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论著

CRE-decoy ODN对慢性吗啡诱导SK-N-SH细胞CCK及fosB mRNA表达上调的抑制作用

苏彦君,丛斌△,张国忠,张瑾,姚玉霞,李淑瑾,付丽红

河北医科大学法医学教研室， 河北 石家庄 050017

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摘要 目的：研究以转录因子cAMP反应元件结合蛋白 (CREB) 为靶点的 CRE-decoy ODN对慢性吗啡诱导SK-N-SH细胞胆囊收缩素 (CCK) 及fosB mRNA表达上调的抑制作用。 方法： 体外合成含cAMP反应元件CRE序列TGACGTCA的单链寡核苷酸，将自身杂交形成发卡结构。将终浓度为150 nmol/L的CRE-decoy ODN与SK-N-SH细胞孵育1 h后，加入终浓度为100 μmol/L的吗啡作用48 h，随后加入终浓度为10 μmol/L的纳络酮，戒断15 min。采用电泳迁移率改变分析 (EMSA) 检测CRE-decoy ODN与CREB结合的序列特异性及其对慢性吗啡诱导的CREB的DNA结合活性升高的影响；细胞掺入的CRE-decoy ODN 用酚：氯仿法提取，经20%非变性聚丙烯酰胺凝胶电泳及放射自显影检测；采用RT-PCR检测CCK及fosB mRNA表达。 结果： 慢性吗啡作用及纳络酮急性戒断使SK-N-SH细胞CREB的DNA结合活性、CCK和fosB mRNA表达明显升高，CRE-decoy ODN可特异抑制其升高。 结论： CRE-decoy ODN通过特异抑制慢性吗啡诱导SK-N-SH细胞CREB的DNA结合活性而下调CCK及fosB mRNA表达。

关键词 吗啡； SK-N-SH细胞； CRE-decoy oligodeoxynucleotide 胆囊收缩素； 基因,fosB

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Inhibitory effect of CRE-decoy ODN on the upregulation of CCK and fosB mRNA induced by chronic morphine administration in SK-N-SH cells

SU Yan-jun, CONG Bin, ZHANG Guo-zhong, ZHANG Jin, YAO Yu-xia, LI Shu-jin, FU Li-hong

Department of Fornesic Science， Hebei Medical University， Shijiazhuang 050017，China

Abstract

<FONT face=Verdana>AIM: To investigate the inhibitory effects of a synthetic CRE-transcription factor decoy oligodeoxynucleotide (CRE-decoy ODN) on the upregulation of the expression of cholecystokinin (CCK) and fosB mRNA induced by chronic morphine administration in SK-N-SH cells. METHODS: The CRE cis-element, TGACGTCA， was palindromic， a synthetic single-stranded phosphorothioate oligodeoxynucleotide composed of the CRE sequence self-hybridizes to form a duplex/hairpin. The CRE-palindromic decoy and control oligodeoxynucleotides were added to the medium (1 h before exposure to morphine) at 150 nmol/L in the presence of cationic lipid DOTAP. After the cells were treated with 100 μmol/L morphine for 48 h， 10 μmol/L naloxone was used for 15 min. The effects of CRE-decoy ODN on the DNA-binding activity of CREB, the expression of CCK and fosB mRNA were detected by electrophoresis mobility shift assay (EMSA) and RT-PCR, respectively. The stability of cell-incorporated ［32P］-labeled CRE-decoy ODN was extracted with phenol:chloroform and then subjected to 20% nondenaturing polyacrylamide gel electrophoresis and autoradiography. RESULTS: Chronic morphine administration and acute naloxone-precipitated withdrawal significantly activated the DNA-binding activity of CREB and the expression of CCK and fosB mRNA in SK-N-SH cells. The CRE-decoy ODN penetrated into the cells， specifically downregulated these indexes. CONCLUSIONS: CRE-decoy ODN can significantly downregulates the expression of CCK and fosB mRNA through specifically suppressing the DNA-binding activity of CREB activated by chronic morphine administration in SK-N-SH cells.</FONT>

Key words Morphine SK-N-SH cells CRE-decoy oligodeoxynucleotide Cholecystokinin Genes

fosB

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通讯作者 丛斌 bincong@263.net