Augmented adrenergic vasoconstriction in hypertensive diabetic obese Zucker rats

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Augmented adrenergic vasoconstriction in hypertensive diabetic obese Zucker rats. Am J Physiol Heart Circ Physiol 282: H816–H820, 2002. First published November 1, 2001; 10.1152/ajpheart.00695.2001.—This study examined skeletal muscle microvessel reactivity to constrictor stimuli in obese (OZR) versus lean Zucker rats (LZR). Gracilis arteries from both rat groups were isolated, cannulated with glass micropipettes, and viewed via television microscopy. Changes in vessel diameter were measured with a video micrometer. Arterial constriction to norepinephrine was elevated in OZR versus LZR, although vasoconstrictor reactivity to endothelin and angiotensin II was unaltered. Differences in reactivity between vessels of LZR and OZR were not explained by the loss of either endothelial nitric oxide synthase or β-adrenergic receptor function. Reactivity of in situ cremasteric arterioles of OZR to norepinephrine was elevated versus LZR. Treatment with prazosin increased the diameter of in vivo gracilis arteries of OZR to levels determined in LZR and also normalized blood pressure in OZR. These results suggest that the constrictor reactivity of skeletal muscle microvessels in OZR is heightened in response to β-adrenergic stimuli and that development of diabetes in OZR may be associated with impaired skeletal muscle perfusion and hypertension due to microvessel hyperreactivity in response to sympathetic stimulation.

skeletal muscle microcirculation; hypertension; norepinephrine

TYPE 2 DIABETES MELLITUS impacts ~11 million Americans and is a potent risk factor for the development of peripheral vascular disease, a debilitating condition impacting nearly 60 million Americans. The effects of diabetes may be exacerbated when combined with hypertension and obesity, a condition known as Syndrome X. Despite this correlation, the pathophysiological processes linking Syndrome X and impaired limb perfusion remain unclear. We hypothesize that a contributing factor to poor muscle perfusion in patients with Syndrome X is altered reactivity of skeletal muscle microvessels to vasoconstrictor stimuli.

The obese Zucker rat (OZR) has been chosen as a model of Syndrome X because it has been previously reported to demonstrate concomitant development of hypertension, type 2 diabetes mellitus, and obesity (9, 18). We examined the constrictor reactivity of skeletal muscle resistance arteries (gracilis muscle) and distal arterioles (cremaster muscle) to norepinephrine of obese Zucker rats compared with their lean counterparts (LZR). The specificity of altered adrenergic reactivity was assessed by comparing skeletal muscle microvessel responses to norepinephrine, endothelin, and angiotensin II. The role of nitric oxide was probed via inhibition of nitric oxide synthase with Nω-nitro-L-arginine methyl ester (L-NAME), and the role of β-adrenoreceptors was examined with the specific antagonist propranolol. The data obtained in these studies suggest that increased microvascular sensitivity to adrenergic stimulation may be associated with hypertension and reduced hindlimb perfusion in the OZR.

MATERIALS AND METHODS

Animals. All experiments used 13- to 15-wk-old male LZR and OZR (Harlan) fed standard rat chow and tap water ad libitum. Rats were housed in an American Association for Accreditation of Laboratory Animal Care-accredited animal care facility at the Medical College of Wisconsin, and all protocols were approved by the Institutional Animal Care and Use Committee. For all experiments, rats were anesthetized with an injection of pentobarbital sodium (60 mg/kg ip), and a carotid artery was cannulated for determination of arterial pressure. Supplemental injections of anesthetic were given via intraperitoneal injection as needed.

Preparation of isolated vessels. Gracilis arteries were surgically dissected from the anesthetized rat, as described previously (5, 6). Arteries were placed in a heated (37°C) chamber that allowed the lumen and exterior of the vessel to be perfused and superfused, respectively, with physiological salt solution (PSS) from separate reservoirs. The PSS used in these experiments was equilibrated with 21% O2-5% CO2-74% N2 and had the following composition (mM): 119 NaCl, 4.7 KCl, 1.17 MgSO4, 1.6 CaCl2, 1.18 NaH2PO4, 24 NaHCO3, 0.026 EDTA, and 5.5 glucose. Vessels were cannulated at both ends with glass micropipettes (~100 μm tip diameter) and secured to the inflow and outflow pipettes using 10-0 nylon suture. Any side branches were ligated with a single strand teased from 6-0 silk suture. The inflow pipette was connected to a reservoir perfusion system that allowed the intraluminal pressure and luminal gas concentrations to be controlled. Vessel diameter was measured using television microscopy and an on-screen video micrometer.

Arteries were extended to their in vivo length (determined before removal of the vessel from the anesthetized rat) and equilibrated at 80% of the mean arterial pressure of the
animal to approximate the perfusion pressure encountered in vivo (LZR = 95 ± 5.4 mmHg; OZR = 118 ± 4.1 mmHg; Ref. 6). Any vessel that did not demonstrate active tone at rest was discarded. Active tone at the equilibration pressure was calculated as (ΔD/ΔDmax) × 100, where ΔD is the diameter increase from rest in response to Ca2+-free PSS and ΔDmax is the maximum diameter measured at the equilibration pressure in Ca2+-free PSS.

**Measurement of cremaster arteriolar diameter.** For each experiment, a cremaster muscle in each rat was prepared for television microscopy, as described previously (4). After completion of the muscle preparation, the tissue was continuously superfused with PSS, equilibrated with a gas mixture containing 5% CO2–95% N2, and maintained at 35°C as it flowed over the muscle. Arteriolar diameter was determined with a video micrometer, accurate to ±1 μm.

**Measurement of gracilis artery diameter in vivo.** In vivo determination of gracilis artery diameter was accomplished using a modification of strobscopic microscopy techniques described previously for the in vivo beating heart (14). For each experiment, the skin was removed over the ventral surface of the thigh, and subcutaneous fat was gently removed. The animal was placed under a stroboscopic microscope (magnification ×100, Leitz), and the surfaces of the femoralis and gracilis muscles were illuminated by a strobed light source (Chadwick-Helmuth) flashed at 120 Hz. Images were acquired, stored, and analyzed using Scion Image Software for the MacIntosh.

**Constrictor agonists.** The constrictor reactivity of isolated and in situ skeletal muscle microvessels from LZR and OZR was assessed in response to 1) norepinephrine (10−10–10−6 M), 2) angiotensin (10−10–10−7 M), and 3) endothelin (10−12–10−9 M). To determine the role of nitric oxide as a contributor to the vascular constrictor reactivity, the concentration versus response curves in response to norepinephrine were also performed in the presence of 10−4 M L-NAME. Finally, to determine the contribution of individual adrenergic receptors to the constrictor reactivity of vessels to norepinephrine, concentration versus response curves were also evaluated in the presence of 10−5 M propranolol (β-adrenergic receptor antagonist) or 10−5 M phentolamine (α-adrenergic receptor antagonist).

**Mathematical and statistical analyses.** All data are presented as means ± SE. Significant differences between experimental conditions for all data were determined using analysis of variance, repeated measures analysis of variance, or Student's t-test, where appropriate. All post hoc analyses were done using Fisher's probable least significant difference test. In all cases, a probability level of P < 0.05 was considered to be statistically significant.

| Table 1. Resting diameter of isolated gracilis arteries from LZR and OZR under the experimental conditions of the present study |
|---------------------------------|--------|-------|
| Lean                           | Obese  |       |
| Control                        | 124 ± 6| 127 ± 4 |
| +10−4 M L-NAME                 | 117 ± 3*| 126 ± 4 |
| +10−3 M Propranolol            | 121 ± 3 | 127 ± 3 |
| +10−3 M Phentolamine           | 125 ± 4 | 130 ± 2 |
| Maximum diameter               | 208 ± 9*| 161 ± 4†|

Values are means ± SE in μm. LZR, lean Zucker rats; OZR, obese Zucker rats. L-NAME, Nω-nitro-L-methyl ester arginine. *P < 0.05 vs. control; †P < 0.05 vs. LZR.

**RESULTS**

Table 1 presents data describing the resting diameter of in vitro gracilis arteries from LZR and OZR under the experimental conditions of the present study. There was no difference in resting diameter of skeletal muscle arterioles of LZR and OZR under control conditions. Treatment with L-NAME reduced arteriolar diameter in LZR only. Application of either propranolol or phentolamine had no effect on basal arterial tone.

Table 2 presents baseline hemodynamic data from LZR and OZR under the experimental conditions of the study. OZR were heavier and were hypertensive relative to LZR, but heart rate was not different. Left ventricular mass, expressed as a percentage of total heart weight, was most increased, further substantiating the presence of hypertension.

Data describing the norepinephrine-induced constriction of isolated gracilis arteries from LZR and OZR are presented in Fig. 1. In OZR, constriction of skeletal muscle resistance arteries following application of norepinephrine was markedly increased across the agonist concentration range compared with responses determined in LZR. This difference is reflected in a 15-fold leftward shift in the ED50 concentration for the.
α-adrenergic agonist (LZR = 6.4 ± 2.4 × 10⁻⁸ M vs. OZR = 4.3 ± 0.7 × 10⁻⁹ M; P < 0.01).

Figure 2 presents data describing the constriction of isolated gracilis arteries in response to challenge with angiotensin II (Fig. 2A) or endothelin (Fig. 2B). In contrast to the shift in the constrictor reactivity of vessels from OZR in response to α-adrenergic stimulation, the vascular response of vessels to either endothelin or angiotensin II was similar in OZR versus to LZR.

Figure 3 presents data describing the effects of treatment of isolated gracilis arteries from LZR with the nitric oxide synthase inhibitor l-NAME, the α-adrenergic antagonist phentolamine, or the β-adrenergic antagonist propranolol on the constriction of these vessels in response to challenge with norepinephrine. Treatment of vessels with l-NAME had no effect on arterial constriction in response to norepinephrine (Fig. 3A), suggesting that loss of endothelial nitric oxide cannot explain the increased adrenergic reactivity observed in OZR vs. LZR. Propranolol (Fig. 3B) was also without effect, suggesting that the loss of β-adrenergic receptor function also could not explain the observed hyperresponsiveness of vessels from OZR to norepinephrine. Treatment of isolated gracilis arteries of LZR with phentolamine nearly abolished vascular reactivity to norepinephrine (Fig. 3B), demonstrating the predominance of α-adrenergic receptors in mediating norepinephrine-induced contraction of these microvessels.

Data describing the effects of blockade of α-adrenergic receptors following intravenous infusion of prazosin (50 μg/kg) on the resting tone of in vivo gracilis arteries of LZR and OZR are presented in Fig. 4. After an intravenous infusion of a bolus of prazosin, the diameter of gracilis arteries in LZR was not altered from their control value. In contrast, blockade of α-adrenergic receptors in OZR caused a significant dilation of gracilis arteries, such that arterial diameter was not different from that determined in LZR under control conditions.
In Fig. 5, data are presented that describe the reactivity of in situ cremasteric arterioles from LZR and OZR in response to application of norepinephrine (Fig. 5A) or angiotensin II (Fig. 5B). Although somewhat less pronounced than upstream effects, cremasteric arterioles from OZR demonstrated a significantly greater reactivity in response to norepinephrine versus responses in distal arterioles from LZR (LZR ED\textsubscript{50} = 5.8 ± 2.2 × 10^{-9} M; OZR ED\textsubscript{50} = 5.7 ± 1.1 × 10^{-10} M). Responses of cremasteric arterioles to following challenge with angiotensin II were not different between LZR and OZR (Fig. 5B).

**DISCUSSION**

The principle new finding in this study is that α-adrenergic reactivity in the microvasculature of the hindlimb is markedly augmented in the OZR, a model of type 2 diabetes and Syndrome X. Whereas altered reactivity to vasodilators is the OZR is well established (10, 12), the effects of this condition on vasoconstrictor reactivity are less clear, because studies reporting increased vascular reactivity to constrictor stimuli (8, 13, 17) have been countered by studies to the contrary (3, 11, 15). Within studies reporting augmented constriction, controversy exists regarding which constrictor stimuli are affected and which stimuli are unaltered (8, 13, 16). Many of these studies are further confounded by restriction of observations to conduit arteries or by competing effects in in vivo systems. To address this final issue, the present study focused primarily on the investigation of isolated skeletal muscle resistance arteries, although we also included additional experiments to examine microvessel constrictor reactivity in both in vivo skeletal muscle resistance arteries and in situ skeletal muscle arterioles.

One significant advantage of collecting data regarding the reactivity of isolated skeletal muscle microvessels is that it allows for the determination of vascular responses, and any alteration therein that may develop in response to an experimental condition or pathology without the potentially confounding effects of the specific agonists on hemodynamic characteristics or non-vascular tissues. Thus the dramatic increase in α-adrenergic reactivity in the isolated skeletal muscle resistance artery observed in OZR (Fig. 1) indicates a fundamental alteration in vascular responsiveness to norepinephrine. Furthermore, the additional experiments performed in the present study demonstrating an increased α-adrenergic sensitivity of in vivo gracilis arteries and in situ cremasteric arterioles provide important information in that these results demonstrate that observations using isolated resistance arteries are also manifested under in vivo conditions and at multiple levels in the skeletal muscle microvascular network. The reduced diameter of in vivo gracilis arteries of OZR versus LZR under control conditions (Fig. 4), as assessed by stroboscopic epiillumination, was restored following administration of the α-adrenergic antagonist prazosin. In addition, under these same conditions, the elevated blood pressure present in OZR was...
normalized to levels seen in LZR, providing further evidence that that vascular α-adrenergic sensitivity is elevated in OZR versus LZR. Finally, the reactivity of distal arterioles of in situ cremaster muscles in response to adrenergic stimulation with norepinephrine was also significantly increased in OZR relative to LZR. When taken together, these data indicate that the development of obesity, type 2 diabetes mellitus, and hypertension, as manifested in OZR, results in marked changes in the contractile behavior of resistance arteries with direct consequences on in vivo microvascular resistance.

The mechanism by which α-adrenergic microvessel reactivity is increased in OZR is presently unknown, although the results of the present study suggest that this altered reactivity is specific to α-adrenergic responses, because microvessel responses following challenge with angiotensin II and endothelin were unaltered in OZR versus LZR (Figs. 2 and 5). This finding is in agreement with the recent study by Carlson et al. (2) in which the authors demonstrated that sympathetic blockade, but not inhibition of angiotensin-converting enzyme, reduced blood pressure in OZR. The results of the present study also suggest that the previously reported loss of endothelial nitric oxide efficacy (7) was not a contributor to the augmented α-adrenergic reactivity of skeletal muscle microvessels of OZR, because this response was unaltered following inhibition of nitric oxide synthase activity with l-NAME. Finally, our data indicate that blockade of β-adrenergic receptors (propranolol) had no effect on skeletal muscle microvessel reactivity in response to norepinephrine, whereas application of the α-adrenergic receptor antagonist phenolamine abolished all responses to norepinephrine. These data suggest that there may be a fundamental alteration in the signaling pathways of α-adrenergic receptor stimulation that accounts for the exaggerated contractile response of skeletal muscle microvessels observed in the present studies. The results of the present study warrant future investigation into potential factors that might alter the sensitivity of α-adrenoceptor behavior in skeletal muscle microvessels of OZR, alterations to the plasma membrane lipid profile (1), or the tonic effects of elevated blood glucose or insulin levels present in Syndrome X (17).

The results of the present study describe a marked alteration to the reactivity of skeletal muscle microvessels in OZR, a model of Syndrome X. Microvessel constrictor responses following α-adrenergic stimulation were significantly enhanced in OZR versus LZR, although reactivity in response to challenge with angiotensin II and endothelin were not altered. In an in vivo setting, pharmacological blockade of α-adrenoceptors normalized both skeletal muscle microvessel diameter and blood pressure in OZR to levels determined in LZR control animals. The data suggest that a fundamental alteration to the behavior of skeletal muscle microvessels develops in the OZR with the progression of type 2 diabetes mellitus, and hypertension that may shift the balance of constrictor and dilator influences to favor compromised skeletal muscle perfusion.

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