Role of reactive oxygen species-sensitive extracellular signal-regulated kinase pathway in angiotensin II-induced endothelin-1 gene expression in vascular endothelial cells

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摘要

Abstract

Background: Circulating angiotensin II (Ang II) increases vascular endothelin-1 (ET-1) tissue levels, which in turn mediate a major part of Ang II-stimulated vascular growth and hypertension in vivo. Ang II also stimulates the generation of reactive oxygen species (ROS) within vascular endothelial cells. However, whether ROS are involved in Ang II-induced ET-1 gene expression, and the related intracellular mechanisms occurring within vascular endothelial cells remain unclear. *Methods:* Cultured endothelial cells were stimulated with Ang II, and the thus elicited ET-1 gene expression was examined by Northern blotting and a promoter activity assay. Antioxidant pretreatment of endothelial cells was performed prior to Ang II-induced extracellular signal-regulated kinase (ERK) phosphorylation in order to elucidate the redox-sensitive pathway for ET-1 gene expression. *Results:* The ET-1 gene was induced with Ang II, which was inhibited with Ang II type 1 receptor antagonist (irbesartan). Ang II-enhanced intracellular ROS levels were inhibited by irbesartan and several antioxidants, and antioxidants also suppressed Ang II-induced ET-1 gene expression. Further, Ang II-activated ERK phosphorylation was also significantly inhibited by certain antioxidants. An ERK inhibitor, U0126, inhibited Ang II-induced ET-1 expression completely. Cotransfection of the dominant negative mutant of Ras, Raf and MEK1 (ERK kinase) attenuated the Ang II-enhanced ET-1 promoter activity, suggesting that the Ras/Raf/ERK pathway is required for Ang II-induced ET-1 gene expression. Ang II-induced activator protein-1 (AP-1) reporter activities were inhibited by antioxidants. Moreover, mutational analysis of the ET-1 gene promoter showed that the AP-1 binding site was an important *cis* element in Ang II-induced ET-1 gene expression. *Conclusions:* Our data suggest that ROS are involved in Ang II-induced ET-1 gene

expression within endothelial cells. The redox-sensitive ERK-mediated AP-1 transcriptional pathway plays an important role in Ang II-induced ET-1 gene expression.