

High glucose impairs endothelium-dependent relaxation in rabbit aorta

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KEY WORDS relaxation; glucose; acetylcholine; phenylephrine; sodium nitroprusside; arginine; superoxide dismutase; vascular endothelium; thoracic aorta

ABSTRACT

AIM: To study the effects of high glucose on endothelium-dependent relaxation (EDR) and the action of *L*-arginine, superoxide dismutase (SOD), or glucose re-normalization in aorta. **METHODS:** Measurement of EDR of the isolated rabbit thoracic aortic rings. **RESULTS:** Elevated glucose (25 mmol·L⁻¹) caused profound impairment of acetylcholine (ACh)-induced relaxation, EC₅₀: 1.6 μmol·L⁻¹ (95% CL: 7.9 nmol·L⁻¹ - 6.3 μmol·L⁻¹) vs normal glucose (5.5 mmol·L⁻¹) EC₅₀: 0.08 μmol·L⁻¹ (95% CL: 0.02 μmol·L⁻¹ - 0.3 μmol·L⁻¹) (*P* < 0.01), which not reversed followed by a further 24 h incubation in normal glucose M199, EC₅₀: 2.0 μmol·L⁻¹ (95% CL: 0.2 pmol·L⁻¹ - 12.5 μmol·L⁻¹). However, aortic rings incubated with mannitol (19.5 mmol·L⁻¹) relaxed to ACh normally. *L*-arginine 1 mmol·L⁻¹ or SOD 150 U·L⁻¹ restored ACh relaxation in elevated glucose to normal, EC₅₀: 0.16 μmol·L⁻¹ (95% CL: 0.04 μmol·L⁻¹ - 0.8 μmol·L⁻¹) and 0.16 μmol·L⁻¹ (95% CL: 0.03 - 0.63 μmol·L⁻¹). The relaxation in response to sodium nitroprusside was not different between rings exposed to normal or elevated glucose. **CONCLUSION:** Hyperglycemia impaired EDR, which was not reversible by glucose re-normalization, increased free radical production and altered *L*-arginine metabolism were involved in this endothelium dysfunction.

INTRODUCTION

The relation between diabetes and premature vascu-

lar disease has been well established. One of the defects involves endothelial dysfunction characterized by impaired endothelium-dependent relaxation responses^[1]. Of the many metabolic disturbances of diabetes, hyperglycemia, has been suggested to be the main cause of endothelial dysfunction^[2]. Several *in vivo* studies of vascular function have shown that the impairment of EDR in normal rabbit aorta caused by a short-term (2 - 6 h) exposure to abnormally raised concentrations of glucose^[3-5]. However, little is known about the effects of a more prolonged exposure to high glucose on EDR and whether endothelium dysfunction is reversible after high glucose was withdrawn.

Several mechanisms have been proposed to involve in hyperglycemia mediated endothelium dysfunction including increased polyol pathway activity^[6], activation of protein kinase C^[3], increased production of constrictor prostanoids^[7], and generation of reactive oxygen species through many biochemical pathways (glucose auto-oxidation, polyol pathway, prostanoid synthesis, protein glycation)^[5,8]. But the underlying mechanisms need further elucidation.

Based on this, we studied the comparative long-term (24 h) effects of high glucose on the EDR in rabbit aortic rings. In addition, whether NO and oxygen free radicals were involved in this event was also studied.

MATERIALS AND METHODS

Aortic rings preparation The thoracic aorta were aseptically dissected from New Zealand rabbits (1.5 - 2.0 kg, supplied by the Animal Center of the Second Affiliated Hospital of Hu-nan Medical University, Certificate No 001) of either sex killed by exsanguination after anesthesia with pentobarbital sodium (30 mg·kg⁻¹, iv) and anticoagulation with heparin sodium (150 U·kg⁻¹ iv). Arterial segments about 4 mm in length were incubated in M 199 medium for indicated times with normal glucose (5.5 mmol·L⁻¹, NG), high glucose (25 mmol·L⁻¹, HG), or with mannitol (19.5 mmol·L⁻¹,

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Received 1999-04-12

Accepted 1999-06-08

Mann) as a hyper-osmotic control, respectively, and gassed with 95 % O₂ and 5 % CO₂. In order to show the substantial change of vascular response induced by high glucose, rings were maintained in an incubator at 37 °C in 95 % humidified air and 5 % CO₂ during the period of incubation. Under this experimental condition, the vascular activity of aortic rings cultured in M 199 with normal glucose showed no changes when compared to the freshly isolated rings.

Organ chamber experiments After incubation for indicated times, aortic rings were suspended in organ chambers containing 5 mL Krebs' solution, composed of (mmol·L⁻¹): NaCl 118, KCl 4.8, CaCl₂ 2.5, MgSO₄ 1.2, NaH₂PO₄ 1.2, NaHCO₃ 24, glucose 11, and edetic acid 0.03, at 37 °C bubbled with 95 % O₂ and 5 % CO₂ gas mixture. The tension of ring was measured via a force-displacement transducer (Model TB-611T, Nihon Kohden, Japan). Aortic ring was loaded with 3 g on resting tension and equilibrated for 90 min. Rings were pre-contracted with KCl (80 mmol·L⁻¹). After a maximal response to KCl was obtained, the vascular rings were washed repeatedly with Krebs' solution and equilibrated again for 30 min.

Experimental protocol In order to measure relaxation responses, rings were contracted with phenylephrine (Phe, 0.1 μmol·L⁻¹) to 40 % - 50 % of their maximal contractions. After the contractions stabilized, a cumulative concentration-response curve to ACh (0.005 - 5 μmol·L⁻¹), or sodium nitroprusside (0.001 - 10 μmol·L⁻¹) was obtained. In order to investigate the action of L-arginine and SOD, rings were incubated with high glucose in the presence of L-arginine or SOD. To verify whether glucose effect was reversible, aortic rings previous exposure to high glucose for 24 h were subject to relaxation response after the high glucose was withdrawn for 24 h.

Statistical analysis Results were expressed as $\bar{x} \pm s$. Statistical analysis was carried out by analysis of variance and the Newman-Keuls test. P values < 0.05 were regarded as significant. The values of EC₅₀ and 95 % CL were calculated according to Bliss and Finney method.

RESULTS

Endothelium-dependent relaxation In the presence of Phe (0.1 μmol·L⁻¹), ACh (0.005 - 5 μmol·L⁻¹) caused a concentration-dependent relaxation in aortic rings with intact endothelium incubated with

NG- and HG-M 199 for 24 h. Under high glucose conditions, the relaxation responses of aortic rings to ACh were profoundly impaired, EC₅₀: HG 1.6 μmol·L⁻¹ (95 % CL: 7.9 nmol·L⁻¹ - 6.3 μmol·L⁻¹) vs NG 0.08 μmol·L⁻¹ (95 % CL: 0.02 - 0.3 μmol·L⁻¹) (n = 7, P < 0.01). However, aortae incubated with Mann-M199 (19.5 mmol·L⁻¹) for 24 h relaxed to ACh normally, EC₅₀: Mann 0.1 μmol·L⁻¹ (95 % CL: 6.3 nmol·L⁻¹ - 1.0 μmol·L⁻¹) (n = 4) (Fig 1 2).

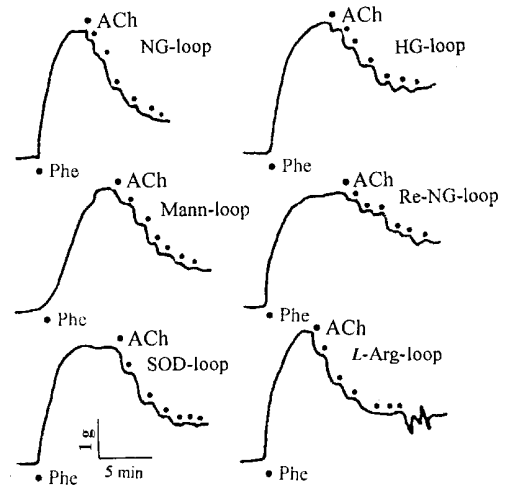


Fig 1. Typical tracings of ACh effect on phenylephrine pre-contracted rings. ● Phe: phenylephrine 0.1 μmol·L⁻¹, ● ACh: Acetylcholine (-lg mol·L⁻¹) 8.5 - 5.5, each dot represents an increment of 0.5.

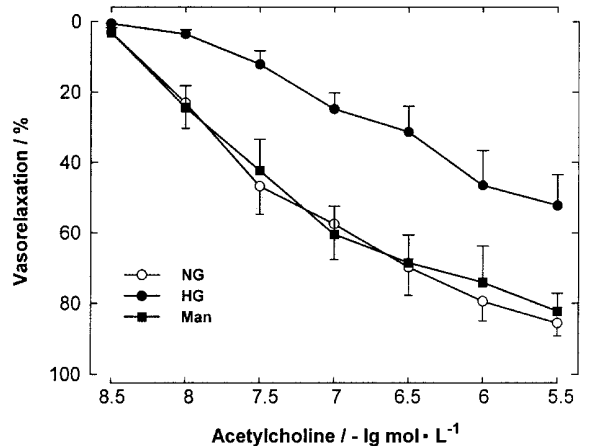


Fig 2. ACh-induced relaxation in aortic rings pre-contracted with phenylephrine, after 24 h incubation in M199 medium containing normal glucose (NG, n = 7), high glucose (HG, n = 7 rabbits) and mannitol (Mann, n = 4 rabbits).

Treatment with *L*-arginine $1 \text{ mmol} \cdot \text{L}^{-1}$ or SOD $150 \text{ U} \cdot \text{L}^{-1}$ prevented the impaired EDR in high glucose, EC_{50} : HG + *L*-arginine $0.16 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ (95 % CL: $0.04 - 0.8 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$), HG + SOD $0.16 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ (95 % CL: $0.03 - 0.63 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$), ($n = 6$), vs NG $0.08 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ (95 % CL: $0.02 - 0.3 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$) ($n = 7$, $P > 0.05$), while no different between NG and NG + *L*-arginine or NG + SOD, EC_{50} : NG + *L*-arginine $0.1 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ (95 % CL: $0.05 - 0.16 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$), NG + SOD $0.1 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ (95 % CL: $0.02 - 0.50 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$) ($n = 5$) (Fig 1 3).

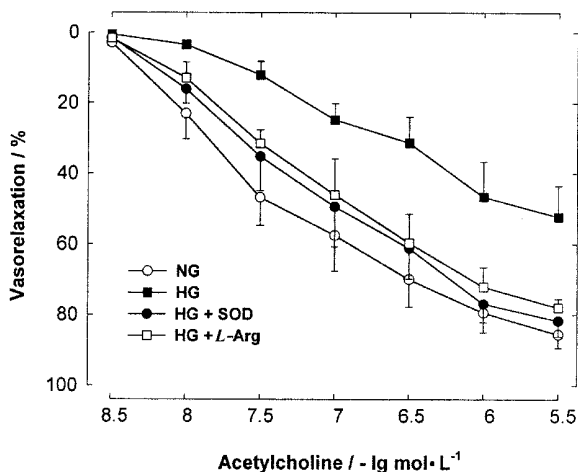


Fig 3. ACh-induced relaxation in aortic rings pre-contracted with phenylephrine, after 24 h incubation in NG-M199 (NG, $n = 6$ rabbits), HG-M199 (HG, $n = 6$ rabbits), and HG-M199 with *L*-arginine $1 \text{ mmol} \cdot \text{L}^{-1}$ or SOD $150 \text{ U} \cdot \text{L}^{-1}$ (HG + *L*-Arg, or HG + SOD, $n = 6$ rabbits).

Rings incubated in high glucose for 24 h were allowed to incubate in normal glucose for another 24 h. Glucose re-normalization did not improve impaired relaxation in high glucose, EC_{50} : $2.0 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ (95 % CL: $0.2 \text{ pmol} \cdot \text{L}^{-1} - 12.5 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$) ($n = 5$), vs HG $1.6 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ (95 % CL: $7.9 \text{ nmol} \cdot \text{L}^{-1} - 6.3 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$) ($n = 7$, $P > 0.05$). Aortae incubated in normal glucose for 48 h showed normal EDR when compared to freshly isolated aortic rings, EC_{50} : $0.1 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ (95 % CL: $15.8 \text{ nmol} \cdot \text{L}^{-1} - 0.5 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$) ($n = 5$), $0.08 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ (95 % CL: $0.01 - 0.63 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$) (Fig 1 A).

Endothelium-independent relaxation The relaxation caused by sodium nitroprusside ($0.001 - 10 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$) was not different between rings after 24 h incubation in NG- and HG-M 199, EC_{50} : $0.08 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ (95 % CL: $6.3 \text{ nmol} \cdot \text{L}^{-1} - 0.6 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$) and 0.1

$\mu\text{mol} \cdot \text{L}^{-1}$ (95 % CL: $7.9 \text{ nmol} \cdot \text{L}^{-1} - 1 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$) ($n = 5$, $P > 0.05$) (Fig 5).

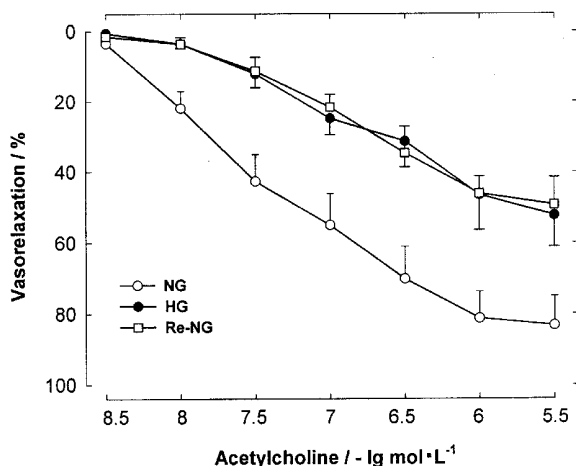


Fig 4. ACh-induced relaxation in aortic rings pre-contracted with phenylephrine, after 48 h incubation in normal glucose (NG, $n = 5$ rabbits), and 24 h in high glucose (HG, $n = 7$ rabbits), or then followed by another 24 h incubation in normal glucose (Re-NG, $n = 5$ rabbits).

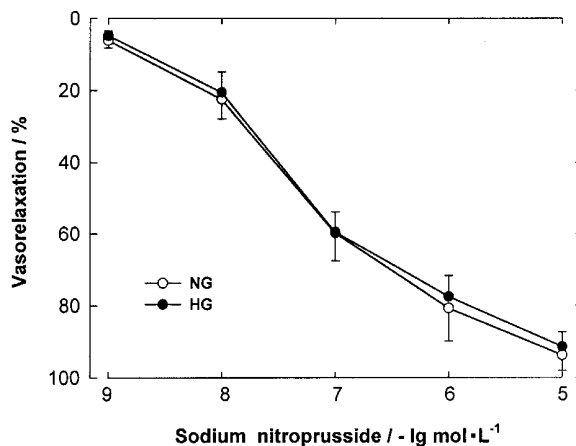


Fig 5. Sodium nitroprusside-induced relaxation of rabbit aorta pre-contracted with phenylephrine after 24 h incubation in NG- and HG-M 199 medium ($n = 5$ rabbits).

DISCUSSION

The present study demonstrated that, after exposure to conditions that mimic pronounced hyperglycemia, EDR induced by ACh was profoundly impaired in rabbit thoracic aorta preparations. The abnormal relaxation observed after incubation with elevated glucose for 24 h was not due to a hyper-osmotic effect, because the same concentration of mannitol had not effect on the re-

laxation induced by ACh. This agreed with the previous observations^[3,6]. However, in this study, aortic rings were dissected aseptically and exposed to high glucose for a more prolonged period, thus, to some extent, our results might better reflect the action of high glucose to EDR. Under our experimental conditions, aortic rings were kept in a cell-culture incubator and continuously gassed with O₂, EDR of aortic rings was not changed by this *in vitro* incubation up to 48 h. Based on this experimental model, we found that glucose re-normalization for 24 h did not restore impaired relaxation to ACh by elevated glucose, suggesting that altered endothelium function will not be restored tightly followed the occurrence of euglycaemia after glucose control in diabetes.

There is substantial increasing evidence that vasodilatation mediated by endothelium-derived NO is impaired in animal models of diabetes and in diabetic patients^[2]. Gupta *et al* have found *L*-arginine supplementation prevents endothelium-dependent inhibition of Na⁺/K⁺ ATPase induced by hyperglycemia, suggesting reduced nitric oxide (NO) release may be responsible for the lowered enzyme activity^[9]. Chakravarthy^[10] demonstrated that the nitric oxide synthase (NOS) expression in vascular endothelial cells was suppressed by high glucose. In the present study, the NO precursor, *L*-arginine incubated along with elevated glucose, prevented impaired relaxation to ACh. This suggested that hyperglycemia could affect NOS activity, decrease the utilization of *L*-arginine, in turn reduced NO releases and resulted in the impaired EDR.

Oxidative stress has been considered to play an important role in the development of vascular complications^[8]. This study showed that SOD, when added along with glucose, prevented impaired relaxation to ACh in high glucose. This suggests a role for oxygen-derived free radicals in glucose-induced impairment of relaxation, consistent with the observation in diabetic rat aorta^[11]. Recently, high glucose has been demonstrated to increase superoxide anion generation in cultured endothelial cells^[12]. Thus, oxygen free radicals may contribute to endothelial dysfunction induced by high glucose.

The relaxation response to the endothelium-independent vasodilator, sodium nitroprusside, was not affected by elevated glucose, which was consistent with the previous studies in diabetic arteries^[13-15]. This suggested that vascular smooth muscle relaxation was not

impaired by high glucose.

In conclusion, this study suggested a central role for glucose in the development of endothelial dysfunction associated with diabetes. Oxygen free radicals generation and altered arginine metabolism may be involved in elevated glucose-induced impairment of EDR.

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高糖损伤兔主动脉内皮依赖性舒张反应

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关键词 松弛; 葡萄糖; 乙酰胆碱; 苯福林; 硝普钠; 精氨酸; 超氧化物歧化酶; 血管内皮; 胸主动脉

目的: 研究高糖对兔胸主动脉内皮依赖性舒张反应(EDR)的影响及 L-精氨酸、超氧化物歧化酶(SOD)和高糖撤除的作用。方法: 以主动脉环 EDR 为检测指标。结果: 高糖可使乙酰胆碱(ACh)诱导的 EDR 明显受损, 高糖撤除 24 h 后不能恢复 ACh 的舒血管作用, 而甘露醇($19.5 \text{ mmol} \cdot \text{L}^{-1}$)不影响血管环 EDR。L-精氨酸 $1 \text{ mmol} \cdot \text{L}^{-1}$ 或 SOD $150 \text{ U} \cdot \text{L}^{-1}$ 可取消高糖对 EDR 的损伤作用, 高糖不影响硝普钠的舒血管作用。结论: 高糖可损伤血管 EDR, 短时间高糖撤除不能逆转, 其机制可能与自由基产生及 L-精氨酸代谢改变有关。

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