Mechanism of reactive oxygen species generation after opening of mitochondrial K_{ATP} channels

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OPENING of the ATP-sensitive K⁺ (K_{ATP}) channel mediates the cardioprotective effect induced by pathophysiological stressors such as ischemic preconditioning (IPC) (15, 29), heat shock (19), and pharmacological agents, including adenosine (5), ACh (26), opioids (12), monophosphoryl lipid A (31), phosphodiesterase 5A (PDE5A) inhibitors (24, 27), and mTOR inhibitor, rapamycin (20). In addition, direct opening of the mitochondrial K_{ATP} (mitoK_{ATP}) channels with diazoxide induces early and delayed preconditioning effects that are abolished by the channel inhibitor 5-hydroxydecanoate (5-HD) (23). The mitoK_{ATP} channels are activated when intracellular ATP levels drop. Within 1–3 min of ischemia, there is a pronounced shortening of the action potential duration (APD) secondary to activation of the K_{ATP} channels (6). Activation of K_{ATP} channels has been reported to be partially responsible for the increase in outward K⁺ currents, shortening of APD, and the increase in extracellular K⁺ concentration during anoxic or globally ischemic conditions (4). Because there was a lack of correlation between the APD shortening and cardioprotection with the pharmacological openers of K_{ATP} channels (17), Garlid et al. (14) first proposed that mitoK_{ATP} channels were involved in the cardioprotective effect. By using reconstituted mitochondrial vesicles or isolated mitochondria and measuring potassium flux, these investigators demonstrated that heart and liver mitoK_{ATP} channels shared pharmacological properties with the channels found in sarcolemma while possessing a distinct profile. Furthermore, it was shown that opening of mitoK_{ATP} channels leads to the generation of reactive oxygen species (ROS) by the mitochondria and represents an important mechanism that is required for the cardioprotective effect of IPC (25, 30). Opening of these channels allowed potassium to enter the mitochondrial inner matrix, which causes generation and release of ROS from the respiratory chain (21). ROS then act as second messengers to activate a downstream pathway of protective kinases, including protein kinase C (PKC), that finally converge on the cardioprotective end effector as shown in Fig. 1. One of the principal downstream effectors of ROS is PKCε (2), which is translocated to the mitochondria (3). Cardioprotection conferred by IPC and pharmacological agents is blocked by ROS scavengers before the index ischemia (11, 25). In most earlier studies, mitoK_{ATP} channels were proposed to be end effectors, and the channels were assumed to open during the index ischemia (13, 15). Recent studies, however, suggest that opening of mitoK_{ATP} channels is also the initial trigger of the cardioprotective effect, promoting the generation of ROS and inducing the activation of PKCε (11, 25). Other studies have suggested that mitoK_{ATP} channels act both as a trigger as well as an end effector of IPC (16). In contrast to these studies, Hanley and coworkers (10) recently challenged that diazoxide does not evoke superoxide (which dismutates to H₂O₂) from the respiratory chain by a direct mechanism. They reported that the stimulatory effects of this compound on mitochondrial respiration and oxidation of dichlorodihydrofluorescein (fluorescent probe for ROS) is not due to the opening of mitoK_{ATP} channels. Moreover, these authors suggested that inhibitory effect of decanoate on diazoxide-induced flavoprotein oxidation supports the notion that 5-HD acts as a metabolic substrate rather than a mitoK_{ATP} channel inhibitor. Clearly, there are controversial issues that need to be resolved in the future.

There has also been considerable interest in the role of the nitric oxide (NO)-cGMP-protein kinase G (PKG)-pathway in protection of the heart against ischemia-reperfusion injury (18). PKG is a serine/threonine protein kinase and is one of the major intracellular receptors for cGMP. Inhibition of cGMP-specific PDE5A with selective potent inhibitors, sildenafil citrate (Viagra) and vardenafil (Levitra), induced protective effects against ischemia-reperfusion injury in the intact heart (24, 28) and adult cardiomyocytes (9). Conceptually, these drugs inhibit the enzymatic hydrolysis of cGMP, which in turn maintains the tissue accumulation of cGMP, leading to downstream protective mechanisms involving activation of PKG and opening of mitoK_{ATP} channels. Our studies showed that sildenafil induces IPC through NO generated from endothelial and/or inducible nitric oxide synthase, activation of PKC, and opening of the mitoK_{ATP} channels (reviewed in Ref. 22). Similarly, bradykinin mimics IPC by generating ROS, and it does so via cGMP activation of PKG (8). Furthermore, Costa et al. showed that PKG + cGMP induce mitoK_{ATP} channel opening via an endogenous PKCε in isolated mitochondria (7).

It is quite obvious from the above review that there is overwhelming evidence for the involvement of ROS in cardioprotection. However, there is no direct demonstration that mitoK_{ATP} channel opening actually leads to ROS production in the mitochondria. Moreover, the mechanism and the site of ROS generation are unknown. What Garlid and associates (1) have proposed in this issue of the American Journal of Physiology-Heart and Circulatory Physiology is a novel mechanism of ROS generation with the opening of mitoK_{ATP} channel. By using a series of fluorescent probes that are sensitive to hydrogen peroxide and pH, they measured their changes in suspensions of isolated rat heart and liver mitochondria. The data suggest that the K⁺-specific ionophore valinomycin quantitatively reproduced the ROS production similar to that observed by the mitoK_{ATP} opens diazoxide and chromakalim. They further show that alkaline pH but not increasing the matrix.
volume by itself enhanced ROS production. These results are supported by carefully and systematically presented data. Neutralization of the matrix pH with acetic acid led to the blockade of the stimulatory effect of valinomycin on ROS production. Furthermore, ROS production exhibited a biphasic dependence on valinomycin concentration, with peak production occurring at valinomycin concentrations that catalyze about the same K⁺ influx as mitoK_{ATP} channel openers. The ROS production decreased with higher concentrations of valinomycin and with all concentrations of a classical protoponothoretic uncoupler. These data suggest that the increase in ROS is due specifically to K⁺ influx into the matrix and is mediated by the attendant matrix alkalinization. The authors went on to demonstrate the applicability of this mechanism with PKG and PKC activator PMA in the isolated mitochondria.Using isoform specific blockers, they show that PKG + cGMP and PMA stimulate mitochondrial ROS production in a PKC-dependent and mitoK_{ATP}-dependent manner. The authors propose that electrons are passed to ubiquinone from complex I (NADH:ubiquinone oxidoreductase) of the electron transport chain. The increase of matrix pH causes retardation of the electron flow at the FMNH₂ or FMN semiquinone sites. This results in the increase in reduction at the flavin site and consequent increase in steady-state superoxide production.

Overall, this study offers a provocative mechanism by which ROS are generated with the opening of the mitoK_{ATP} either directly with the use of pharmacological openers or signaling pathway(s) involving activation of PKG and PKC. Obviously, one of the limitations of this study is that it was performed in vitro in the isolated heart and liver mitochondrial preparations. Therefore, it is far too soon to make predictions of the relevance of these findings in explaining the mechanism of ROS generation in vivo with the cardioprotective modalities, including ischemic and pharmacological preconditioning. Clearly, future investigations are needed to address this important issue.

REFERENCES


