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Carvedilol ameliorates endothelial dysfunction in streptozotocin-induced diabetic rats

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Abstract

The beta-blocker, carvedilol has an additional endothelium-dependent vasodilating properties in patients with hypertension or heart failure. Whether carvedilol can improve endothelium-dependent relaxation in a diabetic animal model and its mechanism of action are unknown. The aim of this study was to investigate the effect of carvedilol on the endothelial-response of aortas from diabetic rats and the underlying mechanism. Acetylcholine-induced endothelium-dependent relaxation, sodium nitroprusside (SNP)-induced endothelium-independent relaxation, and expression of nitric oxide synthase 3 (NOS3) mRNA were measured in aortas isolated from both non-diabetic and streptozotocin-induced diabetic rats. The level of NO in serum was also measured 5 weeks after carvedilol administration (1 or 10 mg/kg/day). Endothelium-dependent relaxation declined along with the decrease of serum NO level in aortas from diabetic rats. Treatment with carvedilol for 5 weeks prevented the inhibition of endothelium-dependent relaxation and the decrease of serum NO levels caused by diabetes. The expression of NOS3 mRNA, protein expression and NOS3 phosphorylation at Ser1177 in diabetic rat aorta was very low in untreated diabetic aortas compared with the healthy group. Administration of carvedilol not only significantly increased the expression of NOS3 mRNA but also protein expression and NOS3 phosphorylation at Ser1177 in the healthy and diabetic groups. In conclusion, chronic carvedilol administration significantly ameliorated the endothelial dysfunction in diabetic rat aortas, in which increased NO level, up-regulated NOS3 mRNA and phosphorylation at Ser1177 may be involved.

Keywords: Carvedilol; Diabetes; Endothelium function; NOS3

1. Introduction

Carvedilol is a nonselective β-adrenoceptor and selective α1-adrenoceptor blocker. It is widely used in the treatment of heart failure and hypertension with or without diabetes. Carvedilol, unlike classic β-blockers, has additional endothelium-dependent vasodilating properties in patients with hypertension or heart failure. These effects are related to its antioxidant and free radical scavenger properties (Yue et al., 1993; Noguchi et al., 2000).

Diabetes is an important cardiovascular risk factor and impaired endothelium-dependent relaxation has been demonstrated in various vascular beds of different models of diabetes (Fahim et al., 2001; Rodriguez-Manas et al., 2003). Thus, we hypothesized that carvedilol enhances endothelium-dependent vasodilatation in diabetic rats by up-regulating NOS3 mRNA and phosphorylation. In the present study, we observed aortic rings isolated from streptozotocin-induced diabetic rats.

2. Materials and methods

2.1. Chemicals

Carvedilol (in troche form) was a gift from Roche. Streptozotocin, acetylcholine, sodium nitroprusside (SNP), phenylephrine were from Sigma Chemical Co.; the antibody of NOS3 was from Santocraz, and phospho-NOS3-specific antibody was from Cell

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Table 1
Various parameters in normal control (C), untreated diabetes (D), normal treated with small dose of carvedilol (1 mg/kg) (CS), normal treated with large dose of carvedilol (10 mg/kg) (CL), diabetes receiving small dose of carvedilol (DS), and diabetes receiving large dose of carvedilol (DL)

	C	CS	CL	D	DS	DL
Body weight (g)	363±56	342±50	378±66	167±12[a,]	247±66[a,b]	255±60[a,b]
Blood glucose (mmol/l)	4.94±0.48	4.62±0.50	5.47±0.49	20.59±2.62[a,]	19.47±1.93[a,]	20.20±1.97[a,]
HR (bpm)	426±45	390±40	348±46[a]	362±49[a]	347±44[a]	326±38[a]
MAP (mmHg)	98±14	94±12	92±14	100±15	94±11	96±13

Mean±S.D. [a] $P<0.05$ *vs.* C, [b] $P<0.05$ *vs.* D.

Signaling Technology; and the kit for measurement of nitrite/nitrate (NO) was from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.2. Animals

Male Sprague–Dawley rats (180–220 g) were housed at a constant temperature of 22±2 °C, with a 12 h light/dark cycle, and given free access to food or water. All procedures were approved by the Ethics Committee for the Use of Experimental Animals in Zhejiang University.

2.3. Experimental groups

Diabetes was induced by i.p. injection of 60 mg/kg streptozotocin as previously described (Bojunga et al., 2004a,b). Age-matched healthy rats received saline only. About 72 h after streptozotocin treatment, blood samples were taken from the caudal vein, and glucose levels were measured with a glucometer (One Touch II glucometer). Rats with glucose levels >15 mmol/l were considered diabetic. Three days after injection of streptozotocin or vehicle, rats were randomly divided into the following seven groups: (1) untreated diabetic rats (D, $n=8$); (2) diabetic rats treated with low dose carvedilol (DS, 1 mg/kg/day) ($n=9$); (3) diabetic rats treated with high dose carvedilol (DL, 10 mg/kg/day) ($n=9$); (4) normal control rats (C, $n=9$); (5) normal control rats treated with low dose carvedilol (CS, 1 mg/kg/day) ($n=7$); (6) normal control rats treated with high dose carvedilol (CL, 10 mg/kg/day) ($n=8$). (7) diabetic rats treated with vitamins E (75 mg/kg) and C (10 mg/kg, s.c.). The carvedilol and vitamin E were administered through an intragastric tube daily for 5 weeks.

2.4. Measurement of blood glucose, carotid arterial pressure and serum NO level

After five weeks, the rats were weighed, and glucose in blood from the caudal vein was measured with a glucometer. Then, the rats were anesthetized with 10% hydrated chloral (350 mg/kg), the right carotid artery was cannulated with a BD angiocatheter (20 GA 1.1 mm×48 mm, Italy) for recording heart rate and mean arterial pressure, after which, the rats were killed by cervical dislocation. The blood was collected, allowed to settle for 15 min, centrifuged (10,000 ×*g*, 10 min, 4 °C), and the supernatant was used for NO measurement. Serum NO level was assayed by the Griess method (Wang et al., 2005).

2.5. Preparation of rat thoracic aortic rings

A section of the thoracic aorta from between the aortic arch and the diaphragm was rapidly dissected out and immersed in chilled Krebs' solution, composed of (mM): NaCl, 118; KCl, 4.7; $MgSO_4 \cdot 7H_2O$, 1.2; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25; glucose, 11; and bubbled with 95% O_2+5% CO_2 (pH 7.4). After the perivascular tissue was carefully removed, aortic rings approximately 4 mm in length were cut. Some rings were used for isometric force recording; others were immediately frozen and stored at −70 °C.

2.6. Bioassay of vasoreactivity

For isometric force recording (Tesfamariam et al., 1991), aortic rings were mounted between two stainless steel hooks and suspended in a 10 ml organ bath containing Krebs' solution at 37 °C bubbled with 95% O_2+5% CO_2 (pH 7.4). An initial load of 2.0 g was applied, and the tension of the aortic ring was monitored by a force transducer and recorded in a data acquisition system (MedLab, Nanjing Medease Co. Ltd., China). After a 90 min equilibration period, cumulative concentration-response curves to phenylephrine (1 nM–30 μM) were generated, and the maximal contraction (E_{max}) and the half-maximal effective concentration (EC_{50}) were calculated. Other rings were precontracted with

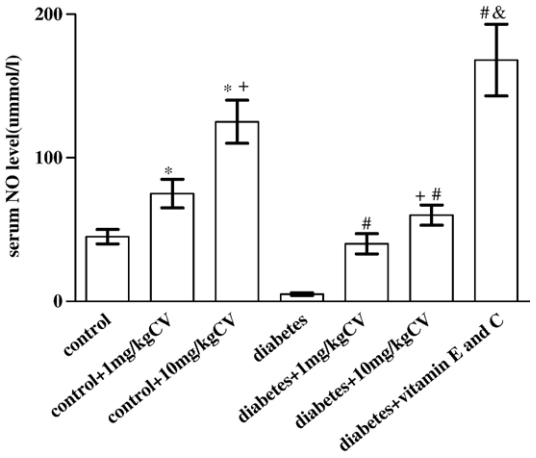

Fig. 1. Serum nitric oxide (NO) levels in normal control rats (C), untreated diabetic rats (D), normal treated with a low dose of carvedilol (1 mg/kg) (CL), normal treated with a high dose of carvedilol (10 mg/kg) (CH), diabetic receiving a low dose of carvedilol (DL), diabetic receiving a high dose of carvedilol (DH), and diabetic receiving vitamin E and C (VD). Mean±S.D. *$P<0.05$ *vs.* C; # $P<0.05$ *vs.* D; + $P<0.05$. DH *vs.* DL; CH *vs.* CL; & $P<0.05$. VD *vs.* DH.

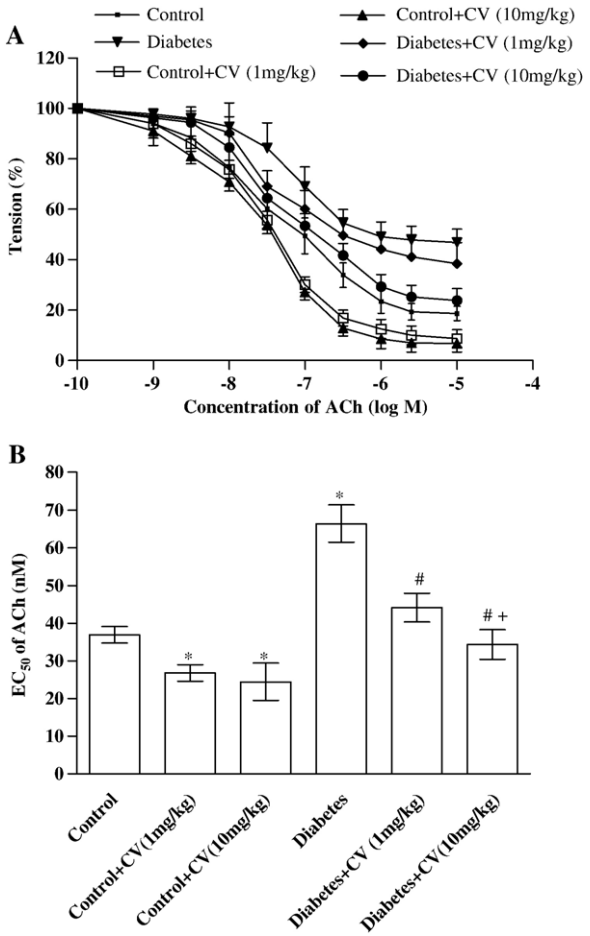

Fig. 2. Acetylcholine-induced endothelium-dependent relaxation (EDR) (A) and EC_{50} values (B) in rat aortic rings in normal control rats (C), untreated diabetic rats (D), normal treated with a low dose of carvedilol (1 mg/kg) (CL), normal treated with a high dose of carvedilol (10 mg/kg) (CH), diabetic receiving a low dose of carvedilol (DL), and diabetic receiving a high dose of carvedilol (DH). Mean±S.D. $n=8$. $P<0.05$ *vs.* C; # $P<0.05$ *vs.* D; + $P<0.05$ DH *vs.* DL; CH *vs.* CL.

phenylephrine (1 μM). After the developed tension attained its peak value, the ring was relaxed by acetylcholine or SNP. Cumulative concentration-response curves to acetylcholine (0.001–10 μM) or SNP (0.001–10 μM) were generated (Qian et al., 2006).

2.7. Reversal transcription polymerase chain reaction (RT-PCR)

Vascular tissue was pulverized to a powder. Total RNA was extracted using Trizol (Life Technologies). RNA quality and integrity was assured by gel visualization and spectrophotometric analysis (OD260/280) and quantified at 260 nm. Experiments were performed using a Light Cycle rapid thermal cycle (Roche Diagnostics). The RT reaction was amplified using TaqDNA polymerase and primers to murine NOS3 cDNA (sense: 5′-TCCAGTAACACAGACAGTGCA-3′; antisense: 5′-CAGGAAGTAAGTGAGAGC-3′). The PCR profile was set at 94 °C melting, 62 °C annealing, and 72 °C extension for 1 min, and semi-quantitation was optimized to 28 cycles. GAPDH transcript abundance was used as an endogenous control to which NOS3 transcript abundance was normalized.

2.8. Western blot analysis

To investigate whether carvedilol treatment could activate NOS3 in aortas, we measured the total protein expression and phosphorylation of NOS3 by Western blot using NOS3 or phospho-NOS3-specific antibody. Vascular proteins were prepared and separated by 10% SDS-polyacrylamide gel and electrotransferred to a polyvinylidene diflouride membrane (Bio-Rad). The membranes were blocked in blocking solution (Tris-buffered solution) containing 0.1% (v/v) Tween 20 and 5% (v/v) BSA for 1 h and then incubated overnight with rabbit polyclonal anti-phospho-eNOS-Ser1177 (1:500) or anti-eNOS antibodies (1:1000). The mumbranes were washed extensively in Tris-buffered saline containing 0.1% (v/v) Tween 20 before incubation for 1 h with a secondary anti-rabbit antibody conjugated to horseradish peroxidase. Protein was then visualized using enhanced chemiluminescence solution (Amersham) and X-ray film.

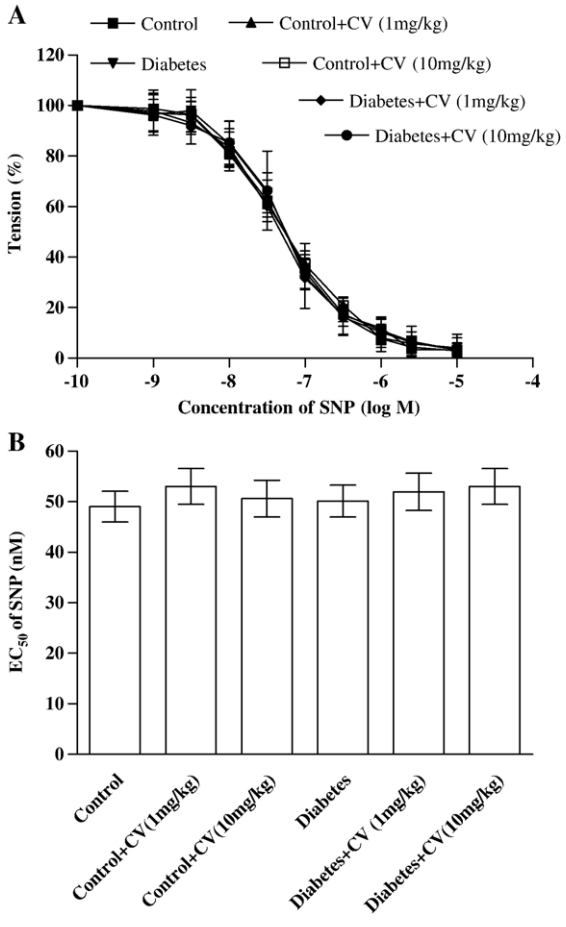

Fig. 3. SNP-induced endothelium-independent relaxation (EIR) (A) and EC_{50} values (B) in rat aortic rings in normal control (C), untreated diabetic (D), normal treated with a low dose of carvedilol (1 mg/kg) (CL), normal treated with a high dose of carvedilol (10 mg/kg) (CH), diabetic receiving a low dose of carvedilol (DL), and diabetic receiving a high dose of carvedilol (DH). Mean± S.D. $n=8$.

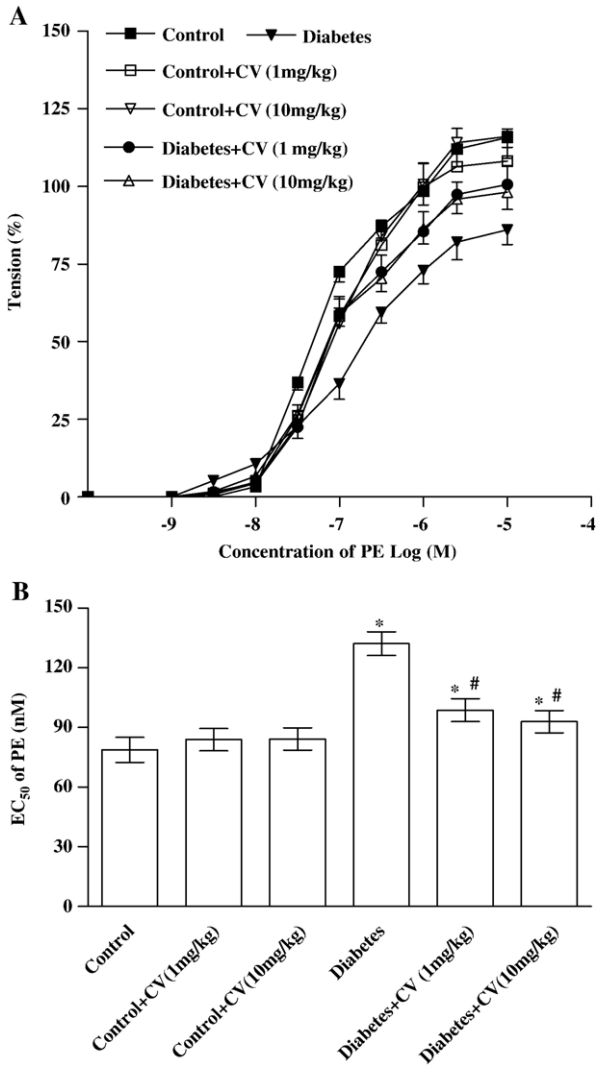

Fig. 4. Phenylephrine-induced contraction (A) and EC_{50} values (B) in rat aortic rings in normal control (C), untreated diabetic (D), normal treated with a low dose of carvedilol (1 mg/kg) (CL), normal treated with a high dose of carvedilol (10 mg/kg) (CH), diabetic receiving a low dose of carvedilol (DL), and diabetic receiving a high dose of carvedilol (DH). Mean±S.D. $n=8$. CV means carvedilol. PE means phenylephrine. *$P<0.05$ *vs.* C; #$P<0.05$ *vs.* D.

2.9. Data analysis

All data were expressed as mean±S.D. The acetylcholine or SNP-induced maximal relaxation (E_{max}) in aortic rings was calculated as a percentage of the contraction in response to phenylephrine (1 μM). The phenylephrine-induced E_{max} in treated aortic rings was calculated as a percentage of the control in response to phenylephrine. The half-maximum effective concentration (EC_{50}) was defined as the concentration of acetylcholine, SNP or phenylephrine that induced 50% of maximal relaxation or contraction, and was calculated from the concentration-response curve by nonlinear regression (curve fit) using GraphPad Prism (Version 4.0). Statistical comparisons were made using one-way ANOVA followed by Newman–Keuls test. $P<0.05$ was considered to be statistically significant.

3. Results

3.1. Weight, blood glucose level, heart rate and mean arterial pressure

There were no significant differences among the six groups at the beginning of the experiment (Table 1). After 5 weeks, animals injected with streptozotocin had lost weight compared with the normal controls ($P<0.0001$), and carvedilol treatment significantly alleviated the weight loss in diabetic rats. There was no significant change in healthy rats treated with carvedilol (Table 1).

Three days after injection of streptozotocin, the average blood glucose levels were more than 20 mmol/l in the diabetic group and remained at this level during the experimental period of 5 weeks. In the diabetic rats, the blood glucose levels did not change after 5 weeks of treatment with low or high doses of carvedilol (Table 1). Blood glucose also did not significantly change in healthy rats treated with carvedilol (Table 1).

Heart rate in untreated diabetic rats significantly decreased ($P=0.0024$). Carvedilol treatment reduced heart rate slightly, but not significantly, in the diabetic rats ($P=0.0604$, Table 1).

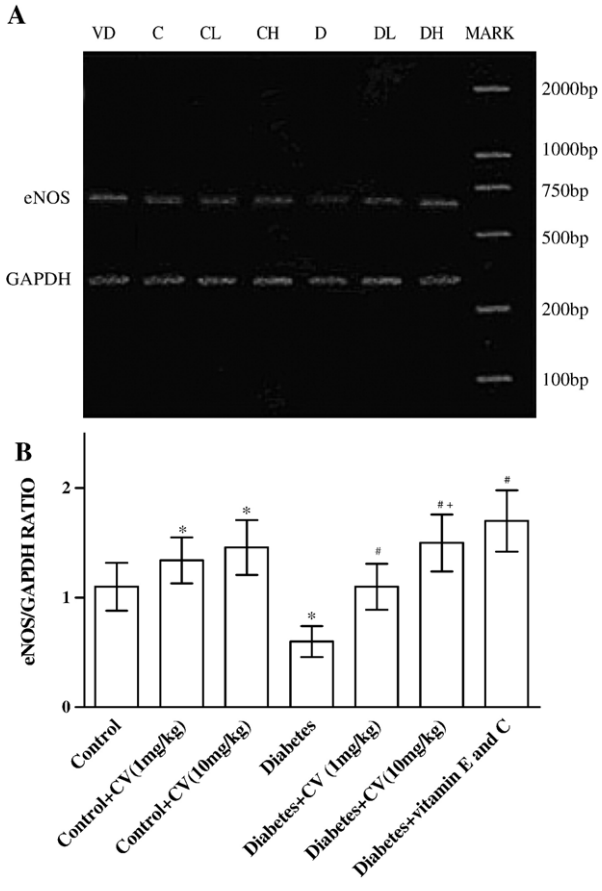

Fig. 5. Semiquantitative RT-PCR analysis of eNOS mRNA in the aorta of normal control rats (C), normal rats treated with a low (CL) or a high (CH) dose of carvedilol, untreated diabetic rats (D), diabetic rats treated with a low (DL) or a high (DH) dose of carvedilol, diabetic rats treated with vitamin E and C(VD). Each bar represents the mean±S.D.; $n=6$ for each group. *$P<0.05$ *vs.* C, # $P<0.05$ *vs.* D, + $P<0.05$ DL *vs.* DH, or CL *vs.* CH.

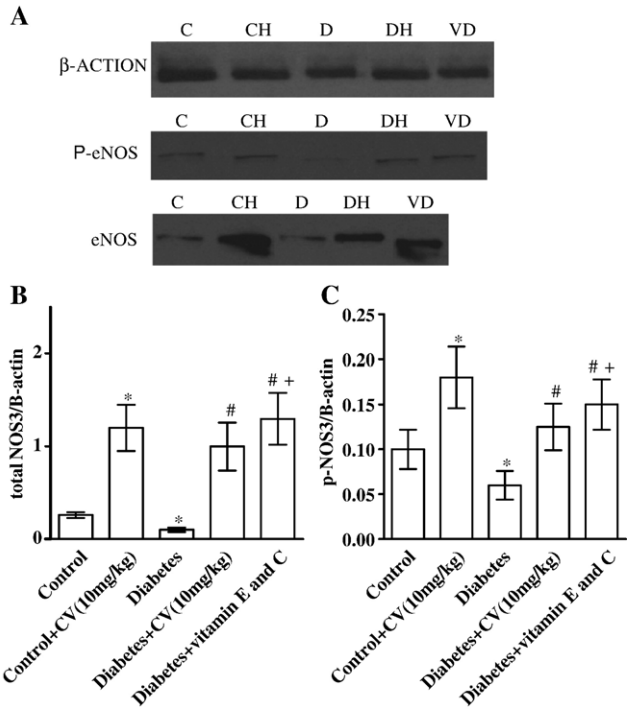

Fig. 6. Protein expression and phosphorylation of NOS3 by Western blot in the aorta of normal control rats (C), normal rats treated with a high (CH) dose of carvedilol, untreated diabetic rats (D), diabetic rats treated with a high (DH) dose of carvedilol, diabetic rats treated with vitamin E and C (VD). B-actin was an endogenous control. Each bar represents the mean±S.D.; $n=6$ for each group. *$P<0.05$ *vs*. C, # $P<0.05$ *vs*. D, + $P<0.05$ DL *vs*. DH, or CL *vs*. CH.

Reduced heart rates were found in control treated with high dose carvedilol ($P=0.0018$, Table 1).

Mean arterial pressure did not significantly change in diabetic rats ($P=0.7202$), and was not decreased by low or high carvedilol treatment (100±15 *vs*. 94±11, $P=0.5101$; 100±15 *vs*. 96±13, $P=0.4448$, Table 1).

3.2. The effect of chronic carvedilol treatment on serum NO level

Serum NO level was significantly lower in the diabetic group than in healthy controls (Fig. 1). Chronic treatment with carvedilol significantly increased serum NO level compared with untreated diabetic rats, and this reduction was dose-related. Carvedilol alone also increased the serum NO level in healthy control rats. Vitamin E and C treatment also increased serum NO level in diabetic rats.

3.3. The effect of chronic carvedilol treatment on vasodilatation of aortic rings from diabetic rats

The endothelium-dependent relaxation induced by acetylcholine in the aortic rings from diabetic rats precontracted with phenylephrine was significantly impaired compared with healthy controls. The E_{max} of aortic rings fell to 53.2±5.3%, and the EC_{50} increased to 66.5±5.0 nM (Fig. 2). Chronic treatment with carvedilol improved the vasodilatation over that in the untreated diabetic group, the E_{max} of aortic rings reaching 60.3±7.5%, and 73.2±5.7%, and the EC_{50} decreasing to 42.2±3.1 nM, and 35.4±4.0 nM, respectively (Fig. 2). Chronic carvedilol treatment of normal rats also significantly increased the acetylcholine-induced endothelium-dependent relaxation compared with normal controls (Fig. 2). Neither diabetes nor carvedilol had any significant effects on the endothelium-independent relaxation induced by SNP (0.001–10 μM) (Fig. 3).

3.4. The effect of chronic carvedilol treatment on constriction of aortic rings from diabetic rats

Phenylephrine-induced vasoconstriction in the aortic rings from diabetic rats was significantly impaired compared with healthy controls. The E_{max} of aortic rings fell to 77.2±6.3%, and the EC_{50} increased to 132.2±5.9 nM (Fig. 4). Chronic treatment with carvedilol improved the vasodilatation over that in the untreated diabetic group, the E_{max} of aortic rings reaching 87.5±7.3%, and 85.2±5.7%, and the EC_{50} decreasing to 98.7±5.8 nM, and 92.9±5.6 nM, respectively (Fig. 4). Low and high dose carvedilol treatment of normal rats did not significantly change the phenylephrine-induced vasoconstriction compared with normal controls (Fig. 4).

3.5. The effect of chronic carvedilol treatment on mRNA expression of NOS3 from diabetic rat aorta

The expression of NOS3 mRNA was compared between untreated and carvedilol-treated aortic rings (Fig. 5). Administration of carvedilol significantly increased the expression of NOS3 mRNA in healthy and diabetic groups. The levels of mRNA encoding for GAPDH were not significantly different between each group. The expression of NOS3 mRNA was very low in untreated diabetic aortas compared with the healthy group. The expression was significantly upregulated in the carvedilol-treated diabetic groups and the vitamin E and C groups, compared with the untreated diabetic group. Expression of NOS3 mRNA was on average 1.7-fold or 2.5-fold higher in diabetic rats with low or high dose carvedilol treatment than in diabetic rats without treatment ($P<0.05$).

3.6. The effect of chronic carvedilol treatment on protein expression of NOS3 and NOS3 phosphorylation from diabetic rat aorta

Protein expression of NOS3 and NOS3 phosphorylation significantly reduced in diabetic rats compared with healthy control. Carvedilol treatment stimulated NOS3 phosphorylation, and significant increase in total NOS3 expression was found (Fig. 6). Both NOS3 phosphorylation and total NOS3 protein expression in the vitamin E and C group increased (Fig. 6).

4. Discussion

Our results showed that carvedilol treatment did not significantly affect blood glucose level (Table 1). Carvedilol has been reported to have antioxidant actions in healthy volunteers treated

with small and moderate doses (6.25–25 mg/day) (Dandona et al., 2000). We selected corresponding low and high doses in healthy and diabetic rats (1 mg/kg/day and 10 mg/kg/day).

It is interesting that carvedilol treatment alleviated the weight loss in diabetic rats despite having no effect on hyperglycemia. This change may be related to improved cardiac function. Previous studies have shown improved cardiac function is associated with higher weight (Xydas et al., 2006; Soga et al., 2006). Increased appetite may also be a contributing factor.

Mean arterial pressure did not significantly differ between diabetic and healthy rats, although there was an increasing trend in diabetic rats. Carvedilol treatment had no significant effect on mean arterial pressure in any group. Heart rate in untreated diabetic rats significantly decreased, while carvedilol treatment reduced heart rate slightly but not significantly in diabetic rats.

Nitric oxide (NO) derived from the endothelial subtype of NOS, NOS3, is an important mediator of vasodilation (Furchgott, 1999). Abnormal release of, or response to, NO has been proposed as a contributor to vascular and endothelial dysfunction in the diabetic state (Cohen, 1995; Huszka et al., 1997). Hyperglycemia itself not only inhibits the endogenous synthesis of NO (Li and Forstermann, 2000) and decreases the activity of vasodilator factors including NO, but also enhances the activity of vasoconstrictor factors such as angiotensin II and endothelin-1 (Brownlee, 2001). In addition, reactive oxygen radicals play a role in endothelial function. Increased levels of reactive oxygen radicals are associated with apoptosis in myocytes, endothelial cells and fibroblasts in diabetes (Turko et al., 2001), and cause uncoupling of NOS, which produces superoxide instead of NO (Maritim et al., 2003). All these reactions contribute to the pathogenesis of endothelial dysfunction, including impairment of endothelium-dependent vasorelaxation.

In our previous study (Qian et al., 2006), we reported that acetylcholine-induced endothelium-dependent relaxation declines along with the decrease of serum NO level in diabetic rats; in the present study, expression of NOS3 mRNA was down-regulated markedly in streptozotocin-induced diabetic rats after 5 weeks. These results are consistent with data from many chronic diabetic models, such as 4-week streptozotocin-induced diabetic rats (Lu et al., 2004; Oyadomari et al., 2001), 4-week diabetic spontaneously hypertensive rats (Ibrahim et al., 2005), and hyperglycemia-induced human aortic endothelial cells *in vitro* (Srinivasan et al., 2004).

On the contrary, the results from some acute and short-term diabetic models (Cosentino et al., 1997; Oyadomari et al., 2001), which reported that NOS3 mRNA and production of NO in human aortic endothelial cells exposed to high glucose levels (22.2 mmol/L) for 5 days are increased. However, upregulation of NOS3 and NO release is associated with a marked concomitant increase of superoxide production (Cosentino et al., 1997). Local overproduction of NO is seen in the early stages of diabetes, and this can react with superoxide to form peroxynitrite ($ONOO^-$) leading to cardiomyocyte contractile or left ventricular dysfunction. Increased expression of inducible NOS (NOS2) mRNA in the early stage may explain the discrepancy. The non-selective NOS inhibitor L-NAME, the NOS2 inhibitor L-*N*6-iminoethyl-lysine, or the reactive oxygen radicals scavenger, thiourea, can improve dysfunction (El-Omar et al., 2003). Oyadomari et al., 2001 reported that NOS3 mRNA and protein expression are increased 1–2 weeks after streptozotocin treatment and are decreased at 4 weeks. The plasma NO levels increased 1–2 weeks after streptozotocin treatment and decreased at 4 weeks. Thus, it seems that expression of NOS3 and NOS2 mRNA and NO level change with the different stages of diabetes.

Carvedilol has demonstrated significant protection against pyrogallol-induced endothelial dysfunction, enhancing vasorelaxation to acetylcholine (Lopez et al., 1995). Kalinowski et al. showed that it is through stimulated NO release from microvascular endothelial cells that carvedilol displays its additional endothelium-dependent vasodilating properties (Kalinowski et al., 2003). In kidney-transplant patients with post-transplant hypertension treated with carvedilol (25–50 mg/day orally for 4 months), monocyte eNOS mRNA levels and plasma nitrite/nitrate were higher than in a normotensive healthy control group (Calo et al., 2002). In our study, endothelium-dependent relaxation was significantly impaired in diabetic rats, and carvedilol attenuated this condition without effecting blood glucose level and mean arterial pressure. In addition, raised NO level, up-regulated NOS3 mRNA, and increased protein expression of NOS3 were consistent with improved endothelium-dependent relaxation. These results implied that raised NO derived from NOS3 was of benefit for diabetic aortic endothelium-dependent relaxation. Interestingly, treatment with 10 mg/kg carvedilol showed the same level of eNOS mRNA in control and diabetic rats (Fig. 5). However, the effect of treatment with 10 mg/kg carvedilol on serum NO levels was much smaller in diabetic than in control rats (Fig. 1). Low phosphorylation level of NOS3 and reduced protein expression of NOS3 in diabetic rats may explain, at least in part, why serum NO levels are not consistent with eNOS mRNA (Fig. 6). There was a lower phosphorylation level of NOS3 in the aortas from diabetic rats compared with healthy rats, even though the diabetic rats were treated with vitamin E and C or carvedilol.

In our study, phenylephrine-induced contraction was impaired in the diabetic aorta. Most studies show that phenylephrine-induced contraction is larger in diabetic than in control aortas (Weber and MacLeod, 1994; Harris and MacLeod, 1988). However, there are conflicting views (Paskaloglu et al., 2004). Xavier et al. found that diabetes induces time-dependent changes in the vascular reactivity to phenylephrine (Xavier et al., 2003). Desensitization of α-adrenergic receptor-mediated smooth muscle contraction and endothelium in aortas may explain this phenomenon (Hiremath et al., 1991). In addition, the progressive decrease of α-adrenoceptor number in diabetic aorta also contributes to this effect (Bucci et al., 2004; Schulingkamp et al., 2005). Carvedilol treatments at both low (1 mg/kg/day) and high doses (10 mg/kg/day) improved the dose-response curve of phenylephrine in diabetic aorta, more significantly at the higher dose. In normal rats, on the contrary, carvedilol at both doses did not inhibit phenylephrine-induced contraction. Thus, the blocking effect of carvedilol on the α1-adrenoceptor seemed not to affect either diabetic or control aortas after chronic treatment. It has

been confirmed that carvedilol antagonizes the myocardial α1-adrenoceptor in many acute models (Qvigstad et al., 2003), but little is known about carvedilol and its actions on myocardial α1-adrenoceptors in chronic models. Kubo et al. found that carvedilol lost its ability to antagonize functional peripheral α1-adrenoceptors during long-term treatment of human congestive heart failure (Kubo et al., 2001). As for the improved response to phenylephrine-induced contraction, its properties as both a β-blocker and an antioxidant may contribute. Since we neither measured the concentration of catecholamines nor compared vitamins E and C with other antioxidants, we cannot yet explain the mechanism. This requires further study.

In conclusion, chronic carvedilol administration improved streptozotocin-induced diabetic endothelium-dependent relaxation. This was associated with its antioxidant activity, since carvedilol increased serum NO level, up-regulated NOS3 mRNA and accelerated NOS3 phosphorylation.

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