that I/R injury significantly increased PKARIα oxidation and disulfide formation. Similar degrees of PKARIα disulfide formation were observed in adult mouse cardiomyocytes exposed to H_2O_2, under normal culture conditions, indicating that this posttranslational modification is common, sensitive, and physiologically relevant.[8]

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Next, the authors evaluated the consequences of disulfide formation on catalytic activity and intracellular localization of PKARIα using a redox-dead knock-in (KI) mouse model in which Cys17 in RIα was replaced by serine (KI; RIα Cys17Ser mutation).[6] In contrast to previous reports, the authors were unable to detect a clear difference in the catalytic activity of PKARIα between wild-type (WT) and KI mice. Fluorescence recovery after photobleaching experiments in embryonic fibroblasts and neonatal cardiomyocytes confirmed previous findings that oxidation of PKARIα promotes AKAP binding.[8]

Then, confocal microscopy was used to determine the subcellular compartment to which oxidized PKARIα translocated. Whereas localization to the mitochondrial and nuclear compartments was not affected, oxidized PKARIα appeared to localize to the lysosome. This observation posed somewhat of a challenge, because lysosomes are too small to be visualized by conventional confocal microscopy. The authors therefore reverted to super-resolution stimulation emission depletion microscopy, which allows fluorescent detection at nanometer resolution. Using this technique in adult and neonatal cardiomyocytes, it was demonstrated that oxidized PKARIα clusters within 70 nm of lysosomes in an AKAP-dependent fashion. Another important discovery made with stimulation emission depletion imaging was that a large proportion of lysosomes were found to be in close proximity of the ryanodine receptor (RyR), both in WT and KI cardiomyocytes.[8]

Lysosomes are multifunctional organelles that are primarily involved in the degradation of macromolecules, yet recent evidence suggests that they participate in cellular Ca^{2+} handling as well.[9] Lysosomes release Ca^{2+} through their 2-pore channels (TPCs) and trigger diastolic Ca^{2+} release from the RyR.[9] In the context of heart disease, lysosomes generate oscillating Ca^{2+} currents that trigger arrhythmias and can cause mitochondrial depolarization and irreversible cell death.[10] Simon et al[8] therefore hypothesized that PKARIα oxidation and lysosomal translocation could affect cardiomyocyte Ca^{2+} handling. In line with the imaging studies, mitochondrial Ca^{2+} handling was not affected by oxidation of PKARIα. Moreover, general Ca^{2+} transients were also grossly similar in WT and KI mice and sarcoplasmic reticulum Ca^{2+} stores were comparable between genotypes. KI mice did, however, display a significant increase in oscillating Ca^{2+} currents and diastolic Ca^{2+} release events, suggestive of enhanced spontaneous opening of the RyR. Furthermore, the oscillating Ca^{2+} currents in KI mice were prevented by RyR inhibition, by depletion of lysosomal Ca^{2+} stores, and by the TPC inhibitor Ned-19. Together these findings support the hypothesis that oxidized PKARIα regulates spontaneous RyR opening by preventing TPC-mediated calcium release from the lysosomal compartment.[8]

In the context of myocardial I/R injury, spontaneous RyR Ca^{2+} release events can trigger cardiac arrhythmias and stimulate apoptosis.[11] The PKARIα-mediated reductions in lysosomal Ca^{2+} release could thus translate into increased resilience to I/R. To test this hypothesis, murine WT and KI hearts were exposed to ex vivo I/R injury using the Langendorff system. Myocardial infarct size was markedly increased in KI compared with WT hearts, associated with substantial reductions in functional recovery. The TPC inhibitor Ned-19 normalized myocardial injury to control values, confirming the hypothesis that PKARIα oxidation affords endogenous cardioprotection through downstream effects on the TPC.[8]

In summary, Simon et al[8] report the first direct evidence that oxidative stress–induced disulfide bonds within the regulatory domain of PKARIα localize the holoenzyme to lysosomes in proximity to the RyR. In this subcellular domain, oxidized PKARIα blocks lysosomal Ca^{2+} release events that trigger RyR Ca^{2+} sparks and promote myocardial I/R injury. Oxidation and subsequent disulfide formation of PKARIα thus appears to reflect an endogenous protective mechanism that limits myocardial damage in response to oxidative stress (summarized in the Figure).

The authors should be applauded for their meticulous work, which combines multiple state-of-the-art imaging techniques, advanced fluorescent biosensors, and established functional readouts in primary cells and mutant mice. In addition to the previously unrecognized cardioprotective effects of PKARIα, they also revealed the lysosome as a surprising new subcellular domain controlled by PKARIα. Lysosomes have only recently been recognized as culprits in cardiac I/R injury.[10] The discovery that oxidation and lysosomal translocation of PKARIα can prevent lysosomal Ca^{2+} release is intriguing and warrants further exploration. Although the authors did not detect a clear difference in arrhythmia burden, a recent detailed analysis suggested that redox-dead KI mice are resistant to oxidative stress–induced arrhythmias.[12] Further research is required to define the physiologic contribution of oxidized PKARIα to cardiac Ca^{2+} handling and arrhythmogenesis.

As often occurs with such pioneering work, important questions remain. First, in contrast to previous reports,[13,14] PKARIα disulfide formation did not appear to influence the catalytic activity of PKA, nor did it alter phosphorylation of established PKA targets involved in cardiac Ca^{2+} handling. Accordingly, a clear explanation as to how oxidized PKARIα prevents lysosomal Ca^{2+} release from the TPC remains enigmatic. It is highly likely that changes in the catalytic activity of oxidized PKARIα were restricted to the lysosomal compartment, which would not have been detectable using confocal microscopy. We therefore propose that the effects of oxidized PKARIα on Ca^{2+} release are specifically governed by phosphorylation of the TPC (Figure [A]).

Whereas lysosomal translocation of oxidized PKAR1α was convincingly demonstrated with stimulation

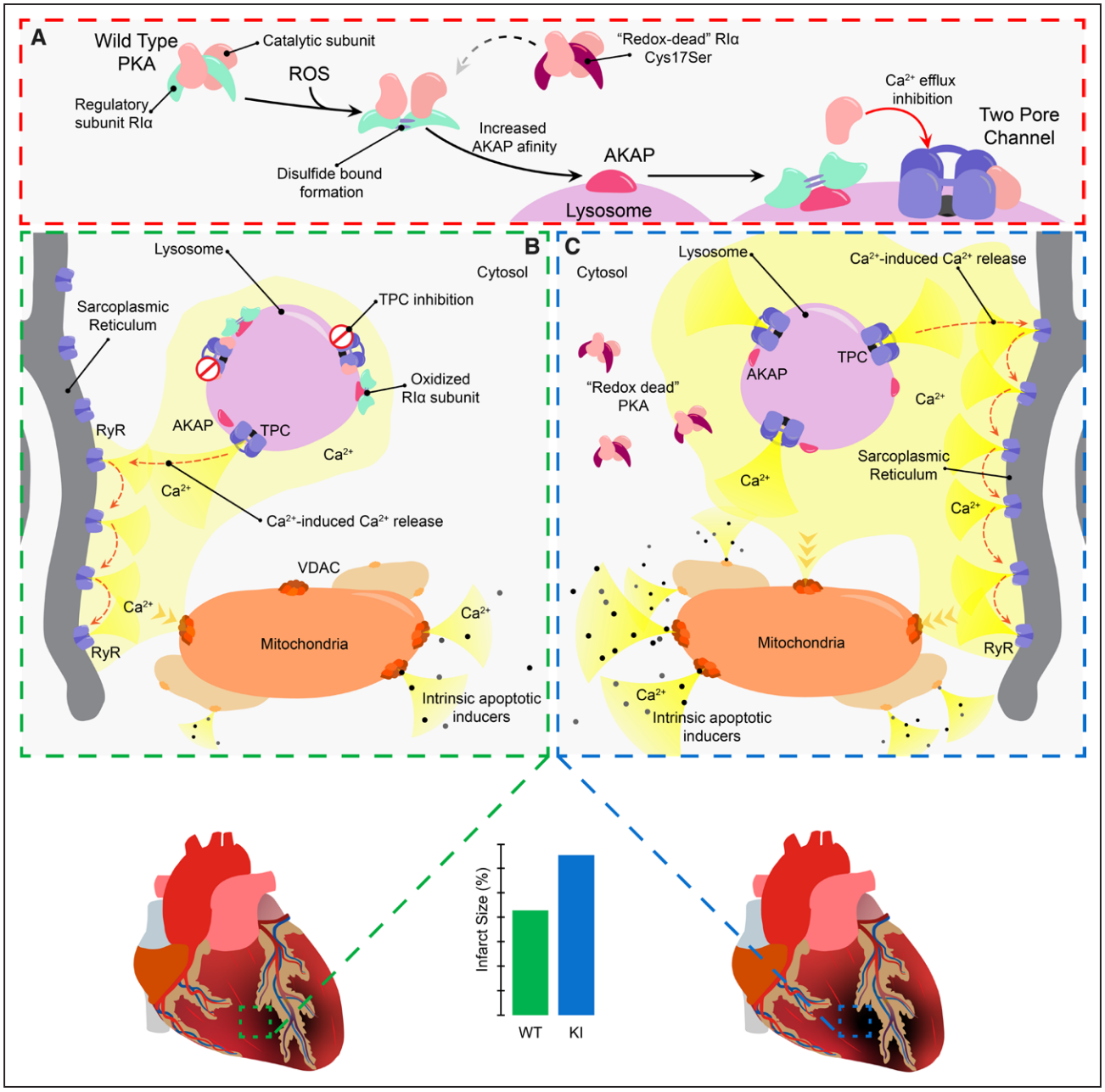

Figure. Oxidation and subsequent disulfide formation of the RIα isoform of PKA (protein kinase A) appears to reflect an endogenous protective mechanism that limits myocardial damage in response to oxidative stress.

A, During ischemia/reperfusion (I/R) injury, oxidation of wild-type PKA occurs in response to reactive oxygen species (ROS) formation, promoting AKAP (A-kinase anchoring protein) binding in the lysosomal microdomain. Binding to AKAP on the lysosome releases the catalytic subunits of PKA that phosphorylate the lysosomal 2-pore channel (TPC) and prevents lysosomal calcium (Ca^{2+}) release. A PKA mutant in which the RIα subunit of PKA is replaced by redox-dead isoform (Cys17Ser) does not localize to the lysosome and does not influence the TPC. **B**, Phosphorylation of the TPC prevents Ca^{2+} release and limits Ca^{2+}-induced Ca^{2+} release from the ryanodine receptor (RyR). The extent of I/R injury is limited at least in part by attenuating Ca^{2+}-induced mitochondrial swelling and the release of apoptotic inducers. **C**, In knock-in mice where the RIα subunit of PKA is replaced by the redox-dead Cys17Ser mutant, PKA can no longer bind to the TPC, which aggravates I/R injury and promotes spontaneous Ca^{2+} release from the lysosome. VDAC indicates voltage-dependent anion channel.

emission depletion microscopy, it was not confirmed by standard biochemical techniques. The relative translocation to the lysosome compared with other subcellular compartments is therefore unknown. Oxidized PKAR1α is known to translocate to myofilaments, and it is conceivable that other subcellular domains may also have contributed to the observed protection offered by oxidized PKAR1α.[7] The majority of the reported work was performed under normal culture conditions, suggesting that the results might have been different in the presence of high concentrations of ROS.

Oxidation of PKAR1α after I/R injury occurred both in the area at risk as well as in the myocardium remote from the infarction, but not in sham-operated hearts. These findings suggest that PKAR1α oxidation not only occurs in the context of oxidative stress but may

also be activated downstream of neurohumoral and hemodynamic signals.[4] The NAADP (nicotinic acid adenine dinucleotide phosphate) signals responsible for TPC-mediated lysosomal Ca^{2+} release are also activated downstream of neurohumoral signaling pathways, providing a possible mechanism for remote oxidation of PKAR1α.[10] Glucagon-like peptide-1 inhibitors specifically activate type 1 PKA and have been shown to attenuate myocardial I/R injury in patients presenting with ST-segment–elevation MI.[8,15]

Simon et al[8] have uncovered a novel endogenous cardioprotective mechanism in which oxidative stress limits myocardial injury via stabilization of lysosomal Ca^{2+} release. Future studies should aim to unravel mechanisms responsible and the extent to which this may translate to other forms of physiologic and pathologic stress. These results reinforce the concept that PKA is not always a villain in cardiac pathologies and provide a clear rationale for strategies to activate PKAR1α to reduce reperfusion injury in patients with an acute MI.

ARTICLE INFORMATION

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