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毒扁豆碱阻断大鼠交感节神经元烟碱乙酰胆碱受体[1]

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关键词 交感神经节；烟碱受体；毒扁豆碱；变构调节；膜片箝技术

目的：研究毒扁豆碱阻断交感节神经元烟碱受体的作用机理。**方法**：以培养的新生大鼠颈上交感节神经元为标本，使用全细胞膜片箝技术，观察毒扁豆碱对交感节烟碱受体选择性激动剂 DMPP 诱发电流的影响。**结果**：毒扁豆碱（5 – 20 μmol·L^{-1}）以浓度依赖性方式抑制 DMPP 诱发电流，促进诱发电流的衰减，其抑制作用没有电压依赖性，毒扁豆碱 200 μmol·L^{-1} 不能激活烟碱受体。**结论**：与骨骼肌烟碱受体相比，交感神经元烟碱受体表现出不同的药理学特性。毒扁豆碱通过作用于变构位点抑制交感神经元烟碱受体，不影响其开放的离子通道和激动剂结合位点。

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Elevation of an endogenous inhibitor of nitric oxide synthase in diabetic rat serum[1]

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KEY WORDS arginine; experimental diabetes mellitus; blood glucose; malondialdehyde; acetylcholine; thoracic aorta

AIM: To study the endogenous inhibitor of NO synthase N^G,N^G-dimethyl-arginine (DMA) in the diabetic rat serum. **METHODS**: In streptozocin-induced diabetic rats, the serum DMA level and endothelium-dependent vasorelaxation to acetylcholine (ACh) were determined. **RESULTS**: The serum DMA concentration was increased in the diabetic rats compared with their age-matched controls (5.4 ± 1.0 *vs* 0.7 ± 0.3 μmol·L^{-1}, $P < 0.01$). The serum malondialdehyde (MDA) level was also increased in the diabetic rats compared with controls (2.5 ± 0.3 *vs* 1.5 ± 0.1 μmol·L^{-1}, $P < 0.01$). Vasodilator response to ACh was impaired in diabetic thoracic aortas, which was improved by preincubation with *L*-arginine 1 mmol·L^{-1}. **CONCLUSION**: Hyperglycemia elevated the endogenous DMA content, which contributed to attenuated endothelium-dependent vasorelaxation in streptozocin-induced diabetic rats.

Nitric oxide (NO), besides regulating vascular tone, possesses a protective effect on endothelial cells and an inhibitory modulation of vascular smooth muscle cell proliferation[1]. NO is synthesized from *L*-arginine by NO synthase in endothelial cells, and *L*-arginine analogues such as N^G,N^G-dimethyl-arginine (DMA), which is present in blood of both human and

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animals, can inhibit NO synthesis *in vivo* and *in vitro*[2,3]. Serum levels of N^G-monomethyl-*L*-arginine (*L*-NMMA) and DMA, which are endogenous inhibitors of NO synthase, are increased in hypertensive humans or atherosclerotic animals[3], and the NO synthase substrate, *L*-arginine, has been shown to be beneficial to the vessel in atherosclerosis and to reduce blood pressure in hypertension[4,5].

Decreases in endothelium-dependent vasodilation were found in diabetic animals and patients[6,7]. Hyperglycemia suppresses the arginine-NO pathway[6], but it remains unclear whether endogenous inhibitors of NO synthase are involved in this defective relaxation in diabetic blood vessels. In this study, we examined the serum DMA level in streptozocin-induced diabetic rats.

MATERIALS AND METHODS

Reagents Streptozocin, N^G,N^G-dimethylarginine, *L*-arginine, phenylephrine, and acetylcholine (ACh) were purchased from Sigma. Thiobarbituric acid was obtained from Fluka.

Rats Sprague-Dawley ♂ rats ($n = 30$, $210 \pm s$ 18 g) were randomly divided into diabetic group and control. Diabetes was induced by ip streptozocin 60 mg·kg^{-1} dissolved in citrate buffer 0.05 mol·L^{-1} (pH 4.5)[8]. Citrate buffer was injected ip in control rats. Blood glucose was measured 1 wk after streptozocin ip to verify hyperglycemia. Diabetic and control rats were housed for 2 months with free food and water.

Vascular reactivity After 2 months, rats were anesthetized with ip sodium pentobarbital 30 mg·kg^{-1}. Blood samples were collected from the carotid artery for biochemical assay. Thoracic aortas were placed in 4 ℃ Krebs' bicarbonate buffer. The aortic segments were carefully cut into rings of 3 mm long. Rings were suspended in 10 mL tissue baths aerated with 95 % O_2 + 5 % CO_2 at 37 ℃. Isometric tension was recorded by force-displacement transducer and a polygraph recorder. After equilibration for 90 min under 2 g resting tension, rings were contracted by phenylephrine 1 μmol·L^{-1}, and relaxation responses to cumulative concentrations of acetylcholine were tested. Relaxation responses to ACh were calculated and expressed as % of contraction elicited by phenylephrine.

To study the effect of *L*-arginine, a precursor of NO, on diabetes-induced endothelial dysfunctions, some rings from diabetic rats were incubated with *L*-arginine 1 mmol·L^{-1} for 30 min after measurement of vasodilator responses to ACh, and then again contracted with phenylephrine followed by addition of ACh in the presence of *L*-arginine.

Biochemical assays Plasma glucose was measured by glucose oxidase-peroxidase method within 1 h. The thiobarbituric acid reaction substance inflecting lipid peroxide was examined by a spectrofluorometer and expressed as the amount of malondialdehyde[9].

Serum 1.0 mL was pipetted into a tube containing 5-sulfosalicylic acid 20 mg, and the mixture was left in ice for 10 min. The precipitated protein was removed by centrifugation at $2500 \times g$ for 20 min (4 ℃), and the supernatant was used for measurement of DMA with high-performance liquid chromatography (HPLC)[10]. HPLC was carried out using a Shimadzu LC-6A liquid chromatograph with Shimadzu SCL-6A system controller and Shimadzu SIC-6A autosampler. *o*-Phthaldiadehyde adducts of methylated amino acids and internal standard DMA produced by pre-column mixing were monitored using a model RF 530 fluorescence detector set at λ_{ex} 338 nm and λ_{em} 425 nm on a resolve C_{18} column. Samples were eluted from the column using a linear gradient containing mobile phase A composed of sodium acetate-methanol-tetrahydrofuran (81∶18∶1 vol∶vol∶vol) 0.05 mol·L^{-1} (pH 6.8) and mobile phase B composed of sodium acetate-methanol-tetrahydrofuran (22∶77∶1 vol∶vol∶vol) 0.05 mol·L^{-1} at a flow-rate of 1.0 mL·min^{-1}.

Statistical analysis The data are presented as $\bar{x} \pm s$. Statistical analysis was performed with unpaired *t* test or paired *t* test.

RESULTS

Body weight After 8 wk, rats treated with streptozocin weighed less than the control rats (203 ± 24 *vs* 327 ± 23 g, $n = 10$, $P < 0.01$).

Blood analysis Plasma glucose levels of diabetic rats were increased compared with control rats. The serum DMA and MDA concentrations in diabetic rats were also markedly raised compared with controls (Tab 1).

Tab 1. Plasma glucose, serum N^G, N^G-dimethylarginine (DMA), and malondialdehyde (MDA) in streptozocin-induced diabetic and age-matched control rats. $n = 10$, $\bar{x} \pm s$. $^cP < 0.01$ *vs* control.

	Control	Diabetes
Glucose/mmol·L^{-1}	8.2 ± 1.5	28 ± 8^c
DMA/μmol·L^{-1}	0.7 ± 0.3	5.4 ± 1.0^c
MDA/μmol·L^{-1}	1.51 ± 0.1	2.5 ± 0.3^c

Vasodilator responses Tension developments in response to phenylephrine in diabetic aortas were not significantly changed compared with controls (1.2 ± 0.3 *vs* 1.2 ± 0.3 g, $n = 10$, $P > 0.05$), and there

was no significant difference between the tension developments in response to phenylephrine in the absence or presence of *L*-arginine in diabetic rings (1.3 ± 0.4 *vs* 1.2 ± 0.4, $n = 5$, $P > 0.05$).

Vasodilator responses to ACh were markedly attenuated in diabetic rings. Preincubation with *L*-arginine 1 mmol · L^{-1} reduced attenuated vasodilator responses in diabetic rings (Fig 1).

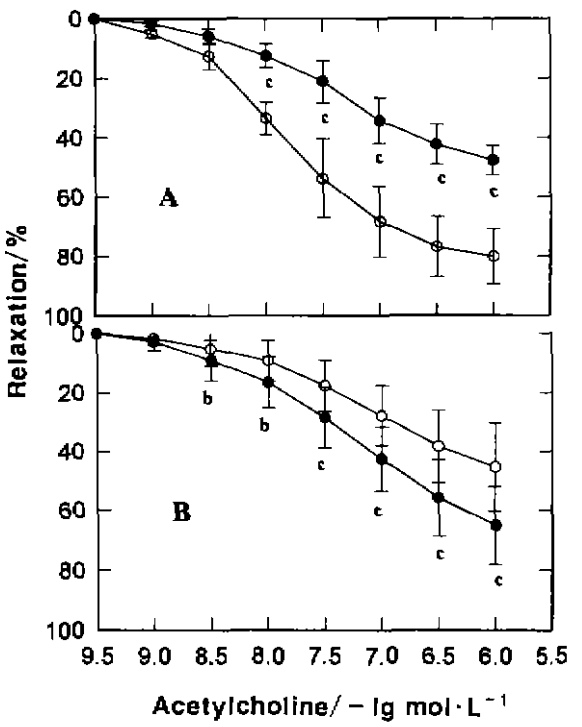

Fig 1. Endothelium-dependent vasorelaxation to ACh. A: Effect of hyperglycemia on vasodilator responses to ACh in diabetic rats (●) and match control rats (○) ($n = 10$). B: Effect of *L*-arginine 1 mmol · L^{-1} on inhibition of vasodilator responses to ACh by hyperglycemia in the absence (○) or presence (●) of *L*-arginine ($n = 5$). $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* match control rats or the rings untreated with *L*-arginine.

DISCUSSION

The present study confirms previous observations that hyperglycemia attenuates endothelium-dependent vasorelaxation to ACh, which is improved by preincubation with *L*-arginine, a precursor of NO[6], and demonstrates for the first time that the serum level of endogenous DMA is raised in streptozocin-induced diabetic rats. Endogenous or exogenous inhibitors of NO synthase attenuated endothelium-dependent vasorelaxation[2,3], and treatment with *L*-arginine *in vivo* or *in vitro* improved endothelium-dependent vasorelaxation in diabetic or hypercholesterolemic animals[5,6]. These results, together with previous studies that endothelium-dependent relaxation to ACh in hypercholesterolemic vessels was attenuated, while the serum level of endogenous DMA was increased in hypercholesterolemic rabbits[11], suggest that endogenous DMA may be a contributor to attenuated endothelium-dependent vasorelaxation in diabetic rats.

The mechanism for the increase of DMA in the serum of diabetic rats is not clear. Several possible mechanisms have been considered. First, blood concentrations of DMA were increased in patients with chronic renal failure, which was ascribed to reduction in excretion of DMA[2]. Kidney damage was shown in streptozocin-induced diabetic rats. Therefore, the possibility that reduction in excretion of DMA was considered in diabetic rats. However, an increase in serum levels of DMA may be due to the stimulation of DMA production rather than the reduction in excretion of DMA in hypercholesterolemic rabbits[3].

Second, it is well established that protein kinase C (PKC) activation plays an important role in the development of vascular disease in diabetes[12]. Activation of PKC can directly cause changes in the actions of various enzymes in addition to altering their expression at the genetic level[13]. Dimethylarginine dimethylaminohydrolase (DDAH) metabolizes methylarginines to citrulline, and it has been suggested that decreased activity of DDAH leads to local accumulation of DMA and inhibition of NO synthase, effects that would be reversed by *L*-arginine[14]. Vitamin E prevented the activation of PKC induced by hyperglycemia[15]. Our recent study showed that supplement with vitamin E decreased DMA content in hypercholesterolemic rabbits[11]. These findings allow us to suppose that the increase of DMA may be secondary to the inhibition of DDAH activity *via* the PKC pathway. However, further studies measuring activity of DDAH and PKC need to be done to establish this hypothesis for the relationship between the level of DMA and PKC activation.

Third, we have recently shown that the increase of DMA is related to the elevation of lipid peroxides, because the increase of DMA is accompanied by an

elevation of malondialdehyde (MDA) level and supplement with antioxidant vitamin E decreases lipid peroxides concomitantly with the reduction of DMA in the serum content in hyperlipidemic rabbits[11]. Oxygen free radicals and lipid peroxide play an important role in the development of diabetic complications such as atherosclerosis. In the present study the concentration of DMA was increased with an elevation of MDA content in diabetic rats. However, the relationship between lipid peroxide and DMA level in diabetic rat serum is a problem for further elucidation.

In summary, this study demonstrated for the first time that the serum level of endogenous DMA was increased in the streptozocin-induced diabetic rat. The present results also suggest that endogenous DMA may be a contributor to attenuated endothelium-dependent vasorelaxation in diabetic rats.

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糖尿病大鼠血中内源性一氧化氮合酶抑制物增高[1]

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关键词 精氨酸; 实验性糖尿病; 血糖; 丙二醛; 乙酰胆碱; 胸主动脉

目的: 测定糖尿病大鼠血中内源性 NO 合酶抑制物二甲基精氨酸(DMA)的含量. **方法**: 在链佐星诱发的糖尿病大鼠测定血清 DMA 的含量和乙酰胆碱(ACh)诱导血管内皮依赖性舒张. **结果**: 与对照组相比, 糖尿病大鼠 DMA 血清浓度显著增加 (5.4 ± 1.0 *vs* 0.7 ± 0.3 $\mu mol \cdot L^{-1}$, $P < 0.01$); 丙二醛含量也高于对照组 (2.5 ± 0.3 *vs* 1.5 ± 0.1 $\mu mol \cdot L^{-1}$, $P < 0.01$); 糖尿病大鼠 ACh 舒血管效应减弱, 其作用可被左旋精氨酸所改善. **结论**: 链佐星诱发糖尿病大鼠高血糖症引起内源性 DMA 含量升高, 同时血管内皮依赖性舒张功能被削弱.