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论著

PKCζ与Raf在Ang Ⅱ引起的大鼠血管平滑肌细胞ERK1/2活化中的作用

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摘要

目的：研究血管紧张素II（Ang Ⅱ）诱导大鼠血管平滑肌细胞（VSMC）肥大的信号转导途径中PKCζ与Raf的作用关系。方法：［^{3}H］-亮氨酸掺入反映VSMC蛋白质合成；Western blotting检测ERK1/2和PKCζ表达；免疫共沉淀实验检测信号分子间的相互作用。结果：Ang Ⅱ刺激可引起VSMC［^{3}H］-亮氨酸掺入显著增加，PKC非特异性抑制剂和PKCζ假底物抑制剂（PS-PKCζ）均明显抑制Ang Ⅱ引起的作用。PS-PKCζ预处理使Ang Ⅱ刺激VSMC的ERK1/2磷酸化水平明显降低。转染dominant negative Raf（Raf S621A）质粒的VSMC中的PKCζ磷酸化水平与转染野生型Raf质粒无明显差异。Ang Ⅱ刺激使Ras与Raf结合增加，但PKCζ抑制剂不影响Ang Ⅱ引起的Raf与Ras的结合。转染Raf S621A抑制Raf活化后，Ang Ⅱ引起的ERK1/2磷酸化水平降低。结论：在VSMC中，PKCζ亚型参与Ang Ⅱ诱导的VSMC蛋白合成，但PKCζ可能通过非依赖Raf的途径激活ERK1/2。

关键词 血管平滑肌细胞； 蛋白激酶C 血管紧张素Ⅱ； 信号转导

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Interaction of PKCζ and Raf in the activation of ERK1/2 in rat vascular smooth muscle cells induced by Ang Ⅱ

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Abstract

<P><FONT face=Verdana>AIM: To investigate the crosstalk of PKCζ isoform with Raf in the signal pathway of vascular smooth muscle cell (VSMC) hypertrophy induced by </FONT><FONT face=Verdana>angiotensin II (Ang Ⅱ).METHODS: The protein synthesis of VSMCs was measured by［³H］-thymidine incorporation. The expression of PKCζ and ERK1/2 proteins were detected by Western </FONT><FONT face=Verdana>blotting. The interaction of the signal molecules was examined by immunoprecipitation.RESULTS: Pretreatment of VSMCs with PKC non-specific inhibitor staurosporine or PKCζ pseudosubstrate inhibitor (PS-PKCζ), the Ang Ⅱ-induced［³H］-</FONT><FONT face=Verdana>thymidine incorporation into VSMCs was decreased markedly. PS-PKCζ pretreatment significantly decreased phosphorylation of ERK1/2 induced by Ang Ⅱ. </FONT><FONT face=Verdana>Compared with VSMCs transfected with wild type Raf, PKCζ phosphorylation was similar in the VSMCs transfected with dominant negative Raf (Raf S621A). </FONT><FONT face=Verdana>Immunoprecipitation analysis showed that Ang Ⅱ stimulated the association of Ras with Raf, but PKCζ inhibitor had no influence on Ang Ⅱ-induced </FONT><FONT face=Verdana>conjugation of Ras with Raf. After Raf activity was inhibited by Raf S621A, Ang Ⅱ-induced ERK1/2 phosphorylation level declined.
CONCLUSION: These results suggest that PKCζ is involved in protein synthese induced by Ang Ⅱ in VSMCs, but PKCζ induces ERK1/2 activation via a Raf-</FONT><FONT face=Verdana>independent pathway. </FONT></P>

Key words Vascular smooth muscle cells Protein kinase C Angiotensin Ⅱ Signal transduction

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