

ACTIVATION OF PROTEIN KINASE C PROMOTES MITOCHONDRIAL TRANSLOCATION OF ATP-SENSITIVE K⁺ CHANNELS

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Activation of ATP-sensitive potassium (K_{ATP}) channels could protect the heart under metabolic stress. Importantly, the modulation of K_{ATP} channels accounts for the ability of brief ischemia and reperfusion to protect the heart against infarction induced by subsequent prolonged ischemia, a phenomenon known as ischemic preconditioning (IPC). Not only do molecularly-defined cell surface K_{ATP} channels (Kir6.2 and SUR2A, sarcK_{ATP}) in the heart activate upon metabolic stress and cause action potential shortening and less energy consumption, K_{ATP} channels in the mitochondrial inner membrane are also implicated in cardioprotection during ischemia. However, despite immense interest and serious attempts to clone mitochondrial K_{ATP} (mitoK_{ATP}) channels, their molecular nature remains elusive.

Since the sarcK_{ATP} shares many features of the mitoK_{ATP} in pharmacology and biophysics, Kir6.2-containing K_{ATP} channels may be simultaneously targeted to both sarcolemmal and mitochondrial inner membrane after synthesis in the cytoplasm, with distinct properties depending on the specific environment that the channel resides in. Given that ion channel activity depends critically on the number of the functional channel, we tested the hypothesis that PKC, a central mediator in IPC, may promote mitochondrial translocation of Kir6.2-containing K_{ATP}. Mitochondrial localization of Kir6.2-containing K_{ATP} channels was analyzed from the K_{ATP} channel-deficient COS-7 cells transiently transfected with Kir6.2 and SUR2A by western blot and fluorescence microscopy. The phorbol ester, Phorbol 12-myristate 13-acetate (PMA) was used to stimulate endogenous PKC. We found that there was significant increase in Kir6.2 protein level ($55.8 \pm 10.27\%$) in mitochondrial fraction after PMA (100 nM) treatment as compared to vehicle treated group, and this increase was inhibited by selective PKC inhibitor chelerythrine. Fluorescence imaging of either COS-7 cells or mitoplasts (mitochondria devoid of outer membrane) shows significant colocalization of Kir6.2 with the mitochondrial marker MitoTracker. Mitochondrial localization of Kir6.2 is further enhanced by PMA treatment (percentage of cells showing significant colocalization: $28.7 \pm 1.0\%$ in control vs 55.5 ± 3.6 in PMA, 100 cells in each group). We conclude that the K_{ATP} channel pore-forming subunit Kir6.2 is indeed localized in mitochondria and this content in mitochondria is increased by PKC activation. PKC-regulated mitochondrial translocation of K_{ATP} channels may have significant implication in cardioprotection of IPC.