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AMPK is critical for mitochondrial function during reperfusion after myocardial ischemia

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ABSTRACT

AMP-activated kinase (AMPK) is a stress responsive kinase that regulates cellular metabolism and protects against cardiomyocyte injury during ischemia–reperfusion (IR). Mitochondria play an important role in cell survival, but the specific actions of activated AMPK in maintaining mitochondrial integrity and function during reperfusion are unknown. Thus, we assessed the consequences of AMPK inactivation on heart mitochondrial function during reperfusion. Mouse hearts expressing wild type (WT) or kinase-dead (KD) AMPK were studied. Mitochondria isolated from KD hearts during reperfusion had intact membrane integrity, but demonstrated reduced oxidative capacity, increased hydrogen peroxide production and decreased resistance to mitochondrial permeability transition pore opening compared to WT. KD hearts showed increased activation of the mitogen activated protein kinase kinase 4 (MKK4) and downstream c-Jun terminal kinase (JNK) and greater necrosis during reperfusion after coronary occlusion. Transgenic expression of mitochondrial catalase (MCAT) prevented the excessive cardiac JNK activation and attenuated the increased myocardial necrosis observed during reperfusion in KD mice. Inhibition of JNK increased the resistance of KD hearts to mPTP opening, contractile dysfunction and necrosis during IR.

Thus, intrinsic activation of AMPK is critical to prevent excess mitochondrial reactive oxygen production and consequent JNK signaling during reperfusion, thereby protecting against mPTP opening, irreversible mitochondrial damage and myocardial injury.

Abbreviations: AA, antimycin A; AMPK, AMP-activated protein kinase; CG5N, calcium green 5 N; CRC, calcium retention capacity; FCCP, carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone; Gapdh, glyceraldehyde 3-phosphate dehydrogenase; IR, ischemia–reperfusion; JNK, c-Jun terminal kinase; KD, kinase-dead AMPK expressor; LAD, left anterior descending coronary artery; LVDP, left ventricular developed pressure; MAPK, mitogen activated kinase; MCAT, mitochondrial catalase expressor; MIF, macrophage migration inhibitory factor; MKK4, mitogen activated protein kinase kinase 4; mPTP, mitochondrial permeability transition pore; NRF, nuclear factor-erythroid 2-related factor; OCR, oxygen consumption rates; Oligo, oligomycin; RPP, rate pressure product; ROS, reactive oxygen species; SH3BP5 (Sab), SH3 domain binding protein 5; Sod2, mitochondrial superoxide dismutase 2; TMRM, tetramethylrhodamine methyl ester; Trxr2, thioredoxin reductase 2; TTC, triphenyl tetrazolium chloride; WT, wild type.

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1. Introduction

AMP-activated protein kinase (AMPK) is activated during low energy cellular states, such as myocardial ischemia, and orchestrates a cellular response that decreases ATP consumption and increases ATP production [1]. High-energy phosphate compound production is critically dependent on mitochondrial function in striated muscles. Chronic stimulation of AMPK is known to promote skeletal muscle mitochondrial biogenesis [2,3], while AMPK inactivation results in decreased skeletal muscle mitochondrial content [4]. In the heart, the role of AMPK in modulating mitochondrial biogenesis is not well understood. Activated AMPK also promotes glucose uptake [5] and prevents ATP depletion [6] during ischemia and improves recovery of ATP during reperfusion [5]. The resumption of mitochondrial oxidative phosphorylation after ischemia–reperfusion is necessary for myocardial contractile recovery [7], however, the actions of activated AMPK in maintaining mitochondrial function during ischemia–reperfusion remain unclear.

Mitochondrial damage during ischemia–reperfusion is primarily caused by opening of the mitochondrial permeability transition pore (mPTP) early during reperfusion. Opening of the pore is inhibited during ischemia by low pH, but this inhibition is lost early during reperfusion and the pore opens in response to low mitochondrial ATP content, calcium influx and oxidative stress [8]. Under aerobic conditions, mitochondrial anti-oxidative processes balance the production of reactive oxygen species (ROS): manganese superoxide dismutase transforms superoxide into hydrogen peroxide, glutathione peroxidase and thioredoxin convert hydrogen peroxide into water [9]. However, during ischemia–reperfusion, increased ROS production can override endogenous scavenging mechanisms resulting in activation of deleterious signaling and leading to mPTP opening [9]. mPTP opening disrupts mitochondrial function and can result in the irreversible loss of mitochondrial oxidative capacity. Although the opening of the pore is inhibited by cyclosporin A binding to cyclophilin D [10], there is still uncertainty regarding the components of the pore and the role of activated AMPK in its regulation.

Mitogen activated protein kinase (MAPK) pathways are activated by oxidative cellular stress during ischemia–reperfusion [11]. JNK (c-Jun N-terminal kinase) is a MAPK family member that modulates multiple cellular functions, including proliferation, differentiation, and apoptosis [12]. JNK activation plays a role in reperfusion injury after ischemia [13], and in the absence of JNK, mouse hearts subjected to ischemia–reperfusion have significantly less necrosis and apoptosis [14]. However, recent evidence suggests that the action of JNK-1 in reperfusion injury can range from protective to injurious with a direct relationship to the length of ischemia [15]. Translocation of JNK to the mitochondrial outer membrane and interaction with SH3 domain-binding protein 5 (Sab) has been proposed as a mechanism for JNK mediated apoptosis in myocardial ischemia–reperfusion [16]. An inhibitory action of AMPK on JNK pathway activation has been suggested in endothelial cells, where chronic AMPK stimulation attenuated JNK activation by hydrogen peroxide [17]. Whether inhibition of JNK activation during reperfusion contributes to the protective role of activated AMPK in the cardiomyocyte remains uncertain.

There has been some controversy regarding the theoretical role of AMPK in the protection of the heart during ischemia and reperfusion due to its effect of increasing fatty acid oxidation and decreasing glucose oxidation with a potential for increased oxygen demand [18]. However, experimental work has shown that myocardial recovery is blunted after ischemia and reperfusion in absence of active AMPK [5,19,20]. There is significant interest in developing molecular strategies that target the AMPK pathway for the treatment of diabetes, cancer and cardiovascular disease. Thus, the purpose of this study was to investigate the intrinsic role of AMPK in regulating myocardial mitochondrial function during ischemia and reperfusion. We tested the hypothesis that activation of the intrinsic AMPK pathway is required to inhibit mPTP opening through modulation of oxidative stress and JNK signaling.

2. Materials and methods

2.1. Animals

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Yale University Animal Care and Use Committee. All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering. We utilized α2 kinase dead (KD) AMPK transgenic mice (C57BL/6 backcross) that express an inactive rat α2 isoform (K45R mutation), which results in a high level of functional inactivation of both α1 and α2 AMPK complexes in the heart [5]. We also crossed KD mice with MCAT transgenic mice that overexpress mitochondrial catalase in the heart [21]. Littermate mice were used as controls and all mice were studied at 10–14 weeks of age.

2.2. In vitro global myocardial ischemia and reperfusion

Hearts from wild type (WT) and KD mice were subjected to retrograde Langendorff perfusion, as previously described, with buffer containing 7 mM glucose, 0.4 mM oleate, 1% BSA and a low-fasting concentration of insulin (10 μU/ml) [5]. Isolated hearts were perfused *ex vivo* for 20 min (baseline), followed by 15 min of ischemia and 10 min of reperfusion. Contractile function was assessed continuously by measuring left ventricular pressure and heart rate. At the end of perfusions, hearts were used for mitochondrial isolation or were frozen in liquid nitrogen. In separate experiments hearts were reperfused for 30 min, then removed from the perfusion system and stained with triphenyl tetrazolium chloride (TTC) to measure the area of necrosis [22].

2.3. In vivo regional myocardial ischemia and reperfusion

WT and KD mice were anesthetized with pentobarbital (60 mg/kg IP) and subjected to left coronary artery occlusion for 15 min, followed by 3 h of reperfusion [23]. Hearts were then excised and stained with Evans blue and triphenyl tetrazolium chloride (TTC) to measure the ischemic area at risk and the area of necrosis, respectively [22]. Serum troponin I was measured by ELISA (Life Diagnostics Inc., West Chester, PA). In separate mice, reperfusion was limited to 10 min and hearts were then removed to study early reperfusion cell signaling.

2.4. Mitochondrial bioenergetic measurements

Mitochondria were isolated from hearts by differential centrifugation [24]. Mitochondrial oxygen consumption rates (OCR) were measured using an XF24 Analyzer (Seahorse Bioscience Inc., North Billerica, MA) [25]. After placing 2.5 μg of mitochondria onto assay plates, OCR was measured with pyruvate/malate (10 mM/2 mM) in the presence of ADP (4 mM, state 3), oligomycin (2.5 μg/mL, respiration due to proton leak, state 4), carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP, 4 μM, uncoupled respiration, state 3 uncoupled) and antimycin A (4 μM, nonspecific respiratory background signal) [25].

2.5. In vitro assay for resistance to mPTP opening

The resistance of mitochondria to mPTP opening was tested during stimulation with progressively increasing concentrations of calcium [10,26,27]. Isolated mitochondria were resuspended in assay buffer containing 130 mM KCl, 10 mM Tris, 5 mM KH_2PO_4, 10 μM EGTA and 5 mM succinate, without or with 2 μM rotenone, pH 7.6 [10]. TMRM (tetramethylrhodamine methyl ester, 50 nM) was used as an indicator for inner mitochondrial membrane potential [26]. Calcium green 5 N (CG5N, 0.2 μM) was used as an indicator for the extramitochondrial