ATP-sensitive K^+Channel Attenuates Mitochondrial Superoxide Production Induced by Ischemia Reperfusion

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Background: Reactive oxygen species have been implicated in ischemic dysfunction. Ischemic preconditioning (IPC) mechanism may protect the heart against ischemia/reperfusion (I/R)-induced injuries, in which ATP-sensitive K^+ (K_{ATP}) channels play as an effector. We investigated the hypothesis that mitochondrial K_{ATP} channels may balance the massive increase of superoxide during reperfusion, thus reduce both nucleus and mitochondrial DNA damages.

Methods and results: Single rat ventricular myocytes isolated by Langendorff system with collagenase were subjected to 25 min anoxic perfusion and then 45 min of reoxygenation (I/R group, n=5), or which were intermittently preconditioned twice for 5 min in an ischemic solution (IPC group, n=5), 100μM diazoxide (Diaz group, n=5) prior to I/R episodes. To monitor the change of mitochondrial superoxide, cells were stained with MitoSOX and visualized under fluorescence confocal microscope. Infarction and viable areas were separated by TTC staining. Oxidative damage of nucleus DNA was measured by comet assay. Mitochondrial DNA damage was evaluated by sizing the DNA fragmentation.

Our results showed that IPC reduced superoxide production during reperfusion in comparison with I/R. Diazoxide playedthe same effect as IPC to reduce DNA damage. Pretreated with 100uM diazoxide reduced H_2O_2-induced superoxide from 171±32% to 111±8.6% (baseline: 100%). We observed that the

mitochondrial DNA damage were significantly different between infarction and viable areas.

Conclusions: mitochondrial K_{ATP} channels might play an important role in IPC phenomenon by reducing superoxide production during reperfusion episode. The balance of these reactive oxygen species, therefore, is crucial for the protection the heart from oxidative stress. This will be the first direct evidence showing the change of superoxide during ischemia and reperfusion and mitoDNA oxidative damage in infarction/viable areas.

Key words: Ischemic Preconditioning, Mitochondrial DNA Oxidative Damage, Superoxide