Crucial Role of Extracellular Signal-regulated Kinase Pathway in Reactive Oxygen Species-mediated Endothelin-1 Gene Expression Induced by Endothelin-1 in Rat Cardiac Fibroblasts.

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摘要

Abstract

Endothelin-1 (ET-1) has been implicated in fibroblast proliferation. However, the mechanism involving ET-1 is not clear. The present study was performed to examine the role of endogenous ET-1 in ET-1 – stimulated fibroblast proliferation and to investigate the regulatory mechanism of ET-1 – induced ET-1 gene expression in cardiac fibroblasts. Both ETA receptor antagonist [(hexahydro-1H-azepinyl)carbonyl-Leu-d-Trp-d-OH (BQ485)] and endothelin-converting enzyme inhibitor (phosphoramidon) inhibited the increased DNA synthesis caused by ET-1. ET-1 gene was induced by ET-1, as revealed with Northern blotting and ET-1 promoter activity assay. ET-1 increased intracellular reactive oxygen species (ROS), which were significantly inhibited by BQ485 and antioxidants. Antioxidants suppressed ET-1 gene expression and DNA synthesis stimulated by ET-1. ET-1 activated mitogen-activated protein kinases (MAPK), including extracellular signal-regulated kinase (ERK), p38 MAPK, and c-Jun N-terminal kinase, which were significantly inhibited by antioxidants. Only ERK inhibitor U0126 could inhibit ET-1 – induced transcription of the ET-1 gene. Cotransfection of dominant-negative mutant of Ras, Raf, and MEK1 decreased the ET-1 – induced increase in ET-1 transcription, suggesting that the Ras-Raf-ERK pathway is required for ET-1 action. Truncation and mutational analysis of the ET-1 gene promoter showed that the activator protein-1 (AP-1) binding site was an importantcis-element in ET-1 – induced ET-1 gene expression. Antioxidants attenuated the ET-1 – stimulated AP-1 binding activity. Our data

suggest that ROS were involved in ET-1 – induced fibroblast proliferation and mediated ET-1 – induced activation of ERK pathways, which culminated in ET-1 gene expression