

论文

M₃受体对体外H₂O₂诱导大鼠心肌细胞凋亡的保护作用

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摘要:

目的探讨M₃受体激动对H₂O₂诱导的大鼠培养心肌细胞凋亡的作用,进一步阐明其机制。方法末端标记法(TUNEL)进行细胞凋亡检测;免疫组化方法检测Bcl-2和Fas的表达;共聚焦显微镜观察[Ca²⁺]_i荧光强度变化。结果M₃受体激动剂胆碱(10 mmol·L⁻¹)可减少H₂O₂诱导的心肌细胞凋亡的数量,并可增加心肌Bcl-2的表达,减少Fas表达,抑制H₂O₂诱导的[Ca²⁺]_i荧光强度的升高。但预先应用4DAMP(10 nmol·L⁻¹)阻断M₃受体可逆转胆碱作用。结论激动M₃受体对H₂O₂诱导的心肌细胞凋亡有保护作用,其机制可能与Bcl-2和Fas表达以及下调[Ca²⁺]_i有关。

关键词: M₃受体 细胞凋亡 培养的心肌细胞 过氧化氢 原位缺口末端标记 共聚焦显微镜 钙

Protective effect of M₃ receptor on H₂O₂-induced apoptosis of rat myocardial cells *in vitro*

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Abstract:

AimTo observe the effect of activation of M₃ receptor on H₂O₂ induced apoptosis in cultured rat myocytes and to investigate its possible mechanisms. MethodsIsolated neonatal cardiomyocytes were cultured. Morphologic changes were observed by microscopy. The apoptosis in cardiomyocyte was detected by terminal deoxynucleotide transferase directed d-UTP nick and end labeling (TUNEL) assay. The expression of apoptosis-related protein in Bcl-2 and Fas was measured by immunohistochemistry assay. [Ca²⁺]_i in single cardiomyocyte loaded with Fluo 3-AM was measured by confocal microscope. ResultsH₂O₂-mediated myocyte apoptosis was attenuated by M₃ receptor agonist choline (10 mmol·L⁻¹). Pretreatment of cardiac myocytes with choline also increased Bcl-2, decreased Fas expression, and inhibited the increase in FI value of [Ca²⁺]_i in H₂O₂-stimulated cardiac myocytes. However, blockade of M₃ receptor by 4DAMP (10 nmol·L⁻¹) completely inhibited the effects of choline on H₂O₂-stimulated cardiac myocytes. ConclusionActivation of M₃ receptor showed protective effect on H₂O₂-induced apoptosis in cultured rat myocytes and this effect might be related to modulating the expression of some genes including Bcl-2 and Fas as well as the downregulation of [Ca²⁺]_i.

Keywords: apoptosis cultured myocyte hydrogen peroxide *in situ* nick-end labeling confocal microscope calcium M₃ receptor

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