Original Article

Impaired Endothelial Alpha-2 Adrenergic Receptor-Mediated Vascular Relaxation in the Fructose-Fed Rat

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To investigate the vascular endothelial dysfunction in the insulin resistance syndrome, muscarinic and α_2 -adrenergic mediated relaxations were studied in the fructose-fed rat. Male Sprague-Dawley rats were fed either fructose-rich chow (FFR, $n=14$) or normal chow (CNT, $n=13$) for 8 weeks. Systolic blood pressure (SBP) was measured by the tail-cuff method. A 3 mm segment of mesenteric artery was cannulated and pressurized, pretreated with prazosin (10^{-6} mol/l) and propranolol (3×10^{-6} mol/l), then pre-contracted with serotonin (10^{-6} mol/l). Endothelium-dependent relaxation was induced by addition of acetylcholine (ACh, 10^{-9} – 10^{-4} mol/l) or a selective α_2 -agonist, B-HT 920 (10^{-9} – 10^{-5} mol/l), with or without the nitric oxide (NO) synthase inhibitor, L-NAME (10^{-4} mol/l). SBP was significantly elevated in FFR but not in CNT. Plasma triglyceride in FFR (241 ± 115 mg/dl) was significantly ($p < 0.01$) higher than in CNT (84 ± 34 mg/dl). Insulin and insulin/glucose ratio were higher but not significantly. Plasma glucose was not different between the two groups. In the dose-response curves to ACh, maximum relaxation and ED_{50} were similar between FFR and CNT. Moreover, L-NAME shifted the dose-response curves similarly to the right in both groups. Dose-response curves to B-HT 920, however, showed less relaxation in FFR than in CNT ($p < 0.05$). B-HT 920-induced relaxations were mostly abolished by L-NAME. It is concluded that endothelial α_2 -adrenergic relaxation, predominantly mediated by NO, is likely more sensitive to the development of insulin resistance than muscarinic receptor relaxation in this 8-weeks FFR model. This early impairment of endothelial α_2 -adrenergic relaxation may contribute to the development of hypertension and insulin resistance in the FFR. (*Hypertens Res* 2002; 25: 197–202)

Key Words: insulin resistance, muscarinic, adrenergic, alpha-2, nitric oxide

Introduction

Insulin resistance is recognized as a major factor in the metabolic syndrome in which the accompanying hyperinsulinemia is associated with hypertension (1–3). Insulin modulates vascular tone, and its acute effect as a vasodilator is well established (4). However, this action of insulin has made it difficult to resolve its association with hypertension. It is possible that with chronic exposure to high-level of insulin as of-

ten observed in obesity and type 2 diabetes, blood vessels become desensitized to insulin's vasodilator effect (5, 6). This resistance to insulin's vasodilator effect could be mediated by reduced efficacy of the endothelial nitric oxide (NO) system. (7–9)

We previously reported (10) that both muscarinic and α_2 -adrenergic receptor-induced relaxations were attenuated in precontracted mesenteric arteries from fructose-fed rats (FFR) treated for 40 weeks, a model of the insulin resistance syndrome.

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Fig. 1. Percentage change in relaxation to increasing doses of B-HT 920 in deendothelialized (a) and idazoxan-treated (b) mesenteric arteries. Data are shown as mean $\pm$ SEM. 5-HT, serotonin.

In this study, we examine the vascular responses in FFR fed for 8 weeks in order to better understand the early stages and progression of endothelial dysfunctions in a model of insulin resistance.

Methods

Animals

Male Sprague-Dawley rats at 8 weeks of age were obtained from Bantin-Kingman Inc. (Laboratory Animal Consultants, Fremont, USA). The animals were housed in the vivarium (American Association for the Accreditation of Laboratory Animal Care approved) on a 12-h light/dark cycle, at 24°C with free access to water and chow. They were randomly divided into two groups, control rats (CNT, $n = 13$) that were fed normal chow and fructose-fed rats (FFR, $n = 14$) that were fed fructose-rich (60%) chow (Harlan Teklad, Madison, USA) for 8 weeks. Systolic blood pressure (SBP) was measured in the beginning and the end of the study period in the conscious rat using tail-cuff plethysmography (IITC Inc., Woodland Hills, USA).

Preparation of Blood Vessels

The rats were anesthetized with methoxyflurane (Schering-Plough, Liberty Corner, USA) inhalation and the abdomen was opened. Five milliliters of arterial blood was drawn from the abdominal aorta and saved for later measurements. The mesenteric arterial branches from a region 50–100 mm distal to the pylorus were dissected and transferred to a dish filled with oxygenated (5% CO₂ and 95% O₂) Krebs-Ringer bicarbonate solution. The Krebs solution consisted of (mmol/l composition), NaCl 124.6, Na₂HPO₄ 1.2, NaHCO₃ 24.9, KCl 5.0, CaCl₂ 1.8, MgSO₄ 1.2, glucose 11.1 and EDTA 0.026. A 3 mm segment (200–300 μ m in intra-arterial diameter) of mesenteric artery was removed from surrounding adipose tissue and cannulated at both ends by micropipettes in the vessel

chamber which was then mounted on a microscope equipped with a video camera (Living Systems Instrumentation, Burlington, USA). To establish the no-flow condition, the distal end of the pipette was closed after flushing out the residual blood inside of the artery. Intra-arterial pressure was maintained with negligible flow, in vessels with no leaks at a constant 40 mmHg by a peristaltic pump that was automatically regulated by a pressure transducer. The vessel chamber was constantly superfused with warmed (37°C) Krebs solution at a velocity of 30 ml/min. Drugs were added to the superfusion reservoir. The changes of intra-arterial diameter and wall thickness were recorded by video-dimension analyzer (Living Systems Instrumentation) and data acquisition system (Dataq Instruments, Akron, USA).

Protocols

The artery was equilibrated for 1 h and pretreated with prazosin (10^{-6} mol/l) and propranolol (3×10^{-6} mol/l) to exclude the influence of α_1 - and β -adrenergic receptors, respectively. Prior to each cumulative dose response curve, the artery was precontracted with serotonin (5-HT, 10^{-6} mol/l). After 5-HT contractions reached a stable plateau, cumulative doses of acetylcholine (ACh) (10^{-9} – 10^{-4} mol/l), or B-HT 920 (10^{-9} – 10^{-5} mol/l) a specific α_2 agonist was added in 2 min interval. From previous experiments ACh at 10^{-4} mol/l produced maximum relaxations.

The same dose-response protocols were repeated after 1-h treatment with L-NAME (10^{-4} mol/l). After each cumulative dose-response curve, the entire superfusion circuit was flushed with fresh Krebs solution. The artery was incubated for 30 min as the recovery period between the measurements. Relaxation was calculated as a percent dilation of the intra-arterial diameter after 5-HT contraction. The concentration that produced half-maximal relaxation (ED₅₀) to ACh was calculated from the dose-response curves.

Although the existence of dopamine D₂ receptor in vascular endothelial cells is not confirmed, it has been reported

Table 1. Body Weight and Metabolic Measurements in Plasma

	CNT	FFR	<i>p</i>
Body weight (g)	486 ± 55	475 ± 47	NS
Plasma triglyceride (mmol/l)	0.95 ± 0.38	2.72 ± 1.30	< 0.01
Plasma insulin (pmol/ml)	498 ± 46	726 ± 87	NS
Plasma glucose (mmol/l)	15 ± 3	15 ± 2	NS
Insulin/glucose ratio	33.2 ± 18.6	48.4 ± 36.4	NS

Data are shown as mean ± SD. CNT, control rats; FFR, fructose-fed rats.

Table 3. Comparison of Vertical Measurements of Mesenteric Artery between Control (CNT) and Fructose-Fed Rats (FFR) When Pressurized to 40 mmHg without Agonists

	CNT	FFR	<i>p</i>
Wall thickness (WT, μ m)	30 ± 4	35 ± 5	NS
Intra-arterial diameter (ID, μ m)	274 ± 30	262 ± 38	NS
WT/ID ratio	11.1 ± 1.0	13.5 ± 2.4	< 0.01

Data are shown as mean ± SD.

that a high concentration of norepinephrine ($> 10^{-5}$ mol/l) relaxes the rat mesenteric artery through the D_1 receptor in the presence of an α_1 receptor blocker and a β blocker (11). Therefore, cross reactivity to dopaminergic receptor subtypes may also relax the artery. We confirmed the specificity of B-HT 920 as a α_2 agonist in two preliminary studies using a similar preparation, because this compound is reported to also have dopaminergic (D_2) agonist activity (12). First, relaxations to B-HT 920 within the dose range tested (10^{-9} to 10^{-5} mol/l) were completely abolished ($p < 0.01$) after chemical deendothelization by 0.3% CHAPS perfusion for 30 s (Fig. 1a). This indicates that B-HT 920-induced relaxations are endothelium-dependent. Additionally, the B-HT 920-induced relaxations were inhibited 70–80% ($p < 0.05$) after pretreatment with a selective α_2 antagonist, idazoxan ($1-3 \times 10^{-5}$ mol/l, Fig. 1b). These studies confirm that B-HT 920-induced relaxations in the rat mesenteric artery are predominantly mediated by endothelial α_2 receptors within the concentration range (10^{-9} – 10^{-5} mol/l) used.

Blood Chemistry

The blood sample was immediately centrifuged and the plasma was collected and stored at -20°C . Plasma insulin was assayed by radioimmunoassay using a ^{125}I insulin kit (Bio-Trak, Amersham-Pharmacia Biotech, Piscataway, USA). Plasma glucose was measured by the glucose oxidase method using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, USA). Plasma triglycerides were measured by radioimmunoassay (Endocrine Sciences, Inc., USA).

Table 2. Changes of Systolic Blood Pressure between 0 and 8 Weeks

	0 week	8 weeks	<i>p</i>
CNT (mmHg)	113 ± 7	125 ± 12	NS
FFR (mmHg)	114 ± 10	132 ± 15	< 0.05

Data are shown as mean ± SD. CNT, control rats; FFR, fructose-fed rats.

Chemicals and Drugs

Prazosin hydrochloride, DL-propranolol hydrochloride and acetylcholine chloride were obtained from Sigma Chemical (St. Louis, USA). N^G -nitro-L-arginine methyl ester was purchased from Cayman Chemical (Ann Arbor, USA). Serotonin hydrochloride was purchased from Research Biochemical International (Natick, USA). B-HT 920 dihydrochloride was a gift from Boehringer Ingelheim Ltd. (Ridgefield, USA).

Statistical analysis

The data are expressed as both mean ± SD (results and tables) and mean ± SEM (figures). Dose-response curves were compared using two-way analysis of variance. Statistical evaluations between diet or drug treatment groups were made by paired and unpaired Student's *t* tests or non-parametric methods, as appropriate. *P* values of less than 0.05 were considered to be statistically significant.

Results

As shown in Table 1, the mean body weight was not different between CNT and FFR. Plasma triglyceride in FFR was significantly higher compared to CNT. There was no significant difference in mean plasma glucose between FFR and CNT. Plasma insulin and the insulin/glucose ratio were higher in FFR than in CNT, however the difference was not significant.

Mean SBP was significantly elevated in FFR at 8 weeks compared to 0 weeks. The rise in SBP in the CNT group was not statistically significant (Table 2).

There was no difference in intra-arterial diameter of mesenteric arteries between 8-week FFR and CNT after 1-h equilibration at 40 mmHg intra-arterial pressure. Wall thickness/intra-arterial diameter ratio was significantly greater in FFR than in CNT at 8 weeks (Table 3), suggesting an early effect of insulin resistance on arterial wall morphometry.

Dose-response curves to ACh-induced relaxation are shown in Fig. 2. The degree of maximum relaxation attained was similar in both FFR and CNT both with (b, $88 \pm 15\%$ and $93 \pm 15\%$) and without (a, $91 \pm 12\%$ and $95 \pm 4\%$) L-NAME. The ED_{50} of ACh was similar between FFR and CNT, and was similarly increased by L-NAME.

Fig. 2. Percentage change in relaxation in response to increasing doses of acetylcholine (ACh) in the mesenteric arteries from control rats (CNT) and fructose-fed rats (FFR) with (b) and without (a) the addition of L-NAME. Relaxation was expressed as percent change from pre-contraction with serotonin (5-HT) (10^{-6} mol/l). L-NAME shifted the curve to right similarly in both FFR and CNT. Data are shown as mean $\pm$ SEM.

Fig. 3. Percentage change in relaxation to increasing doses of the selective α_2 -agonist B-HT 920 in the mesenteric arteries from control rats (CNT) and fructose-fed rats (FFR). Relaxation was expressed as percent change from pre-contraction with serotonin (5-HT) (10^{-6} mol/l). The relaxation response to B-HT 920 was markedly decreased by L-NAME in both groups. Data are shown as mean $\pm$ SEM.

Figure 3 shows the dose-response relaxation to B-HT 920 in CNT and FFR. The relaxation response to B-HT 920 was less ($p < 0.05$) in the FFR than in the CNT and almost abolished by L-NAME.

Discussion

The results of this study demonstrate that endothelium-dependent relaxation to the selective α_2 agonist, B-HT 920, in

precontracted rat mesenteric arteries was impaired at an early stage of insulin resistance in the 8-weeks FFR, while ACh-induced relaxations were still normal at this early stage. These data suggest that endothelial α_2 -adrenergic receptor-induced vascular relaxation responses are more sensitive to the metabolic changes in this model, whereas the muscarinic receptor-induced vascular relaxation is preserved in this early stage.

As a model of insulin resistance, several distinctive features were demonstrated in the FFR in the current study. Blood pressure rose significantly in the FFR while not in CNT at 8 weeks of fructose feeding and emerging insulin resistance. Plasma triglycerides were significantly higher in FFR vs. CNT at the end of 8-week experimental period. Plasma insulin was higher in the FFR as was the insulin/glucose ratio at 8-weeks of feeding suggesting the presence of developing insulin resistance, although these changes did not reach statistical significance. However, more substantive insulin resistance was seen in the 40-week FFR (10). This sequence of events suggest that lipid abnormalities may be one of the first metabolic changes in this model. Randle *et al.* (13) showed that increased fatty acids impair glucose uptake by muscle and Svedberg *et al.* (14) demonstrated that they also affected insulin clearance in the liver. Alpha-2 adrenergic receptors may also influence the hyperlipidemia as they have been shown to influence lipoprotein lipase activity (15). Thus, the sequence of events in the development of the metabolic changes in this model require much further study.

Impaired endothelial responses have been described in studies using different protocols for fructose feeding. Richey *et al.* (16) reported that there was an impaired acetylcholine-induced relaxation in the mesenteric vessel with 10% fructose administered in the drinking water. Katakam *et al.* (17) noted that endothelial dysfunction preceded the hypertension in FFR. Modulation of ACh-induced relaxation in mesenteric arteries is also known to vary among animal models (SHR, SHR-SP, reduced renal mass hypertensive rats), the

experimental design (isometric, isobaric contractions) and the type of precontraction drugs that were used (norepinephrine, phenylephrine, 5-HT) (18–21).

Precise mechanism(s) for the impaired endothelial relaxation responses in FFR are being elucidated. As alluded to above, fructose feeding is reported to alter hepatic glucose metabolism and cause hyperinsulinemia by elevating free fatty acids and triglyceride levels (13, 14). These metabolic changes, which are also the features of the insulin resistance syndrome, are known to impair endothelial function (22–24). The mesenteric artery of the FFR demonstrated a higher wall thickness/internal diameter ratio than in CNT, which suggests vascular remodeling and/or hypertrophy. This morphological change may also be related to the endothelial dysfunction found in the later stages in the FFR. Alpha-adrenergic receptors may also be involved in the vascular changes. Dubey *et al.* (25) reported that adenosine inhibited cardiac fibroblast growth through activation of α -2B receptors. Decreased α -2B activity, therefore, could be a mechanism for local growth stimulation.

Attenuation of ACh-induced relaxation has been reported in the mesenteric artery in diet-induced hypertension (16, 26, 27). However, in the current study, 8-weeks fructose feeding did not attenuate ACh-induced relaxation whereas 40-weeks of fructose feeding did attenuate the maximum relaxation to ACh with L-NAME (10). These observations suggest that the ACh-induced relaxation was not critical to the early rise in blood pressure. As the ACh-induced relaxation mechanisms were normally maintained after 8-weeks fructose feeding, the early changes in blood pressure and vascular morphology were probably mediated by a different mechanism.

The endothelial α -adrenergic receptor has been characterized in the rat mesenteric artery, and can be a major modulator for the release of endothelial-derived relaxation factors (26, 27). Evidence is now strong that NO mediates the α -adrenergic receptor-induced relaxation (28–31). These relaxations tend to be larger in the mesenteric artery of the SHR (30) and are impaired in the aorta of the SHRSP (32) but its status in the FFR has not been previously examined. The endothelial α -adrenergic relaxation mechanism, which is mostly mediated by NO, is impaired at 40 weeks feeding in the FFR (10). In this study, the impairment of α -adrenergic relaxation at 8 weeks much before the impairment of muscarinic relaxation suggests an evolution of vascular defects that ultimately lead to increased vascular tone and vessel hypertrophy in insulin resistance as seen in the FFR. However, from the literature it can be said in general, that NO-mediated mechanisms are impaired earlier than the NO-independent mechanisms (EDHF, prostaglandins) in the emergence of insulin resistance.

In conclusion, 8-weeks of fructose feeding in rats created a dysfunction of endothelium-dependent relaxation to α -adrenergic agonists (B-HT 920) but not to ACh relaxation in rat resistance arteries. This finding suggests that the impairment of endothelial α -adrenergic receptor-mediated vascular

relaxation previously reported in 40-weeks fructose feeding was not due solely to an aging effect. Moreover, the endothelial α -adrenergic receptor function is more sensitive to alteration with insulin resistance and high triglyceride states than are the muscarinic receptor-induced mechanisms. This early dysfunction in the endothelial α -adrenergic mechanism may initiate the early stages of hypertension and alter vascular wall morphology in the FFR.

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