CaMKII Determines Mitochondrial Stress Responses in Heart
Joiner et al

Although the recent identification of the mitochondrial Ca\(^{2+}\) uniporter (MCU) has resolved a long-standing mystery as to how Ca\(^{2+}\) freely enters the mitochondria, it has also evoked additional questions such as its mode of regulation and the identity of other associated factors. In an article recently published in *Nature*, Joiner et al provide data demonstrating that in the heart, matrix-localized Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaMKII) can upregulate MCU activity in a manner requiring phosphorylation of the channel N terminus. They showed that inhibition of CaMKII-dependent MCU activity protected the heart from ischemic injury by presumably reducing Ca\(^{2+}\) influx and desensitizing the mitochondrial permeability transition pore (MPTP) to opening. Although these results demonstrate convincingly that CaMKII plays an important role in MCU regulation and subsequent response to cardiac injury, several questions remain unanswered.

The ability of mitochondria to take up and sequester Ca\(^{2+}\) plays an important role in the buffering of cytosolic Ca\(^{2+}\), regulation of ATP production via the citric acid cycle, and regulation of apoptotic and necrotic cell death pathways. Although mitochondrial Ca\(^{2+}\) uptake was first described in the 1960s and the electrophysiological properties of the MCU were reported in 2004, it was not until 2011 that 2 articles were published revealing the genetic identity of the MCU. This pioneering work has initiated a search for additional members of the MCU complex (such as MICU1 and the recently discovered MCU1), as well as an attempt to understand how MCU-mediated Ca\(^{2+}\) influx participates in the regulation of whole-cell Ca\(^{2+}\) signaling and whether well-described pathways that regulate other Ca\(^{2+}\) handling processes can similarly modulate MCU-dependent Ca\(^{2+}\) uptake. In the featured article, Joiner et al show that CaMKII serves as an important endogenous regulator of MCU in the heart.

CaMKII proteins are represented by 3 isoforms in the heart: CaMKII\(\delta\) (the most prevalent), CaMKII\(\gamma\), and CaMKII\(\beta\). These CaMKII proteins assemble to form hetero-dodecamers or tetradecamers activated by binding to Ca\(^{2+}\)/calmodulin. Sustained stimulation of CaMKII with Ca\(^{2+}\) induces autophosphorylation, which then leads to Ca\(^{2+}\)-independent activity. In addition to the known Ca\(^{2+}\)-dependent and autophosphorylation-dependent activity, CaMKII is activated by the oxidation of specific methionine residues in the regulatory region. In the heart, CaMKII is a prominent regulator of several Ca\(^{2+}\) influx and efflux pathways, including, among others, Ca\(^{2+}\)-dependent facilitation of the L-type Ca\(^{2+}\) channel Ca\(_{V1.2}\); phosphorylation of the type 2 ryanodine receptor (RyR2), which increases open probability; and phosphorylation of the sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\) ATPase 2–regulatory protein phospholamban, which enhances Ca\(^{2+}\) uptake into the sarcoplasmic reticulum (SR) after cardiac contraction. Thus, CaMKII is a nodal regulator of Ca\(^{2+}\) handling within the cardiac myocyte.

Previous studies have indicated that CaMKII inhibition protects against a variety of pathological insults, including ischemia/reperfusion (I/R) injury, myocardial infarction, adrenergic insult via isoproterenol infusion, and pressure overload–induced hypertrophy induced via transverse aortic constriction. In addition, transgenic overexpression of CaMKII\(\delta\) in the heart results in cardiac hypertrophy and dilated cardiomyopathy. Structural defects observed with CaMKII\(\delta\) overexpression have been linked to distortions in CaMKII-regulated Ca\(^{2+}\) signaling pathways. CaMKII activation results in increased mitochondrial Ca\(^{2+}\) in a manner dependent on SR Ca\(^{2+}\) load, and inhibition of CaMKII prevents endoplasmic reticulum stress–mediated increases in mitochondrial Ca\(^{2+}\). Although CaMKII is normally characterized as a protein localized to the cytosol, SR, and nucleus (with ratios depending on isoform and splice variant examined), a subpopulation of CaMKII\(\delta\) (10%) is localized to mitochondria, where it could be positioned to play a role in regulating mitochondrial Ca\(^{2+}\) handling.

To determine whether mitochondria-localized CaMKII could directly participate in MCU-mediated mitochondrial Ca\(^{2+}\) uptake and activation of the MPTP, Joiner et al created transgenic mice with cardiac-specific overexpression of CaMKII, a protein that inhibits CaMKII with remarkable specificity, modified so that it partitioned to all membranes, including the mitochondria. CaMKII transgenic hearts displayed...
significant protection after I/R injury that was accompanied by resistance to MPTP opening, which was associated with preserved mitochondrial membrane potential (ΔΨm) after a Ca2+ challenge. To achieve even greater subcellular specificity, the investigators created a second transgenic mouse model overexpressing CaMKII with a mitochondrial localization motif (mtCaMKII). mtCaMKII transgenic hearts demonstrated protection similar to CaMKII-expressing hearts after injury, albeit to a slightly lesser extent, perhaps suggesting the contribution of extramitochondrial CaMKII-regulated pathways to post-I/R remodeling. However, the protection that was observed definitely showed that CaMKII functions within the mitochondria to mediate myocyte death during I/R injury. Thus, CaMKII inhibition protects the heart not only at the level of the mitochondria but also outside this organelle. Mechanistically, cardiac myocytes expressing the mitochondria-localized CaMKII inhibitor displayed slowed Ca2+ uptake and were resistant to MPTP opening after serial Ca2+ injections, suggesting that mitochondrial Ca2+ capacity was increased. Although addition of cyclosporine A, an inhibitor of the MPTP, could also increase mitochondrial Ca2+ uptake capacity, mtCaMKII showed a more significant elevation that was increased even further with cyclosporine A addition. These observations suggest that cyclosporine A and mtCaMKII enhance mitochondrial Ca2+ uptake capacity via mutually exclusive mechanisms and that CaMKII likely does not participate in direct modulation of the MPTP. Instead, whole-mitoplast patch-clamp studies demonstrated lower MCU current (I_{MCU}) in the presence of mtCaMKII, suggesting regulation of the MCU by matrix-localized CaMKII. Addition of constitutively active (but not kinase-dead) CaMKII in the pipette solution (matrix side) increased I_{MCU} and immunoprecipitation studies confirmed binding of CaMKII to the MCU complex (Figure). Thus, CaMKII directly binds the MCU complex in the matrix and regulates its activity as a mechanism for altering mitochondrial Ca2+ and cell death propensity.

The notion that CaMKII inhibition decreases MCU activity while simultaneously increasing mitochondrial Ca2+ uptake capacity seems contradictory. Hypothetically, mitochondrial Ca2+ capacity could be upregulated by inhibiting MPTP-dependent Ca2+ efflux, by increasing Ca2+ entry through the MCU, or by altering the Ca2+ buffering environment in the matrix. Application of Ru360, a selective inhibitor of MCU, eliminates mitochondrial swelling,25 suggesting that MCU-dependent Ca2+ influx is required for MPTP formation. Indeed, previous work using shRNAs targeted against MCU showed loss of mitochondrial Ca2+ uptake.3 Because CaMKII inhibition reduces MCU activity, one would suppose that mitochondrial Ca2+ uptake capacity would be reduced as well; however, the authors report that CaMKII inhibition increases mitochondrial Ca2+ capacity as much as or more than cyclosporine A alone. Metabolic flux through the electron transport chain is also regulated by Ca2+ such that higher matrix Ca2+ increases the activity of key dehydrogenases, although the authors reported no change in respiratory activity in isolated mitochondria from mtCaMKII hearts. These results collectively suggest that despite the change in MCU activity caused by CaMKII, total matrix Ca2+ is probably not altered. Thus, the secondary effect on MPTP opening and cell death must be attributable to an unexplained aspect of MCU-dependent Ca2+ regulation such as a rapid change in free (unbuffered) Ca2+ within the matrix or a change in the finite buffering capacity in the microenvironment of the MCU near the inner membrane, which then affects the MPTP. Alternatively, CaMKII phosphorylation may regulate other, as yet unknown matrix proteins that influence Ca2+ uptake, efflux, or buffering.

The authors extended their findings on CaMKII regulation of MCU by identifying 2 putative phosphorylation sites. Mutation of these sites to alanine prevented upregulation of MCU after constitutively active CaMKII was introduced in the patch pipette. Because these sites were present on the N terminus and CaMKII added to the bath solution (intermembrane space side) failed to upregulate I_{MCU}, these data suggest that the N terminus of MCU is located in the mitochondrial matrix, agreeing with the conclusions of the study published by the Mootha laboratory in 2011.5 Despite the known centrality of CaMKII as a nodal effector of cardiac Ca2+ handling, many important questions remain. For example, how is CaMKII efficiently localized to the mitochondrial matrix? Although CaMKII isosforms share a great deal of similarity, each contains a variable region that is responsible for the majority of sequence divergence.27 Some variable
regions have been suggested to provide unique nuclear or SR localization signals for CaMKII complexes,27 and other, more exotic forms of CaMKII (such as the kinase-dead splice variant αKAP that is derived from CaMKIIα)28 may serve solely to regulate localization. Work on the δ isoform has demonstrated 2 primary splice variants in the heart, δh and δl, which were initially reported to have varying localization, with δh carrying a nuclear localization sequence. However, a recent study has suggested that although there are differences in the proportions of each CaMKIIδ splice variant found in nuclear, SR, cytosol and mitochondrial fractions, both δh and δl are present at some level in each of these compartments, and activation of these splice variants seems to proceed similarly.23 Hence, it remains unclear whether select isoforms of CaMKII are partitioned to the mitochondria, how they are imported into the matrix, and whether they take on the classic dodecamer configuration.

Another important question concerns the nature of the CaMKII/MCU interaction and how it might be regulated. It is unclear whether CaMKII levels in the mitochondrial matrix are maintained at stable levels or whether those levels fluctuate during disease conditions (such as I/R injury) or stress. Compounding this is the question of how CaMKII is actually partitioned into the mitochondrial matrix in the first place because this requires specific N-terminal or internal mitochondrial localization signals recognized by the TIM23 or TIM22 complex, respectively.29 In addition to understanding whether mitochondrial levels of CaMKII are changed during disease (such as by increased import), it is even more unclear how it might be regulated by Ca2+ and calmodulin. CaMKII is activated by association with Ca2+/calmodulin when Ca2+ levels reach a certain threshold, often resulting in autophosphorylation and Ca2+-independent activation. However, how this might happen in the mitochondrial matrix is a mystery, in part because reports of maximum steady-state free mitochondrial matrix Ca2+ concentrations obtained in vitro are quite variable, ranging from the low micromolar range in isolated brain mitochondria30 up to 50 μmol/L in isolated heart mitochondria,31 whereas in HeLa and primary chromaffin cells, matrix free Ca2+ has been shown to approach up to 1 mmol/L.32 In actively beating adult myocytes, conditions would seem to favor constitutive activity of CaMKII, so it is uncertain how the system is regulated. However, Joiner et al demonstrated that calyculin A applied to the matrix side of the inner mitochondrial membrane can enhance Im, presumably via inhibition of endogenous phosphatases associated with the CaMKII/MCU complex, thus increasing its net phosphorylated state. This result suggests that CaMKII reserve capacity exists in the matrix so that not all CaMKII is constitutively active. Although recent studies have indicated that the mitochondrial matrix is capable of incredible feats of Ca2+ buffering,33 the ability to examine microdomain matrix Ca2+ concentrations is beyond the state of the art. In addition, depending on the true local Ca2+ concentration, CaMKII inhibitory autophosphorylation may be capable of tuning its activity to the demands of its matrix environment, suggesting the possibility that matrix-localized CaMKII may be regulated primarily via microdomain Ca2+ generated in the vicinity of the MCU.

Although several questions remain, the work of Joiner et al has established yet another nodal integration point whereby CaMKII controls Ca2+ handling, but this time at the level of the mitochondria. They show that CaMKII directly controls MCU activity by phosphorylation, thereby regulating the rate of Ca2+ influx across the inner mitochondrial membrane to influence MPTP and subsequent cell death during disease. Their observations have profound implications for human disease and heart failure and suggest that Ca2+ dysregulation at the level of the mitochondria could be obviated by inhibiting CaMKII and by selectively downregulating MCU activity.

Disclosures

None.

References


CaMKII Does It Again: Even the Mitochondria Cannot Escape Its Influence
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