High concentration of glucose inhibits endothelium-dependent vasorelaxation of rabbit aortic artery

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ABSTRACT

AIM: To determine whether high concentration of glucose inhibits endothelium-dependent vasorelaxation of rabbit aortic artery and possible mechanisms. METHODS: The organ-bath of rings of rabbit aorta was used to determine changes of tension of vessel in response to different concentrations of acetylcholine (ACH) and sodium nitroprusside (SNP) after removal of endothelium, coinoculated with nitric oxide synthase (NOS) inhibitor L-NMMA, different concentrations of glucose, vitamin C, and cell-permeable superoxide dismutase mimetic and oxygen-derived free radical scavenger manganese (III) tetrakis (1-methyl-4-pyridyl) porphyrin (MnTMPyP). RESULTS: ACh induced concentration-and endothelium-dependent aortic vasorelaxation. High concentration of glucose markedly inhibited this vasorelaxation, and vitamin C and MnTMPyP could not antagonize this inhibitory effect by high concentrations of glucose. SNP induced concentration-dependent and endothelium-independent vasorelaxation, and high concentration of glucose had no effect on the vasorelaxation by SNP. CONCLUSION: High concentration of glucose inhibited endothelium-dependent vasorelaxation and this effect was unlikely mediated through activating oxygen-derived free radical production.

INTRODUCTION

The leading cause of morbidity and mortality in patients with diabetes mellitus is accelerated small and large-vessel disease. Recent trials have understood the importance of elevated glucose levels as an independent risk factor. The mechanisms by which high glucose concentrations may induce vascular changes in diabetic patients are not well understood.

The normal endothelium plays a very important role in maintaining vessel wall homeostasis, synthesizes biologically active substances that modulate vascular tone, prevents thrombosis, and influences smooth muscle growth. Important one among these vasoactive substances is endothelium-derived relaxing factor, identified as nitric oxide. Reduced levels of nitric oxide could contribute to vascular injury and disease by facilitating platelet-vessel wall interaction, adhesion of circulating monocytes to endothelial surface, and proliferation of vascular smooth muscle cells. There is now substantial evidence that endothelium-dependent vasorelaxation is abnormal in animal models of diabetes mellitus and diabetic patients. This has been attributed to abnormal lipid and advanced glycation end product levels in plasma. However, it is still unclear whether high concentration of plasma glucose, a very important metabolic abnormality in diabetes, inhibits endothelium-dependent vasorelaxation. The purpose of present study is to determine whether high concentration of plasma glucose inhibits endothelial dependent vasorelaxation of rabbit aortic artery and possible mechanisms.

MATERIALS AND METHODS

Protocol Fresh thoracic aortic artery were obtained and cut into 2-3 mm rings from healthy New England rabbits (Experimental Animal Center of Railway Ministry, Grade II, Certificate No 9600019). Rings were placed under 2 g tension in 3 mL organ baths containing Krebs' solution (composition in mmol/L: NaCl 118.3, KCl 4.7, CaCl2 2.5, MgSO4 1.2,
NaHCO₃ 25.0, edetic acid 0.026, and glucose 11.1) bubbled with 95 % O₂ and 5 % CO₂. Repeated contractions were elicited to noradrenaline 0.1 µmol/L, with washouts in between, until stable and reproducible contractions were obtained. Relaxant concentration-effect curves were performed to ACh 10 mmol/L – 10 µmol/L in the absence or presence of glucose 7.5 – 30 mmol/L. In separate aortic rings, relaxant concentration-effect curves were determined to SNP 10 mmol/L – 10 µmol/L (an endothelium-independent vasodilator which degrades to form NO), in the absence or presence of glucose 7.5 – 30 mmol/L. In parallel experiments, aortic rings were co-incubated with MnTMPyP 100 µg/L, prior to adding glucose and determining responses to ACh.

**Drugs** The following drugs were used: ACh (Sigma), MnTMPyP (Alexis Biochemicals, UK), glucose (Sigma), L-NMMA (Sigma), and SNP (Sigma). Solutions of drugs were freshly prepared daily. All drugs were dissolved in the distilled water. The drugs were added to the bath in volumes less than 10 µL.

**Statistical analysis** Relaxation of vessel rings was expressed as percent of developed tension induced by noradrenaline. All data were expressed as $\bar{x} \pm s$ and statistically analyzed using one-way ANOVA. Differences were considered significant at a value of $P < 0.05$ (two-sided).

**RESULTS**

ACh elicited a concentration-dependent vasorelaxation in precontracted rings with intact endothelium [$E_{max}: 61.6 \% \pm 4.8 \%; EC_{50}: (4.2 \pm 1.3) \mu$mol/L (95 % confidence limits: 3.2 – 5.7)], which was abolished by prior denudation of the endothelium or co-incubation with L-NMMA 1 mmol/L (Fig 1A). SNP also elicited a concentration-dependent vasorelaxation [$E_{max}: 67.8 \% \pm 5.3 \%; EC_{50}: (4.6 \pm 0.3) \mu$mol/L (95 % confidence limits: 4.1 – 5.2), Fig 2], but denudation of endothelium and L-NMMA did not prevent this effect (data not shown).

**Fig 1.** Percentage changes of tension in rabbit aortic rings in response to increasing concentrations of ACh. ○ = control; ● = endothelium removed; × = L-NMMA; ⊗ = glucose 7.5 mmol/L; ◇ = glucose 15 mmol/L; ∗ = glucose 30 mmol/L; □ = glucose 30 mmol/L + vitamin C 0.1 g/L; □ = glucose 30 mmol/L + MnTMPyP 0.1 g/L. n = 7. $\bar{x} \pm s$.

Baseline tension = (2.3 ± 0.4) g.
Fig 2. Percentage changes of tension in rabbit aortic rings in response to increasing concentrations of SNP. △ = control; ▲ = glucose 30 mmol/L. n = 7. ± s. Baseline tension = (2.4 ± 0.3) g.

High concentration of glucose induced a concentration-dependent inhibition to vasorelaxation induced by ACh (Fig 1B), but had no effect on vasorelaxation induced by SNP (Fig 2).

Vitamin C 0.1 g/L and MnTMPyP 100 μg/L had no marked effect on inhibition of vasorelaxation induced by high concentration of glucose (Fig 1C, D).

DISCUSSION

Abnormal endothelium-dependent vasodilatation has been shown repeatedly in animal models and patients of diabetes. Studies using drugs such as ACh, ADP, and histamine to stimulate release of NO have found that endothelium-dependent vasodilatation was abnormal in both conduit arteries and resistance vessels of diabetic rats. Also, cGMP levels are low in aortas of diabetic rats, suggesting that basal concentration of NO are reduced in these vessels. Another study of diabetic rats found that the rate of NO synthesis in coronary arterial endothelial cells was severely impaired (85% reduction in the production of nitrite and nitrate compared with normal rats). These data suggest that the production of NO in vascular endothelium be decreased in diabetes. However, until now, this decrease of NO production is still not very clear.

Our present study found that high concentration of glucose inhibited endothelium-dependent vasorelaxation to ACh in rabbit aorta in a concentration-dependent manner. These concentrations of glucose are also very similar to those found in the serum of diabetic patients. By contrast, high concentration of glucose have no effect on endothelium-independent vasorelaxation to SNP. These suggest that high concentration of glucose inhibit endothelium-dependent vasorelaxation through reducing endogenous NO production in vascular endothelial cells, and not through increasing NO degradation. Some studies have found that high concentration of glucose increased radical formation. Since radicals can inactivate NO, it seems that high concentration of glucose reduces endothelium-dependent vasorelaxation through increasing radical production. But, in our present study, we found that antioxidant vitamin C and cell-permeable superoxide dismutase mimetic MnTMPyP had no great effects on glucose response. It is more likely, therefore, that high concentration of glucose is exerting an inhibitory effect on NO production. Since high concentration of glucose had no effect on SNP-induced vasorelaxation, it is also unlikely that glucose increases radical production.

REFERENCES

高浓度葡萄糖抑制兔胸主动脉内皮依赖的血管舒张

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关键词 血管舒张；血管平滑肌；血管内皮；
葡萄糖；一氧化氮；胸主动脉；自由基

目的：研究高浓度葡萄糖是否能抑制兔胸主动脉内
皮依赖的血管舒张以及可能的机制。方法：利用器
官组织浴动脉环法测定血管张力，观察去除内皮、
NO 合酶抑制剂 L-NMMA，不同浓度的葡萄糖对乙
酰胆碱（ACh）产生的内皮依赖的和硝普钠（SNP）产生
的非内皮依赖的血管舒张功能的影响以及维生素 C
和膜渗透性抗氧化剂 MnTMPyP 对高浓度葡萄糖作
用的影响。结果：ACh 和 SNP 均产生浓度依赖的血
管舒张效应，高浓度葡萄糖明显抑制 ACh 产生的血
管舒张，而对 SNP 的作用无明显影响，维生素 C 和
MnTMPyP 不能逆转高浓度葡萄糖对 ACh 产生的血
管舒张的抑制作用。结论：高浓度葡萄糖抑制内皮
依赖的血管舒张，这种作用不是通过增加氧自由基
的产生介导的。

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