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论著

NADPH氧化酶亚单位nox-1在心肌细胞急性缺氧复氧损伤时的变化及心肌营养素-1的作用

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摘要 目的: 探讨心肌细胞急性缺氧复氧损伤时NADPH氧化酶亚单位nox-1的变化及心肌营养素-1的作用。方法: 用改良的方法培养出生1-3 d的乳鼠心肌细胞，分为6组：(1)对照组；(2)缺氧复氧组；(3)缺氧复氧+CT-1组；(4)缺氧复氧+CT-1+LY294002组 (PIK3/Akt 阻断剂)；（5）缺氧复氧+CT-1+PD98059组（ERK 阻断剂）；（6）缺氧复氧+CT-1+助溶剂DMSO组。 CT-1 的浓度为10 μg/L。MTS法测定心肌细胞的存活率，四氯四乙基苯丙咪唑基羰化青碘化物（JC1）检测心肌细胞线粒体膜电位(Δψm)，二氯荧光黄双乙酸盐（DCFH-CA）检测细胞活性氧（ROS），流式细胞仪检测心肌细胞凋亡率。Nox-1蛋白采用Western blotting检测。结果: 缺氧复氧培养后心肌细胞凋亡率及细胞内ROS较对照组明显增加，分别是(19.7%±1.4% vs 2.1%±0.5%, 14.07%±1.25% vs 3.54%±0.86%, P<0.05)，而心肌细胞存活率显著降低，线粒体膜电位（Δψm）下降；nox-1表达明显升高。CT-1处理的心肌细胞，较缺氧复氧组心肌细胞存活率明显上升(87.0%±7.3%),而心肌细胞凋亡率及细胞内ROS 显著减少，Δψm水平增加，nox-1蛋白表达下调。而CT-1的这些作用能被PIK3/Akt和ERK阻断剂抑制。结论: 心肌细胞急性缺氧复氧损伤时NADPH氧化酶亚单位nox-1表达上调，而心肌营养素-1能通过下调nox-1表达，发挥对心肌细胞保护作用。

关键词 Nox-1 心肌细胞 缺氧复氧 心肌营养素-1

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Change of subunit of NADPH oxidation enzyme complex nox-1 protein in cardiocyte hypoxia-reoxygenation injury and the role of cardiotrophin-1

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Abstract

<FONT face=Verdana>AIM: To observe the change of subunit of NADPH oxidation enzyme complex nox-1 protein in cardiocyte hypoxia-reoxygenation injury and the role of cardiotrophin-1.METHODS: Cardiomyocytes from the hearts of 1-3 d old neonatal rats were prepared by a modified method. Five groups were included in the study: control; hypoxia/reoxygenation; hypoxia/reoxygenation+CT-1; CT-1+hypoxia/reoxygenation+LY294002 (PIK3/Akt inhibitor); CT-1+hypoxia/reoxygenation+PD98059 (ERK inhibitor); CT-1+hypoxia/reoxygenation+DMSO. The concentration of CT-1 was 10 μg/L. The survival rate of myocytes was evaluated by MTS method. Apoptosis, mitochondrial permeability transition pore (Δψm) and reactive oxygen species (ROS) were detected by flow cytometry. Nox-1 protein was determined by Western blotting.RESULTS: Apoptosis of cardiomyocytes and the level of ROS (19.7%±1.4% vs 2.1%±0.5%, 14.07%±1.25% vs 3.54%±0.86%, P<0.05) increased markedly after hypoxia/reoxygenation, but cardiomyocyte survival rate and the level of Δψm (40.55%±4.25% vs 86.28%±7.15%, P<0.01) decreased significantly. The expression of nox-1 protein was upregulated markedly. With CT-1 intervention, cardiomyocyte survival rate increased markedly, apoptosis, both ROS and expression of nox-1 protein reduced significantly. The level of Δψm increased obviously. The effect of CT-1 was inhibited by LY294002. No significant effect was observed on cells survival in DMSO group, which confirmed that LY294002 was specifically involved in blocking the protective effect of CT-1.CONCLUSION: The expression of subunit of NADPH oxidation enzyme complex nox-1 protein is upregulated markedly in cardiocyte hypoxia-reoxygenation injury. CT-1 protects

cardiac cells against hypoxia-reoxygenation injury by downregulating the expression of nox-1 protein to decrease the level of ROS.</FONT>

Key words Nox-1 Cardiomyocytes Hypoxia-reoxygenation Cardiotrophin-1

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