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论著

二甲双胍对葡萄糖-6-磷酸酶基因表达的抑制作用及其机制

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摘要 目的 探讨二甲双胍对葡萄糖-6-磷酸酶(G6Pase)基因表达作用及其分子机制。方法 应用稳定表达G6Pase的鼠肝细胞瘤H4ⅡE M1.3细胞，一组细胞分别给予二甲双胍0.1~5.0 mmol·L^{-1}孵育16 h；另一组细胞先加入化合物C 20 μmol·L^{-1}，Bay11-7085 5 μmol·L^{-1}或雷帕霉素25 nmol·L^{-1}作用30 min后，再加入二甲双胍2 mmol·L^{-1}共育16 h，采用荧光素酶报告基因检测方法测定G6Pase基因表达水平；细胞加入化合物C 20 μmol·L^{-1}作用30 min后，再分别加入二甲双胍2 mmol·L^{-1}、5-氨基-4-甲酰胺咪唑核糖核苷酸(AICAR)1 mmol·L^{-1}孵育15 min，Western印迹法检测腺苷酸活化蛋白激酶(AMPK)蛋白表达及其磷酸化水平；细胞加入二甲双胍2 mmol·L^{-1}和胰岛素1 μmol·L^{-1}作用15 min，Western印迹法检测蛋白激酶B(Akt)蛋白表达及其磷酸化水平。结果 二甲双胍0.5，1，2和5 mmol·L^{-1}作用16 h可以显著抑制G6Pase基因表达(P<0.05，P<0.01)，二甲双胍0.5和5 mmol·L^{-1}时，分别抑制G6Pase基因表达26%(P<0.05)和85%(P<0.01)。AMPK抑制剂化合物C可部分逆转二甲双胍的抑制作用(P<0.05)；二甲双胍可诱导AMPK磷酸化，与AICAR作用相似，但这一作用可被化合物C抑制。结论 二甲双胍抑制G6Pase基因表达，其作用机制可能与激活AMPK有关，而可能与Akt，雷帕霉素靶蛋白(mTOR)及核因子-κB(NF-κB)介导的通路无关。

关键词 二甲双胍 葡萄糖-6-磷酸酶 蛋白激酶类 分子作用机制

分类号 R977.1

Inhibitory effect of metformin on glucose-6-phosphatase gene expression and its possible mechanism

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Abstract

OBJECTIVE To investigate effect of metformin (Met) on glucose-6-phosphatase(G6Pase) gene expression and its molecular mechanism. **METHODS** H4ⅡE M1.3 cells were incubated with Met 0.1-5.0 mmol·L^{-1}, respectively; cells in another group was preincubated with the AMPK inhibitor Compound C 20 μmol·L^{-1}, Bay11-7085 5 μmol·L^{-1} or Rapamycin 25 nmol·L^{-1} for 30 min, then incubated with Met 2 mmol·L^{-1} for 16 h, respectively. The expression levels of G6Pase were detected by luciferase assay. After either Met 2 mmol·L^{-1} or 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR) 1 mmol·L^{-1} for 15 min, cells were cultured with Compound C 20 μmol·$L^{-1}$for 30 min, then phosphorylation of AMP-activated protein kinase (AMPK) was detected by Western blot. After cells were incubated with Met 2 mmol·L^{-1} or insulin 1 μmol·L^{-1} for 15 min, phosphorylation of Akt was detected by Western blotting. **RESULTS** Met 0.5, 1, 2 and 5 mmol·L^{-1} significantly inhibited G6Pase gene expression(P<0.05). The inhibitory rate of Met 0.5 and 5 mmol·L^{-1} on G6Pase promoter activity were 26%(P<0.05) and 85%(P<0.01), respectively, and the inhibitory effect could partly be reversed by Compound C, but not by rapamycin and Bay11-7085. The increase of AMPK phosphorylation by Met was similar to that of AICAR, which could be reversed by the AMPK inhibitor Compound C, while it could not be observed to the effect on Akt phosphorylation. **CONCLUSION** Met can inhibit G6Pase gene expression, and the molecular mechanism of inhibitiory effect may be related to the activitation of AMPK, rather than Akt, mammalian target of rapamycin (mTOR) and NF-κB.

Key words metformin glucose-6-phosphatase protein kinases molecular mechanisms of action

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