Oxidant stress and constrictor reactivity impair cerebral artery dilation in obese Zucker rats

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Phillips, Shane A., Francis A. Sylvester, and Jefferson C. Frisbee. Oxidant stress and constrictor reactivity impair cerebral artery dilation in obese Zucker rats. Am J Physiol Regul Integr Comp Physiol 288: R522–R530, 2005. First published October 28, 2004; doi:10.1152/ajpregu.00655.2004.—This study tested the hypothesis that evolution of the metabolic syndrome in obese Zucker rats (OZR) leads to impaired dilator reactivity of cerebral resistance arteries vs. responses determined in lean Zucker rats (LZR). Middle cerebral arteries (MCA) from 17-wk-old male LZR and OZR were isolated and cannulated with glass micropipettes. Vascular reactivity was assessed in response to challenge with ACh, sodium nitroprusside (SNP), reductions and elevations in PO2, 5-HT, and increased intraluminal pressure. Vessels were treated with the free radical scavenger 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl (tempol) to assess the role of superoxide production in altering reactivity, and passive vascular wall mechanics was assessed in each vessel. Vascular superoxide production was assessed in isolated arteries using fluorescence microscopy. Vessel dilation to ACh and hypoxia was impaired in OZR vs. LZR, although responses to SNP were normal. Vessel constriction to 5-HT, elevated PO2, and elevated intraluminal pressure was enhanced in OZR vs. LZR. Fluorescence microscopy demonstrated an increased superoxide production in arteries of OZR vs. LZR, correctable by incubation with tempol. Although treatment of vessels from OZR with tempol improved dilation to ACh and hypoxia, constrictor responses to 5-HT, elevated PO2, and pressure were not altered by tempol treatment. Indexes of vessel wall mechanics were comparable between groups. These results suggest that vasodilator reactivity of MCA of OZR in response to endothelium-dependent dilator stimuli is impaired vs. LZR and that this may represent a reduced bioavailability of signaling molecules due to oxidative scavenging. However, oxidative stress-independent increases in myogenic tone and constrictor reactivity may contribute to blunted dilator responses of cerebral microvessels.

THE METABOLIC SYNDROME is defined as the combined presentation of obesity, insulin resistance, hypertension, and dyslipidemia and currently impacts more than 40 million Americans (2, 3, 46). Additionally, the expression of these multiple pathological conditions represents potent risk factors for development of numerous cardiovascular disease states and impairments, including atherosclerosis, myocardial infarction, and chronic demand-based ischemia (2, 3). We have previously employed obese Zucker rats (OZR) as a model for the metabolic syndrome, caused by a dysfunctional leptin receptor gene causing chronic hyperphagia (9, 10). OZR rapidly develop insulin resistance, hypertriglyceridemia, and a moderate, clinically relevant hypertension (9, 10, 39). Our previous studies examining alterations to the structure and function of the skeletal muscle microcirculation subsequent to the evolution of the metabolic syndrome in OZR have clearly demonstrated impaired dilator responses to numerous vasoactive stimuli (24, 26). While these impairments in arteriolar dilator reactivity are partially dependent on an elevated vascular oxidant tone reducing the bioavailability of dilator signaling molecules (26), additional studies have suggested that increased vasoconstrictor reactivity (21, 25), and a reduced distensibility of the microvessel wall (22) also represent contributing elements to the impaired dilator responses in the skeletal muscle of OZR.

Given the predisposition for individuals afflicted with the metabolic syndrome to suffer from an increased incidence of cerebrovascular dysfunction and stroke (2, 3), we elected to extend our efforts to examine alterations to the dilator reactivity of cerebral resistance arteries within adult OZR exhibiting the full manifestation of metabolic syndrome. The recent study by Erdos et al. (19) demonstrated that the dilator reactivity of the cerebral arteries was impaired in 12-wk-old insulin-resistant OZR vs. responses in control lean Zucker rats (LZR) and that this was almost entirely mediated by an elevation in oxidant tone. The purpose of the present study was to evaluate the role of oxidant tone in contributing to alterations in reactivity of the middle cerebral arteries (MCA) of diabetic OZR and to assess whether alterations in vascular tone, constrictor reactivity, and/or vascular wall distensibility also act in parallel to any demonstrated oxidant stress-based impairments in vasodilator reactivity.

MATERIALS AND METHODS

Animals. Seventeen-week-old male LZR and OZR (Harlan, Indianapolis, IN), fed standard chow and tap water ad libitum, were used for all experiments. Rats were housed in an American Association for Accreditation of Laboratory Animal Care-accredited animal care facility, and all protocols received prior Institutional Animal Care and Use Committee approval. Initially, all rats were anesthetized with injections of pentobarbital sodium (50 mg/kg ip), and received tracheal intubation to facilitate maintenance of a patent airway. In all rats, a carotid artery and an external jugular vein were cannulated for determination of arterial pressure and for intravenous infusion of additional anesthetic, if necessary. Data describing the baseline characteristics of LZR and OZR used in the present study are summarized in Table 1. At 17 wk of age, OZR were significantly heavier than

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Table 1. Baseline characteristics describing animals used in the present study

<table>
<thead>
<tr>
<th></th>
<th>LZR</th>
<th>OZR</th>
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</thead>
<tbody>
<tr>
<td>Mass, g</td>
<td>364±12</td>
<td>688±15*</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>105±4</td>
<td>128±6*</td>
</tr>
<tr>
<td>[Glucose]plasma, mg/dl</td>
<td>106±14</td>
<td>202±34*</td>
</tr>
<tr>
<td>[Insulin]plasma, ng/ml</td>
<td>2.4±1.1</td>
<td>16.9±2.0*</td>
</tr>
<tr>
<td>[Cholesterol]plasma, mg/dl</td>
<td>66.9±8.1</td>
<td>84.5±10.4</td>
</tr>
<tr>
<td>[Triglycerides]plasma, mg/dl</td>
<td>75.8±9.8</td>
<td>348±158*</td>
</tr>
<tr>
<td>[8-Isoprostanep]plasma, ng/ml</td>
<td>72.0±5.8</td>
<td>142±78*</td>
</tr>
</tbody>
</table>

Values are means ± SE. For lean Zucker rats (LZR), n = 21; for obese Zucker rats (OZR), n = 23. *P<0.05 vs. LZR.

age-matched LZR and also demonstrated faster hyperglycemia, hyperinsulinemia, elevated plasma triglyceride levels, and hypertension. Furthermore, OZR had elevated plasma levels of 8-epi-prostaglandin F2α, an in vivo marker of lipid peroxidation and oxidant stress (35), compared with values in LZR.

Investigation of isolated vessels. After the initial surgery, each rat was decapitated, and the brain was removed from the skull case and placed in cold physiological salt solution (PSS; 4°C). Subsequently, an MCA was dissected from its origin at the circle of Willis, as described previously (36). Arteries were placed in a heated chamber (37°C) that allowed the lumen and exterior of the vessel to be perfused and superfused, respectively, with PSS equilibrated with 21% O2:5% CO2:74% N2 from separate reservoirs. Vessels were cannulated at both ends with glass micropipettes and were tied (10–0 nylon suture) both ends with glass micropipettes and were tied (10–0 nylon suture) to the inflow and outflow pipettes, which were connected to a reservoir perfusion system that allowed intraluminal pressure and gas concentrations to be controlled. Any side branches were ligated using a single strand teased from 6-0 suture. Vessel diameter was measured using a single strand teased from 6-0 suture. Vessel diameter was measured using television microscopy and an on-screen video micrometer.

Arteries were extended to their in situ length and were equilibrated at 80% of the animal’s mean arterial pressure (MAP) (84 ± 4 mmHg for LZR; 102 ± 5 mmHg for OZR). Active tone for vessels in the present study, calculated as \( \frac{D}{D_{\text{max}}} \), where \( D \) is the diameter increase from rest in response to \( \text{Ca}^{2+} \)-free PSS, and \( D_{\text{max}} \) is the maximum diameter measured at the equilibration pressure in \( \text{Ca}^{2+} \)-free PSS, averaged 24 ± 2% in LZR (n = 21) and was significantly elevated in OZR 32 ± 2% (n = 23; P = 0.018). The reactivity of isolated arteries was assessed in response to \( J \) acetylcholine (10⁻⁹ M–10⁻⁶ M), 2) sodium nitroprusside (10⁻⁹ M–10⁻⁶ M), 3) hypoxia (\( \Delta P_{\text{O2}} \) from ~140 to ~35 mmHg), 4) elevated \( P_{\text{O2}} \) (from ~140 to ~660 mmHg), 5) 5-hydroxytryptamine (5-HT; 10⁻⁹ M–10⁻⁶ M), and 6) altered intravascular pressure (myogenic activation; 0 to 140 mmHg in randomized 20-mmHg increments). To ensure that a negative intraluminal pressure was not exerted on the vessel, 5 mmHg was used as the “0 mmHg” intraluminal pressure point; all other active intraluminal pressure was not exerted on the vessel, 5 mmHg for OZR). Active tone for vessels in the present study exposed to varied levels of intraluminal pressure was calculated as \( \Delta T = -1.333 \times P_{\text{L}} \times (0.5 \times ID_{\text{Active}} - 0.5 \times ID_{\text{Passive}}) \times 0.0001 \) where \( \Delta T \) (dynes/cm) represents the difference in wall tension between passive conditions (\( \text{Ca}^{2+} \)-free PSS) vs. active conditions (normal PSS) at a given intraluminal pressure (\( P_{\text{L}} \); mmHg). ID represents arterial inner diameter (\( \mu \)m) under “active” (calcium present) or “passive” (zero calcium) conditions; and the constants 1.333 and 0.0001 are factors for converting pressure in millimeters of mercury to dynes per centimeters squared and from micrometers to centimeters, respectively. As such, \( \Delta T \) represents the absolute value describing the amount by which the passive tension in the vascular wall would be increased if all active contractile mechanisms were inhibited at a given intraluminal pressure (34). In the present study, this difference in vessel wall tension between the passive and active condition is designated as “wall tension difference.”

All calculations of passive arteriolar wall mechanics (used as indicators of structural alterations to the individual microvessel) are based on those described previously (6), with minor modification.

Vessel wall thickness was calculated as

\[
WT = \frac{(OD - ID)}{2}
\]

where WT represents wall thickness (\( \mu \)m) and OD and ID represent arteriolar outer and inner diameter, respectively (\( \mu \)m).

Incremental arteriolar distensibility (DIST\(_{\text{INC}}\); % change in arteriolar diameter/mmHg) was calculated as

\[
\text{DIST}_{\text{INC}} = \frac{\Delta ID}{ID \times \Delta P_{\text{L}}) \times 100}
\]

where \( \Delta ID \) represents the change in internal arteriolar diameter for each incremental change in intraluminal pressure (\( \Delta P_{\text{L}} \)).
For the calculation of circumferential stress, intralumenal pressure was converted from millimeters of mercury to newtons per meter squared, where 1 mmHg = 1.334 × 10^2 N/m². Circumferential stress (σ) was then calculated as

\[ \sigma = (P_{\text{IL}} \times ID) / (2WT) \]

Circumferential strain (ε) was calculated as

\[ \varepsilon = (1D - ID) / ID \]

where ID\text{\_}m represents the internal arteriolar diameter at the lowest intralumenal pressure (i.e., 5 mmHg).

Arteriolar reactivity data to all pharmacological agonists, intralumenal pressure, and the data describing the passive diameter and incremental distensibility of the vessel were analyzed using repeated-measures ANOVA. Statistically significant differences in arteriolar responses to altered oxygen tension hypoxia and DHE fluorescent intensity data were assessed using ANOVA only. Circumferential stress vs. strain curves were fit with exponential regression equations, and statistically significant differences between slope coefficients were evaluated using Student’s t-test. Student-Newman-Keuls test was employed post hoc as appropriate.

RESULTS

As presented above, the baseline level of vascular tone in MCA from OZR was significantly elevated compared with that in LZR (at their respective equilibration pressures). At this level of vascular tone, equilibrated MCA diameter was 174 ± 5 μm in LZR and 160 ± 4 μm in OZR.

Figure 1 presents data describing the response of isolated MCA from LZR and OZR in response to challenge with acetylcholine (A), hypoxia (B), sodium nitroprusside (C), 5-HT (D), or elevated PO2 (E). In response to a challenge with either acetylcholine or hypoxia, cerebral arteries from OZR not only failed to dilate, but demonstrated a consistent constriction in response to these stimuli, compared with responses observed in vessels from LZR. In contrast, direct application of the nitric oxide donor sodium nitroprusside (Fig. 1C) resulted in a dilator response that was comparable between cerebral vessels of both strains. In terms of constrictor reactivity, application of increasing concentrations of 5-HT or exposure to acute elevations in oxygen tension resulted in a stronger vasoconstrictor response in vessels of OZR, compared with that determined in MCA from LZR.

Myogenic activation of isolated cerebral arteries from OZR and LZR in response to altered intralumenal pressure is presented in Fig. 2. In response to incremental elevation in intralumenal pressure, vessels from OZR demonstrated a significantly stronger pressure-induced constriction than vessels from LZR (Fig. 2A). This response was evident in the generated wall tension difference, as vessels from OZR exhibited a greater difference than vessels from LZR (Fig. 2B).

Figure 3 presents data describing the passive mechanical characteristics of the arterial wall in cerebral vessels of OZR and LZR. Under Ca\textsuperscript{2+}-free conditions, vessels of OZR and LZR exhibited a very similar increase in diameter in response to incremental elevations in intralumenal pressure (Fig. 3A). As a result of this similarity, the calculated incremental distensibility (Fig. 3B) and beta coefficients describing the circumferential stress vs. strain relationship (Fig. 3C) were not different between MCA from LZR and OZR.

Superoxide production in cerebral arteries of LZR and OZR used in the present study, as determined using DHE fluorescence microscopy, is presented in Fig. 4. In untreated control conditions, cerebral arteries from OZR (Fig. 4C) exhibited a statistically significant increase in the rate of superoxide production compared with vessels from LZR (Fig. 4A). Incubation of cerebral arteries with tempol (Fig. 4D) ameliorated this increased production to the extent that the generation of superoxide in treated arteries from OZR was not different from that determined in arteries from LZR (Fig. 4, A or B).

Figure 4, E and F presents the effects of treating vessels from LZR and OZR, respectively, with menadione and DETC, as a positive control experiment. The summarized data on the rate of change in superoxide production are presented graphically in Fig. 4H.

Treatment of isolated MCA with tempol had no effect on basal diameter vs. untreated conditions in either LZR (178 ± 5 μm; P = 0.649) or OZR (167 ± 5 μm; P = 0.293). Data describing the effects of tempol treatment on alterations to the reactivity of isolated MCA from LZR and OZR are presented in Fig. 5. After incubation with tempol, arteries from OZR demonstrated a partial improvement in the dilator responses to acetylcholine (Fig. 5A) and reduced Po2 (Fig. 5B). In contrast, treatment of vessels from either animal strain with tempol had no effect on constrictor responses to 5-HT (Fig. 5C) or elevated Po2 (Fig. 5D) compared with responses determined in control conditions. Further, tempol treatment had a tendency to blunt the enhanced myogenic activation (Fig. 5E) and wall tension difference (Fig. 5F) determined in vessels from OZR vs. LZR, although these differences did not reach statistical significance.

The dilation of MCA in response to challenge with sodium nitroprusside was not significantly altered from control levels in response to treatment of vessels with tempol in either LZR or OZR (n = 3 for each; data not shown).

DISCUSSION

The central observation of the present study is that, with evolution of the metabolic syndrome, the dilator reactivity of isolated MCA of OZR is compromised. This impaired dilator reactivity appears to reflect multiple contributing elements, including an elevated vascular oxidant tone that reduces the bioavailability of dilator-signaling molecules, an increase in vascular tone and myogenic activation representing a potentially competing influence on dilator reactivity and an enhanced reactivity of cerebral microvessels in response to constrictor stimuli, which may present additional competition against vasodilation.

Using somewhat younger OZR (13–15 wk), Schwaninger et al. (48) demonstrated that dilator responses of cerebral arterioles were impaired vs. that determined in LZR after challenge with acetylcholine or ADP but that responses to application of nitric oxide donors elicited a normal dilation. A recent study by Erdos et al. (19) supported and extended these previous results by demonstrating that the dilator reactivity of basilar arteries in 12-wk-old OZR was impaired in response to acetylcholine, prostacyclin analogs, and to direct activation of ATP-sensitive potassium (K\textsubscript{ATP}) channels, although responses to nitric oxide donors were not different from those in LZR. The authors further demonstrated that treatment of vessels from OZR with the oxidative free-radical scavenger superoxide dismutase completely restored any impairment in dilator reactivity. While supporting these previous results, our data also suggest that
mechanisms underlying the impaired cerebrovascular reactivity in OZR may become more multifaceted as the extent of the metabolic syndrome worsens in older OZR. In the present study, a reduction in vascular superoxide production, through treatment of vessels with tempol, resulted in only a partial improvement in dilator reactivity, not the complete recovery determined previously (19). Previous studies have suggested that likely sources for the generation of superoxide may include NADH/NADPH oxidase (16, 33, 50) and protein kinase C (8, 16), although the additional contributions from the polyol pathway (12, 13) and through mitochondrial metabolism (16) must also be considered. Recent study suggests that NOS dysfunction does not contribute to the increased generation of superoxide in OZR (27). Our results suggest that although an increased oxidative stress may contribute to a reduction in signaling molecule bioavailability, or possibly in potassium channel function (19, 20), an increased vascular tone and enhanced constrictor reactivity may also contribute to the impaired dilator responses determined in MCA from OZR.

Fig. 1. Data describing the change in arterial diameter of isolated middle cerebral arteries (MCA) of lean Zucker rats (LZR; n = 5–7) and obese Zucker rats (OZR; n = 6–7) in response to challenge with increasing concentrations of ACh (A), reduced PO2 (B), increasing concentrations of sodium nitroprusside (C), increasing concentrations of 5-HT (D) and elevated PO2 (E). Data are presented as means ± SE. *P < 0.05 vs. responses in LZR.
those identified in peripheral vascular beds (21, 22, 52), is protected from others. Specifically, an alteration in vascular wall mechanics did not develop in MCA of OZR, despite the presence of a significant elevation in MAP, generally considered to be a strong stimulus for remodeling cerebral resistance arteries, leading to a reduction in incremental distensibility (6, 18, 30). Although this observation supports our earlier preliminary work in the cerebral circulation of OZR (52), it is in striking contrast to the microvessel remodeling that occurs in the skeletal muscle of OZR, leading to profound reductions in the distensibility of the vessel wall (22, 52). As such, although it appears unlikely that any alteration in vascular structure plays a significant role in altering vascular reactivity in the cerebral circulation of OZR, future investigation as to the underlying mechanisms contributing to this “protection” of resistance arteries from remodeling in the metabolic syndrome may be warranted.

Our previous work in peripheral microvessels demonstrated that elevated vascular oxidant tone not only contributes to impaired dilator reactivity but also may play a role in an enhanced myogenic activation (25). In contrast, although the present results suggest a role for elevated superoxide produc-
Cerebral arteries of OZR exhibited an increased constrictor reactivity to both 5-HT and elevated oxygen tension. As such, our results suggest that an impaired dilator reactivity of cerebral resistance arteries in OZR could, in part, reflect an increase in vascular tone, myogenic activation, or constrictor reactivity. Previous studies have demonstrated that an increased vascular tone and myogenic activation can be present in the MCA of rats afflicted with type I diabetes mellitus. Zimmermann et al. (56) demonstrated that myogenic depolarization and constriction were enhanced in MCA of streptozotocin-treated rats and that this was associated with a decreased bioavailability of endothelium-derived nitric oxide, acting as a buffer to pressure-induced constriction. More recently, Dumont et al. (17) demonstrated that this increased myogenic activation of isolated cerebral arteries of type I diabetic rats may have been associated with an increased constrictor reactivity mediated via endothelin receptors. To our knowledge, however, our results present the first evidence that myogenic activation of cerebral resistance arteries is enhanced in OZR model of the metabolic syndrome. Further, our results suggest that increased myogenic activation of these vessels may be more complicated in a model of type II diabetes mellitus, because treatment of vessels with tempol, increasing the bioavailability of endothelium-derived nitric oxide, was not associated with a significant alteration in the patterns of myogenic activation in OZR.

Cerebral arteries of OZR exhibited an increase in the constrictor reactivity in response to challenge with 5-HT or elevated oxygen tension. Previous studies using vessels from numerous tissue beds in type I diabetic rats or rabbits have produced contrasting results with regard to the constrictor reactivity of vessels to 5-HT. While several studies have demonstrated that progression of type I diabetes has no effect on 5-HT-induced vasoconstriction of cerebral (41) and coronary arteries (49) of rats, and in cerebral (1) and coronary arteries of rabbits (4), additional studies have suggested that an enhanced constrictor reactivity of rat basilar arteries (55), pial arterioles (42), and rabbit renal arteries (45) in response to 5-HT is associated with progression of non-insulin-dependent diabetes mellitus. To our knowledge, our study represents initial observations of altered 5-HT-induced constriction of cerebral arteries in a model of type II diabetes, although these observations are not without precedent in the literature. The recent study by Janiak et al. (32) that used diabetic OZR...
demonstrated that collateral vessel recruitment after hindlimb ligation-induced ischemia was strongly impaired with the development of the metabolic syndrome as a result of chronic vasoconstrictor tone mediated by 5-HT. Our results also suggest that a comparable 5-HT-based vasoconstriction may contribute to an impaired dilator reactivity of MCA in OZR.

Attempts to broadly define a role for increased constrictor reactivity, vascular tone, and myogenic activation in contributing to impaired dilator reactivity can be problematic, owing to an incomplete understanding of how competing relationships between constrictor and dilator influences interact. Despite our observation that structural alterations to MCA of OZR do not appear to contribute to impairments in dilator reactivity, an enhanced myogenic activation and constrictor reactivity to agonists and stimuli can result in unpredictable alterations to dilator responses. A similar conclusion was also reached previously by Mayhan (40) in a study evaluating how vasoconstrictor responses could contribute to the impaired dilator responses of the basilar artery of the type I diabetic rat.

Predicting how alterations to multiple vasoactive influences contributing to net vascular tone will integrate to produce an appropriate response has been attempted under constrained...
conditions previously. However, these previous results have been demonstrated to be highly dependent on the specific tissue under investigation, and also a function of not only the ability to quantitatively determine the magnitude of the interactions studied, but also the impact of unknown variables which will also impact on the system under investigation (23, 28, 36, 37, 43).

However, it is clear that impairments to vascular dilator reactivity, and enhanced constrictor responses, could contribute to an inability to appropriately increase cerebral perfusion during periods of elevated metabolic demand or ultimately lead to a gradual reduction in baseline cerebral blood flow. There is increasing evidence demonstrating that alterations in cerebral perfusion occur in patients suffering with diabetes and the metabolic syndrome (11, 14, 15, 38, 54). Given the demonstrated role for diabetes as a risk factor for not only vascular disease per se (2, 3), but also for cerebral pathologies associated with vascular impairments (5, 11, 14, 47), continued investigation into the effects of the metabolic syndrome on the regulation of cerebrovascular reactivity and perfusion is warranted.

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