ELSEVIER

European Journal of Pharmacology 412 (2001) 51–59

ejp

www.elsevier.nl/locate/ejphar

Relaxation of rat aorta by adenosine in diabetes with and without hypertension: role of endothelium

Mohammad Fahim *, Tahir Hussain, S. Jamal Mustafa

Department of Pharmacology, School of Medicine, East Carolina University, Greenville, NC 27858-4354, USA

Received 14 June 2000; received in revised form 7 November 2000; accepted 10 November 2000

Abstract

Effects of diabetes on the responses of aortic rings of normotensive Wistar–Kyoto (WKY) and spontaneously hypertensive (SHR) rat to adenosine analogues were examined. Streptozotocin-induced diabetes caused an increase in blood glucose and plasma levels of cholesterol and triglycerides in normotensive (diabetic-WKY) as well as hypertensive (diabetic-SHR) rats. In diabetic-SHR group, the body weight was significantly low (50%) as compared to SHR (non-diabetic). Diabetic-SHR group showed the largest heart weight-to-body weight ratio indicating cardiac enlargement. The relaxation responses to adenosine analogues were obtained in endothelium-intact and -denuded aortic rings precontracted with phenylephrine. The IC_{50} values of adenosine analogues were lower in endothelium-intact aortic rings of WKY as compared to diabetic-WKY and -SHR. Aortic rings from diabetic-SHR showed the greatest attenuation in adenosine analogue-mediated relaxation. Removal of endothelium from the aortic rings inhibited the relaxant response of adenosine analogues and abolished the differences among the groups. Nitric oxide (NO) synthase inhibitor L-monomethylarginine (L-NMMA) caused a significant rightward shift in the concentration–response curves in WKY and diabetic-WKY groups, only a small shift in SHR and no change in diabetic-SHR group indicating that it is primarily the inhibition of NO release which is responsible for attenuation of adenosine receptor responses in SHR and diabetic-WKY and there was absence of NO release in diabetic-SHR. Forskolin and sodium nitroprusside equally relaxed the aortic rings in all the groups. This suggested that there was no abnormality in the relaxant property of vascular smooth muscle due to hypertension and/or diabetes. Therefore, it is concluded that streptozotocin-induced diabetes in SHR aggravates the severity of vascular endothelial dysfunction which led to impairment in adenosine receptor-mediated vascular responses.

Keywords: Adenosine receptor; Smooth muscle, vascular; Vasorelaxation; Nitric oxide (NO); Hypertension; Diabetes; EDRF (endothelium-derived relaxing factor)

1. Introduction

The cardiac problems generally associated with diabetes mellitus include hypertension, coronary artery disease, congestive heart failure, and diabetic cardiomyopathy (Laurent et al., 1999; Raptis, 1982). Morphological and functional alterations of the circulatory system occur during hypertension (Azevedo and Osswald, 1992; Dai and McNeill, 1992) and diabetes mellitus (Dai and McNeill, 1992; Garvey et al., 1993; Mayhan, 1992; Pieper et al., 1992; Ruderman and Haudenschild, 1984; Tesfamariam et al., 1989). Hypertension is also known to aggravate the occurrence of cardiovascular complications associated with diabetes in human (Bell, 1989) and experimental animals (Fein et al., 1990). Since hypertension is known to be more prevalent in diabetes (Raptis, 1982), the two conditions added together may lead to increase pathological changes which may accelerate the onset, or increase the severity of cardiomyopathy. In spontaneously hypertensive rats (SHR), combination of hypertension and experimentally induced diabetes caused greater depression in cardiac function with time (Rodrigues and McNeill, 1986).

The vascular endothelium, which is known to modulate vascular smooth muscle tone (Furchgott et al., 1990) through endothelium-derived relaxing factor (EDRF) (Palmer et al., 1988), is reported to be impaired in hypertension (Azevedo and Osswald, 1992; Biaggioni, 1992;

* Corresponding author. Present address: Department of Physiology, V.P. Chest Institute, University of Delhi, P.O. Box 2101, Delhi-110 007, India. Tel.: +91-11-7257102, 7257441, 7257667; fax: +91-11-7257420.

E-mail addresses: vpci@delnet.ren.nic.in, vpciphysiology@yahoo.com (M. Fahim).

0014-2999/01/$ - see front matter
PII: S0014-2999(00)00869-4

Fahim et al., 1993; Li and Bukoski, 1993; Lockette et al., 1986) and diabetes (Laurent et al., 1999; Mayhan, 1992; Tesfamariam et al., 1989). This impairment of endothelium in diabetes is described to be specific for receptor-dependent release of EDRF, since calcium ionophore A23187 response is not impaired (Pieper and Gross, 1988). However, Durante et al. (1988) have reported attenuated response to A 23187 in diabetes. The impairment in the receptor-mediated release of EDRF in diabetes could be due to various factors including a decrease in the release of EDRF, a decreased sensitivity of diabetic vascular smooth muscle to EDRF, inactivation of EDRF by oxygen-derived free radicals, and release of endothelium-derived contracting factor (EDCF). Contradictory results have been reported regarding endothelium-dependent responses to vasorelaxants in diabetes (Gebremedlin et al., 1988; Mayhan, 1992; Pieper and Gross, 1988; Wakabayashi et al., 1987).

Adenosine produces vasorelaxation (Azevedo and Osswald, 1992; Biaggioni, 1992; Laurent et al., 1999; Monopoli et al., 1994), suppresses noradrenergic transmission, inhibits renin release (Kuan et al., 1990) and decreases blood pressure (Azevedo and Osswald, 1992; Biaggioni, 1992; Homma et al., 1992). The vasodilatory effects of adenosine are mediated through A_2 adenosine receptors linked to adenylate cyclase via Gs proteins (Cushing et al., 1991; Makujina et al., 1992). The presence of two types of A_2 receptors, A_{2A} and A_{2B}, in the rat aorta has been demonstrated (Prentice and Hourani, 1996). The vasorelaxation to 5-*N*-ethylcarboxamidoadenosine (NECA), a non-selective adenosine receptor agonist, is known to be mediated by A_{2A} receptors (Laurent et al., 1999; Lewis et al., 1994; Monopoli et al., 1994; Prentice and Hourani, 1996) and A_{2B} receptors (Merkel et al., 1992). The vasorelaxant responses to adenosine are partly endothelium dependent by releasing EDRF (Furchgott et al., 1990; Lewis et al., 1994). There are several reports showing diminished vasorelaxation response to adenosine (Azevedo and Osswald, 1992; Biaggioni, 1992) and impairment of endothelium-dependent vasomotor control (Bassenge, 1992; Toda et al., 1993) in hypertension. Antihypertensive activity of adenosine (Homma et al., 1992) and hypertensive action of adenosine receptor antagonists (Biaggioni, 1992) indicate the significance of the role of adenosine in the regulation of vascular tone and consequently blood pressure. The combined effects of diabetes and hypertension on endothelium-dependent adenosine receptor-mediated vasorelaxant response have not been investigated. Therefore, the present study investigated the effects of streptozotocin-induced diabetes alone and in combination with genetic hypertension on the adenosine receptor-mediated responses of rat aorta.

2. Materials and methods

Normotensive Wistar–Kyoto (WKY) and age-matched (15–17 weeks) spontaneously hypertensive rats (SHR) were used. Rats were purchased from Taconic (New York) and housed in plastic cages at room temperature. They were kept on a 12-h light/dark cycle and were allowed free access to standard laboratory food and water. All experiments were approved by the School of Medicine, East Carolina University Institutional Animal Care and Use Committee, and were carried out under the Guidelines for the Care and Use of Experimental Animals. Rats were divided randomly into non-diabetic normotensive (WKY), normotensive diabetic (diabetic-WKY), non-diabetic hypertensive (SHR) and hypertensive diabetic (diabetic-SHR) groups. Diabetic-WKY and diabetic-SHR groups were injected with 654 mg/kg intraperitoneal streptozotocin to induce diabetes (Mayhan, 1992). The other two groups (WKY, SHR) of rats (non-diabetic) were injected intraperitoneally with vehicle (10 ml/kg citrate buffer, pH 4.5). Rats were weighed and blood samples for the measurement of glucose were obtained before and every week after injections till the day of experiment (8 weeks). Blood pressure was monitored in the conscious state by using tail cuff pump (model 20-NW), pulse amplifier (model 59) and heart rate-pressure meter (model 72 of IITC, Woodland Hills, CA) using a polygraph (model 7D, Grass Inst., Quincy, MA). Blood glucose was measured by using glucose sensor (ExacTech, Medsense, Cambridge, MA). Rats with a blood glucose concentration of greater than 300 mg/dl were considered diabetic. Blood was collected for measurement of plasma levels of total cholesterol and triglycerides using the diagnostic kits (Sigma, St. Louis, MO).

2.1. Organ bath experiments

Rats were sacrificed by decapitation and aortae were dissected and cleaned of fat and connective tissue. Four to five rings of approximately 4 mm in length from each animal were prepared and mounted in 10-ml organ baths filled with Krebs-Henseleit solution and oxygenated with 95% O_2 + 5% CO_2 (pH 7.4, 37°C). The composition of Krebs-Henseleit buffer was (mM): NaCl, 118; KCI, 4.8; $MgSO_4$, 1.2; $NaHCO_3$, 25; $CaCl_2$, 2.5; and glucose, 11. Changes in isometric tension were measured with force transducers (Grass FT.03) connected to Sensomedics dynographs (R 611). Rings were equilibrated for 1 h under an initial tension of 2 g (determined separately from the length–tension relationship); the buffer being changed every 15 min. After equilibration, rings were challenged with (5×10^{-7} M) phenylephrine (ED 75) until constant reproducible contractions were achieved. Phenylephrine at 5×10^{-7} M concentration produced significant contraction in the aortic rings obtained from all the four groups of animals. The increase in tension produced by phenylephrine was not significantly different in the tissues from the different groups of animals (WKY 780 + 55 mg; diabetic WKY 815 + 72 mg; SHR 830 ± 85 mg; diabetic-SHR

810 ± 79 mg). While dissecting the aortic rings, care was taken to have the same size of tissues from the individual animals of all the groups to eliminate the effect of large differences in the eight animals between the groups. The dry weight of aortic rings from different groups of animals was not significantly different. The integrity of endothelium was assessed by the ability of acetylcholine (10^{-6} M) to relax the contracted vascular rings. After achieving sustained and reproducible contractions, various agonists were added in a cumulative fashion to the bath to obtain the concentration–response curves. Agonists additions were made only after the previous response had stabilised. Concentration–response curves (10^{-9}–10^{-4} M), to 2-chloroadenosine, 5-*N*-ethylcarboxamidoadenosine (NECA), 2-*p*-(2-carboxyethyl) phenethylamino-5′-*N*-ethylcarboxamidoadenosine (cgs-21680) and N^6-cyclopentyladenosine (CPA) were generated in endothelium-intact and -denuded aortic rings from rats of all the four groups in order to study the role of endothelium on adenosine receptor-mediated relaxations. Relaxation response to 2-chloroadenosine and NECA was studied before and after addition of L-monomethylarginine (L-NMMA) (30×10^{-5} M) in order to investigate the role of EDRF (NO) in adenosine receptor-mediated relaxation of vascular smooth muscle from different groups. L-arginine (100×10^{-5} M) was used to reverse the effects of L-NMMA on NO release. Concentration–response curves for forskolin (10^{-9}–10^{-5} M) and sodium nitroprusside (10^{-9}–10^{-5} M) were obtained to test the specificity of adenosine-mediated responses of all the groups.

2.2. Drugs

Streptozotocin, phenylephrine hydrochloride, acetylcholine, 2-chloroadenosine, cgs-21680, N^6-cyclopentyl adenosine (CPA), L-arginine, sodium nitroprusside, forskolin were obtained from Sigma, 5-*N*-ethylcarboxamidoadenosine (NECA), L-monomethylarginine (L-NMMA) from Research Biochemicals (Wayland, MA). Streptozotocin was freshly dissolved in citrate buffer (pH 4.5) immediately before use at a concentration of 65 mg/ml. 2-chloroadenosine, CPA and NECA were dissolved in 1 ml of 50% ethyl alcohol and diluted in 9 ml water to make a stock solution of 10^{-2} M. Serial dilutions were done in water. All other drugs were solubilized in water. The maximum concentration (10^{-4} M) of adenosine analogues used contained 0.05 % ethyl alcohol. Same amount of alcohol added to 10 ml organ bath did not produce any significant effect on the tension in the aortic rings.

2.3. Statistical analysis

The relaxation responses to adenosine analogues were expressed as a decline (percent) in steady-state contraction obtained with 5×10^{-7} M phenylephrine. All values are expressed as mean ± S.E.M. Two-way analysis of variance (ANOVA) was used for concentration–response curves. The IC_{50} was calculated (Tesfamariam and Cohen, 1992) in the endothelium-intact aortic rings precontracted with 5×10^{-7} M phenylephrine. Tukey–Kramer multiple comparison test was used for analysis between the groups. The P values for statistical significance were set at the 0.05 level.

3. Results

3.1. Animal model

Eight weeks after streptozotocin injection, blood glucose levels (Table 1) were significantly ($P < 0.05$) higher in diabetic rats. (normotensive and hypertensive) Diabetic normotensive (diabetic-WKY) and diabetic hypertensive (diabetic-SHR) rats showed significantly ($P < 0.05$) lower body weights but greater heart weight-to-body weight ratios as compared to corresponding non-diabetic rats. The hypertensive rats had significantly low body weight. The average body weight of SHR of same age was 2.5 times greater than that of rats treated with streptozotocin (Table 1). Heart weight-to-body weight ratio was higher in SHR as compared to WKY. Diabetic-SHR showed the highest

Table 1
Effects of diabetes on blood glucose, plasma cholesterol and triglycerides, systolic blood pressure, body weight, heart weight and heart weight-to-body weight ratio in SHR and WKY rats

	n	Blood glucose (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Systolic blood pressure (mm Hg)	Body weight (g)	Heart weight (g)	Heart weight/ body weight (g/kg)
WKY	5	96 ± 3	65 ± 4	90 ± 7	124 ± 3	528 ± 9	1.58 ± 0.03	2.99 ± 0.02
diabetic-WKY	8	425 ± 12^{a}	85 ± 6^{a}	264 ± 13^{a}	126 ± 3	295 ± 13^{a}	1.07 ± 0.04^{a}	3.63 ± 0.03^{a}
SHR	5	98 ± 4	35 ± 4^{a}	48 ± 6^{a}	194 ± 3^{a}	314 ± 4^{a}	1.26 ± 0.03^{a}	3.93 ± 0.07^{a}
diabetic-SHR	8	$497 \pm 15^{b,c}$	83 ± 5^{b}	$935 \pm 21^{b,c}$	196 ± 4^{b}	$126 \pm 2^{b,c}$	$0.57 \pm 0.03^{b,c}$	$4.54 \pm 0.06^{b,c}$

Values are means ± S.E.M.; *n*, number of animals in each group; WKY (non-diabetic normotensive); diabetic-WKY (diabetic normotensive); SHR (non-diabetic spontaneously hypertensive); diabetic-SHR (diabetic hypertensive).

[a] $P < 0.05$ WKY vs. diabetic-WKY or WKY vs. SHR.

[b] $P < 0.05$ SHR vs. diabetic-SHR.

[c] $P < 0.05$ diabetic-WKY vs. diabetic-SHR.

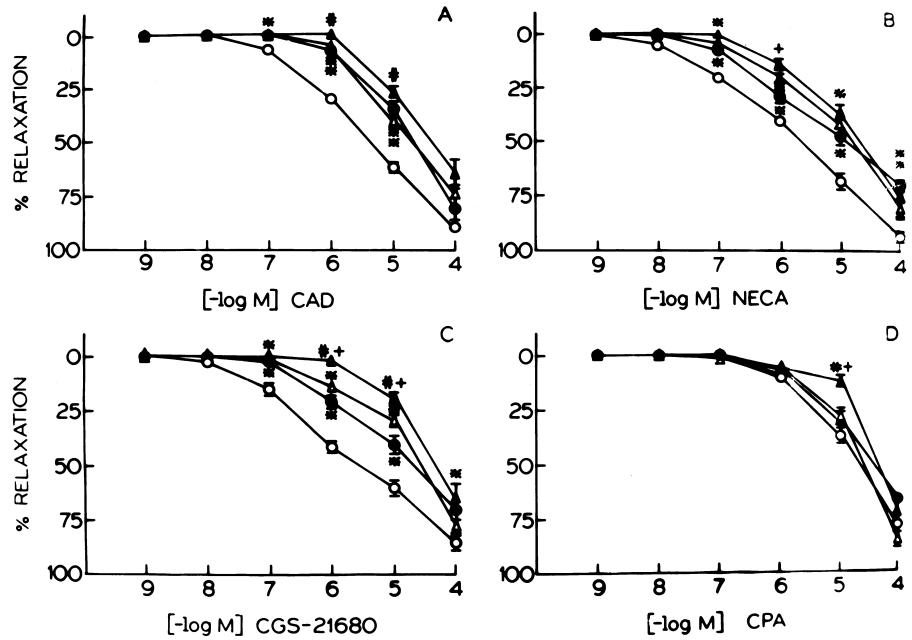

Fig. 1. Cumulative concentration–response curves to adenosine analogues in phenylephrine contracted endothelium-intact rat aortic rings from WKY (○), diabetic-WKY (●), SHR (△) and diabetic-SHR (▲) rats. Each point represents mean ± S.E.M. from 4–6 rings from each animal WKY (5), diabetic-WKY (8), SHR (5) and diabetic-SHR (8) rats. Number in parentheses represents the number of animals in each group. $^{*}P < 0.05$ WKY vs. diabetic-WKY or WKY vs. SHR, $\#P < 0.05$ SHR vs. diabetic-SHR, $+P < 0.05$ diabetic-WKY vs. diabetic-SHR.

heart weight-to-body weight ratio among all the groups. Diabetic rats showed significantly ($P < 0.05$) elevated plasma cholesterol and triglycerides levels as compared to non-diabetic rats. Hypertensive non-diabetic rats (SHR) had lower cholesterol and triglycerides as compared to normotensive non-diabetic (WKY) rats. Systolic blood pressure of SHR was significantly ($P < 0.05$) higher than those of WKY. Streptozotocin-induced diabetes did not affect the systolic blood pressure of either WKY or SHR rats.

3.2. Relaxant response to acetylcholine (10^{-6} m) in endothelium-intact and-denuded aortic rings

In endothelium-intact aortic rings precontracted with 5×10^{-7} M phenylephrine, 10^{-6} M acetylcholine produced 89 ± 22% (five animals) fall in tension produced by phenylephrine in WKY; 78 ± 22% in diabetic-WKY (eight animals); 70 ± 21% (five animals) in SHR and 56 ± 11% (eight animals) in diabetic-SHR. Endothelium was denuded by rotating a wet cotton swab inside the rings. Acetylcholine (10^{-6} M) did not relax the denuded rings.

3.3. Concentration–response curves of adenosine analogues in rings with intact endothelium

2-Chloroadenosine, NECA and cgs-21680 produced a concentration-dependent relaxation in all the groups (Fig. 1). However, N^6-cyclopentyladenosine (CPA) produced significant ($P < 0.05$) relaxation only at high concentrations. Streptozotocin-induced diabetes caused a significant ($P < 0.05$) shift to the right in the concentration–response

Table 2
IC_{50} (μM) values for various adenosine analogues in non-diabetic and diabetic SHR and WKY rats

Adenosine analogue	WKY ($n = 5$)	Diabetic-WKY ($n = 8$)	SHR ($n = 5$)	Diabetic-SHR ($n = 8$)
2-chloro-adenosine	3.45 ± 0.1	7.90 ± 0.2[a]	9.70 ± 0.3[a]	15.5 ± 0.1[b]
NECA	0.96 ± 0.3	4.40 ± 0.2	7.60 ± 0.2[a]	11.7 ± 0.2[b]
cgs-21680	0.93 ± 0.2	6.70 ± 0.2[a]	16.9 ± 0.1[a]	34.5 ± 0.1[b]
CPA	13.4 ± 0.1	20.6 ± 0.1	27.6 ± 0.2	31 ± 0.2[c]

Values are means ± S.E.M.; *n*, number of animals in each group; WKY (non-diabetic normotensive); diabetic-WKY (diabetic-normotensive); SHR (non-diabetic spontaneously hypertensive); diabetic-SHR (diabetic hypertensive).

[a] $P < 0.05$ WKY vs. diabetic-WKY or WKY vs. SHR.

[b] $P < 0.05$ SHR vs. diabetic-SHR.

[c] $P < 0.05$ diabetic-WKY vs. diabetic-SHR.

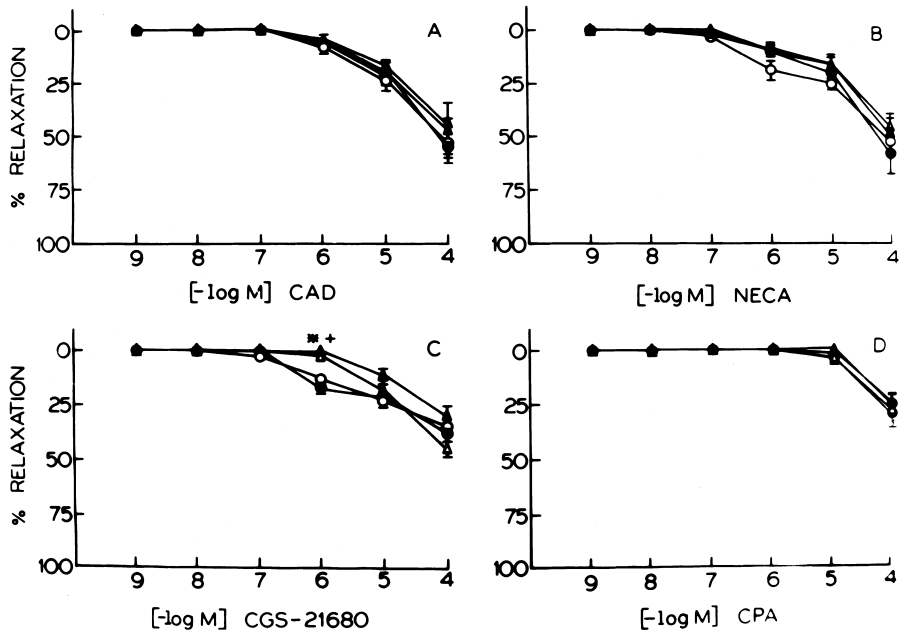

Fig. 2. Cumulative concentration–response curves to adenosine analogues in phenylephrine contracted endothelium-denuded rat aortic rings from WKY (○), diabetic-WKY (●), SHR (△) and diabetic-SHR (▲) rats. Each point represents mean ± S.E.M. from 4–6 rings from each animal WKY (5), diabetic-WKY (8), SHR (5) and diabetic-SHR (8) rats. Number in parentheses represents the number of animals in each group. $^{*}P < 0.05$ WKY vs. SHR, $+P < 0.05$ diabetic-WKY vs. diabetic-SHR.

curves for 2-chloroadenosine, NECA and cgs-21680 for WKY and cgs- 21680 and 2-chloroadenosine for SHR (Fig. 1). IC^{50} values for all the adenosine analogues from the four groups of rats are given in Table 2. The rank order of potency of adenosine analogues for various groups were: WKY, cgs-21680 ≥ NECA > 2-chloroadenosine CPA; diabetic-WKY, NECA > cgs-21680 > 2-chloroadenosine > CPA; SHR, NECA > 2-chloroadenosine >

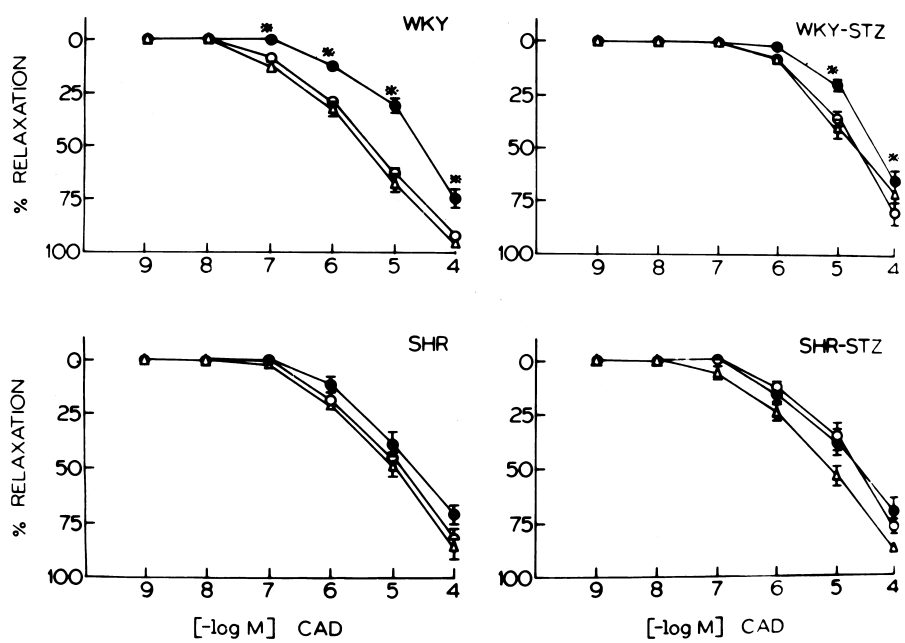

Fig. 3. Cumulative concentration–response curves to 2-chloroadenosine in phenylephrine contracted endothelium-intact rat aortic rings before (○), and after incubation of tissues with 30 μM L-NMMA (●) or 30 μM L-NMMA plus 100 μM L-arginine (△). Each point represents mean ± S.E.M. from 4–6 rings from each animal WKY (5), diabetic-WKY (8), SHR (5) and diabetic-SHR (8) rats. Number in parentheses represents the number of animals in each group. $^{*}P < 0.05$ WKY vs. diabetic-WKY.

cgs-21680 > CPA and diabetic-SHR, NECA > 2-chloroadenosine > cgs-21680 > CPA. In diabetic groups, 2-chloroadenosine (10^{-6} M) produced very small (< 10 %) relaxation.

3.4. Concentration–response curves of adenosine analogues in endothelium-denuded aortic rings

Endothelium removal attenuated the relaxation responses to the adenosine analogues in all groups and furthermore abolished the differences in relaxation observed in the presence of endothelium (Fig. 2).

3.5. Effect of L-NMMA on relaxation response to adenosine analogues

L-NMMA (30 μM) caused a significant ($P < 0.05$) rightward shift in the concentration–response curves for 2-chloroadenosine (Fig. 3) and NECA (Fig. 4) in WKY and diabetic-WKY groups. Rightward shift in SHR was significant ($P < 0.05$) only in the case of NECA (Fig. 4) but not 2-chloroadenosine. L-NMMA-induced rightward shift in the 2-chloroadenosine response curve was smaller in SHR (Fig. 3). Diabetic-SHR rings did not show any significant ($P > 0.05$) change in the concentration–response curves to 2-chloroadenosine and NECA on exposure to L-NMMA (Figs. 3 and 4). L-Arginine (100 μM) reversed the inhibitory responses of L-NMMA (Figs. 3 and 4) in groups which had a shift to the right.

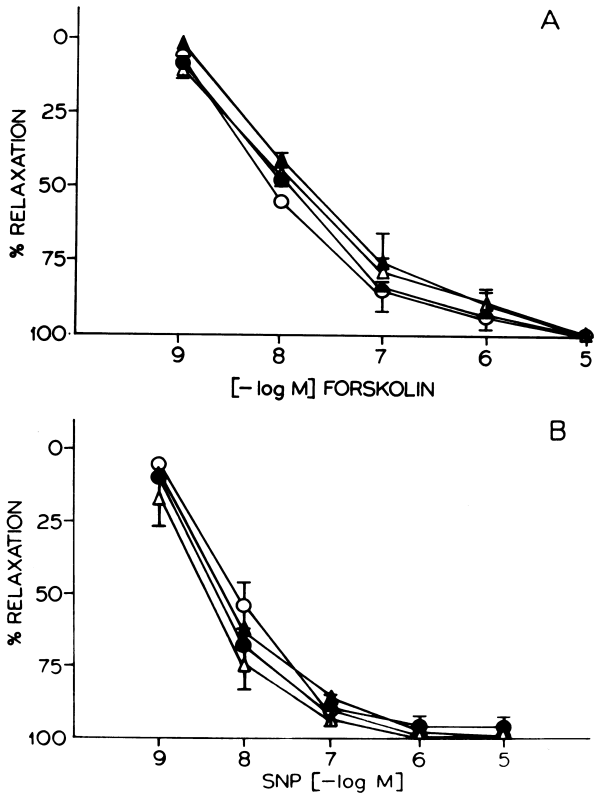

Fig. 5. Cumulative concentration–response curves to forskolin (A) and sodium nitroprusside (B) in phenylephrine contracted endothelium-intact rat aortic rings from WKY (○), diabetic-WKY (●), SHR (△), and diabetic-SHR (▲) rats. Each point is the mean ± S.E.M. of observations on four rings from aorta of each animal WKY (5), diabetic-WKY (8), SHR (5) and diabetic-SHR (8) rats. Number in parentheses represents the number of animals in each group.

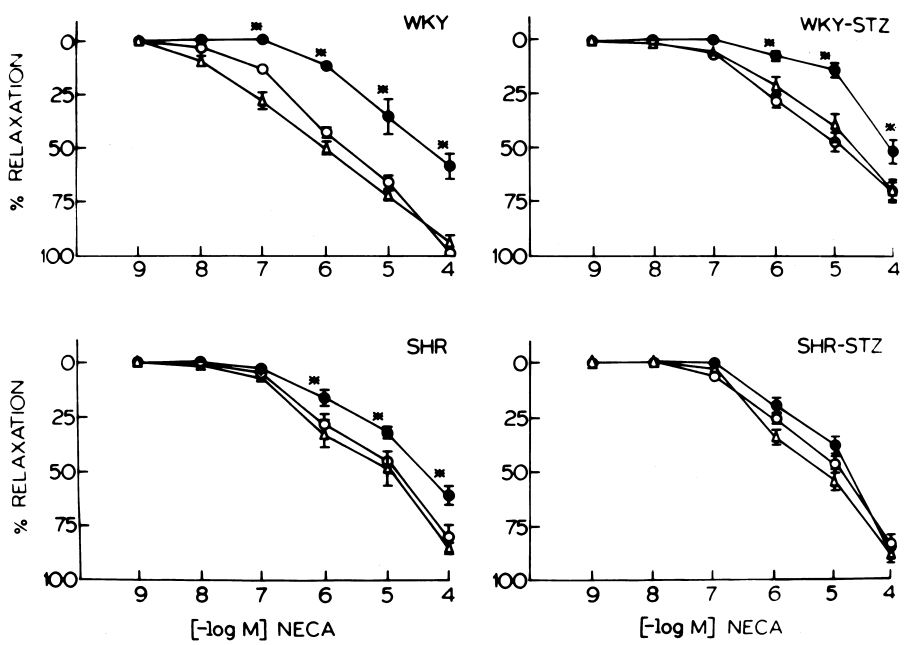

Fig. 4. Cumulative concentration–response curves to NECA in phenylephrine contracted endothelium-intact rat aortic rings before (○), and after incubation of tissues with 30 μM L-NMMA (●) or 30 μM L-NMMA plus 100 μM L-arginine (△). Each point represents mean ± S.E.M. of four rings from aorta of each animal of WKY (5), diabetic-WKY (8), SHR (5) and diabetic-SHR (8) rats. Number in parentheses represents the number of animals in each group. * $P < 0.05$ WKY vs. diabetic-WKY.

3.6. Concentration–response curves for forskolin and sodium nitroprusside

Forskolin (10^{-9}–10^{-5} M) (Fig. 5A) and sodium nitroprusside (10^{-9}–10^{-5} M) (Fig. 5B) showed concentration-dependent relaxations similar in the endothelium-intact and -denuded (data not shown) aortic rings from all the groups of rats. There was no significant difference in the concentration–response curves between the groups.

4. Discussion

The present study shows that endothelium-dependent vasorelaxation responses to adenosine analogues were significantly attenuated in the aorta of age-matched streptozotocin-induced diabetic WKY and SHR rats. The differences between the diabetic and nondiabetic SHR and WKY were abolished after the removal of vascular endothelium indicating impairment of endothelium-dependent mechanism(s) in diabetic and hypertensive rat aortae. The rank order of potency of adenosine analogues fits the typical A_2 receptor classification in WKY. However, in the other groups there may be slight changes in this order due to attenuated endothelial responses to adenosine analogues. Adenosine analogue cgs-21680 is known to have high potency and selectivity for A_{2A} receptors. NECA, a potent vasodilatory adenosine receptor agonist with nearly equal affinity at A_1 and A_2 receptors, stimulates adenylate cyclase via both the A_{2A} and A_{2B} receptors in vascular tissues, dilation induced by adenosine analogues is believed to be mediated mainly by A_2 receptors. However, the vasorelaxation caused by nonselective adenosine analogue CAD with only modest A_2 selectivity over A_1 and CPA, a potent A_1 selective adenosine analogue, may be partly mediated by stimulation of an A_1-like receptor coupled to potassium channels (Merkel et al., 1992).

Diabetes mellitus impairs endothelium-dependent relaxation in isolated blood vessels (Laurent et al., 1999; Mayhan et al., 1991; Oyama et al., 1986; Pieper and Gross, 1988). Oxygen-derived free radicals have been reported to abolish the endothelium-dependent relaxation in diabetic rat aorta (Pieper and Gross, 1988). Attenuation of the vasorelaxant response in diabetes has been demonstrated to be related to the enhanced release of oxygen-derived free radicals in diabetic animals (Pieper et al., 1992) including the in vitro exposure of vascular tissue to elevated glucose (Tesfamariam et al., 1990; Tesfamariam and Cohen, 1992). Hypertension is also known to inhibit endothelium-dependent adenosine receptor-mediated vasorelaxant responses (Azevedo and Osswald 1992; Biaggioni, 1992; Fahim et al., 1993). The present study provides the first evidence of aggravated malfunction of endothelium-dependent relaxation of aorta from SHR following streptozotocin-induced diabetes. Attenuation of adenosine receptor-mediated relaxation by streptozotocin-induced diabetes was more prominent in the vessels of WKY which had normal endothelial function and normal relaxation responses to adenosine analogues. SHR aorta, which had already attenuated responses to adenosine analogues, showed further attenuation in the responses after diabetes. IC_{50} values clearly demonstrate the attenuated vasorelaxant responses in diabetic groups to all the adenosine analogues used in this study. Larger IC_{50} values for SHR as compared to WKY confirm the reduced responsiveness of SHR vascular tissues to adenosine analogues reported earlier from this laboratory (Fahim et al., 1993). In all the groups, NECA was still the most potent adenosine analogue relaxing the aortae from different groups. However, cgs-21680 was equally effective in WKY and WKY with diabetes but not in SHR with and without diabetes. cgs-21680 in these two groups was least potent in relaxing the aortae. These data indicated an altered endothelial response to A_{2A} receptor analogue due to the fact that cgs-21680, an A_{2A} selective adenosine receptor analogue, was less effective than the nonselective analogue NECA with nearly equal affinity to A_1 and A_2 receptors, which is in agreement with Prentice and Hourani (1996). It is suggested that the vasorelaxant response to NECA is mediated by adenosine A_{2A} receptors (Lewis et al., 1994; Monopoli et al., 1994; Prentice and Hourani, 1996), which are located at least in part on the endothelium (Prentice and Hourani, 1996) and partly at other sites (Lewis et al., 1994; Prentice and Hourani, 1996). NECA also has high affinity at A_{2B} receptors (Merkel et al., 1992). Our observations with adenosine analogue CPA, a potent A_1 selective adenosine analogue, clearly demonstrated that in comparison to other analogues of adenosine the response of CPA was much less affected by hypertension or streptozotocin treatment. The vasorelaxant response of CPA is known to be partly non-receptor mediated and endothelium independent (Lewis et al., 1994; Prentice and Hourani, 1996). The response to CPA was least affected by hypertension and/or diabetes as compared to other adenosine analogues used in this study which are known to have endothelium-dependent vasorelaxant response. This supports our findings that the inhibition of vasorelaxant response to adenosine in hypertension and streptozotocin-induced diabetes is largely due to impairment of endothelium. These results suggest that diabetes accompanied by hypertension aggravates the circulatory disorder not only by causing depression of myocardial function (Fein et al., 1990; Rodrigues and McNeill, 1986), but it also influences the vascular responsiveness.

Cardiac hypertrophy in diabetic-WKY rats and larger heart weight-to-body weight ratio in diabetic-SHR as compared to SHR or diabetic-WKY observed in this study indicated the greater degree of cardiac hypertrophy due to added stress of streptozotocin-induced diabetes in hypertensive rats. In an earlier study, it has been reported that WKY rats do not exhibit myocardial impairment even at 12 weeks after streptoztocin injection (Rodrigues and Mc-

Neill, 1986). In Sprague–Dawley rats, myocardial dysfunction started at 9 weeks after streptozotocin injection (Dai and McNeill, 1992). Besides strain differences another possible explanation for the discrepancy in these findings may be due to variation in the severity of diabetes. In the present study, streptozotocin-induced diabetes was severe from 1 week after streptozotocin injection, which might have caused severe impairment in vascular function and significant cardiac hypertrophy. Furthermore, the present study also revealed that 8 weeks of severe diabetes did not affect the blood pressure of normotensive or hypertensive rats. Previous studies in renal hypertensive rats and SHR have shown that the combination of hypertension and streptozotocin-induced diabetes causes histological pathology, and functional impairment in the myocardium (Fein et al., 1990; Rodrigues and McNeill, 1986). Whereas, deoxycorticosterone acetate (DOCA)-induced hypertension did not cause myocardial dysfunction as did streptozotocin-induced diabetes per se (Dai and McNeil, 1992). However, in the present study we observed more cardiac hypertrophy and larger attenuation in the vascular responsiveness to adenosine analogues in rats having both hypertension and diabetes.

A NO synthase inhibitor, L-NMMA shifted the concentration–response curves for 2-chloroadenosine and NECA to the right and L-arginine reversed the inhibition in the relaxation caused by L-NMMA. The rightward shift to 2-chloroadenosine and NECA was most prominent in WKY as compared to other groups. This was further confirmed by the fact that the endothelium removal significantly attenuated these responses. This could be attributed to normal endothelium-dependent mechanism(s) involved in adenosine receptor-mediated vasorelaxant responses. A significant rightward shift in the concentration–response curves in diabetic-WKY aorta by L-NMMA was observed only at higher concentrations of 2-chloroadenosine and NECA. This indicated either an impairment in NO release by adenosine analogues in diabetes and/or attenuated action of NO on vascular smooth muscle. The latter has been supported by a previous study (Pieper et al., 1992) showing that diabetic rat aorta releases similar levels of EDRF in response to acetylcholine, but the action of EDRF was attenuated by enhanced release of oxygen-derived free radicals. Diabetic-SHR aorta did not show any significant ($P > 0.05$) effect of L-NMMA on the concentration–response curves of 2-chloroadenosine and NECA suggesting further impairment of endothelium and total loss of NO-mediated vasorelaxant responses due to added influence of diabetes in SHR. Laurent et al. (1999) have shown that NO synthase inhibitor, L-NAME, reduced the dilator response to adenosine in aortic rings from diabetic rats, but not in those from normal rats suggesting the involvement of NO in the vasorelaxant response to adenosine in diabetic rats. Elevated levels of plasma cholesterol and triglycerides in streptozotocin-treated rats could possibly be another contributing factor in the attenuation of adenosine receptor-mediated vasorelaxation in diabetic rat aorta. Previous studies have shown that oxidized-low density lipoproteins inhibit endothelium-dependent relaxation (Matsuda et al., 1993). The possibility of involvement of other mechanisms, e.g. alteration in EDHF, EDCF, cyclooxygenase products from the endothelium and other unknown factors in diabetes also exists.

As indicated earlier, removal of endothelium abolished the inter-group differences of relaxation mediated by adenosine analogues. This suggested that the relaxing property of vascular smooth muscle was not impaired, which was further supported by the observation that forskolin and sodium nitroprusside produced similar relaxations in aortic rings from all the four groups of rats.

Inhibition of vasorelaxant response to adenosine analogues in SHR has been attributed to the release of EDCF and/or cyclooxygenase products from the vascular endothelium and the possibility of inhibition of release of EDRF (Li and Bukoski, 1993). Involvement of these mechanisms also in diabetes is possible.

In summary, our results showed that endothelium plays a vital role in the adenosine receptor-mediated relaxation of rat aorta. In diabetic or hypertensive rats, vasorelaxant responsiveness to adenosine receptor stimulation is attenuated. Induction of diabetes in SHR caused further reduction in the vascular smooth muscle relaxant responses to adenosine analogues indicating the aggravation of vascular disorder due to the combined effects of hypertension and diabetes. The alterations due to diabetes and hypertension in vascular relaxation were endothelium-dependent suggesting endothelial impairment (possibly NO release) in the presence of normal relaxing response of smooth muscle cells.

Acknowledgements

This work was supported by NIH grant HL 27339. The authors are thankful to Ms. Pam Wynne and Ms. Maninder for secretarial assistance.

References

Azevedo, I., Osswald, W., 1992. Does adenosine malfunction plays a role in hypertension? Pharmacol. Res. 25, 227–236.

Bassenge, E., 1992. Clinical relevance of endothelium-derived relaxing factor (EDRF). Br. J. Clin. Pharmacol. 34, 375–425.

Bell, D.S., 1989. Hypertension in the person with diabetes. Am. J. Med. Sci. 297, 228–232.

Biaggioni, I., 1992. Contrasting excitatory and inhibitory effects of adenosine in blood pressure regulation. Hypertension 20, 457–465.

Cushing, D.J., Brown, G.L., Sabouni, M.H., Mustafa, S.J., 1991. Adenosine receptor-mediated coronary artery relaxation and cyclic nucleotide production. Am. J. Physiol. 261, H343–H348.

Dai, S., McNeill, J.H., 1992. Myocardial performance of STZ-diabetic DOCA-hypertensive rats. Am. J. Physiol. (Heart Circ. Physiol. 32) 263, H1798–H1805.

Durante, W., Sen, A.K., Sunahara, F.A., 1988. Impairment of endothelium-dependent relaxation in aortae from spontaneously diabetic rats. Br. J. Pharmacol. 94, 463–468.

Fahim, M., Makujina, S.R., Mustafa, S.J., 1993. Vasorelaxant response to adenosine analogs in SHR and WKY rats. FASEB J. 7 (4), A547.

Fein, F.S., Zola, E., Malhotra, A., Cho, S., Factor, S.M., Scheuer, J., Sonnenblick, E.H., 1990. Hypertensive-diabetic cardiomyopathy in rats. Am. J. Physiol. (Heart Circ. Physiol. 27) 258, H793–H805.

Furchgott, R.F., Khan, M.T., Jothianandan, D., 1990. Comparison of properties of nitric oxide and endothelium-derived relaxing factor, some cautionary findings. In: Rubanyi, G.S., Vanhoutte, P.M. (Eds.), Endothelium-Derived Relaxing Factors. Karger, Basel, pp. 8–21.

Garvey, S.T., Hardin, H.D., Juhaszova, M., Dominiguez, J.H., 1993. Effects of diabetes on myocardial glucose transport system in rats: implications for diabetic cardiomyopathy. Am. J. Physiol. (Heart Circ. Physiol. 33) 264, H837–H844.

Gebremedlin, D., Koltai, M.Z., Poga'tsa, G., Magyar, K., Hadha'zy, P., 1988. Influence of experimental diabetes on the mechanical responses of canine coronary arteries: role of endothelium. Cardiovasc. Res. 22, 537–544.

Homma II., Watanabe, Y., Abiro, T., 1992. Nucleosides and nucleotides: 112. 2-(Hexyn-1-yl) adenosine-5-uronamides: a new entry of selective A_2 adenosine receptor agonists with potent antihypertensive activity. J. Med. Chem. 35, 2881–2890.

Kuan, C.J., Wells, J.N., Jackson, E.K., 1990. Endogenous adenosine restrains renin release in conscious rats. Circ. Res. 66, 637–646.

Laurent, M., Hillarie-Buys, D., Chapal, J., Dietz, S., Portet, K., Gros, G., Michel, A., 1999. Contrasting effects of streptozotocin-induced diabetes on the in vitro relaxant properties of adenosine in rat pancreatic vascular bed and thoracic aorta. Naunyn-Schmiedeberg's Arch. Pharmacol. 360 (3), 309–316.

Lewis, C.D., Hourani, S.M., Long, C.J., Collis, M.G., 1994. Characterization of adenosine receptors in the rat aorta. Gen. Pharmacol. 25 (7), 1381–1387.

Li, J., Bukoski, R.D., 1993. Endothelium-dependent relaxation of hypertensive resistance arteries is not impaired under all conditions. Circ. Res. 72, 290–296.

Lockette, W., Osaka, Y., Carreter, O., 1986. The loss of endothelium-dependent vascular relaxation in hypertension. Hypertension 8 (Suppl. II), II61–II66.

Makujina, S.R., Sabouni, M.H., Bhatia, S., Mustafa, S.J., 1992. Vasodilatory effects of adenosine A_2 receptor agonists CGS 21680 and CGS 22492 in human vasculature. Eur. J. Pharmacol. 222, 243–247.

Matsuda, Y., Hirata, K., Inoue, N., Svenmatsu, M., Kawishima, S., Akita, H., Yokoyama, M., 1993. High density lipoprotein reverses inhibitory effect of oxidized low density lipoprotein on endothelium-dependent arterial relaxation. Circ. Res. 72, 1103–1109.

Mayhan, W.G., 1992. Impairment of endothelium-dependent dilatation of the basilar artery during diabetes mellitus. Brain Res. 58, 297–302.

Mayhan, W., Simmons, L.K., Sharpe, G.M., 1991. Mechanism of impaired responses of cerebral arterioles during diabetes mellitus. Am. J. Physiol. (Heart Circ. Physiol. 29) 260, H319–H326.

Merkel, L.A., Rivera, L.M., Colussi, D.J., Spada, A.P., Lappe, R.W., Perrone, M.H., Cox, B.F., 1992. Demonstration of vasorelaxant activity with an A_1 selective agonist in porcine coronary artery: involvement of potassium channels. J. Pharmacol. Exp. Ther. 260, 437–443.

Monopoli, A., Conti, A., Dionisotti, S., Casati, C., Camaioni, E., Cristalli, G., Ongini, E., 1994. Pharmacology of the highly selective A_1 adenosine receptor agonist 2-chloro-N^6-cyclopentyladenosine. Arzneim.-Forsch. 44 (12), 1305–1312.

Oyama, Y., Kawasaki, H., Hattori, Y., Kanno, M., 1986. Attenuation of endothelium-dependent relaxation in aorta from diabetic rats. Eur. J. Pharmacol. 131, 75–78.

Palmer, R.M.J., Ashton, D.S., Moncada, S., 1988. Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature 333, 664–666.

Pieper, G.M., Gross, G., 1988. Oxygen-derived free radicals abolish endothelium-dependent relaxation in diabetic rat aorta. Am. J. Physiol. (Heart Circ. Physiol. 24) 255, H825–H833.

Pieper, G.M., Mei, D.A., Langenstroer, P., O'Rourke, S.T., 1992. Bioassay of endothelium derived relaxing factor in diabetic rat aorta. Am. J. Physiol. (Heart Circ Physiol. 32) 263, H676–H680.

Prentice, D.J., Hourani, S.M., 1996. Activation of multiple sites by adenosine analogues in the rat isolated aorta. Br. J. Pharmacol. 118 (6), 1509–1517.

Raptis, S., 1982. Hypertension and diabetics mellitus. In: Rosenthan, J. (Ed.), Arterial Hypertension. Springer, New York, NY, pp. 93–99.

Rodrigues, B., McNeill, J.H., 1986. Cardiac function in spontaneously hypertensive diabetic rats. Am. J. Physiol. (Heart Circ. Physiol. 20) 251, H571–H580.

Ruderman, N.B., Haudenschild, C., 1984. Diabetes as an atherogenic factor. Prog. Cardiovasc. Res. 26, 373–412.

Tesfamariam, B., Cohen, R.A., 1992. Free radicals mediate endothelial cell dysfunction caused by elevated glucose. Am. J. Physiol. (Heart Circ. Physiol. 32) 263, H321–H326.

Tesfamariam, B., Jakubowski, J.A., Cohen, R.A., 1989. Contraction of diabetic rabbit aorta caused by endothelium-derived PHH_2-TxA_2. Am. J. Physiol. (Heart Circ. Physiol. 26) 257, H1327–H1333.

Tesfamariam, B., Brown, M.L., Deykin, D., Cohen, R.A., 1990. Elevated glucose promotes generation of endothelium-derived vasoconstrictor prostanoids in rabbit aorta. J. Clin. Invest. 85, 929–932.

Toda, N., Kitamura, Y., Okamura, T., 1993. Neural mechanism of hypertension by nitric oxide synthase inhibitor in dogs. Hypertension 21, 3–8.

Wakabayashi, I., Hatake, K., Kimura, N., Kakishita, E., Nag, K., 1987. Modulation of vascular tones by the endothelium in experimental diabetes. Life Sci. 40, 643–648.