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论著

高表达受体活性修饰蛋白1对血管紧张素Ⅱ和降钙素基因相关肽诱导的A10细胞降钙素受体样受体膜分布的影响

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摘要 目的 探索受体活性修饰蛋白1(RAMP1)对血管紧张素Ⅱ(AngⅡ)和(或)降钙素基因相关肽(CGRP)诱导的降钙素受体样受体(CRLR)在血管平滑肌细胞(VSMC)的表达和分布的影响，进一步揭示CGRP抑制VSMC增殖的机制。方法 通过酶切、连接、转化等方法构建pCDNA3.1(+)-RAMP1真核表达载体并稳定转染至鼠源性血管平滑肌细胞株A10中，获得稳定高表达RAMP1的细胞系。无转染细胞、转染空载体[pCDNA3.1(+)]细胞和RAMP1高表达组细胞[pCDNA3.1(+)-RAMP1]分别用AngⅡ 100 nmol·L^{-1}、CGRP 100 nmol·L^{-1}和CGRP+AngⅡ处理24 h，用MTT法检测细胞存活；逆转录PCR、Western印迹和免疫荧光法分别检测CRLR mRNA含量、蛋白表达及细胞膜分布。结果 单纯转染空质粒或RAMP1对细胞增殖无明显影响。AngⅡ处理对3种细胞存活的影响无显著差异。pCDNA3.1(+)-RAMP1细胞经CGRP处理24 h后，细胞存活明显高于其他两组细胞(P<0.05)；经CGRP+AngⅡ处理，细胞存活明显低于其他两组(P<0.05)。AngⅡ，CGRP和CGRP+AngⅡ处理对3种细胞CRLR mRNA表达无明显影响，但CGRP处理使pCDNA3.1(+)-RAMP1细胞中CRLR蛋白明显高于其他两种细胞(P<0.05)，而CGRP+AngⅡ处理使pCDNA3.1(+)-RAMP1细胞中CRLR蛋白明显低于其他两种细胞(P<0.05)。免疫荧光结果显示，经无血清或CGRP处理后，无转染细胞和pCDNA3.1(+)细胞中的RAMP1和CRLR主要分布在胞浆区域；经AngⅡ或CGPR+AngⅡ处理后，pCDNA3.1(+)-RAMP1细胞中RAMP1和CRLR在细胞膜上的分布多于无转染细胞和pCDNA3.1(+)细胞。结论 高表达RAMP1能增强CGRP抑制AngⅡ诱导的血管平滑肌细胞增殖，其机制可能是通过RAMP1增加CRLR的膜分布，从而增强CGRP受体对CGRP的敏感性有关。

关键词 血管紧张素Ⅱ 降钙素基因相关肽 降钙素受体样受体 受体活性修饰蛋白1

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Effect of overexpression of RAMP1 on membrane distribution of CRLR induced by angiotensinⅡ and calcitonin gene-related peptide in A10 cell line

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Abstract

OBJECTIVE To investigate the effect of overexpression of receptor activity modifying protein 1 (RAMP1) on distribution of the calcitonin receptor like receptor (CRLR) in vascular smooth muscle cell (VSMC) in order to reveal the antiproliferative mechanism of calcitonin gene-related peptide(CGRP) for VSMC. **METHODS** pCDNA3.1(+)-RAMP1 eukaryon expression vector was successfully constructed by digestion, ligation, transform and transfected to the mouse VSMC cell line A10. After that the normal cells, pCDNA3.1(+) cells and pCDNA3.1(+)-RAMP1 cells were treated by AngⅡ, CGRP and CGRP+AngⅡ for 24 h. The proliferation of cell line A10 was determined by MTT assay while mRNA and proteins levels of CRLR and RAMP1 were determined by RT-PCR and Western blotting, respectively. The distribution of RAMP1 and CRLR in cell line A10 was observed by immunofluorescence. **RESULTS** Proliferation was not significant in three kinds of cells treated by 0.1%

FBS or Ang Ⅱ. Proliferation in RAMP1 overexpression cell higher than in normal cells and the pCDNA3.1(+) cells treated by CGRP groups(P<0.05), but lower than treated by CGRP+Ang Ⅱ (P<0.05). Cells treated with CGRP and Ang Ⅱ decreased the CRLR proteins expression in RAMP1 overexpression group (P<0.05) while the difference of mRNA levels of CRLR in each group had no significance. However, after treated with 0.1%FBS or CGRP, the RAMP1 and CRLR proteins were distributed into cytoplasm in normal cells and pCDNA3.1(+) cells, but the membrane distribution of RAMP1 and CRLR in pCDNA3.1(+)-RAMP1 cells were higher than that of normal and pCDNA3.1(+)