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论著

亚硝基谷胱甘肽对大鼠肝微粒体谷胱甘肽转移酶的激活及机制

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摘要 目的 探索一氧化氮供体亚硝基谷胱甘肽(GSNO)能否在体外通过*S*-亚硝酰化机制激活大鼠肝微粒体谷胱甘肽转移酶(mGST)。方法 微粒体粗提物与GSNO体外共孵育，测定mGST催化动力学改变，结合*N*-乙基马来酰亚胺（NEM）再激活实验和二巯基苏醇（DTT）逆转实验，以及酶蛋白游离巯基和酶*S*-亚硝酰化蛋白的改变，研究酶的激活机制。结果 GSNO在0.125～2 mmol·L^{-1}浓度范围内呈浓度和时间(3～15 min)依赖性地激活mGST，NEM对酶的再激活效应消失，DTT可以逆转上述激活作用，同时酶蛋白游离巯基浓度依赖性减少，而*S*-亚硝酰化蛋白浓度依赖性增多。结论 GSNO体外可激活大鼠肝mGST，激活机制可能与mGST第49位半胱氨酸(Cys^{49})的巯基被亚硝酰化形成*S*-亚硝基硫醇结构有关。

关键词 微粒体 谷胱甘肽转移酶类 酶激活 亚硝基谷胱甘肽

分类号 R963

Mechanism of the activation of rat liver microsomal glutathione transferases by nitrosoglutathione in vitro

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Abstract

AIM To explore if nitrosoglutathione(GSNO) can activate rat liver microsomal glutathione transferases(mGST) *in vitro* via cysteine *S*-nitrosylation. **METHODS** Partially purified mGST was incubated with GSNO *in vitro*, kinetic parameters of mGST were measured. *N*-ethylmaleimide(NEM) reactivation and dithiothreitol(DTT) reversibility tests were performed, combined with the changes of free sulfhydryl group and *S*-nitrosylated protein, to demonstrate the relevant mechanism. **RESULTS** Rat mGST was activated by GSNO in a concentration and time dependent manner, within the concentrations of 0.125－2 mmol·L^{-1}. NEM failed to reactivate the GSNO pretreated mGST, while DTT almost completely reversed the effect of GSNO. After incubated with GSNO, free sulfhydryl group in partially purified mGST significantly reduced, and *S*-nitrosylated protein increased, both in a concentration- dependent manner. **CONCLUSION** Rat liver microsomal glutathione transferases could be activated by GSNO, possibly *via S*-nitrosylation of the single cysteine(Cys^{49}) in mGST.

Key words microsomes glutathione transferases enzyme activation nitrosoglutathione

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