Impaired arachidonic acid-mediated dilation of small mesenteric arteries in Zucker diabetic fatty rats

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Zhou, Wei, Xiao-Li Wang, Terry L. Kaduce, Arthur A. Spector, and Hon-Chi Lee. Impaired arachidonic acid-mediated dilation of small mesenteric arteries in Zucker diabetic fatty rats. Am J Physiol Heart Circ Physiol 288: H2210–H2218, 2005. First published December 30, 2004; doi:10.1152/ajpheart.00704.2004.—Arachidonic acid (AA) is a precursor of important vasoactive metabolites, but the role of AA-mediated vasodilation in Type 2 diabetes is not known. Using Zucker diabetic fatty (ZDF) rats, we examined the effects of AA in small mesenteric arteries preconstricted with endothelin. In ZDF rat mesenteric arteries, 1 μM AA produced only one-third the amount of dilation as in vessels from lean control animals. In lean control rats, the effect of AA was significantly and predominantly inhibited by the lipoxygenase inhibitors baicalein and cinnamyl-3,4-dihydroxy-cyanocinnamate (CDC). However, baicalein and CDC had no effect on AA-mediated dilation in ZDF rat mesenteric arteries. The major [3H]AA metabolite produced by isolated mesenteric arteries in both lean and ZDF rats was 12-hydroxyeicosatetraenoic acid (12-HETE), but the amount of [3H]12-HETE produced by ZDF rat vessels was only 36% of that of control vessels. In addition, 12-HETE produced similar amounts of dilation in lean and ZDF rat mesenteric arteries. Immunoblot analysis showed an 81% reduction in 12-lipoxygenase protein in ZDF rat mesenteric arteries that colocalized with 12-lipoxygenase in endothelium, and 12-lipoxygenase coprecipitation with anti-nitrotyrosine antibodies was enhanced in ZDF rat vessels. We conclude that AA-mediated relaxation in ZDF rat mesenteric arteries is impaired due to reduced 12-lipoxygenase protein and activity. Increased oxidative stress and nitrination of 12-lipoxygenase may underlie the impairment of AA-mediated relaxation in small mesenteric arteries of diabetic rats.

Type 2 diabetes; 12-hydroxyeicosatetraenoic acid; lipoygenase; cinnamyl-3,4-dihydroxy-cyanocinnamate; baicalein

Arachidonic acid (AA) is the most abundant and important precursor of vasoactive eicosanoids. Free AA is converted by 1) cyclooxygenase into prostaglandins and thromboxanes; 2) lipoxygenase into leukotrienes, lipoxins, and intrachain hydroxyicosatetraenoic acids (HETEs); and 3) cytochrome P-450 monoxygenase into epoxygenosatrienoic acids and chain-terminal HETEs. These products are important regulators of vasoreactivity. AA produces potent dilation in human coronary arterioles that is dependent on the cytochrome P-450 pathway, whereas the dilation produced in rat mesenteric microvessels is mediated mainly through the lipoxygenase pathway.

Type 2 diabetes has become a disease of epidemic proportions and is known to be associated with abnormal regulation of blood vessels. Type 2 diabetes-associated vascular dysfunction has been extensively studied in humans and various animal models. Endothelial dysfunction with impaired activity of various endothelium-derived factors plays an integral role in Type 2 diabetes. The Zucker diabetic fatty (ZDF) rat is a useful animal model for Type 2 diabetes, although it has certain limitations including slight hyperglycemia and higher serum insulin levels in lean control animals as compared with Sprague-Dawley rats. It has been reported that in obese Zucker rats, NO-dependent flow-induced arteriolar dilation was attenuated with impaired arteriolar ability to regulate wall shear rate, and these changes were associated with increases in vascular superoxide levels. In addition, these animals have altered skeletal muscle arteriolar reactivity including increased myogenic activation and α-adrenergic vasoconstriction. Recently, NO- and K⁺-channel-mediated dilator responses in basilar arteries were found to be greatly diminished as a result of enhanced oxidative stress and PKC activation. It appears that reactive oxygen species play a central role in the pathogenesis of vasculopathy in Type 2 diabetes. In addition to alterations in pharmacological responses, reduced microvessel density and arteriolar distensibility and remodeling of the microcirculation also contribute substantially to impaired vascular function in diabetes.

AA is converted to potent vasodilator substances in the endothelium including prostacyclin, endothelium-derived hyperpolarizing factors, and 12-HETE. However, the role of AA in the regulation of vasoreactivity in Type 2 diabetes is not clear. The goal of this study was to examine AA-mediated relaxation in lean and ZDF rat small mesenteric arteries to determine the metabolic pathways mediating the effects of AA and the mechanism of abnormal AA-mediated vasodilation in ZDF rats.

Materials and Methods

Animals. Male ZDF rats (ZDFGmi-fa/fa) and their age-matched genetic controls were obtained from Charles River Laboratories at 6–8 wk of age. All rats received Purina 3008 modified mouse/rat diet and were studied after 4 wk of hyperglycemia (>300 mg/dl) in ZDF rats according to a protocol approved by the Animal Use Committee of the Mayo Foundation.

Vasoreactivity Measurements. Animals were anesthetized with pentobarbital sodium (50 mg/kg). The bowels and mesentery were rapidly excised and placed in ice-cold Krebs solution that contained (in mM) 118.3 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 25 NaHCO₃, and 11.1 dextrose. Reactivity of isolated small mesenteric arterioles to determine the metabolic pathways mediating the effects of AA was defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
arteries (100–250 μm in diameter) was determined by video microscopy (23, 44). All vessels were equilibrated for at least 30 min in Krebs solution at 37°C before the start of the experiment.

To study the endothelium-dependent and -independent components of vasorelaxation, endothelium was removed by passing an air bubble through the isolated vessels. Vessels were used only if they did not relax with administration of acetylcholine (10–4 M) but had normal responses to nitroprusside (10–4 M) and KCl (60 mM).

**Pharmacological interventions.** All compounds were added to the circulating bath, and the cumulative dose responses were determined at 3- to 5-min intervals between doses. Vessels were constricted to ~40% of baseline diameter using endothelin-1, and the effects of AA (1 × 10–10 to 3 × 10–5 M) on vasorelaxation were examined. Vessels were discarded if they failed to produce 85% relaxation with 100 μM sodium nitroprusside or 30% constriction with 60 mM KCl.

To identify the metabolic pathways responsible for mediating the effects of AA, small mesenteric arteries were preincubated for 30 min with indomethacin (10–6 M) to inhibit the cyclooxygenase pathway, baicalein (10–6 M) or cinnamyl-3,4-dihydroxy-cyanocinnamate (CDCA; 10–6 M) to inhibit the 12-lipoxygenase pathway, or miconazole (10–6 M) to inhibit the cytochrome P-450 pathway.

To determine the tonic mechanism through which AA mediates its vasodilatory effects, vessels were preincubated for 30 min with ibetrixin (IBTX; 10–7 M) to block the large-conductance Ca2+-activated K+ (BKCa) channels or 4-aminopyridine (4-AP; 1 mM) to block the voltage-gated K+ (Kv) channels.

**Eicosanoid production in small mesenteric arteries of lean and ZDF rats.** Small mesenteric arteries from two lean and two ZDF rats were dissected and collected in 1 ml of modified DMEM that contained 0.1 μM BSA (DMEM-BSA) on ice. The vessels were incubated for 1 h at 37°C with 1 ml of DMEM-BSA that contained 5 μM [5,6,8,9,11,12,14,15-3H]AA (1 μCi/mmol). Incubations were terminated by removing the media and extracting the lipids. Radioactive metabolites of AA were separated by reverse-phase high-performance liquid chromatography (44). The elution profile consisted of water and formic acid at pH 4.0 and an acetonitrile gradient that increased from 30 to 57% over 30 min, increased from 57 to 65% over 20 min, remained at 65% for 5 min, and then increased to 100% acetonitrile for 6 min. Radioactivity of the column effluent was measured by an in-line flow scintillation detector. Retention times of the radiolabeled components were compared with those of standards.

**Immunoblot analysis.** Isolated mesenteric arteries were homogenized in ~300 μl of T-PER tissue protein-extraction reagent (Pierce), resolved on a 4–15% Tris-HCl polyacrylamide gradient gel, and transferred electrothermally onto a polyvinylidene difluoride membrane. Membranes were reversibly stained with Ponceau S, blotted against rabbit anti-murine 12-lipoxygenase antibodies (1:100 dilution; Cayman) and monoclonal anti-nitrotyrosine antibodies (1:70 dilution; Upstate) for 1.5 h and then with goat anti-mouse-fluorescein- and goat antirabbit-Texas red-conjugated secondary antibodies for 1 h. Sections were mounted in ProLong antifade reagent (Molecular Probes) and visualized using an LSM-510 confocal laser-scanning microscope (Carl Zeiss).

**Immunoprecipitation.** Mesenteric artery homogenates were precleared by a 2-h incubation with 0.25 μg of normal mouse IgG and 20 μl of protein G-agarose. Precleared supernatants were incubated with 4 μg of monoclonal anti-nitrotyrosine antibodies at 4°C overnight and then with protein G-agarose (30 μl) for 2 h. The immune complexes were collected by centrifugation at 2,000 g for 5 min, resolved by SDS-PAGE, and blotted against anti-12-lipoxygenase antibodies.

**Statistical analysis.** Data were analyzed using one-way ANOVA with post hoc Tukey test and are presented as means ± SE. P < 0.05 was considered significant.

### RESULTS

At the time of the experiment, the ZDF rats were significantly heavier (412 ± 4 vs. 371 ± 4 g, n = 29; P < 0.0001) and more hyperglycemic (493 ± 9 vs. 164 ± 8 mg/dl, n = 29; P < 0.0001) than age-matched lean control rats.

**Vasoactive effects of AA.** AA produced dose-dependent relaxation in small mesenteric arteries from lean rats. At a 1 μM concentration, AA produced 54.3 ± 5.4% relaxation, and at 30 μM, AA produced 88.5 ± 5.1% relaxation (n = 12), which is similar to previous reports (23). In ZDF rat preparations, 1 μM AA produced only 18.6 ± 3.6% relaxation and 30 μM produced 51.2 ± 4.2% relaxation (n = 11; P < 0.001 vs. lean rats for both; Fig. 1A). Most of the AA-mediated relax-

### Table 1. Sequences of primers used for real-time RT-PCR

<table>
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<tr>
<th>Protein</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Positions</th>
</tr>
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<tr>
<td>12-Lipoxygenase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet type</td>
<td>tgacctgcctcactgcatacc</td>
<td>aaaccatccagtcgccccgtag</td>
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<td>856–875</td>
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<tr>
<td>5-Lipoxygenase</td>
<td>catgtgaggttcaccattgc</td>
<td>atgtcctcggtgtgtccat</td>
<td>1286–1305</td>
</tr>
<tr>
<td>Ribosomal L32</td>
<td>gaaactggcggaaacc</td>
<td>ggatctggccccgtgaatcttc</td>
<td>16–39</td>
</tr>
</tbody>
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With the use of these primers, PCR products of the expected size were amplified, and quantitative results were obtained by normalizing the target signal to the L32 signal.
vasodilation was lost with removal of the endothelium in lean vessels but not in ZDF rat vessels. Nitroprusside ($10^{-4}$ M) produced similar relaxation in lean and ZDF rat vessels (Fig. 1B). These results indicate that AA-mediated relaxation is impaired in small mesenteric arteries of ZDF rats.

In lean rats, incubation with miconazole or indomethacin had no significant effect on vasorelaxation produced by AA at concentrations of $\leq 3$ μM (Fig. 1C). In contrast, after incubation with baicalein, AA-mediated vasorelaxation was inhibited at all concentrations. Incubation with baicalein plus indomethacin had no additional inhibitory effects, and incubation with all three inhibitors together also had no additional inhibitory effects except at 30 μM of AA (Fig. 1D). Inhibition of the lipoxygenase pathway with another inhibitor, CDC (1 μM), produced results similar to those with baicalein (Fig. 1, D and F). These results suggest that AA-mediated mesenteric relaxation occurs predominantly through metabolites produced by the lipoxygenase pathway. The cyclooxygenase and cytochrome P-450 epoxygenase pathways may also participate in AA-mediated vasorelaxation but only in the presence of high
AA concentrations. Incubation with the pathway inhibitors in ZDF rat vessels did not affect AA-mediated vasorelaxation except at the highest AA dose with baicalein and miconazole treatments (Fig. 1, E and F), which suggests that the production of vasoactive AA metabolites formed by the lipoxygenase pathway might be impaired.

Role of K⁺ channels on AA-mediated vasorelaxation. Incubation of small mesenteric arteries from lean rats with IBTX produced a significant reduction in relaxation at AA concentrations ≥10⁻⁸ M with only 29.1 ± 4.6% relaxation at 1 μM AA and 56.5 ± 4.3% relaxation at 30 μM AA (P < 0.001 for both vs. baseline; Fig. 2A). In contrast, incubation of ZDF rat vessels with IBTX had no effect on AA-mediated relaxation except at 30 μM (Fig. 2B). Similarly, 4-AP produced a significant reduction in mesenteric relaxation in lean rats at all AA concentrations tested, with 23.9 ± 3.2% relaxation at 1 μM AA and 58.8 ± 4.9% relaxation at 30 μM AA (P < 0.005 for both vs. baseline; Fig. 2C). However, 4-AP did not alter AA-mediated relaxation in ZDF rat vessels (Fig. 2D). These results suggest that AA has lost its ability to produce mesenteric relaxation through activation of BKCa and Kv channels in ZDF rats.

Eicosanoid production by small mesenteric arteries. The main radiolabeled metabolite produced by lean and ZDF rat mesenteric arteries was 12-HETE (retention time, 42 min; Fig. 3). The ZDF rat vessels produced only 36% as much radiolabeled 12-HETE as the lean rat vessels (12-HETE produced: lean, 903.4; ZDF, 331.5 pmol/g of wet wt; total wet weight of mesenteric arteries from two rats: lean, 115.9; ZDF, 125.8 mg). ZDF rat vessels also showed a reduction in the radiolabeled component with a retention time of 30 min, which is identical to the 12-HETE β-oxidation product that was previously identified as 8-hydroxyhexadecatrienoic acid [(8-OH)16:3] in endothelial and vascular smooth muscle cells (16, 27). Thus the lean rat mesenteric arteries converted 7% of the available AA to 12-HETE, whereas the ZDF rat mesenteric arteries converted only 3.4%. These results demonstrate that conversion to 12-HETE, a lipoxygenase product, is the major pathway for AA metabolism in small mesenteric arteries in lean rats, and the production of this lipoxygenase product is impaired in ZDF rats.

Response of small mesenteric arteries to 12-HETE. Both lean and ZDF rat vessels had similar dose-dependent relaxation responses on exposure to 12-HETE (Fig. 4A). The effects of 12-HETE were significantly attenuated by IBTX and 4-AP in both lean and ZDF rat vessels (Fig. 4, B and C), which suggests that both BKCa and Kv channels mediate the 12-HETE effects. These results indicate that impaired AA-mediated relaxation in ZDF rat small mesenteric arteries is not due to a reduced response of the vessels to 12-HETE.

Expression of 12-lipoxygenase. Immunoblot analysis showed reduced 12-lipoxygenase protein expression in ZDF rat mesenteric arteries (Fig. 5A, top). This difference was not due to the amount of protein loaded (Fig. 5A, bottom). The relative amount of 12-lipoxygenase protein was reduced by 81% in ZDF rat mesenteric arteries compared with lean rat vessels (1.55 ± 0.15 in lean vs. 0.30 ± 0.04 in ZDF rats, n = 4; P < 0.005; Fig. 5B), which indicates that impaired AA-mediated relaxation in ZDF rat mesenteric arteries is due to reduced amounts of 12-lipoxygenase protein. Because this antibody cross-reacts with both platelet- and leukocyte-type 12-lipoxygenase standards (Fig. 5C), we are not able to distinguish between them immunologically.

We then measured 12-lipoxygenase mRNA levels of both platelet and leukocyte types in lean and ZDF rat mesenteric
arteries. The mRNA levels for the platelet-type 12-lipoxygenase were not significantly different between lean and ZDF rat mesenteric arteries (Fig. 6A). However, the leukocyte-type 12-lipoxygenase mRNA levels were 4.6 ± 1.0-fold higher in ZDF than lean control rat mesenteric arteries (n = 9; P = 0.002 vs. lean control; Fig. 6B). The platelet-type 12-lipoxygenase mRNA level in lean rat vessels was fourfold higher than the leukocyte-type counterpart (n = 9; P < 0.005; Fig. 6C), but the platelet- and leukocyte-type 12-lipoxygenase mRNAs were equally expressed in ZDF rat mesenteric arteries (Fig. 6D). These results indicate that expression of the 12-lipoxygenase gene was not suppressed in ZDF rat vessels. Rather, the reduction in 12-lipoxygenase protein in ZDF rat vessels was due to either impaired translation or increased turnover. We also found that ZDF rat vessels had a 4.19 ± 0.99-fold increase in 5-lipoxygenase mRNA compared with lean rat vessels (n = 8; P < 0.05), which suggests that 5-lipoxygenase gene expression also was activated and not suppressed in ZDF rat vessels.

Tyrosine nitration of 12-lipoxygenase. Both lean and ZDF rat mesenteric arteries were strongly labeled in Texas red with anti-12-lipoxygenase antibodies (Fig. 7A). In contrast, the fluorescein signals of anti-nitrotyrosine antibodies were very weak in lean rat vessels but strong in ZDF rat vessels (Fig. 7B).

Merging the Texas red and the fluorescein images produced strikingly strong yellow signals in endothelium of ZDF rat vessels (Fig. 7C) but not from lean control rat vessels. These results suggest that ZDF vessels have higher levels of protein tyrosine nitration, and 12-lipoxygenase in the ZDF vessel is strongly tyrosine nitrated, especially in endothelium.

Immunoprecipitation experiments showed that more 12-lipoxygenase is coprecipitated with an anti-nitrotyrosine antibody in ZDF than in lean rat preparations, which again suggests that 12-lipoxygenase in ZDF rat vessels is more strongly tyrosine nitrated (Fig. 8). This finding is especially significant considering that the amount of 12-lipoxygenase protein in ZDF rat mesenteric arteries is only one-third of that in lean control animals, and it confirms the results of immunofluorescence labeling.
DISCUSSION

We found that small mesenteric arteries in rats with Type 2 diabetes have impaired AA-mediated relaxation. This impairment is due to reduced production of 12-HETE, a potent vasoactive lipoxygenase metabolite of AA. The reduced 12-HETE production in turn is due to reduced 12-lipoxygenase enzyme protein in ZDF rat mesenteric arteries. However, lipoxygenase mRNA levels were not reduced in ZDF rats, which suggests that there might be increased inactivation and degradation of lipoxygenase due to the observed increase in tyrosine nitration.

We found that AA is a potent dilator of small mesenteric arteries in lean rats, and this effect was dependent on lipoxygenase activity. These results are in agreement with those reported by Miller et al. (23). Likewise, 12-HETE formed by

Fig. 5. Immunoblot analysis of 12-lipoxygenase (12-LOX) in small mesenteric arteries from lean and ZDF rats. Immunoblot using anti-12-lipoxygenase antibodies (75-kDa band; A, top). Same membrane is shown, but blotted against anti-actin antibodies (A, bottom). Group data from four pairs of lean and ZDF rats (B). Densitometric measurements of 12-lipoxygenase were normalized against those of actin. Immunoblot using anti-12-lipoxygenase antibodies against porcine leukocyte and human platelet 12-lipoxygenase standards and rat aorta homogenate (included as reference control; C). *P < 0.05 vs. lean.

Fig. 6. Relative abundance of 12-lipoxygenase mRNA in small mesenteric arteries from lean and ZDF rats. Mesenteric mRNA was isolated from nine pairs of lean and ZDF rats, and both platelet- and leukocyte-type 12-lipoxygenase mRNAs were measured using real-time PCR techniques. Results are expressed as means ± SE showing the relative abundances of the platelet-type 12-lipoxygenase mRNA between lean and ZDF rat vessels (A), the leukocyte-type 12-lipoxygenase mRNA between lean and ZDF rat vessels (B), the platelet- and leukocyte-type 12-lipoxygenase mRNAs in lean rat vessels (C), and the platelet- and leukocyte-type 12-lipoxygenase mRNAs in ZDF rat vessels (D).
the lipoxygenase pathway produced vasodilation in porcine coronary microvessels (44). However, in human coronary microvessels, the effects of AA were mediated via a cytochrome P-450 epoxygenase product (24), which suggests that the metabolic fate of AA varies between species and vascular beds.

AA-mediated relaxation is significantly impaired in ZDF rats. In ZDF rat mesenteric arteries, 1 μM AA generated only 34% of the relaxation seen in lean control rats. Blockade of 12-lipoxygenase resulted in significant reduction of the effects of AA in lean but not ZDF rat mesenteric arteries, which suggests that the lipoxygenase metabolite is the major contributor to AA-mediated relaxation in lean rats. Our failure to observe any increase in the formation of cyclooxygenase or cytochrome P-450 products when ZDF rat small mesenteric arteries were incubated with [3H]AA indicates that the reduced lipoxygenase activity was not associated with a compensatory increase of activity in the other two pathways.

AA is mainly converted to 12-HETE by vessels from lean rats, but ZDF rat vessels only produced one-third as much. This was further confirmed by immunoblot analysis, which demonstrated that ZDF rat mesenteric arteries contain only 19% of the amount of lipoxygenase protein as lean rat vessels. Hence, the reduced lipoxygenase activity could be attributed to the reduced lipoxygenase protein in ZDF rats.

Real-time RT-PCR indicated that the small mesenteric arteries of lean rats expressed both platelet- and leukocyte-type 12-lipoxygenase, but the platelet type was the predominant form. This is consistent with previous findings that the 12-HETE produced by mouse brain cerebral microvessels, rat basilar arteries, and porcine coronary microvessels is the S-enantiomer (9, 26, 44), the stereoisomer produced by the platelet enzyme. The reduced levels of lipoxygenase in mesenteric arteries, however, are not due to downregulation of lipoxygenase transcription. In fact, mRNA levels of platelet-type 12-lipoxygenase were unchanged, and those of leukocyte-type 12-lipoxygenase were elevated four- to fivefold in ZDF rats. Platelet 12-lipoxygenase deficiencies have been reported in myeloproliferative disorders (31). Although platelet 12-lipoxygenase is activated in some forms of malignancies such as prostate cancer (38), it is suppressed in others such as cervical cancer (29). The upregulation of leukocyte-type 12-
lipoxigenase mRNA expression that occurred in ZDF rat vessels could be induced by hyperglycemia (28) or by cytokines and other stress signals associated with diabetes (4). Recently, db/db diabetic mice were found (17) to have increased urinary 12-HETE excretion and increased 12/15-lipoxygenase expression in endothelial cells isolated from these animals. Results from human studies are mixed. Reduced 12-lipoxygenase activity has been observed in platelets of patients with non-insulin-dependent diabetes (39), but an increase in urinary 12-HETE in female diabetic patients also has been reported (36). However, whole body 12-lipoxygenase product measurements may not reflect what is occurring in small mesenteric arteries. Reduced 12-HETE production in mesenteric arteries of ZDF rats may account for the blunted vasodilator responses of the mesenteric microvasculature in diabetes.

In addition, our results indicate that at high concentrations of AA (≥10 μM), cyclooxygenase and cytochrome P-450 pathways also contribute to AA-mediated mesenteric relaxation in lean rats (see Fig. 1C). Products of these pathways are important modulators of vascular reactivity. The major cyclooxygenase product of AA in the vascular endothelium is PGI2, a powerful vasodilator and inhibitor of platelet aggregation (25). PGI2 produces autocrine and paracrine effects by activating the IP prostanoid receptor, which leads to an increase in intracellular cAMP (1). Abnormalities in PGI2 regulation have been reported in diabetes. For example, PGI2-induced vasorelaxation in mesentry artery is impaired in Type 1 diabetes (37); serum PGI2 levels are decreased in Type 2 diabetic patients with angiopathy (22); and endothelial dysfunction in diabetic rats can be overcome by oral administration of a PGI2 analog. The defective PGI2-dependent vasodilator response in obese Zucker rats is due to PGI2 inactivation by superoxide anion (11), and an increase in superoxide and protein kinase C (PKC) activity in the basilar arteries of obese Zucker rats causes impaired PGI2-dependent vasodilation and cerebrovascular dysfunction (8). Recently, decreased cytochrome P-450 epoxygenase and increased epoxide hydrolase expression were also observed in the mesenteric arteries of obese Zucker rats (42). Therefore, all three AA metabolic pathways involved in vascular regulation have the propensity to become abnormal in diabetes and may contribute to impairment of vasodilator function. However, in the ZDF rat mesenteric arteries that we studied, the abnormality in the lipoxigenase pathway appears to have a greater effect under ordinary conditions, although all three pathways contribute to impaired AA-mediated vasorelaxation at high AA concentrations.

Oxidative stress is increased and may account for the vascular dysfunction that occurs in diabetes (15). In obese Zucker rats, basal plasma levels of isoprostane 8-epi-PGF2α, an in vivo marker of lipid peroxidation, were elevated by fivefold and markedly reduced by dietary vitamin E (21). As indicated by the superoxide-sensitive dye hydroethidine, production of superoxide was found to be significantly increased in basilar arteries of obese compared with lean Zucker rats (8). Increased superoxide production facilitates its reaction with NO to form peroxynitrite. This reaction would not only inactivate and decrease the bioavailability of NO but would also produce a highly potent oxidant and nitrating agent that has an array of biological actions that contribute to endothelial dysfunction (32). Detection of nitrotyrosine in proteins is considered to be a biomarker for endogenous peroxynitrite formation (6, 30, 40). Hyperglycemia has been shown to promote tyrosine nitration via peroxynitrite, producing inactivation of prostacyclin synthase in human endothelial cells (5). Aortic segments from obese Zucker rats showed significantly more nitrotyrosine staining (increased by 51%) than lean Zucker rat aorta (13). We found that mesenteric arteries from ZDF rats had high levels of protein tyrosine nitration, and the strong nitrotyrosine signals were colocaled with 12-lipoxygenase in endothelium. Furthermore, 12-lipoxygenase was coprecipitated with antityrosinylated immune complexes, which suggests that 12-lipoxygenase in ZDF rat mesenteric arteries is a substrate of tyrosine nitration by peroxynitrite. The most common consequence of protein tyrosine nitration is loss of function (40), and this could contribute to the reduced 12-lipoxygenase activity in ZDF rat mesenteric arteries. In addition, protein tyrosine nitration has been shown to enhance susceptibility to proteolytic degradation (33) and may cause increased 12-lipoxygenase turnover, which would lead to reduced enzyme protein in ZDF rat mesenteric arteries. Our findings add to the growing list of proteins that are modified by tyrosine nitration, which contributes to the progression of cardiovascular diseases (40). These novel observations suggest that enhanced oxidative stress could be a common denominator for the mechanism of vascular and endothelial dysfunction in diabetes.

GRANTS

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