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Characteristics of impaired endothelium-dependent relaxation of rat aorta after streptozotocin-induced diabetes

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KEY WORDS vascular endothelium; thoracic aorta; experimental diabetes mellitus; nitric oxide; calcium; acetylcholine; bradykinin; cyclopiazonic acid; calcimycin; nitroarginine

ABSTRACT

AIM: To study whether impaired endothelium-dependent relaxation (EDR) in early diabetic mellitus in response to different receptor-mediated and nonreceptor-mediated vasodilators ran parallel and its possible mechanism. **METHODS**: Isometric tension recording in aortic rings from streptozotocin (Str)-induced diabetic and age-matched nondiabetic rats. **RESULTS**: EDR induced by receptor agonist acetylcholine (ACh), histamine (His) or bradykinin (BK) were all significantly reduced in diabetic rings compared with control rings, whereas nonreceptor agonist calcimycin-induced EDR was well reserved in diabetic rings [IC_{50} control: (0.13 ± 0.07) $\mu mol \cdot L^{-1}$ diabetic: (0.14 ± 0.06) $\mu mol \cdot L^{-1}$, $P > 0.05$, $n = 7$]. Cyclopiazonic acid (CPA) which also is a nonreceptor mediated endothelium-dependent vasorelaxant and cells' capacitative Ca^{2+} entry stimulant, failed to trigger EDR in diabetic rings. Pretreatment with N^{ω}-nitro-*L*-arginine methylester (*L*-NAME, 0.3 mmol · L^{-1}) not only abolished all of the EDR elicited by above mentioned vasodilators in either of diabetic or control rings, but also leveled responses triggered by each of the agonists between diabetic and control rings. Upon the maximal EDR induced by ACh (1 $mol \cdot L^{-1}$) or CPA (3 $\mu mol \cdot L^{-1}$) in phenylephrine (1 $\mu mol \cdot L^{-1}$) precontracted rings, calcimycin (1 $\mu mol \cdot L^{-1}$) further relaxed diabetic rings, but contracted control preparations. When endothelium was denuded, relaxation evoked by sodium nitroprusside and contractions triggered by CPA or His were all identical between diabetic and control rings. **CONCLUSION**: Receptor agonists but not nonreceptor agonists-induced EDR are commonly impaired in 4-wk Str-induced diabetic rat aorta, and this defective effect is attributable to the low formation of EDRF/NO which is related to impaired capacitative Ca^{2+} entry pathway in endothelium.

INTRODUCTION

According to the WHO 1 % of diabetics die of diabetic comas, but as many as 74 % die of vascular complications. It has also been recognized that endothelial cells play a pivotal role in the development of many vascular disease including diabetes mellitus[1].

The endothelium adapts to the local environment by releasing vasodilators and/or vasoconstrictors. Among these, endothelium-derived relaxing factor (EDRF), a substance believed to be nitric oxide (NO) or a closely related compound, not only is a potent vasorelaxant, but also has other important cardiovascular protective effects, eg, inhibition of platelet aggregation, leukocyte adhesion, and smooth muscle proliferation[2]. Its synthesis can be triggered by two different mechanisms, either of receptor independent mechanism such as shear stress or by receptor mediated processes in response to several endogenous substances such as acetylcholine or bradykinin[3]. In the past decade, many investigators provide evidence that impaired endothelium-dependent relaxation (EDR) is a common feature known to occur in both experimental diabetic animals and diabetic patients[1]. However, up to now, a single unifying mechanism accounting for such a dysfunction in diabetes is still missing, though

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several possible mechanisms have been proposed, including at least (a) high destruction of NO by O_2^--derived free radicals, (b) release of endothelium-derived vasoconstrictors, (c) or arginine deficiency in endothelium. Considering that many previous studies used only receptor agonists' pharmacological tools (eg, mostly for acetylcholine) to observe EDR in diabetes, and that evidence of well-reserved EDR in diabetes via nonreceptor mediated signaling pathway was also presented[4,5], we therefore speculate that such an apparent discrepancy may imply a new important mechanism about endothelial dysfunction in early diabetes, eg, decreased production of NO due to any alteration of Ca^{2+} mobilization triggered by receptor agonist in diabetic endothelium.

Thus, the present study was to systematically compare these two signaling pathway-mediated EDR in a 4-wk diabetic rat model, and provid new insights to early diabetic endothelial dysfunction.

MATERIALS AND METHODS

Drugs and chemicals Streptozotocin (Str), cyclopiazonic acid (CPA), calcimycin, bradykinin (BK), N^{ω}-nitro-*L*-arginine methylester (*L*-NAME), acetylcholine chloride (ACh), histamine phosphate (His), phenylephrine hydrochloride (PE), sodium nitroprusside (SNP), and indometacin were purchased from Sigma. All drugs were dissolved in Krebs' solution or distilled water except for CPA and calcimycin in Me_2SO. The final bath concentration of Me_2SO never exceeded 0.1 %. Indometacin was first dissolved in 2 % Na_2CO_3, then diluted with Krebs' solution.

Rats Male Sprague-Dawley rats (Grade Ⅱ, Zhejiang Medical Laboratory Animal Center, Certificate № 22-9601018 conferred by Animal Management Committee, Chinese Academy of Sciences), weighing 215 g ± *s* 5 g were induced diabetes by intravenous tail-vein injection of streptozotocin (Str, 50 mg·kg^{-1} in citrate buffer 0.1 mol·L^{-1}, pH 4.5). Blood glucose was measured with a glucometer (Upjohn) at d 2, wk 2 and wk 4 after Str administration to verify hyperglycemia in diabetic animals. Diabetic and age-matched control rats were housed for 4 wk before we conducted the experiments.

Tissue preparations On the day of experiment, rats were killed by bleeding. The thoracic aorta was isolated and placed in Krebs' solution at pH 7.4 containing (mmol·L^{-1}): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, KH_2PO_4 1.0, $NaHCO_3$ 25, and glucose 11. Fat and connective tissues were removed and the aorta was cut into 3 – 4 mm rings. In some rings, the endothelium was intentionally removed by gently rubbing against the teeth of a pair of forceps. The successful removal of endothelium was assessed by showing that ACh 1 μmol·L^{-1} failed to relax the rings precontracted with PE 1 μmol·L^{-1}. The rings of control and diabetic group in one experiment were from two rats.

Tissue-bath experiments The aortic rings were mounted on a 3-mL organ bath, connected to a force transducer and a pen recorder. The organ bath containing Krebs' solution was bubbled continuously with 95 % O_2 + 5 % CO_2 at 37 ℃. The solution in the baths was changed every 15 min. The rings were equilibrated for 20 min before stretching them to approximate 2 g and were allowed to further equilibrate for 90 min. Before data collection, stimulation of the rings with KCl 60 mmol·L^{-1} was repeated every 20 min until a reproducible contractile response was obtained.

To test the relaxing response to individual agonist, the rings were first precontracted with PE 1 μmol·L^{-1} which we found no significant difference between control and diabetic aortic rings in our preliminary experiment. After steady state contraction induced by PE, different endothelium-dependent or independent vasodilators were respectively added to the bath in a cumulative fashion. Only one vasodilator was tested for any ring preparation and indometacin (10 μmol·L^{-1}) was present in all protocols. In some experiments, *L*-NAME (0.3 mmol·L^{-1}) was added to the bath 20 min before the addition of PE. In this case, PE concentration was adjusted to 0.5 μmol·L^{-1} to keep the contractile responses identity between *L*-NAME treated and untreated rings. To test the contractile response to CPA or His, the endothelium was removed.

Data analysis Relaxation of vessel rings was expressed as percent of developed tension. All values were expressed as $\bar{x} \pm s$. One-way analysis of variance (ANOVA) was performed, followed by *t* test.

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RESULTS

Characteristics of diabetic animals A total of 27 diabetic and 29 nondiabetic matched rats were used for this study. Body weight in control rats increased from (215 ± 5) g (initial) to (310 ± 17) g (at the conclusion of the study). In contrast, body weight in diabetic rats at the initial stage (220 ± 8) g was similar to that at the conclusion of the study (241 ± 21) g.

Blood glucose levels were significantly elevated in diabetic rats compared with nondiabetic rats in the d 2 [(16 ± 2) mmol·L^{-1} *vs* (4.5 ± 0.3) mmol·L^{-1}], wk 2 [(21 ± 2) mmol·L^{-1} *vs* (4.4 ± 0.6) mmol·L^{-1}] and the day of sacrifice [(24 ± 3) mmol·L^{-1} *vs* (4.7 ± 0.6) mmol·L^{-1}].

EDR induced by receptor agonists ACh, BK, and His in both control and diabetic aortic rings ACh and BK caused a concentration-dependent relaxation in PE (1 μmol·L^{-1})-precontracted aortic rings from control and diabetes. Relaxation induced by ACh or BK was significantly attenuated in aortas from diabetic rats compared with control animal with intact endothelium. Pretreatment with *L*-NAME (0.3 mmol·L^{-1}) almost abolished the relaxation induced by ACh or BK in either control or diabetic rings, but this treatment did not differentiate the responses of ACh or BK between diabetic and control rings (Fig 1A, B).

Similarly, in control preparation with intact endothelium, His also produced well sustained concentration-dependent relaxation with maximal amplitude over 50 %; however, dual responses to His were observed in diabetic rings, with slight relaxation in low concentration and vasoconstriction in high concentration. His produced similar contraction when both control and diabetic rings were pretreated with *L*-NAME 0.3 mmol·L^{-1} (Fig 1C).

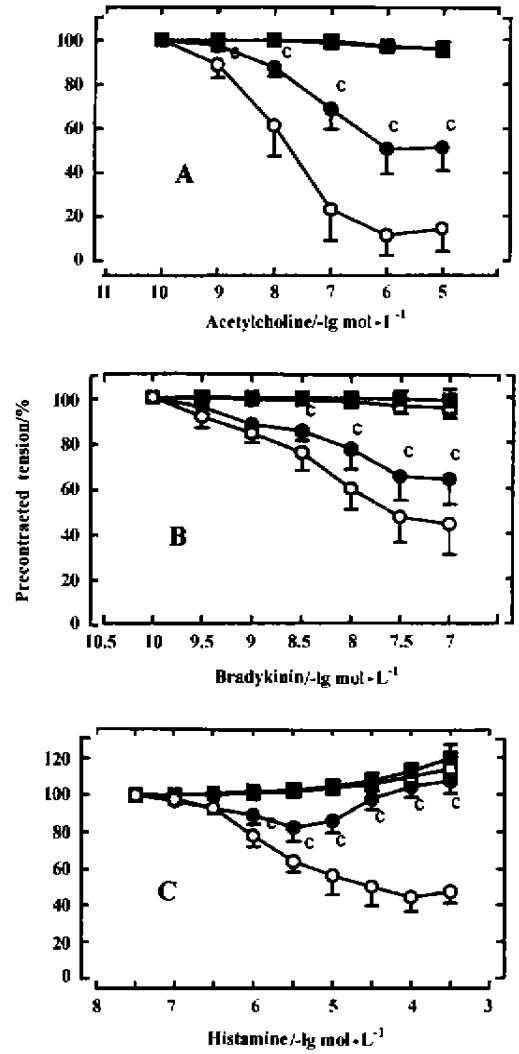

Fig 1. Concentration-dependent relaxation to the endothelium-dependent vasodilator of ACh (A, *n* = 10), BK (B, *n* = 8 or 9) or His (C, *n* = 7) in control (○) and diabetic (●) aortic rings precontracted with PE (1 μmol·L^{-1}). Note that responses triggered by each agonist in both control (□) and diabetic (■) rings were leveled by *L*-NAME (0.3 mmol·L^{-1}) pretreatment (*n* = 6 pairs of aortic rings respectively). $^{c}P < 0.01$ *vs* control.

EDR induced by nonreceptor agonist calcimycin or CPA in both control and diabetic aortic rings Concentration and endothelium-dependent relaxation induced by Ca^{2+} ionophore-calcimycin were not significantly different between control and diabetic groups [IC_{50} control: (0.13 ± 0.07) μmol·L^{-1} diabetic: (0.14 ± 0.06) μmol·L^{-1}, $P > 0.05$, $n = 7$, Fig 2A]. Pretreatment with *L*-NAME (0.3 mmol·L^{-1}) almost abolished the relaxation induced by calcimycin in either of diabetic or control rings, but this treatment did not differentiate the responses of calcimycin between diabetic and control preparations (Fig 2A).

A well sustained relaxation was concentration-dependently induced by cells' capacitative Ca^{2+} entry stimulant-CPA in endothelium intact control rings; but, in diabetic rings, CPA, like His, triggered dual effects in which a slight relaxation at low concentration and a

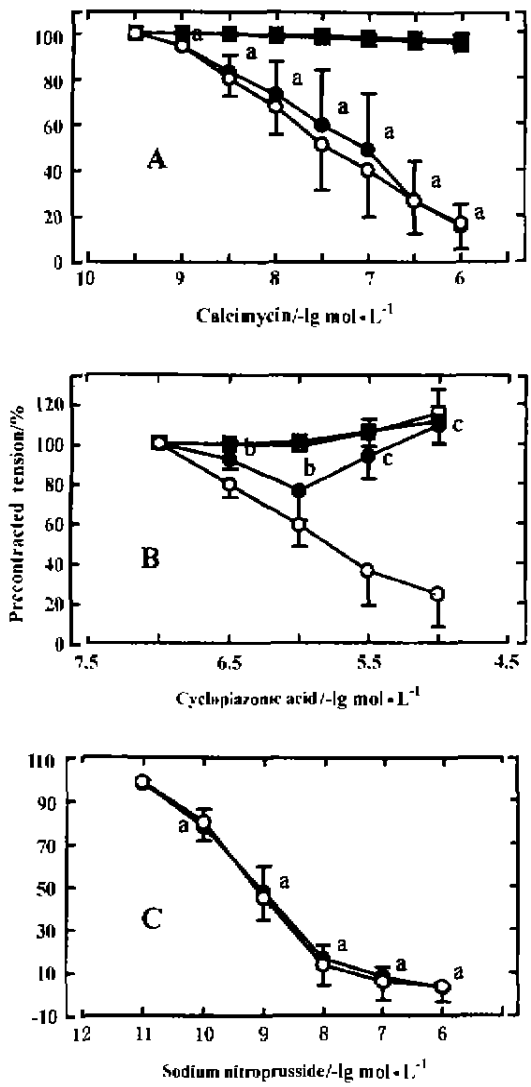

Fig 2. Concentration-dependent relaxation to the nonreceptor mediated endothelium-dependent vasodilator of calcimycin (A, $n=7$) and CPA (B, $n=6$), or to the endothelium-independent vasodilator of SNP (C, $n=10$) in control (○) and diabetic (●) aortic rings precontracted with PE (1 μmol·L^{-1}). Note that responses triggered by calcimycin or CPA in both control (□) and diabetic (■) rings were leveled by *L*-NAME (0.3 mmol·L^{-1}) pretreatment (n = 5 - 6 pairs of aortic rings respectively). For group of SNP, endothelium denuded. $^{a}P>0.05$, $^{b}P<0.05$, $^{c}P<0.01$ *vs* control.

sustained contraction at high concentration were observed. When control or diabetic preparations were pretreated with *L*-NAME (0.3 mmol·L^{-1}), an identical vasoconstrict effect was triggered by CPA (Fig 2B).

Effect of calcimycin on EDR induced by ACh or CPA in both control and diabetic aortic rings Upon the maximal EDR induced by ACh (1 μmol·L^{-1}) in PE (1 μmol·L^{-1})-precontracted rings, calcimycin (1 μmol·L^{-1}) further relaxed diabetic rings, but contracted control preparations (Fig 3A). Similar results were observed for calcimycin on CPA (3 μmol·L^{-1})-induced EDR in both control and diabetic rings (Fig 3B).

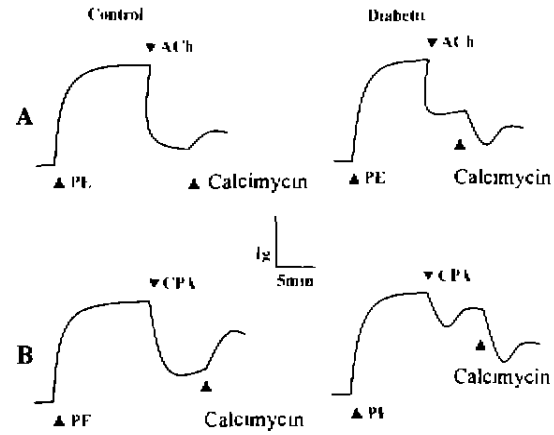

Fig 3. Effects of calcimycin (1 μmol·L^{-1}) on ACh (1 μmol·L^{-1}, A) or CPA (3 μmol·L^{-1}, B) induced endothelium-dependent relaxation in PE (1 μmol·L^{-1}) precontracted-control or diabetic rings. Tracings are typical of 5 separate experiments.

Effect of CPA or His on resting tone in both control and diabetic endothelium-denuded rings In endothelium-denuded and PE-free rings, CPA produced concentration-dependent contraction. Though the contractile response developed slowly, the tension magnitude triggered by CPA was not significantly different between control and diabetic rings (Fig 4A). Note, that PE (1 μmol·L^{-1}) produced similar contraction in both control and diabetic rings [control (0.9±0.1) g, diabetic (0.8±0.1) g, $P>0.05$, n = 5]. Similar results were observed for His (Fig 4B).

Endothelium-independent relaxation induced by SNP in both control and diabetic aortic rings Compared with control group, dose-dependent relaxation produced by SNP in diabetic aorta showed no shift and no depression of maximal relaxation capacity, whether endothelium was intact (data not shown) or denuded (Fig 2C).

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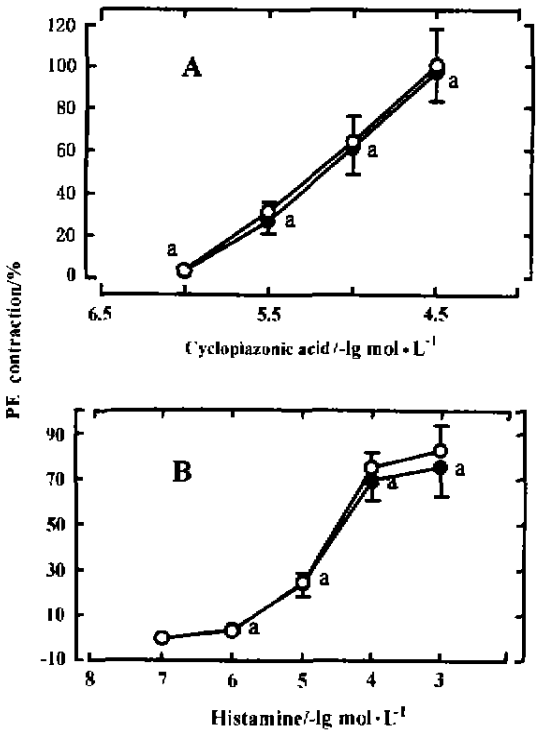

Fig 4. Concentration-dependent contraction of CPA (A, $n = 5$) or His (B, $n = 5$) in unstimulated but endothelium-denuded control (○) or diabetic (●) rings. Data are expressed as percentage contraction of maximal response induced by PE ($1\ \mu mol \cdot L^{-1}$). $^aP > 0.05$ *vs* control.

DISCUSSION

This is the first study that systematically compared receptor-mediated and nonreceptor-mediated EDR in an early (4 wk) diabetic rat model. We found that impaired EDR due to diabetes not only happened to cholinergic receptor agonist ACh, but also to histamine receptor agonist His and bradykinin receptor agonist BK; while EDR induced by Ca^{2+} ionophore calcimycin in diabetic preparations was well reserved. All these results suggest that early diabetic mellitus may mainly impair receptor-mediated EDR. These results are in accordance with previous reports which also showed a decrement of EDR induced by ACh in different diabetic models, and further confirmed recent findings that calcimycin-induced EDR was resistant to diabetes[4] or to the high glucose incubation model *in vitro*[5].

The most important finding of this study is that unlike calcimycin, CPA, a selective ER/SR-Ca^{2+}-ATPase inhibitor, which is also a nonreceptor-mediated endothelium-dependent vasodilator[6-8], presents a significant incapability of inducing EDR in diabetic rings at high concentrations. This indicates that well reserved nonreceptor-mediated EDR is not a common feature in diabetic mellitus. Surprisingly, our result is contrasted by a recent study in which a noted but not statistically different EDR induced by CPA was reported[9]. The precise reasons for this discrepancy are unclear, but several factors could potentially account for the difference, eg, difference in experimental methodology and animal species. However, the most important factor which could represent such a difference is that Kamata *et al*[9] used preparation of mesenteric resistance artery in which both EDRF and EDHF all participate in the regulation of the tone[10]; furthermore, for some disease state such as early diabetes, EDHF may serve as a backup to a defective NO-dependent relaxation system in resistance arteries[10]; whereas in our aorta preparation, it is well known that EDHF play no or minor role of tone regulation[10]. Indeed, report by Fukao *et al*[13] has showed that approximate half of the EDR triggered by CPA in rat mesenteric artery attributed to the EDHF release. Our previous reports[6,7], combined with others[8] and the present data that *L*-NAME abolished EDR induced by CPA in both control and diabetic preparations, strongly support that decrement of CPA-induced EDR in diabetic aortic rings is attributable to the defective EDRF/NO-mediated vasorelaxation mechanism.

In the present study, *L*-NAME not only abolished CPA but also other receptor agonists (eg, ACh, BK, His) and nonreceptor agonist (eg, calcimycin) mediated EDR. In addition, the relaxation induced by SNP was not different between diabetic and control preparations. All these results indicate that the depression of EDR triggered by some receptor agonists (eg, ACh, BK, His), which was similar to that of CPA, could also be attributed to the low availability and/or high destruction of NO rather than a generalized decreased relaxing capacity for diabetic smooth muscle. Yet, one may argue that the decreased EDR could result from the increased release of endothelium-derived vasoconstrictors or from increased direct smooth muscle contractile effects produced by some agonists due to diabetes. However, this might be not the case for the following reasons: 1) indometacin was included in our

experiments, thus endothelium-derived vasoconstrict and vasorelaxant prostanoids may not be involved; 2) Surely, endothelium may release more indometacin-resistant vasoconstrictors due to diabetes, however, we did not find significant differences of the responses triggered by each of the agonists between diabetic and control rings when preparations were pretreated with *L*-NAME($0.3\ mmol \cdot L^{-1}$); 3) given the fact that both CPA and His triggered dual effects due to diabetes, we compared their direct smooth muscle contractile effects between diabetic and control rings respectively, but no significant differences were observed.

The precise step at which diabetic mellitus impairs EDR induced by receptor stimulation remains to be elucidated. In the endothelial cells, EDRF/NO is synthesized through oxidation of *L*-arginine by a Ca^{2+}-activated NADPH-dependent enzyme-ecNOS[3]. If early diabetic mellitus impairs EDR via rapid destruction of NO, then a general impairment of EDR would be observed regardless the signaling pathways, eg, receptor- or nonreceptor-mediated EDR. In view of the fact that EDR induced by calcimycin (which is known to release NO by raising the intracellular Ca^{2+} in a manner unrelated to any receptor mechanisms) was resistant to diabetes, it is tempting to speculate that impaired EDR triggered by receptor agonists might be due to the decreased Ca^{2+} elevation in endothelium. It was reported that NO production was not so related to the intracellular Ca^{2+} release, but was more dependent on the sustained extracellular Ca^{2+} influx[11]. In this respect, diabetic mellitus may mainly target the extracellular Ca^{2+} entry pathway (s). However, since intracellular Ca^{2+} release is generally accompanied by transmembrane Ca^{2+} influx, the linkage between these two events is proposed as the capacitative Ca^{2+} entry mode[12], in which transmembrane Ca^{2+} influx is controlled by the filling state of intracellular Ca^{2+} stores. Such a mechanism was confirmed in vascular endothelial cells[12]. From these points of view, combined with our finding that CPA, a typical capacitative Ca^{2+} entry stimulant, also failed to induce EDR in diabetic aortic rings, it was reasonable to speculate that defective EDR triggered by receptor agonists in diabetic mellitus may relate to the impaired capacitative Ca^{2+} entry mechanism in endothelium. This hypothesis was supported by our another important finding that calcimycin further relaxed diabetic rings in the presence of ACh or CPA, whereas somehow compromised EDR induced by ACh or CPA in control rings, suggesting that decreased extracellular Ca^{2+} influx may play an important role in early diabetic endothelium dysfunction. Recent studies demonstrate that Ca^{2+} entry activated by CPA is indistinguishable from the influx activated by receptor-mediated mechanisms in endothelial cells[14] and diabetic mellitus not only failed to impair NaF-induced intracellular Ca^{2+} elevation, but also failed to alter the expression and function either of G-protein or ecNOS in endothelium[15], suggesting that G-protein coupled or receptor-operated Ca^{2+} channel (ROCC) may not be impaired. All these results seem to further substantiate our speculations.

In summary, our results provide evidence for the first time to suggest that in 4-wk Str-induced diabetic rat aorta, receptor agonists but not nonreceptor agonists-mediated EDR are commonly impaired, and this defective effect is attributable to the low formation of EDRF/NO which is related to impaired capacitative Ca^{2+} entry pathway in endothelium.

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REFERENCES

1 Helmut D. Endothelial dysfunction: clinical implications. Prog Cardiovasc Dis 1997; 39: 287 – 324.
2 Furchgott RF. The 1996 albert lasker medical research awards. The discovery of endothelium-derived relaxing factor and its importance in the identification of nitric oxide. JAMA 1996; 276: 1186 – 8.
3 Moncada S, Palmer RMJ, Higgs A. Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacol Rev 1991; 43: 109 – 42.
4 Pieper GM, Siebeneich W, Roza AM, Jordan M, Adams MB. Chronic treatment *in vivo* with dimethylthiourea, a hydroxyl radical scavenger, prevents diabetes-induced endothelial dysfunction. J Cardiovasc Pharmacol 1996; 28: 741 – 5.
5 Fraccarollo PDD, Santostasi G, Maragno I. Impairment of endothelium-dependent but not of endothelium-independent dilatation in guinea-pig aorta rings incubated in the presence of elevated glucose. Br J Pharmacol 1997; 121: 972 – 6.

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E-mail aps@server.shcnc.ac.cn Phn Fax 86-21-6474-2629

6 Zheng XF, Guan YY, Kwan CY. Cyclopiazonic acid causes endothelium-dependent relaxation in rat aorta. Acta Pharmacol Sin 1993; 14: 21 – 6.
7 Zheng XF, Kwan CY, Daniel EE. Role of intracellular Ca^{2+} in EDRF release in rat aorta. J Vasc Res 1994; 31: 18 – 24.
8 Moritoki H, Hisayama T, Takeuchi S, Kondoh W, Imagawa M. Relaxation of rat aorta induced by the Ca^{2+}-ATPase inhibitor, cyclopiazonic acid, possibly through nitric oxide formation. Br J Pharmacol 1994; 111: 655 – 62.
9 Kamata K, Hayashi T, Umeda F. Persistence of cyclopiazonic acid-induced endothelium-dependent vasodilatation in spontaneously hypertensive and streptozotocin-induced diabetic rats. Jpn J Pharmacol 1997; 73: 247 – 50.
10 Quilley J, Fulton D, McGiff JC. Hyperpolarizing factors. Biochem Pharmacol 1997; 54: 1059 – 70.
11 Wang Y, Shin WS, Kawaguchi H, Inukai M, Kato M, Sakamoto A, *et al*. Contribution of sustained Ca^{2+} elevation for nitric oxide production in endothelial cells and subsequent modulation of Ca^{2+} transient in vascular smooth muscle cells in coculture. J Biol Chem 1992; 267: 20877 – 82.
12 Putney JWJr, Bird GStJ. The signal for capacitative calcium entry. Cell 1993; 75: 199 – 201.
13 Fukao M, Hattori Y, Kanno M, Sakuma I, Kitabatake A. Thapsigargin- and cyclopiazonic acid-induced endothelium-dependent hyperpolarization in rat mesenteric artery. Br J Pharmacol 1995; 115 (6): 987 – 92.
14 Wang X, van Breemen C. Multiple mechanisms of activating Ca^{2+} entry in freshly isolated rabbit aortic endothelial cells. J Vasc Res 1997; 34: 196 – 207.
15 Mancusi G, Hutter C, Baumgartner Parzer S, Schmidt K, Schutz W, Sexl V. High-glucose incubation of human umbilical vein endothelial cells does not alter expression and function of G-protein alphar-subunits or of endothelial NO synthase. Bjochem J 1996; 315 (Pt 1): 281 – 7.

链佐星所致糖尿病大鼠主动脉内皮依赖舒张反应损伤的特征

R587.102

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链佐星

关键词 血管内皮；胸主动脉；糖尿病；一氧化氮；钙；乙酰胆碱；缓激肽；环匹阿尼酸；卡西霉素；硝基精氨酸甲酯

目的：研究早期糖尿病大鼠内皮依赖舒张反应（EDR）损伤的机制. **方法**：离体主动脉环张力实验. **结果**：乙酰胆碱（ACh），组胺（His），缓激肽、环匹阿尼酸（CPA）在糖尿病组 EDR 均比对照组明显减弱. 而卡西霉素诱导的 EDR 未见损伤. *L*-NAME（0.3 mmol·L^{-1}）预处理取消所有 EDR、并使两组间效应均一化. ACh 或 CPA 诱导最大 EDR 时，卡西霉素（1μmol·L^{-1}）进一步扩张糖尿病而非正常组血管环. 硝普钠扩血管及 CPA 或 His 缩血管效应均无组间差异. **结论**：在 4 周链佐星糖尿病大鼠主动脉，受体而不是非受体介导的 EDR 普遍损伤，其机制与内皮细胞电容性钙内流信号通路受损从而使 NO 合成减少有关.

（责任编辑 周向华）