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论著

内洋地黄素拮抗剂上调缺血再灌注损伤大鼠心肌细胞膜钠泵亚基的表达

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摘要 目的： 观察内洋地黄素特异性拮抗剂地高辛抗血清对心肌缺血再灌注（MIR）损伤大鼠心肌组织内洋地黄素水平、钠泵活性、线粒体总钙浓度以及钠泵各亚基基因表达的影响，探讨内洋地黄素在心肌缺血再灌注损伤中的作用及其机制。

方法: 将56只雄性SD大鼠随机分成7组，每组8只。假手术对照组（sham）：丝线穿过左冠状动脉前降支，但不结扎；缺血再灌注组（MIR）：结扎左冠状动脉前降支30 min，再灌注45 min；生理盐水组（NS）、维拉帕米组（Ver）、小剂量、中剂量、大剂量地高辛抗血清组（ADA）：于再灌注前5 min经股静脉分别注射生理盐水、维拉帕米5 mg·kg^{-1}、地高辛抗血清8.6 mg·kg^{-1}、 17.3 mg·kg^{-1}、34.5 mg·kg^{-1}，容积均为5 mL·kg^{-1}，5 min内注射完毕，其余同MIR模型组。再灌注结束后，立即取缺血区左室心肌检测心肌匀浆内洋地黄素水平、心肌细胞膜Na+ K+$_{ATP}$酶和Ca2+$_{Mg}$2+$_{ATP}$酶活性、线粒体总钙浓度；分别采用RT-PCR及Western blotting方法和免疫组化方法检测心肌钠泵α_1、α_2、α_3和β_1亚基mRNA及蛋白水平基因表达的改变。结果: 心肌缺血再灌注损伤时，心肌组织内洋地黄素水平明显升高，心肌细胞膜钠泵和Ca2+$_{Mg}$2+$_{ATP}$酶活性显著下降，线粒体总钙浓度升高，钠泵α_1、α_2、α_3和β_1亚基在mRNA及蛋白水平基因表达均明显下降；维拉帕米除具有降低线粒体总钙浓度外，对其它各项指标无明显影响。地高辛抗血清呈剂量依赖性地显著降低心肌组织内洋地黄素水平，恢复细胞膜钠泵和Ca2+$_{Mg}$2+$_{ATP}$酶活性，降低线粒体总钙浓度，上调钠泵α_1、α_2、α_3和β_1亚基mRNA及蛋白水平的基因表达。

结论: 心肌缺血再灌注促进机体内洋地黄素分泌增加，后者通过下调心肌细胞膜上的钠泵α_1、α_2、α_3和β_1亚基基因表达抑制钠泵活性，进而抑制Ca2+$_{Mg}$2+$_{ATP}$酶活性，导致线粒体内钙超载，介导心肌缺血再灌注损伤。内洋地黄素特异性拮抗剂地高辛抗血清通过阻断内洋地黄素的生物学作用，上调钠泵各亚基的基因表达，发挥其抗心肌缺血再灌注损伤的作用。

关键词 内洋地黄素 心肌再灌注损伤 Na(+)K(+)交换ATP酶 抗体,地高辛

分类号 R542.2+2 R965.2

Endoxin antagonist up-regulates gene expression of sodium pump isoforms in rats with myocardial ischemia reperfusion injury

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Abstract

<FONT face=Verdana>AIM: To observe the effect of endoxin antagonist,anti-digoxin antiserum,on endoxin level,ATPase activities,intramitochondrial total calcium concentration and gene expression of sodium pump isoforms in myocardium of rats with myocardial ischemia reperfusion (MIR).
METHODS: Fifty-six male Sprauge Dawley rats were randomly divided into 7 groups.Sham operation group: silk suture threading the left anterior descending coronary artery without ligature;MIR group: left anterior descending coronary artery was subjected to 30 min ligation followed by 45 min reperfusion;normal saline group: MIR model was given 5 mL/kg normal saline;verapamil group: MIR model was given 5 mg/kg verapamil;low dose antidigoxin antiserum group: MIR model was given 8.6 mg/kg antidigoxin antiserum;middle dose antidigoxin antiserum group: MIR model was given 17.3

mg/kg antidigoxin antiserum;high dose antidigoxin antiserum group: MIR model was given 34.5 mg/kg antidigoxin antiserum.All drugs were injected into vessel via femoral vein within 5 min before reperfusion,respectively.After reperfusion,left ventricle myocardium samples were processed immediately in order to measure the activity of Na+K+_{ATP}ase and Ca2+_{Mg}2+_{ATP}ase,endoxin level,intramitochondrial total calcium concentration and the experssion of α₁,α₂,α₃ and β₁ isoforms of sodium pump on mRNA and protein levels by RT-PCR and Western blotting and immunohistochemical assay,respectively.
RESULTS: After MIR,the level of endoxin in myocardium was obviously increased.The activities of Ca2+_{Mg}2+_{ATP}ase and Ca2+Mg2+ATPase in myocardial membrane were significantly decreased while intramitochondrial total calcium concentration increased.The gene expression of the α₁,α₂,α₃ and β₁ isoforms of sodium pump at both mRNA and protein levels were reduced markedly.Only the effect of verapamil on reducing intramitochondrial total calcium concentration was observed.Antidigoxin antiserum significantly reduced the level of endoxin in myocardium,restored the activities of Na+K+_{ATP}ase and Ca2+_{Mg}2+_{ATP}ase,reduced intramitochondrial total calcium concentration,and up-regulated the expression of α₁,α₂,α₃ and β₁ isoforms of sodium pump at both mRNA and protein levels.
CONCLUSION: MIR results in increase of endoxin secretion.The latter depresses the activity of Na+K+_{ATP}ase by down-regulating the gene expression of α₁,α₂,α₃ and β₁ isoforms of sodium pump in myocardial membrane,and also induces intramitochondrial calcium overload,thereby mediates MIR injury.</FONT>

Key words Endoxin Myocardial reperfusion injury Na(+)-K(+)-exchanging ATPase Antibodies digoxin

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