ELSEVIER

European Journal of Pharmacology 428 (2001) 241–249

ejp

www.elsevier.com/locate/ejphar

Mechanisms underlying attenuated contractile response of aortic rings to noradrenaline in fructose-fed mice

Katsuo Kamata *, Noriyasu Kanie, Akemi Inose

Department of Physiology and Morphology, Institute of Medicinal Chemistry, Hoshi University, Shinagawa, Tokyo, 142-8501, Japan

Received 18 June 2001; received in revised form 27 July 2001; accepted 31 July 2001

Abstract

We hypothesized that an impairment of endothelial dysfunction and an increased response to α-adrenoceptor agonists may occur in fructose-fed, insulin-resistant mice. The aim of the present study was to assess the relationship between endothelial dysfunction and agonist-induced contractile responses in such mice. The acetylcholine-induced relaxation was significantly attenuated in streptozotocin-diabetic and fructose-fed mice. The contractile response to noradrenaline was significantly weaker than the control in fructose-fed but not in streptozotocin-diabetic mice; treatment with N^G-nitro-L-arginine effectively restored this response. Incubating aortic rings with noradrenaline increased the NO_x [nitrite (NO_2^-) and nitrate (NO_3^-)] level and this level was significantly higher in fructose-fed mice than in control mice. Clonidine induced a dose-dependent relaxation in aortic rings pre-contracted with prostaglandin $F_{2\alpha}$ that was completely abolished by N^G-nitro-L-arginine; this relaxation was markedly enhanced in fructose-fed mice. In both control and fructose-fed mice, the clonidine-induced relaxation was significantly attenuated and the noradrenaline-induced contraction augmented by pertussis toxin. These results suggest that endothelial function is attenuated in both fructose-fed and streptozotocin-diabetic mice. It is suggested that the decreased noradrenaline contractile response in fructose-fed mice (compared to both controls and streptozotocin-diabetic mice) may be due to an increase in nitric oxide formation mediated by endothelial GTP-binding-coupled α_2-adrenoceptors.

Keywords: Fructose-fed, mouse; Noradrenaline; Contraction; Endothelium; Insulin-resistance; Aorta

1. Introduction

Insulin resistance and the resulting hyperinsulinaemia are closely related to hypertension in a number of species, including humans (Lucas et al., 1985; Modan et al., 1985; Tedde et al., 1989; Brands et al., 1991; Reaven and Chang, 1991; Sechi and Bartoli, 1997; Abe et al., 1998). Fructose feeding in rats results in hyperinsulinaemia, insulin resistance, hypertriglyceridaemia and hypertension (Hwang et al., 1987; Verma et al., 1996; Richey et al., 1998; Cosenzi et al., 1999). In general, depending on its type, hypertension may be linked to insulin resistance and hyperinsulinaemia (Modan et al., 1985; Lucas et al., 1985; Tedde et al., 1989; Brands et al., 1991; Reaven and Chang, 1991; Sechi and Bartoli, 1997; Abe et al., 1998) or not linked to insulin resistance and hyperinsulinaemia (Manolio et al., 1990; Cambien et al., 1987).

While the endothelium-dependent relaxation of vessels induced by acetylcholine is impaired in blood vessels of the fructose-induced hypertensive rat (Verma et al., 1996; Richey et al., 1998; Kamata and Yamashita, 1999), the contractile responses to vasoactive agents may be unchanged (Kotchen et al., 1991; Verma et al., 1996, 1997; Iyer and Katovich, 1996), although some reports suggest attenuation (Bunnag et al., 1997; Berger et al., 1998). It has been reported that mice lacking the gene for endothelial nitric oxide synthase are hypertensive (Huang et al., 1995) and that insulin resistance plays an essential role in the hypertension and hypertriglyceridaemia and in the reduced endothelium-dependent vascular relaxation seen in mice lacking insulin-receptor substrate-1 (Abe et al., 1998). Thus, we hypothesized that an impairment of endothelial dysfunction and an increased response to α-adrenoceptor agonists may occur in fructose-fed, insulin-resistant mice. However, there have as yet been no reports of any change in such contractile responses in fructose-fed mice. As soon as we began to investigate changes in noradrenaline-induced contractile response in fructose-fed mice, we noted

* Corresponding author. Tel./fax: +81-3-5498-5856.
E-mail address: kamata@hoshi.ac.jp (K. Kamata).

0014-2999/01/$ - see front matter
PII: S0014-2999(01)01262-6

a marked decline in the concentration-dependent contraction of aortic rings. In these fructose-fed mice, blood pressure was significantly increased. We therefore decided to make a more detailed examination of the decreased contractile response of aortic rings to noradrenaline and to compare responses between fructose-fed mice and streptozotocin-induced diabetic mice.

2. Materials and methods

2.1. Experimental design

Male ICR mice aged 5 weeks and weighing 27.8 ± 1.4 g were housed under constant climatic conditions (temperature 21°–22 °C, relative air humidity $50 \pm 5\%$). The appropriate diet and water were available ad libitum to each animal. This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the Committee on the Care and Use of Laboratory Animals of Hoshi University, which is accredited by the Ministry of Education, Science and Culture, Japan.

Mice were divided into three groups: controls, fructose-fed mice and streptozotocin-induced diabetic mice. Starting at 5 weeks of age, male mice were fed a fructose-rich diet (containing 60% fructose), whereas age-matched control animals received standard mouse chow. Five-week-old male ICR mice received a single injection of streptozotocin (200 mg/kg) in the tail vein in order to induce diabetes. Age-matched controls were injected with a similar volume of citrate buffer. Streptozotocin-induced diabetic mice were fed a normal diet. The experiments described here were performed 12 weeks after the start of fructose feeding or the injection of streptozotocin. Blood pressure was recorded by the tail-cuff method.

2.2. Treatment with pertussis toxin

Some mice were treated with pertussis toxin for 3 days prior to the experiment. The mice were anaesthetized with an i.p. injection of sodium pentobarbitone (50 mg/kg) and then injected i.v. either with 10 μg/kg pertussis toxin in 0.0125 M Tris buffer containing 25% glycerin and 0.25 M NaCl or with the buffer alone as a control.

2.3. Measurement of isometric force

At the end of the 12-week feeding period, the age-matched controls, fructose-fed mice and streptozotocin-induced diabetic mice were anaesthetized with ether, a midline incision was made and blood was obtained from the abdominal aorta to be used to estimate plasma cholesterol and glucose levels. The blood was centrifuged at 3000 rpm for 10 min at 4 °C and the plasma was isolated and stored at −80 °C. The thoracic aorta was then rapidly dissected out and placed in ice-cold modified Krebs–Henseleit solution (composition in mM: NaCl, 118.0; KCl, 4.7; $NaHCO_3$, 25.0; $CaCl_2$, 1.8; NaH_2PO_4, 1.2; $MgSO_4$, 1.2; dextrose, 11.0). Each aorta was separated from the surrounding connective tissue and cut into rings (3 mm long), special care being taken not to damage the endothelium. The rings were then suspended in organ-baths, between a clip and a force-displacement transducer (TB-611T; Nihon Kohden, Japan), by means of two stainless-steel wires inserted into the lumen. A resting tension of 1.5 g (determined to be optimum in preliminary studies) was applied to facilitate the measurement of isometric force. The organ chamber was filled with 10 ml of Krebs–Henseleit solution at 37 °C and gassed with 95% O_2–5% CO_2. Following a 1-h equilibration period, prostaglandin $F_{2\alpha}$ was added to the organ bath at a concentration high enough (10^{-6}–3×10^{-6} M) to induce contraction. After this contraction had reached a plateau, 10^{-5} M acetylcholine was added to the organ bath to confirm the integrity of the endothelium. The aortic rings were completely relaxed at this concentration of acetylcholine. The effects of drugs were then tested. The tissue was allowed to relax and equilibrate for 40 min between drug applications. For the relaxation studies, the aortic rings were precontracted with an equieffective concentration of prostaglandin $F_{2\alpha}$ (10^{-6}–3×10^{-6} M). This concentration produced about 95% of the maximal response, each ring developing a tension of about $0.8\,g$ whether it was from a control, streptozotocin-treated, fructose-fed, or agent-treated mouse. Relaxant agents (acetylcholine and clonidine) were added cumulatively once the prostaglandin $F_{2\alpha}$-induced contraction had reached a plateau. For the contraction studies, noradrenaline (10^{-9}–10^{-5} M) was added cumulatively to the bath until a maximal response was achieved. After the addition of sufficient aliquots of the agonist to produce the chosen concentration, a plateau response was allowed to develop before the addition of the next dose of the same agonist. To investigate the influence of 10^{-4} M N^G-nitro-L-arginine and 10^{-6} M yohimbine on the noradrenaline-induced contractile responses, the rings were incubated for 30 min in the appropriate medium before the cumulative addition of the agonist.

2.4. Measurement of serum cholesterol and glucose

Plasma cholesterol levels were determined using a commercially available enzyme kit (Wako, Osaka, Japan). The concentration of glucose in plasma was determined by the *O*-toluidine method.

2.5. Measurement of NO_2^- and NO_3^-

The concentration of nitrite plus nitrate in the effluent from each type of tissue was assayed by the method described by Kamata and Yamashita (1999). Briefly, the NO_2^- and NO_3^- in the perfusate were separated on a reverse-phase separation column packed with polystyrene

Table 1
Plasma levels of cholesterol, triglyceride, glucose and insulin in control, fructose-fed and streptozotocin-induced diabetic mice

Groups	Total cholesterol (mg/dl)	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	Triglyceride (mg/dl)	Plasma glucose (mg/dl)	Plasma insulin (μU/ml)
Control	148.2 ± 9.3	78.9 ± 5.6	66.7 ± 7.0	110.5 ± 14.6	220.0 ± 13.3	18.5 ± 3.0
Fructose-fed	209.3 ± 13.0[a]	114.8 ± 7.8[a]	96.3 ± 7.0[a]	249.4 ± 34.7[b]	202.7 ± 10.7	47.7 ± 10.1[b]
Streptozotocin-diabetic	277.8 ± 36.3[a]	131.5 ± 7.4[c]	142.6 ± 30.0[b]	289.4 ± 38.7[b]	882.7 ± 86.7[c]	3.1 ± 0.8[c]

Values are the mean ± S.E.M. from 12 animals.
[a] $P < 0.01$.
[b] $P < 0.05$.
[c] $P < 0.001$.

polymer (NO-PAK, 4.6 × 50 mm; Eicom), after which NO_3^- was reduced to NO_2^- in a reduction column packed with copper-plated cadmium filings (NO-RED; Eicom). The NO_2^- was mixed with Griese reagent, which was 1.25% HCl containing 5 g/l sulphanilamide with 0.25 g/l *N*-naphthylethylenediamine, to form a purple azo dye in a reaction coil. The separation and reduction columns and the reaction coil were then placed in a column oven set at 35 °C. The absorbance of the coloured product at 540 nm was measured with a flow-through spectrophotometer (NOD-10; Eicom). The mobile phase, which was delivered by a pump at a rate of 0.33 ml/min, was 10% methanol containing 0.15 M NaCl/NH_4Cl and 0.5 g/l 4 Na-ethylenediaminetetraacetic acid. The Griese reagent was delivered at a rate of 0.1 ml/min. For the determination of NO_2^- and NO_3^-, the samples were collected over a 0- or 40-min period during stimulation with 10^{-6} M acetylcholine or 3×10^{-7} M noradrenaline. The concentration of NO_2^- plus NO_3^- in the medium and the reliability of the reduction column were checked in each experiment.

2.6. Drugs

Clonidine, Na-ethylenediaminetetraacetic acid, N^G-nitro-L-arginine, noradrenaline, pertussis toxin, serotonin, sodium nitroprusside and streptozotocin were all purchased from Sigma (St. Louis, MO, USA). Acetylcholine was from Daiichi Pharmaceutical (Tokyo, Japan) and prostaglandin $F_{2\alpha}$ from Ono Pharmaceutical (Osaka, Japan). Prostaglandin $F_{2\alpha}$, sodium nitroprusside and acetylcholine were dissolved in 0.9% saline immediately before each experiment. Isotonic high-K^+ solution was prepared by replacing the NaCl in Krebs–Henseleit solution with KCl. All drugs were dissolved in saline, except where otherwise noted. All concentrations are expressed as the final molar concentration of the base in the organ bath.

2.7. Statistics

Data are expressed as the means ± S.E.M. In some experiments, statistical significance of differences was evaluated with Dunnett's test for multiple comparisons after a one-way analysis of variance, a probability level of $P < 0.05$ being regarded as significant. Statistical comparisons between concentration–response curves were made by means of a two-way analysis of variance (ANOVA) with a post-hoc Bonferroni correction for multiple comparisons. A two-tailed value of $P < 0.05$ was considered significant.

3. Results

3.1. General

As indicated in Table 1, plasma glucose levels were significantly elevated above the control in streptozotocin-induced diabetic mice but not in fructose-fed mice. Plasma insulin levels were significantly lower than the control in streptozotocin-induced diabetic mice and significantly higher in fructose-fed mice. Plasma total cholesterol, high-density lipoprotein and low-density lipoprotein cho-

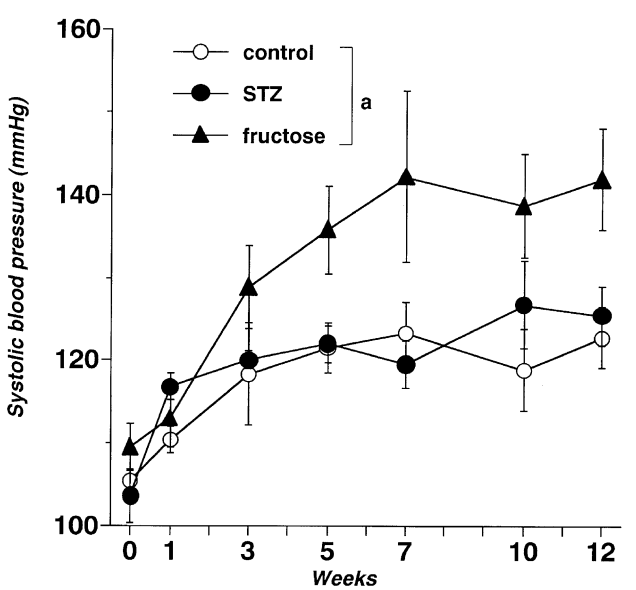

Fig. 1. Changes in blood pressure in fructose-fed mice (fructose) and streptozotocin-induced diabetic mice (STZ) over the 12-week experimental period. Each point represents the mean ± S.E.M. for eight animals. [a] $P < 0.05$, control vs. fructose-fed.

lesterol levels were all significantly increased in both streptozotocin-induced diabetic mice and fructose-fed mice.

The blood pressure in the fructose-fed mice was higher than that of the controls at 3 weeks and thereafter rose progressively though more gradually (Fig. 1). In contrast, the blood pressure of the streptozotocin-diabetic mice was never significantly different from that of the controls. The controls for fructose-fed mice and those for streptozotocin-induced diabetic mice were very similar with respect to blood parameters, blood pressure, acetylcholine-induced relaxation and noradrenaline-induced contraction. Consequently, we pooled the control groups throughout the experiments.

3.2. Relaxation responses induced by acetylcholine and sodium nitroprusside

When the contraction induced by prostaglandin $F_{2\alpha}$ (10^{-6} to 3×10^{-6} M) had reached a plateau, acetylcholine (10^{-9}–10^{-5} M) or sodium nitroprusside (10^{-9}–10^{-5} M) was added cumulatively. The results are summarized in Fig. 2. In aortic rings from control mice, acetylcholine (10^{-9}–10^{-5} M) caused dose-dependent relaxation. This relaxation was significantly weaker in rings from both fructose-fed and streptozotocin-induced diabetic mice (Fig. 2A). The pD_2 values for acetylcholine were

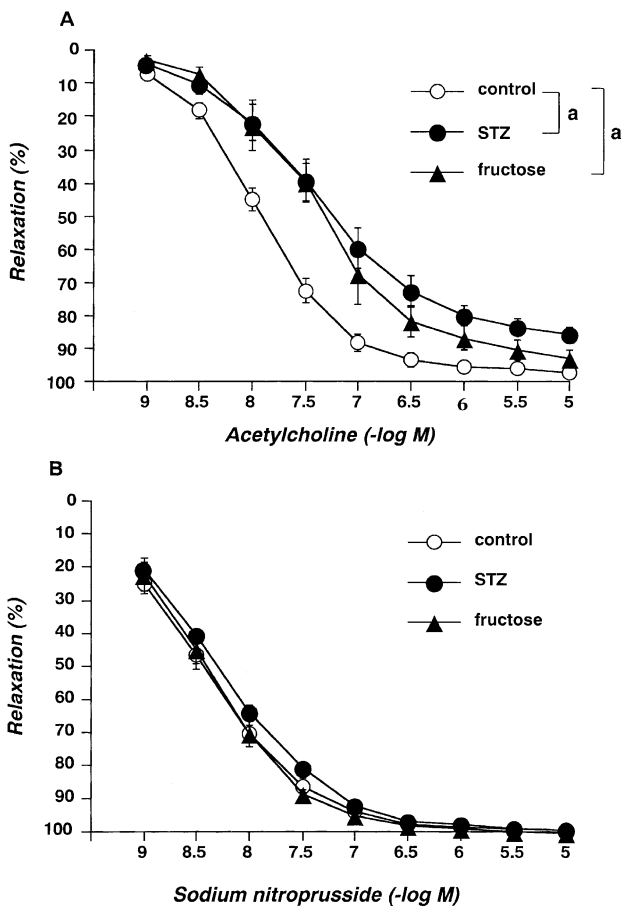

Fig. 2. Dose–response curves for acetylcholine-induced and sodium nitroprusside-induced relaxations of aortic rings from age-matched controls, fructose-fed mice (fructose) and streptozotocin-induced diabetic mice (STZ). (A) Acetylcholine-induced relaxation. (B) Sodium nitroprusside-induced relaxation. Each point represents the mean ± S.E.M. for 8–10 animals. $^{a}P < 0.05$, control vs. STZ or fructose.

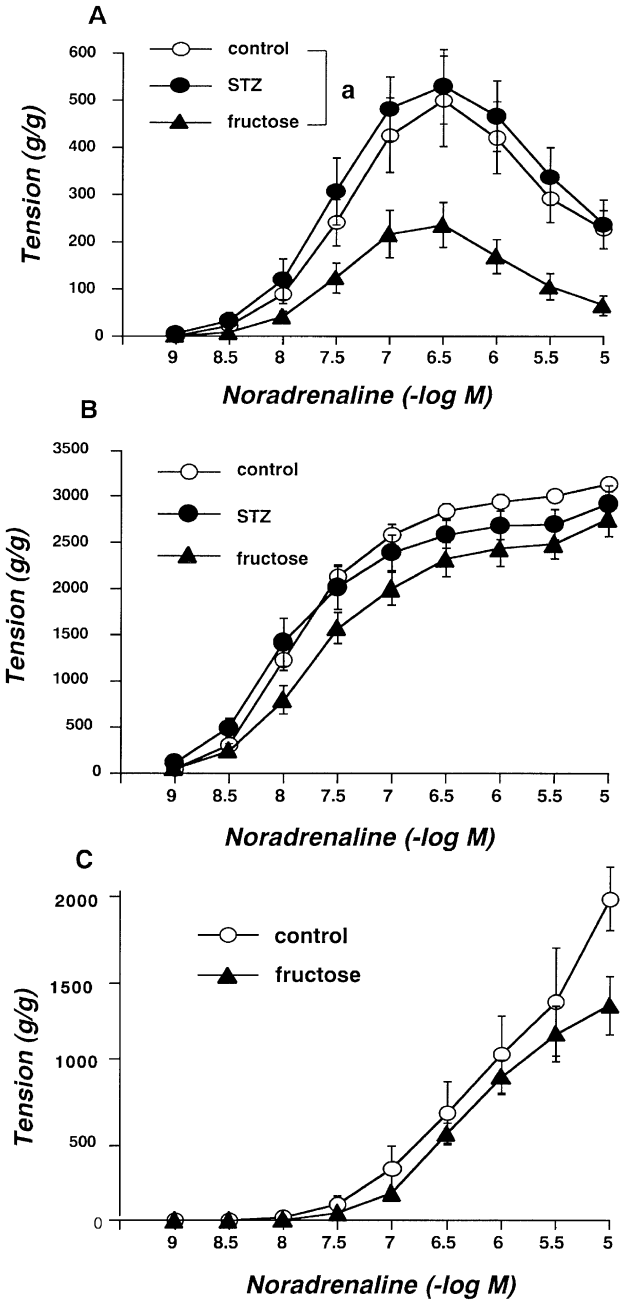

Fig. 3. Dose–response curves for noradrenaline-induced contractions of aortic rings from age-matched controls, fructose-fed mice (fructose) and streptozotocin-induced diabetic mice (STZ). (A) Noradrenaline-induced contractile response. (B) Noradrenaline responses in the presence of N^{G}-nitro-L-arginine (10^{-4} M). (C) Noradrenaline responses in the presence of yohimbine (10^{-6} M). Note different ordinate scales in A, B and C. Each point represents the mean ± S.E.M. for 8–10 animals. $^{a}P < 0.05$, control vs. fructose.

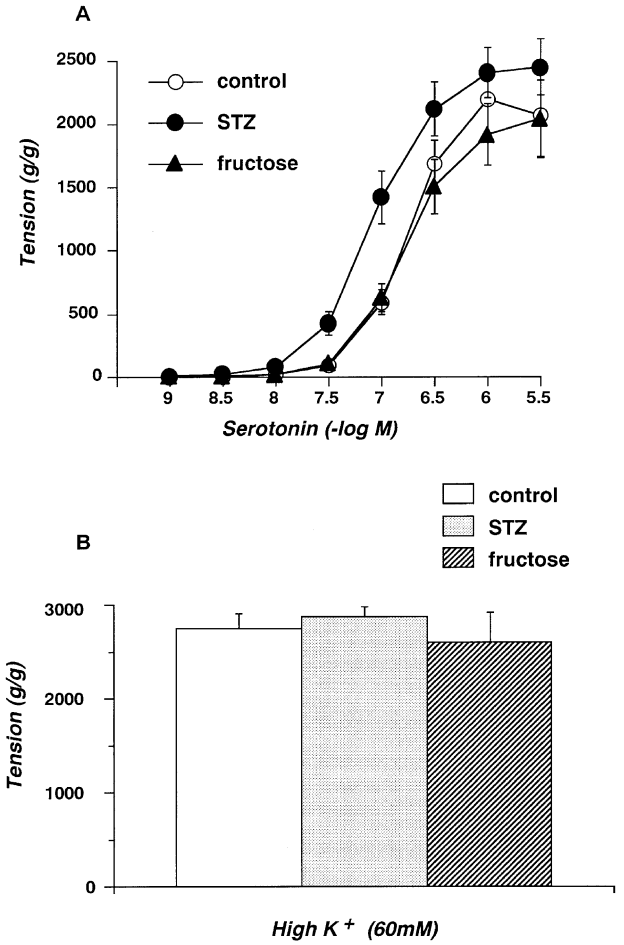

Fig. 4. Dose–response curves for serotonin- and isotonic high-K^+-induced contractions of aortic rings from age-matched controls, fructose-fed mice (fructose) and streptozotocin-induced diabetic mice (STZ). (A) Serotonin-induced contraction. (B) Contraction induced by isotonic high-K^+. Each point represents the mean ± S.E.M. for 8–10 animals.

7.93 ± 0.08, 7.44 ± 0.10 ($P < 0.01$) and 7.39 ± 0.09 ($P < 0.001$) in controls, fructose-fed mice and streptozotocin-diabetic mice. In contrast, the aortic relaxation caused by sodium nitroprusside (10^{-9} ~ 10^{-5} M) did not differ among the three groups (Fig. 2B), the pD_2 values being 8.4 ± 0.10, 8.4 ± 0.09 and 8.3 ± 0.08 in controls, fructose-fed mice and streptozotocin-diabetic mice, respectively.

3.3. Contractile responses induced by noradrenaline, serotonin and isotonic high K^+

Fig. 3A shows dose–response curves for the contractile responses of aortic rings to noradrenaline. The contractile response of the mouse aorta was slight in comparison with that reported for rat aorta [maximal contraction in mouse aorta, 0.5 g; in rat aorta, > 1.0 g (Kamata and Makino, 1997)]. In aortic rings from control mice, the dose–response curve to noradrenaline (10^{-9} to 10^{-5} M) was a bell-shaped. A much smaller dose-dependent contraction was seen in fructose-fed mice. The pD_2 values for noradrenaline were 7.45 ± 0.09, 7.53 ± 0.08 and 7.56 ± 0.09 in controls, fructose-fed mice and streptozotocin-diabetic mice (no significantl differences), respectively. When aortic rings were incubated with N^G-nitro-L-arginine (10^{-4} M) or yohimbine (10^{-6} M) the noradrenaline-induced bell-shaped dose–response curves were changed to sigmoid-shaped curves and the responses were greatly increased at all concentrations in all groups (Fig. 3B,C). The pD_2 values for noradrenaline in the presence of N^G-nitro-L-arginine were 7.79 ± 0.09, 7.61 ± 0.06 and 7.89 ± 0.09 in controls, fructose-fed mice and streptozotocin-diabetic mice (no significant differences).

The contractile response of aortic rings to serotonin (10^{-9} to 3×10^{-6} M) was greater than that of the control (though not significantly) in streptozotocin-induced diabetic mice but not in fructose-fed mice (Fig. 4A). The pD_2 values for serotonin were 6.78 ± 0.08, 6.79 ± 0.06 and 7.05 ± 0.05 ($P < 0.05$) in controls, fructose-fed mice and streptozotocin-diabetic mice, respectively. In contrast, the isotonic K^+ (60 mM)-induced contractile response did not differ among the three groups (Fig. 4B).

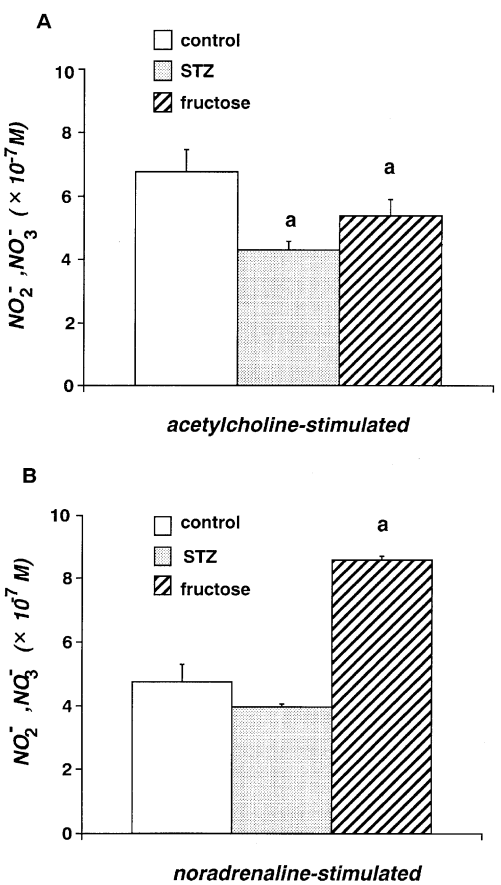

Fig. 5. Acetylcholine (10^{-5} M)- and noradrenaline ($10^{-3} \times 10^{-7}$ M)-stimulated release of NO_2^- plus NO_3^-. Each column represents the mean ± S.E.M. for six animals. [a] $P < 0.05$, control vs. STZ or fructose.

3.4. Measurement of NO_2^- plus NO_3^-

Acetylcholine increased the NO_2^- plus NO_3^- level in the perfusate from aortic strips (Fig. 5A). This response to acetylcholine (10^{-6} M) was weaker in both streptozotocin-induced diabetic mice and fructose-fed mice than in the controls. In contrast, the noradrenaline (3×10^{-7} M)-stimulated NO_2^- plus NO_3^- level was significantly higher in fructose-fed mice than in either the controls or the streptozotocin-induced diabetic mice (Fig. 5B).

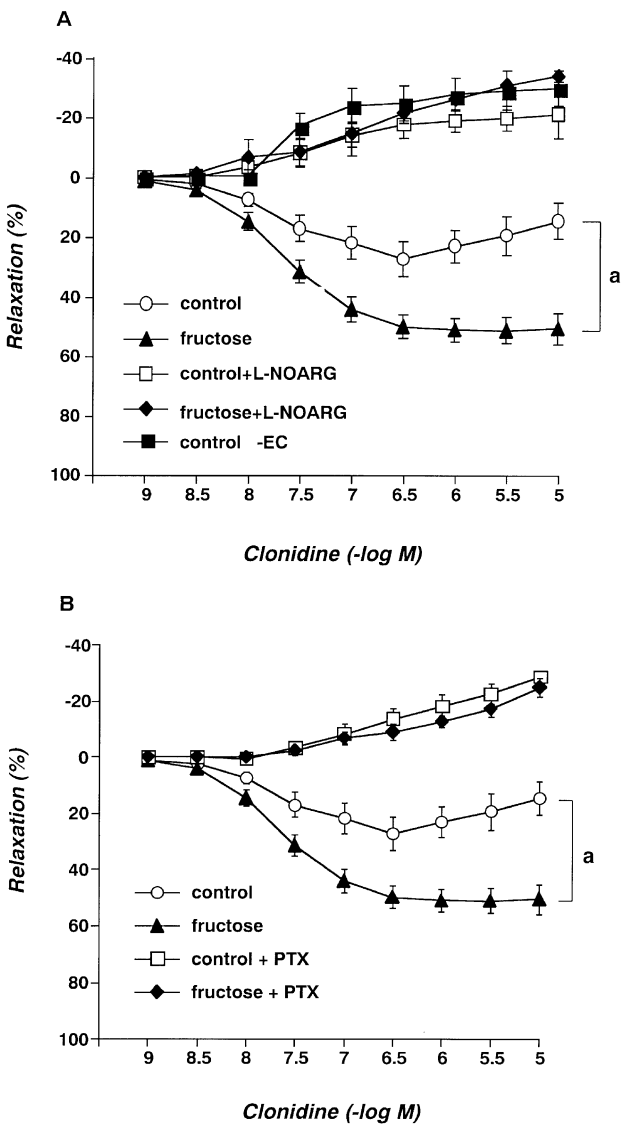

Fig. 6. Dose–response curves for clonidine-induced and relaxations of aortic rings from age-matched controls, and fructose-fed mice (fructose). (A) Clonidine-induced relaxation in the absence or presence of 10^{-4} M N^G-nitro-L-arginine and in an endothelium-denuded preparation from control mice. (B) Effects of pretreatment of mice with pertussis toxin (PTX, 10 μg/kg) on clonidine-induced relaxations. Each point represents the mean ± S.E.M. for 8–10 animals. $^aP < 0.05$, control vs. fructose.

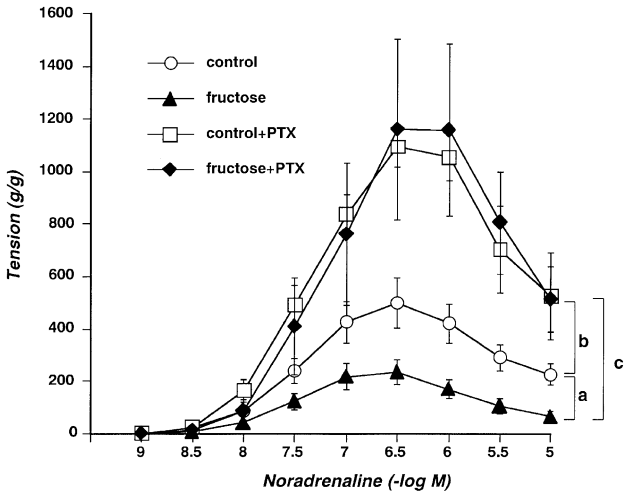

Fig. 7. Effects of pertussis toxin on dose–response curves for noradrenaline-induced contractions of aortic rings from age-matched controls, and fructose-fed mice (fructose). Pertussis toxin (PTX) was given to mice at 10 μg/kg. Each point represents the mean ± S.E.M. for 8–10 animals. $^aP < 0.05$ control vs. fructose; $^bP < 0.01$, controls vs. controls treated with pertussis toxin; $^cP < 0.01$, fructose-fed mice vs. fructose-fed mice treated with pertussis toxin.

3.5. Relaxation response induced by clonidine

When the contraction induced by prostaglandin $F_{2\alpha}$ (10^{-6}–3×10^{-6} M) had reached a plateau, clonidine (10^{-9}–10^{-5} M) was added cumulatively. In aortic rings from control mice, clonidine (10^{-9}–10^{-5} M) caused a dose-dependent relaxation. This relaxation was significantly greater in rings from fructose-fed mice (Fig. 6A). The pD_2 values for the clonidine-induced relaxation were 7.59 ± 0.06 and 7.63 ± 0.09 in controls and fructose-fed mice, respectively (not significantly different). When aortic rings were pretreated with N^G-nitro-L-arginine (10^{-4} M), the clonidine-induced dose-dependent relaxation was completely abolished in both control and fructose-fed mice (Fig. 6A). The clonidine-induced dose-dependent relaxation was changed to a contractile response when endothelium-denuded, rather than endothelium-intact, aortas from control mice were examined. In both control and fructose-fed groups, the clonidine-induced relaxation response was significantly inhibited in aortic rings from mice pretreated with pertussis toxin (10 μg/kg) (Fig. 6B).

3.6. Effects of pertussis toxin on noradrenaline-induced contraction

The noradrenaline-induced dose-dependent contraction was significantly and markedly increased by pretreatment of mice with pertussis toxin (10 μg/kg) and, under the influence of this toxin, the dose–response curve for fructose-fed mice was comparable to that for control mice (Fig. 7). The pD_2 values for noradrenaline were 7.44 ± 0.06 and 7.24 ± 0.07 in controls treated with pertussis

toxin and fructose-fed mice treated with pertussis toxin (not significantly different).

4. Discussion

The main conclusion to be drawn from the present results is that the contractile response of aortic rings to noradrenaline is significantly attenuated in fructose-fed mice and that this attenuation may be mainly due to an increased activity of the GTP-binding protein that is coupled to α_2-adrenoceptors on the endothelium.

The plasma levels of triglyceride, total cholesterol, low density lipoprotein cholesterol and high density lipoprotein cholesterol were all significantly raised in both fructose-fed mice and streptozotocin-diabetic mice. If these factors affect blood pressure, the blood pressure of both the fructose-fed and the streptozotocin-diabetic mice should have been higher than that of the age-matched controls. However, only the fructose-fed group had a blood pressure above that of the controls (see below). The plasma insulin levels were much lower in the streptozotocin-induced diabetics than in the age-matched controls. Furthermore, the plasma glucose level was markedly elevated in streptozotocin-diabetic mice in comparison with that of the controls. In contrast to those seen in streptozotocin-diabetic mice, the plasma glucose levels were similar in controls and fructose-fed mice even though the plasma insulin level of the latter was much higher than that of the controls, suggesting that fructose-fed mice are insulin-resistant. The main differences between fructose-fed mice and streptozotocin-diabetic mice would seem to be the relative insulin resistance and high blood pressure of the former group. Indeed, recent evidence indicates that insulin resistance plays an essential role in the hypertension and hypertriglyceridaemia and the reduced endothelium-dependent vascular relaxation seen in mice lacking insulin-receptor substrate-1 (Abe et al., 1998).

The endothelium-dependent relaxation was attenuated in both fructose-fed mice and streptozotocin-diabetic mice. This is consistent with previous observations of a dysfunction of the endothelium in both fructose-fed animals (Verma et al., 1996; Richey et al., 1998; Kamata and Yamashita, 1999) and streptozotocin-induced diabetic animals (Oyama et al., 1986; Pieper and Gross, 1988; Kamata et al., 1989a,b, 1996; Cohen, 1993; Poston and Taylor, 1995; Pieper, 1998; Kamata and Nakajima, 1998; Kobayashi and Kamata,1999a,b, Makino et al., 2000). The noradrenaline-induced contractile response was markedly increased in all groups by treatment with N^G-nitro-L-arginine, an inhibitor of nitric oxide synthase, suggesting that the noradrenaline-induced contractile response is regulated by nitric oxide released from the endothelium. Since endothelium-dependent relaxation was impaired in both fructose-fed mice and streptozotocin-diabetic mice, the noradrenaline-induced contractile response would be expected to be enhanced in both groups. However, it was significantly attenuated in fructose-fed mice but no different from the control in streptozotocin-diabetic mice. The serotonin-induced contractile response was slightly increased in streptozotocin-diabetic mice and unchanged in fructose-fed mice, while the contractile response to isotonic high K^+ (60 mM) did not differ among the three groups. These results suggest that the contractility of aortic smooth muscle is not different between control and fructose-fed mice and that whether any changes in contractile responses are seen between groups depends on the agonist used.

The acetylcholine-stimulated NO_x [nitrite (NO_2^-) and nitrate (NO_3^-)] level was significantly lower in both fructose-fed and streptozotocin-induced diabetic mice than in the controls. This result was consistent with our finding that the endothelium-dependent relaxation induced by acetylcholine was attenuated in both groups. In marked contrast, the noradrenaline-stimulated NO_x level was significantly raised in fructose-fed mice. It has been reported that there are functional α_2-adrenoceptors on the endothelium and nitric oxide has been shown to inhibit the contractile effects of α-adrenoceptor agonists in vascular smooth muscle (Egleme et al., 1984; Carrier and White, 1985; Martin et al., 1986; Alosachie and Godfraind, 1988; Kaneko and Sunano, 1993). Thus, in mouse aortic rings, the observed noradrenaline-induced contractile response may be the result of summation of a contraction mediated by α_1-adrenoceptors on the smooth muscle and a relaxation mediated by α_2-adrenoceptors on the endothelium. In the next experiment, therefore, we examined the relaxation response to clonidine, an α_2-adrenoceptor agonist, in fructose-fed and control mice. The clonidine-induced relaxation response was greater in fructose-fed mice and was completely inhibited by N^G-nitro-L-arginine, a nitric oxide synthase inhibitor, indicating that the clonidine-induced relaxation is dependent on nitric oxide release from the endothelium. The clonidine-induced relaxation was also inhibited in rings from mice pretreated with pertussis toxin, an inhibitor of the GTP-binding protein coupled to the α_2-adrenoceptor (Flavahan et al., 1989; Boulanger and Vanhoutte, 1997). This suggests that the α_2-adrenoceptor of mouse aorta is coupled to a GTP-binding protein that is activated in fructose-fed mice. If this is the case, then the contractile response of mouse aortic rings to noradrenaline should be enhanced by pertussis toxin, because the noradrenaline-induced contractile response is negatively regulated via α_2-adrenoceptors on the endothelium. In fact, the contraction induced by noradrenaline was markedly enhanced by pertussis toxin in both fructose-fed and control mice. These results suggest that α_2-adrenoceptor-mediated endothelium-dependent relaxation exerts an important modulating influence over α-adrenoceptor-induced contraction. It is interesting that there are of selective dysfunction of the pertussis-toxin-sensitive release of nitric oxide in animal models of hyperlipidaemia and atherosclerosis (Shimokawa et al., 1989a,b; Flavahan, 1993; Shibano et

al., 1994; Liao and Clark, 1995; Boulanger and Vanhoutte, 1997). In our fructose-fed mice, plasma total cholesterol, high-density lipoprotein and low-density lipoprotein cholesterol levels were all significantly increased, indicating that the animal is hyperlipidaemic. However, as mentioned above, our data suggest (i) that the activity of the pertussis toxin-sensitive GTP-binding protein in the aorta may be increased in fructose-fed mice, (ii) that these mice are not only hyperlipidaemic but also insulin-resistant and (iii) that this insulin resistance may be linked to activation of GTP-binding protein.

α_2-Adrenoceptors are closely linked to GTP-binding protein (Flavahan et al., 1989; Boulanger and Vanhoutte, 1997), whereas acetylcholine receptors may not be linked to such a protein (Kamata et al., 1996; Lembo et al., 1997). This difference may explain why acetylcoline decreased the NO_2^- plus NO_3^- level in fructose-fed mice, while noradrenaline increased it.

If the activity of the GTP-binding protein on the endothelium is increased in all blood vessels, then the blood pressure should be lower than normal in fructose-fed mice. This was not the case in the present study. It has been reported that mice lacking the gene for endothelial nitric oxide synthase are hypertensive (Huang et al., 1995). Together, the above observations seem to suggest that the activity of endothelial GTP-binding protein may differ among blood vessels in fructose-fed mice.

In summary and conclusion, we found that the function of the endothelium was attenuated in both fructose-fed and streptozotocin-diabetic mice but that the contractile response to noradrenaline was much weaker in the former than in the latter. It is suggested that the impaired noradrenaline contractile response seen in fructose-fed mice may be due to an increased activity of the GTP-binding protein coupled to endothelial α_2-adrenoceptors.

Acknowledgements

This study was supported in part by the Ministry of Education, Science, Sports and Culture, Japan.

References

Abe, H., Yamada, N., Kamata, K., Kuwaki, T., Shimada, M., Osuga, J., Shionoiri, F., Yahagi, N., Kadowaki, T., Tatemoto, H., Ishibashi, S., Yazaki, Y., Makuuchi, M., 1998. Hypertension, hypertriglyceridemia, and impaired endothelium-dependent vascular relaxation in mice lacking insulin receptor substrate-1. J. Clin. Invest. 101, 1784–1788.

Alosachie, I., Godfraind, T., 1988. The modulatory role of vascular endothelium in the interaction of agonists and antagonists with α-adrenoceptors in the rat aorta. Br. J. Pharmacol. 95, 619–629.

Berger, M.E., Ormsby, B.L., Bunnag, P., Hori, M.T., Tuck, M.L., Golub, M.S., 1998. Increased functional Na^+–K^+ pump activity in the vasculature of fructose-fed hyperinsulinemic and hypertensive rats. Hypertens. Res. 21, 73–80.

Boulanger, C.M., Vanhoutte, P.M., 1997. G protein and endothelium-dependent relaxations. J. Vasc. Res. 34, 175–185.

Brands, M.W., Hildebrandt, D.A., Mizelle, H.L., Hall, J.E., 1991. Sustained hyperinsulinemia increases arterial pressure in conscious rats. Am. J. Physiol. 260, R764–R768.

Bunnag, P., Hori, M.T., Ormsby, B., Berger, M.E., Golub, M.S., Tuck, M.L., 1997. Impaired in vivo adrenergic responses in diet-induced hypertensive rats. Hypertens. Res. 20, 17–21.

Cambien, F., Warnet-Eschwege, E., Jacqueson, A., Richard, J.L., Rosselin, G., 1987. Body mass, blood pressure, glucose, and lipids: does plasma insulin explain their relationship? Arteriosclerosis 7, 197–202.

Carrier, G.O., White, R.E., 1985. Enhancement of alpha-1 and alpha-2 adrenergic agonist-induced vasoconstriction by removal of endothelium in rat aorta. J. Pharmacol. Exp. Ther. 232, 682–687.

Cohen, R.A., 1993. Dysfunction of vascular endothelium in diabetes mellitus. Circulation 87 (suppl. V), V67–V76.

Cosenzi, A., Bernobich, E., Plazzotta, N., Seculin, P., Bellini, G., 1999. Bosentan reduces blood pressure and the target-organ damage induced by a high-fructose diet in rats. J. Hypertens. 17, 1843–1848.

Egleme, C., Godfraind, T., Miller, R.C., 1984. Enhanced responsiveness of rat isolated aorta to clonidine after removal of the endothelial cells. Br. J. Pharmacol. 81, 16–18.

Flavahan, N.A., 1993. Lysophosphatidylcholine modifies G-proteins dependent signaling in porcine endothelial cells. Am. J. Physiol. 264, H722–H727.

Flavahan, N.A., Shimokawa, H., Vanhoutte, P.M., 1989. Pertussis toxin inhibits endothelium-dependent relaxations to certain agonists in porcine coronary arteries. J. Physiol. (London) 408, 549–560.

Huang, P.L., Huang, Z., Mashimo, H., Bloch, K.D., Moskowitz, M.A., Bevan, J.A., Fishman, M.C., 1995. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. Nature 377, 239–242.

Hwang, J.-S., Ho, H., Hoffman, B.B., Reaven, G.M., 1987. Fructose-induced insulin resistance and hypertension in rats. Hypertension 10, 512–516.

Iyer, S.N., Katovich, M.J., 1996. Vascular reactivity to phenylephrine and angiotensin II in hypertensive rats associated with insulin resistance. Clin. Exp. Hypertens. 18, 227–242.

Kamata, K., Makino, A., 1997. A comparative study on the rat aorta and mesenteric arterial bed of the possible role of nitric oxide in the desensitization of the vasoconstrictor response to an α_1-adrenoceptor agonist. Br. J. Pharmacol. 120, 1221–1228.

Kamata, K., Nakajima, M., 1998. Ca^{2+} mobilization in the aortic endothelium in streptozotocin-induced diabetic and cholesterol-fed mice. Br. J. Pharmacol. 123, 1509–1516.

Kamata, K., Yamashita, K., 1999. Insulin resistance and impaired endothelium-dependent renal vasodilatation in fructose-fed hypertensive rats. Res. Commun. Mol. Pathol. Pharmacol. 103, 195–210.

Kamata, K., Miyata, N., Kasuya, Y., 1989a. Impairment of endothelium-dependent relaxation and changes in levels of cyclic GMP in aorta from streptozotocin-induced diabetic rats. Br. J. Pharmacol. 97, 614–618.

Kamata, K., Miyata, N., Kasuya, Y., 1989b. Involvement of endothelial cells in relaxation and contraction responses of the aorta to isoproterenol in naive and streptozotocin-induced diabetic rats. J. Pharmacol. Exp. Ther. 249, 890–894.

Kamata, K., Numazawa, T., Kasuya, Y., 1996. Mechanisms of desensitization of vasodilatation induced by platelet-activating factor in hypertensive rats. Eur. J. Pharmacol. 301, 121–128.

Kaneko, K., Sunano, S., 1993. Involvement of α-adrenoceptors in the endothelium-dependent depression of noradrenaline-induced contraction in rat aorta. Eur. J. Pharmacol. 240, 195–200.

Kobayashi, T., Kamata, K., 1999a. Relationship among cholesterol, superoxide anion and endothelium-dependent relaxation in diabetic rats. Eur. J. Pharmacol. 367, 213–222.

Kobayashi, T., Kamata, K., 1999b. Effect of insulin treatment on smooth muscle contractility and endothelium-dependent relaxation in rat aortae from established STZ-induced diabetes. Br. J. Pharmacol. 127, 835–842.

Kotchen, T.A., Zhang, H.Y., Coveli, M., Blehschmit, N., 1991. Insulin resistance and blood pressure in Dahl rats and in one-kidney, one-clip hypertensive rats. Am. J. Physiol. 261, E692–E697.

Lembo, G., Iaccarino, G., Vecchione, C., Barbato, E., Morisco, C., Monti, F., Parrella, L., Trimarco, B., 1997. Insulin enhances enodthelias α2-adrerenergic vasorelaxation by a pertussis toxin mechanism. Hypertension 30, 1128–1134.

Liao, J.K., Clark, S.L., 1995. Regulation of G-protein α2i subunit expression by oxidized low-density lipoprotein. J. Clin. Invest. 95, 1457–1463.

Lucas, C.P., Estigarribia, J.A., Darga, L.L., Reaven, G.M., 1985. Insulin and blood pressure in obesity. Hypertension 7, 702–706.

Makino, A., Ohuchi, K., Kamata, K., 2000. Mechanisms underlying the attenuation of endothelium-dependent vasodilatation in the mesenteric arterial bed of the streptozotocin-induced diabetic rat. Br. J. Pharmacol. 130, 549–556.

Manolio, T.A., Savage, P.J., Burke, J.L., Jacob, D.R., Sidney, S., Wagenkulkt, L.E., Allman, R.M., Tracy, R.P., 1990. Association of fasting insulin with blood pressure and lipids in young adults: the CARDIA study. Arteriosclerosis 10, 430–436.

Martin, W., Furchgott, R.F., Villani, G.M., Jothianandan, D., 1986. Depression of contractile responses in rat aorta by spontaneously released endothelium-derived relaxing factor. J. Pharmacol. Exp. Ther. 237, 529–538.

Modan, M., Halkin, H., Almog, S., Lusky, A., Eshkil, A., Shefi, M., Shitrit, A., Fuchs, Z., 1985. Hyperinsulinemia: a link between hypertension, obesity and glucose intolerance. J. Clin. Invest. 75, 809–817.

Oyama, Y., Kawasaki, H., Hattori, Y., Kanno, M., 1986. Attenuation of endothelium-dependent relaxation in aorta from diabetic rats. Eur. J. Pharmacol. 132, 75–78.

Pieper, G.M., 1998. Review of alterations in endothelial nitric oxide production in diabetes. Protective role of arginine on endothelial dysfunction. Hypertension 31, 1047–1060.

Pieper, G.M., Gross, G.J., 1988. Oxygen free radical abolish endothelium-dependent-relaxation in diabetic rat aorta. Am. J. Physiol. 255, H825–H833.

Poston, L., Taylor, P.D., 1995. Endothelium-mediated vascular function in insulin-dependent diabetes mellitus. Clin. Sci. 88, 245–255.

Reaven, G.M., Chang, H., 1991. Relationship between blood pressure, plasma insulin and triglyceride concentration, and insulin action in SHR and WKY rats. Am. J. Physiol. 4, 34–38.

Richey, J.M., Si, X., Halter, J.B., Webb, R.C., 1998. Fructose perfusion in rat mesenteric arteries impairs endothelium-dependent vasodilation. Life Sci. 62, PL55–PL62.

Sechi, L.A., Bartoli, E., 1997. Mechanisms of insulin resistance leading to hypertension: what we can learn from experimental models. J. Invest. Med. 45, 238–251.

Shibano, T., Codina, J., Birnbaumer, L., Vanhoutte, P.M., 1994. Pertussis toxin sensitive G-proteins in regenerated endothelial cells after balloon denudation of porcine coronary artery. Am. J. Physiol. 267, H979–H981.

Shimokawa, H., Flavahan, N.A., Shepard, J.T., Vanhoutte, P.M., 1989a. Endothelium-dependent inhibition of ergonovine-induced contraction is impaired in porcine coronary arteries with regenerated endothelium. Circulation 80, 643–650.

Shimokawa, H., Flavahan, N.A., Vanhoutte, P.M., 1989b. Natural course of the impairment of endothelium-dependent relaxation in regenerating porcine endothelial cells. Role of a pertussis toxin sensitive G-protein. Circ. Res. 65, 740–753.

Tedde, R., Sechi, L.A., Marigliano, A., Pala, A., Scano, L., 1989. Antihypertensive effect of insulin reduction in diabetic-hypertensive patients. Am. J. Hypertens. 3 (Pt 1), 163–170.

Verma, S., Bhanot, S., Yao, L., McNeill, J.H., 1996. Defective endothelium-dependent relaxation in fructose-hypertensive rats. Am. J. Hypertens. 9, 370–376.

Verma, S., Skarsgard, P., Bhanot, S., Yao, L., Laher, I., McNeill, J.H., 1997. Reactivity of mesenteric arteries from fructose hypertensive rats to endothelin-1. Am. J. Hypertens. 10, 1010–1019.