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H_2O_2致WB-F344细胞内活性氧的产生及机理

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以双氢罗丹明123(DHR123)作为荧光探针，采用激光共聚焦扫描显微镜研究小剂量(800 nmol/L)H2O2诱导大鼠肝卵圆细胞株WB-F344细胞内活性氧产生的动态变化过程及其机理。 结果发现： (1)小剂量H_2O_2的一次作用可以引起胞内活性氧的产生； (2)胞内活性氧清除剂N-乙酰-L-半胱氨酸(NAC)处理2 h时后， 再加入小剂量H_2O_2，发现胞内活性氧的产生明显减少；(3)用广谱的蛋白激酶抑制剂2-氨基嘌呤（2-AP）、Ca^{2+}依赖性蛋白激酶(PKC)抑制剂Bisindolylmaleimide I、酪氨酸蛋白激酶(TPK)抑制剂Tyrphostin 25分别预处理15 min后，H_2O_2诱导的胞内活性氧的产生现象均消失；(4)细胞在无外钙环境下，小剂量H_2O_2诱导的胞内活性氧的产生明显减少；(5)细胞在无外钙环境下用NAC预处理后，H_2O_2诱导的胞内活性氧的产生现象消失。结果表明， H_2O_2可以通过胞内信号转导系统诱使WB细胞胞内活性氧产生，这可能与小剂量H_2O_2调控细胞生物学功能(如增殖、转化)相关。

EFFECTS OF HYDROGEN PEROXIDE ON PRODUCING INTRACELLULAR ROS OF WB-344 RAT LIVER EPITHELIAL CELL

To study the role and mechanism of hydrogen peroxide(To study the role and mechanism of hydrogen peroxide(H_2O_2) on intracellular reactive oxygen species(ROS) generation of WB-F344 rat liver epithelial cell, DHR123 was used as fluorescence probe to detect the intracellular ROS generation and observed on laser scanning confocal microscope. The results showed that: (1)The generation of intracellular ROS of the cell stimulated by H_2O_2 increased slowly and gradually. (2)The intracellular ROS production stimulated by H_2O_2 decreased when pretreated with ROS scavenger NAC. (3)There were no production of intracellular ROS pretreated with 2-Aminop-urine, a broader spectrum protein kinase inhibitor, or pretreated with Tyrphostin 25, a specific inhibitor of tyrosine protein kinase, or with Bisindolylmaleimide I, a PKC inhibitor. (4)The intracellular ROS could still be generated but the level decreased slightly by incubated in D-Hanks solution containing EG-TA, the chelate of extracellular Ca^{2+}. (5)There were no generation of intracellular ROS pretreated with NAC and incubated WB cell in D-Hanks solution containing EGTA. These results suggested that the pathway of intracellular ROS generation stimulated by H_2O_2 may be related to PKC, TPK and Ca^{2+} signal transduction.) on intracellular reactive oxygen species(ROS) generation of WB-F344 rat liver epithelial cell, DHR123 was used as fluorescence probe to detect the intracellular ROS generation and observed on laser scanning confocal microscope. The results showed that: (1)The generation of intracellular ROS of the cell stimulated by H_2O_2 increased slowly and gradually. (2)The intracellular ROS production stimulated by H_2O_2 decreased when pretreated with ROS scavenger NAC. (3)There were no production of intracellular ROS pretreated with 2-Aminop-urine, a broader spectrum protein kinase inhibitor, or pretreated with Tyrphostin 25, a specific inhibitor of tyrosine protein kinase, or with Bisindolylmaleimide I, a PKC inhibitor. (4)The intracellular ROS could still be generated but the level decreased slightly by incubated in D-Hanks solution containing EG-TA, the chelate of extracellular Ca^{2+}. (5)There were no generation of intracellular ROS pretreated with NAC and incubated WB cell in D-Hanks solution containing EGTA. These results suggested that the pathway of intracellular ROS generation stimulated by H_2O_2 may be related to PKC, TPK and Ca^{2+} signal

transduction.

关键词

过氧化氢(Hydrogen peroxide); 胞内活性氧(Intracellular reactive oxygen species generatio); 信号转导(Signal transduction)