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Attenuating Effect of Angiotensin-(1-7) on Angiotensin II-Mediated Reactive Oxygen Species Induced Apoptosis Through Regulation of Mitochondrial NOX4

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Background: Angiotensin II (Ang II)-mediated reactive oxygen species (ROS) are important second messengers for the transcriptional effects of Ang II, and NOX4 is the central enzyme of Ang II-induced ROS. Recent evidence suggests mitochondrial NOX4 has a remarkable role. In this study, we examined the hypothesis that angiotensin-(1-7) (Ang-(1-7)) attenuates Ang II-induced mitochondrial NOX4 mediated ROS injury in proximal tubular epithelial cells.

Methods: The normal rat kidney tubular epithelial cells (NRK-52E) were cultured, and then stimulated with Ang II (10-6M) with or without pre-incubation with 10-6M of Ang-(1-7). The mitochondrial NOX4 activation was determined to isolation of subcellular fraction by Western blotting. Intracellular ROS generation was measured using DCF-DA and MitoSOX. Mitochondrial membrane potential ($\Delta\Psi$) was detected using JC-1 by flow cytometry and confocal microscopy. Apoptosis was measured using a TUNEL assay and FITC-Annexin V staining.

Results: The mitochondrial and membrane NOX4 were activated in response to Ang II stimuli for 24 hours, however, pre-incubation of Ang-(1-7) inhibited both activation of NOX4. Pre-incubation with Ang-(1-7) in addition to Ang II significantly inhibited the Ang II-induced ROS production as the level of control. Ang-(1-7) attenuated the Ang II induced depolarization of mitochondrial membrane potential, and release of AIF and cytochrome C from mitochondria to cytosol. Ang II -induced apoptotic cell death was attenuated by Ang-(1-7).

Conclusion: Ang-(1-7) attenuated the Ang II-stimulated activation of NOX4 in both mitochondria and membrane. These findings ware related to improved mitochondrial dysfunction and apoptosis in response to Ang II and suggest that Ang-(1-7) may attenuate Ang II-stimulated ROS-mediated apoptosis NRK-52E cells.

Key Words: NOX4, Ang-(1-7), Ang II, ROS
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