Decreased epoxygenase and increased epoxide hydrolase expression in the mesenteric artery of obese Zucker rats

Xueying Zhao, Aparajita Dey, Olga P. Romanko, David W. Stepp, Mong-Heng Wang, Yiqing Zhou, Liming Jin, Jennifer S. Pollock, R. Clinton Webb, and John D. Imig

Vascular Biology Center, Department of Physiology, and Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, Georgia

Submitted 12 January 2004; accepted in final form 26 August 2004

EPOXIESOSATRIENOIC ACIDS (EETs) and 20-hydroxyeicosatetraenoic acid (20-HETE) are the major arachidonic acid products of cytochrome P-450 (CYP) epoxygenase and hydroxylase enzymes. EETs can be further metabolized to their corresponding dihydroxyeicosatrienoic acids (DHETs) by soluble epoxide hydrolase (sEH). 20-HETE and EETs play important roles in the regulation of vascular function (4, 5, 7, 9, 13, 16, 17). The ω-hydroxylation product of 20-HETE causes vasoconstriction in the renal vasculature (18). On the other hand, 11,12-EET and 14,15-EET are potent vasodilators, whereas DHETs are devoid of renal vascular activity (16, 17). EETs are potential candidates for endothelium-derived hyperpolarizing factor (EDHF) because they hyperpolarize vascular smooth muscle cells by activating calcium-sensitive potassium channels (2, 9, 28, 33). Renal, cerebral, and coronary microvessels dilate in response to EETs (2, 5, 9, 17). EETs also induce a concentration-dependent relaxation of small mesenteric arteries and increase the open probability of the vascular smooth muscle BKCa channels (large calcium-sensitive potassium channels) in myocytes from rat mesenteric arteries (10, 11). Nevertheless, the epoxygenase enzymes responsible for EET generation in the mesenteric arteries remain unknown.

Impaired endothelium-dependent relaxation is associated with hypertension and diabetes. Due to a nonfunctional leptin receptor gene, the obese Zucker rat provides a valuable animal model for examining the effects of obesity and type 2 (non-insulin-dependent) diabetes (12). In obese Zucker rats, previous studies have indicated that mesenteric microvessel endothelium-dependent dilation was significantly reduced compared with responses in lean Zucker rats (19, 30, 31). Recent studies also show that the impairment of the endothelium-dependent dilation of the mesenteric arterial bed seen in streptozotocin-induced diabetic rats may be largely due to a defective vascular response to EDHF (20, 29). However, the mechanisms involved in this impaired endothelium-dependent relaxation are not clear. Our laboratory’s recent study (34) indicates that an inability to upregulate CYP2C and maintain CYP2J is associated with impaired vasodilation in rat kidney during salt-sensitive hypertension. Furthermore, renal P-450-derived eicosanoid synthesis is downregulated in rats with high fat diet-induced hypertension (27). We hypothesized that epoxygenase and sEH are also inappropriately regulated in obese Zucker rats. Therefore, the goals of the present study were to determine which epoxygenase isoforms may contribute to formation of EETs in mesenteric arteries and to determine whether changes in mesenteric arterial CYP enzymes are associated with impaired vasodilation in obese Zucker rats.

MATERIALS AND METHODS

Animals. Experiments used male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 250–300 g and 17- to 21-wk-old male lean (420 ± 13 g) and obese (685 ± 17 g) Zucker rats (Harlan Laboratories, Indianapolis, IN). Animals were fed standard chow and drank tap water ad libitum. Rats were housed in an animal care facility at the Medical College of Georgia approved by the American Association for the Accreditation of Laboratory Animal Care.

The costs of publication of this article were 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.
Care. All protocols were approved by the Institutional Animal Care and Use Committee at the Medical College of Georgia. We measured blood glucose levels using a commercially available kit (Roche) by tail vein blood sampling in the morning after 3–4 h of food deprivation; blood glucose levels were 109 ± 7 and 155 ± 8 mg/dl in lean and obese Zucker rats, respectively.

Preparation of renal microvessels and mesenteric arteries. Renal microvessels were isolated according to a method described previously (18). Briefly, rats were anesthetized with an injection of pentobarbital sodium (50 mg/kg ip). The kidneys were infused with a physiological salt solution, and the renal microvessels were separated from the rest of the cortex with the aid of sequential sieving, a digestion period, and collection under a stereomicroscope. Renal microvessels were quickly frozen in liquid N2 and kept at −80°C in a freezer until assayed for mRNA and protein levels.

Mesenteric arteries were harvested as previously described (24). The mesenteric bed, including arteries and veins, was cut away from the intestinal wall. The superior mesenteric bed, including the superior mesenteric artery, was placed in a dissecting dish containing ice-cold homogenization buffer (50 mmol/l Tris-HCl, pH 7.4; 0.1 mmol/l EDTA, 0.1 mmol/l EGTA, 250 mmol/l sucrose, 10% glycerol). The fat was carefully separated from the vessels, and then the veins were removed, using a dissecting microscope. Mesenteric arteries were snap-frozen in liquid N2 and stored at −80°C. In one set of experiments, mesenteric arteries were incubated in DMEM containing β-naphthoflavone (3 µmol/l, 24 h) and then snap frozen for determination of expression levels. In separate experiments, rats were treated with 10 mg·kg−1·day−1 6-(2-propargyloxyphenyl)hexanoic acid (PPOH, Sigma) twice per day for 4 days or with vehicle by tail vein injection for 4 days. After the 4-day treatment period, mesenteric arteries were collected and kept at −80°C until assayed for protein levels. Slightly thawed arteries were homogenized on ice in the presence of fresh protease inhibitors (1 mmol/l PMSF, 1 µmol/l pepstatin A, 2 µmol/l leupeptin, and 0.1% aprotinin) with a glass-glass homogenizer for 10 strokes. Protein concentration was determined by standard Bradford assay, with bovine serum albumin as the standard.

Activities of arachidonic acid metabolism in renal microvessels and mesenteric arteries. The production of arachidonic acid metabolites in intact renal microvessels and mesenteric arteries was determined as described previously (21, 27). Freshly isolated renal microvessels or mesenteric arteries were preincubated with 0.1% Tween 80 in 100 mmol/l MgCl2 and 1 mmol/l EDTA for 15 min at 4°C. This step results in the permeabilization of the tissue and ensures free access of exogenous arachidonic acid and NADPH to CYP enzymes located in the endoplasmic reticulum. Renal microvessels were washed twice with buffer, spun down by centrifugation, and incubated with [1-14C]arachidonic acid (50 µCi/µmol, 30 µmol/l final concentration) in 500 µl of potassium phosphate buffer containing 1 mmol/l NADPH in a shaking bath for 60 min at 37°C as described previously (27). The reactions were terminated by acidification to pH 4.0 with 2 mol/l formic acid, and renal microvessels or mesenteric arteries were homogenized. Extraction and HPLC analyses were carried out as described (24, 25).

Immunoblot analysis. Western blotting was performed as previously described (34). Samples were separated by electrophoresis on a 10% stacking Tris-glycine gel, and proteins were transferred electrophoretically to a nitrocellulose membrane. The primary antibodies used were goat anti-rat CYP2C11 polyclonal antibody (1:1,000; Gentest), rabbit CYP2C23 polyclonal antibody (1:5,000; a gift from Dr. Jorge H. Capdevila, Vanderbilt University, Nashville, TN), rabbit anti-human CYP2J2 antibody (1:2,000; a gift from Dr. Bruce D. Hammock, University of California at Davis, Davis, CA). The CYP2J2 antibody was made against purified recombinant human CYP2J2 protein, which cross-reacts with all CYP2J isoforms (23, 32). The blots were then washed in a PBS-0.1% Tween 20 solution and incubated with the secondary antibody (anti-goat 1:5,000 for CYP2C11 and CYP4A; anti-rabbit 1:100,000 for CYP2C23, CYP2J2, and sEH) conjugated to horseradish peroxidase for 1 h at room temperature and washed. Detection was accomplished with enhanced chemiluminescence Western blotting (Amersham), and blots were exposed to X-ray film (Hyperfilm-ECL, Amersham). We performed densitometry using a digital imaging system (Alpha Innotech). The intensities of the epoxygenase and sEH enzymes were normalized to the β-actin internal controls and are expressed as relative densitometric units (du).

Reverse transcription-polymerase chain reaction. We prepared total RNA from isolated renal microvessels using an ultra-pure TRizol reagent according to the procedure described by the manufacturer (GIBCO-BRL, Grand Island, NY). Random hexanucleotide primers were used for reverse transcription (RT) with equal amounts of RNA (2 µg). Oligonucleotide primers were designed from the published cDNA sequences of CYP2C11, CYP2C23, CYP2J, CYP4A, CYP1A, sEH, and GAPDH. GAPDH was used as an internal standard. The sequences of the CYP2C11, CYP2C23, CYP2J3, CYP2J4, CYP4A, and CYP1A1, sEH, and GAPDH primers are shown in Table 1. RT-polymerase chain reaction (PCR) was performed as previously described (25). After 30 cycles of amplification, 15 µl of each PCR reaction mixture were electrophoresed through a 1.5% agarose gel with ethidium bromide (0.5 µg/ml). We scanned the gel with ultraviolet illumination using Digital Imaging and Analysis (Alpha Innotech). The intensities of the epoxygenase isoforms and sEH were normalized to the GAPDH internal control and are expressed as relative densitometric units.

Measurements of vascular reactivity in isolated mesenteric arteries. In selected experiments, small mesenteric arteries (150–200 µm passive ID at 60 mmHg) were dissected free from connective tissue and internal elastic lamina. Measurements of the contractile effect of serotonin (10−6 mol/l) and noradrenaline (10−5 mol/l) were made at baseline and with the addition of increasing concentrations of pinocembrin (10−9–10−5 mol/l) or citrin (10−9–10−5 mol/l) (Sigma, St. Louis, MO). After each concentration of either serotonin or noradrenaline was added, recordings were continued for 10 min and then the next concentration of the drug was added. The difference in the slope of the cumulative concentration-response curves before and after the addition of pinocembrin or citrin was used to calculate the pA2 value of the drugs. Data are expressed as mean ± SE unless stated otherwise. The pA2 values were compared by one-way analysis of variance with Tukey’s post hoc test.

Table 1. Designation of rat CYP2C, CYP2J, sEH, and GAPDH gene primer sets for RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence (5′-3′)</th>
<th>Product Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C11</td>
<td>Forward GTG TGA GAA TGG CAT AAA</td>
<td>463 bp</td>
</tr>
<tr>
<td></td>
<td>Reverse GTA TCC GCA TGC TGA GTT</td>
<td></td>
</tr>
<tr>
<td>CYP2C23</td>
<td>Forward TCA CTA CCG TGG CTT CGT</td>
<td>325 bp</td>
</tr>
<tr>
<td></td>
<td>Reverse TAA CCC TTA TGA GTC TCT TC</td>
<td></td>
</tr>
<tr>
<td>CYP2J3</td>
<td>Forward TTT CTG TTC CTG GCT CAT GAT</td>
<td>412 bp</td>
</tr>
<tr>
<td></td>
<td>Reverse GGT AGG CCT CCT GTC TTA</td>
<td></td>
</tr>
<tr>
<td>CYP2J4</td>
<td>Forward GAA CGC ATC ACC AAT AAA</td>
<td>464 bp</td>
</tr>
<tr>
<td></td>
<td>Reverse GTC AGG GTC CCA GTC TTT</td>
<td></td>
</tr>
<tr>
<td>sEH</td>
<td>Forward TGG CTT AGG CTG AAG TGG A</td>
<td>408 bp</td>
</tr>
<tr>
<td></td>
<td>Reverse GGT TCC AGT GAC CAG AGT C</td>
<td></td>
</tr>
<tr>
<td>CYP4A1</td>
<td>Forward GTA TCC AAG TCA CAG TCT CCA</td>
<td>827 bp</td>
</tr>
<tr>
<td></td>
<td>Reverse CAG GAC ACT GGA CAC TTT ATT G</td>
<td></td>
</tr>
<tr>
<td>CYP4A2</td>
<td>Forward AGA TCC AAA GGC TTA TCA ATC</td>
<td>317 bp</td>
</tr>
<tr>
<td></td>
<td>Reverse CAG CCT TGG TGT AGG ACC T</td>
<td></td>
</tr>
<tr>
<td>CYP4A3</td>
<td>Forward CAA AGG CCT CTG GAA TTA ATC</td>
<td>321 bp</td>
</tr>
<tr>
<td></td>
<td>Reverse CAG CCT TGG TGT AGG ACC T</td>
<td></td>
</tr>
<tr>
<td>CYP1A1</td>
<td>Forward TTG TGG GAG CTT GGT TTG ACA C</td>
<td>286 bp</td>
</tr>
<tr>
<td></td>
<td>Reverse TTG ACA AAG ACA CAG TCT CG</td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td>Forward AAT GCA TCC TGC ACC ACC AA</td>
<td>515 bp</td>
</tr>
<tr>
<td></td>
<td>Reverse GTA GCC ATA TTC ATT GTC ATA</td>
<td></td>
</tr>
</tbody>
</table>

CYP, cytochrome P-450; sEH, soluble epoxide hydrolase.
and fat in cold Krebs-physiological salt solution with the following composition (in mM, pH 7.4): 119.0 NaCl, 25.0 NaHCO3, 4.6 KCl, 1.2 KH2PO4, 1.2 MgSO4, 1.8 CaCl2, and 11.0 glucose. Individual arteries were mounted on glass micropipettes, pressurized to 60 mmHg in Krebs-physiological salt solution at 37°C, and allowed to equilibrate for 30 min. Because myogenic tone is limited in mesenteric small arteries (~10%), preconstriction was performed with phenylephrine (0.5–1.0 μM) to 50% of resting diameter. Endothelium-dependent vasodilation was examined with serial doses of acetylcholine (10 nM to 10 μM). The contribution of nitric oxide (NO) was determined by repeating acetylcholine doses in the presence of 500 μM nitro-L-arginine methyl ester (L-NAME). Percent relaxation curves are expressed as the difference in diameter at each dose relative to the baseline, preconstricted diameter (0%), and the passive diameter (100%).

Statistics. All data are presented as means ± SE. The mRNA and protein data were analyzed by unpaired t-test. Changes in diameter in response to acetylcholine were normalized by its passive diameter. Statistical significance was calculated by repeated-measures ANOVA followed by the Tukey-Kramer multiple-comparison test. A value of P < 0.05 was considered statistically significant.

RESULTS

Arachidonic acid metabolism in renal microvessels and mesenteric arteries. CYP-derived eicosanoid syntheses were measured in renal microvessels and mesenteric arteries. Renal microvessels and mesenteric arteries permeabilized with 0.1% Tween 80 and incubated with [14C]arachidonic acid (30 μmol/l in final concentration) in the presence of NADPH resulted in the production of detectable levels of DHETs, 20-HETE, and EETs (Fig. 1, A and B). Epoxide-ase activity was determined from the sum of DHET and EET formation. ω-Hydroxylase activity was determined from 20-HETE formation. Hydroxylase activities were 80.2 ± 19.7 pmol·mg⁻¹·min⁻¹ (n = 3) in renal microvessels and 8.1 ± 0.3 pmol·mg⁻¹·min⁻¹ (n = 3) in mesenteric arteries, respectively. Renal and mesenteric arterial epoxygenase activities were 34.2 ± 10.0 pmol·mg⁻¹·min⁻¹ (n = 3) and 13.7 ± 1.4 pmol·mg⁻¹·min⁻¹ (n = 3), respectively. The formation of EETs and 20-HETE was completely dependent on the addition of exogenous NAPDH to the reactions. There was no detectable formation of these products in renal vessels incubated in the absence of added NADPH (Fig. 1C). A similar result was obtained in mesenteric arteries.

CYP4A, CYP2C, and CYP2J isoform expression in mesenteric arteries. Western blot and RT-PCR were used to examine CYP enzyme expression in mesenteric arteries. Representative Western blot and RT-PCR data for CYP4A, CYP2C11, CYP2C23, and CYP2J isoforms in mesenteric arteries and renal microvessels are shown in Figs. 2 and 3. Compared with an abundant expression in renal microvessels, CYP2C23 mRNA and protein were not detected in mesenteric arteries isolated from Sprague-Dawley rats. CYP2C11 mRNA and protein were expressed in mesenteric arteries and renal microvessels. In addition, CYP2J3 and CYP2J4 mRNA were abundantly expressed in mesenteric arteries and renal microvessels. Consequently, CYP2J protein was detectable in mesenteric and renal vasculatures. The CYP4A1, 4A2, and 4A3 isoforms were expressed in renal vessels, but only CYP4A3 mRNA was detected and CYP4A protein expression was relatively low in mesenteric arteries.

Experiments were performed to further establish whether CYP2C11 could be regulated in mesenteric arteries. Incubation of endothelium-intact rat mesenteric arteries with β-naphthoflavone enhanced the expression of CYP2C11 mRNA (Fig. 4). Administration of β-naphthoflavone (3 μmol/l, 24 h) significantly increased CYP2C11 mRNA expression (7.5 ± 2.3 vs. 1.2 ± 0.3 du ratio). β-Naphthoflavone also increased CYP1A1 mRNA expression (6.5 ± 1.4 vs. 3.1 ± 0.9 du ratio) in mesenteric arteries. In addition, the CYP2C11 protein level was evaluated in PPOH-treated rat mesenteric arteries. Con-
Fig. 2. Cytochrome P-450 (CYP) isoform CYP2C23, CYP2C11, CYP2J, and CYP4A mRNA expressions in renal microvessels and mesenteric arteries. Representative RT-PCR shows CYP2C23, CYP2C11, CYP2J, and CYP4A mRNA bands in mesenteric arteries (MA, lane 1) and in renal microvessels (RV, lane 2) isolated from Sprague-Dawley rats.

Fig. 3. CYP2C23, CYP2C11, CYP2J, and CYP4A protein expression in renal microvessels and mesenteric arteries. Representative Western blots show CYP2C23, CYP2C11, CYP2J, and CYP4A bands in renal microvessels and mesenteric arteries isolated from Sprague-Dawley rats; 10 μg of protein were loaded per lane.
sistent with studies in rat kidney cortex (unpublished data), chronic PPOH treatment significantly decreased CYP2C11 protein in mesenteric arteries (Fig. 5). In contrast, the mesenteric artery CYP2J protein level was not changed after PPOH treatment (Fig. 5). These data suggest that CYP2C11 is regulated in mesenteric arteries and that CYP2C11 may be one of the main isoforms responsible for EET formation in mesenteric arteries.

CYP isoform and sEH expression in obese Zucker rats. Obese Zucker rats weighed significantly more than lean Zucker rats (685 ± 17 g vs. 420 ± 13 g). Blood glucose was also elevated in obese compared with lean Zucker rats (155 ± 8 vs. 109 ± 7 mg/dl). We further studied the expression of CYP2C11, CYP2J, and sEH in the mesenteric arteries isolated from obese and lean Zucker rats to determine the changes in CYP and sEH enzymes associated with obesity (Figs. 6 and 7).
CYP2C11 and CYP2J proteins were decreased by 38 and 43%, respectively, in mesenteric arteries isolated from obese Zucker rats (Fig. 6). Protein for the sEH enzyme was consistently expressed in mesenteric arteries. Obese Zucker rats demonstrated increased sEH mRNA expression in mesenteric arteries (17.3 ± 3.3 vs. 6.8 ± 0.9 du ratio, Fig. 7). Consequently, sEH protein levels were increased by 82% in obese Zucker rat mesenteric arteries (Fig. 7).

Acetylcholine-induced vasodilation in obese Zucker rats. To investigate the NO-independent dilation evoked by acetylcholine in rat mesenteric artery, we performed a series of experiments in which acetylcholine (0.1–10 μM) was added cumulatively to mesenteric arteries precontracted by phenylephrine in the absence or presence of L-NAME. As shown in Fig. 8, acetylcholine produced a dose-dependent vasodilation. Vasodilation to acetylcholine was impaired in obese Zucker rats compared with age-matched lean Zucker rats. The nitric oxide synthase inhibitor L-NAME moderately diminished relaxation to acetylcholine, but the response to 10 μM acetylcholine in obese Zucker rats (17 ± 7%) was still significantly reduced compared with lean Zucker rats (58 ± 5%). These data suggest that NO-independent mesenteric artery dilation is impaired in obese Zucker rats.

DISCUSSION

In the present study, activity and expression of CYP monooxygenase enzymes in mesenteric arteries were compared with those in renal microvessels. Consistent with previous studies, CYP2C23 is the main isoform expressed in renal microvessels (14, 34); however, CYP2C23 mRNA and protein were not expressed in mesenteric arteries. CYP2C11 was present in mesenteric arteries, induced by β-naphthoflavone and reduced by PPOH treatment. Additionally, CYP2C11 and CYP2J protein expressions were decreased in obese Zucker rats. In contrast, sEH protein and mRNA were significantly increased in mesenteric arteries of obese Zucker rats. Furthermore, NO-independent vasodilation was significantly attenuated in mesenteric arteries of obese Zucker rats. These findings demonstrate that the main epoxygenase isoforms expressed in mesenteric artery are different from those expressed in renal microvessels and that decreased epoxygenases and increased sEH may contribute to impaired mesenteric artery dilation in obese Zucker rats.

Detectable CYP ω-hydroxylase and epoxygenase activities were observed in renal and mesenteric arteries. It is well established that CYP4A enzymes are the major arachidonic acid ω-hydroxylase in the rat kidney and thereby the primary contributors to 20-HETE synthesis (6, 2). Renal microvessels expressed CYP4A1, CYP4A2, and CYP4A3 isoforms; however, mesenteric arteries only expressed CYP4A3 mRNA, and CYP4A protein was not readily evident. These results suggest that 20-HETE may play a lesser role in regulating the mesenteric artery tone under normal conditions.

Although the role of EETs as EDHF is has been widely investigated (4, 5, 9), our knowledge of CYP isoforms involved in biosynthesis and regulation of EETs in mesenteric arteries is limited. Many CYP enzymes can carry out the epoxidation of arachidonic acid, and several reports have suggested that CYP2C and CYP2J isoforms are responsible for renal EET production (14, 25). For example, Holla et al. (14) demonstrated that CYP2C11 has the highest epoxygenase activity, whereas CYP2C23 has a lower epoxygenase activity. Even with this being the case, kidney EET production profiles and antibody inhibition studies have established CYP2C23 as
the major arachidonic acid epoxygenase in the rat kidney (14). Our present results indicate that the main epoxygenase isoforms present in mesenteric arteries are different from those found in renal microvessels. Compared with the abundant expression in renal microvessels, CYP2C23 mRNA and protein were absent in mesenteric arteries. We were able to establish the presence of CYP2J and CYP2C11 enzymes in rat mesenteric arteries.

We also evaluated the regulation of CYP2C11 in mesenteric arteries by inducing and inhibiting the enzyme. Consistent with a previous study (1), incubation with the CYP inducer β-naphthoflavone increased CYP1A1 mRNA expression in mesenteric arteries. In addition, CYP2C11 mRNA expression was also significantly increased in mesenteric arteries by β-naphthoflavone, which is also consistent with one previous report (8). These CYP2C11 mRNA results should be interpreted with caution because standard RT-PCR is subject to error in the efficiency of the RT and the PCR steps and sample preparation (3). We used the suicide substrate inhibitor of epoxygenase, PPOH, because enzyme destruction and degradation often

---

**Fig. 7.** Soluble epoxide hydrolase (sEH) mRNA and protein in mesenteric arteries isolated from LZR and OZR. A: total RNA was extracted and analyzed by RT-PCR for the expression of sEH. B: protein was subjected to Western blot analysis with a specific antibody directed against sEH; and recombinant sEH was used as a positive control (rsEH). The densitometric evaluations of mRNA (15 µl/lane) and protein (10 µg/lane) levels were obtained from 4 different animals. *P < 0.05 vs. control.

---

**Fig. 8.** Vasorelaxation response curve to acetylcholine for the mesenteric artery of LZR and OZR before (113 ± 10 and 150 ± 13 µm, respectively) and after nitro-L-arginine methyl ester (l-NAME; 113 ± 11 and 122 ± 12 µm, respectively) treatment. Percent relaxation curves are expressed as the difference in diameter at each dose relative to the baseline, preconstricted diameter (0%), and the passive diameter (100%). Values are means ± SE, n = 5 mesenteric arteries from different animals per group. *Significant difference vs. lean control group; #significant difference vs. lean + l-NAME group.
accompany irreversible or suicidal inhibition of CYP activity (26). In our present study, chronic treatment with PPOH significantly decreased CYP2C11 protein expression in mesenteric arteries. In contrast, PPOH treatment did not change the CYP2J protein level in mesenteric arteries. These data suggested that CYP2C11 can be modulated in mesenteric arteries.

Obesity, type 2 diabetes, and hypertension are frequently associated with one another. Although the exact mechanisms that mediate obesity, type 2 diabetes, and hypertension are not fully understood, endothelial dysfunction and a blunted vascular response to endogenous vasodilators are thought to play a role (10, 11). Due to a nonfunctional leptin receptor gene, the obese Zucker rat has an extremely high and uncontrolled appetite (12). As a result of extreme food consumption, obese Zucker rats rapidly develop numerous pathological conditions that are highly relevant to public health issues challenging Western society; among these conditions are type 2 diabetes, moderate hypertension, and obesity (12). Obese Zucker rats have been widely used to investigate changes in responsiveness of the vasculature to vasoconstrictor and vasodilator hormones in an attempt to more clearly characterize the vascular dysfunction associated with obesity-induced hypertension. A number of studies have reported that NO-dependent relaxation to acetylcholine is impaired in skeletal muscle arterioles (10, 11) and small mesenteric arteries (19, 31) of obese Zucker rats. On the other hand, recent studies show that the impairment of the endothelium-dependent dilation of the mesenteric arterial bed seen in streptozotocin-induced diabetic rats may be largely due to a defective vascular response to EDHF (20, 29). In the rat mesenteric artery, a clear component of acetylcholine-induced relaxation is resistant to blockers of NO and prostacyclin synthesis, suggesting a prominent role for EDHF in this vessel. NO-dependent responses are preserved in diabetes, whereas endothelial-dependent responses on EDHF appear to be impaired (28). These differences have likely arisen in part because of differences between studies in the arterial preparation and the age of the animals investigated. In the present study, acetylcholine-induced vasodilation was significantly attenuated in mesenteric arteries of obese Zucker rats compared with their lean controls. Furthermore, the nitric oxide synthase inhibitor L-NAME moderately diminished relaxation to acetylcholine, but the response in obese Zucker rats was still significantly reduced compared with lean Zucker rats. Thus these data suggest that NO-independent vasodilation was impaired in mesenteric arteries of obese Zucker rats.

In small mesenteric arteries, EDHF plays an important role in acetylcholine-induced NO-independent vasodilation (20, 29). In coronary, cerebral, renal, and skeletal muscle circulations, EDHF has been characterized as CYP epoxygenase metabolites of arachidonic acid (2, 5, 9, 15, 17). In the present study, we investigated the CYP2C and CYP2J expressions in mesenteric arteries in obese and lean Zucker rats. CYP2C11 and CYP2J protein levels were significantly decreased in mesenteric arteries isolated from obese Zucker rats. We also evaluated the sEH enzyme that is primarily responsible for conversion of EETs to their corresponding DHETs, which are devoid of effects on the pregglomerular vasculature. In obese Zucker rats, sEH mRNA and protein expression were significantly increased in mesenteric arteries compared with lean Zucker rats. Taken together, decreased epoxygenase and increased sEH enzymes are associated with mesenteric artery endothelial dysfunction observed in obese Zucker rats. Additional studies should be designed to determine whether the changes in CYP enzyme expression are primary alterations or whether these changes in gene expression are secondary to the various pathologies that develop in obesity-induced hypertension.

This study demonstrates that the main epoxygenase isoforms expressed in the rat mesenteric arteries are different from those expressed in renal microvessels. In addition, epoxygenase and sEH expression were altered in obese Zucker rat mesenteric arteries. These changes in mesenteric artery EET biosynthetic and degradative enzymes could contribute to vascular pathologies associated with obesity-related type 2 diabetes and hypertension. Thus manipulation of the synthesis of these CYP metabolites by pharmacological inhibitors or inducers and gene transfer methods in obese rats will be important to elucidate the role of these CYP isoforms in the regulation of microvascular function and blood pressure in this animal model.

GRANTS

This work was supported by National Institutes of Health Grants HL-70887, HL-59699, HL-18575, HL-74167, and DK-38226. J. D. Imig is an Established Investigator of the American Heart Association, and X. Zhao is supported by an American Heart Association Southeast Affiliate Postdoctoral Fellowship.

REFERENCES


