Myogenic Tone and Reactivity of Rat Ophthalmic Artery in Acute Exposure to High Glucose and in a Type II Diabetic Model

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PURPOSE. To evaluate the effect of acute exposure to high glucose on myogenic tone and reactivity of the rat ophthalmic artery and to compare the observations with that in ophthalmic artery from a diabetic rat model.

METHODS. Ophthalmic arteries from Sprague-Dawley rats were pressurized at 70 mm Hg in an arteriograph, and outer diameter was monitored. Myogenic tone was assessed over a range of intraluminal pressures in the presence and absence of high glucose or mannitol. The effects of high glucose on reactivity to carbachol and phenylephrine were determined. Arteries from type II diabetic BBZDR/Wor rats and age-matched control rats were evaluated for myogenic tone and reactivity.

RESULTS. Myogenic tone was enhanced by 25 mM, but decreased by 40 mM glucose, and carbachol-mediated dilation was unaffected. Effects of high glucose were not observed in the absence of endothelium. Miconazole, a nonselектив inhibitor of cytochrome-P450 enzymes or dihydro-ouabain, an inhibitor of Na+/K+ATPase blocked the effect of 40 mM but not 25 mM glucose. Arteries from diabetic rats showed decreased myogenic tone compared with control arteries, and this decrease was not observed in the absence of endothelium.

CONCLUSIONS. Acute exposure to high glucose has a concentration-and endothelium-dependent effect on the myogenic tone of rat ophthalmic artery. Attenuation of tone by high glucose is probably due to the activation of smooth muscle Na+/K+ATPase by endothelial cytochrome-P450 metabolite. Pressure-mediated autoregulation in ophthalmic artery in type II diabetic BBZDR/Wor rat operates at lower resistance, probably due to hyperglycemia. (Invest Ophthalmol Vis Sci. 2006;47: 683–692) DOI:10.1167/iovs.05-1012

Diabetes is associated with micro- and macrovascular diseases. Diabetic microangiopathy increases the risk of blindness, end-stage renal disease, and neuropathy. Hyperglycemia is a major causative factor in the vascular dysfunction associated with diabetes, and the endothelium is the primary target of hyperglycemia.1

Diabetes-specific microvascular dysfunction in the ocular vasculature eventually results in retinal damage and most likely involves hyperglycemia-induced abnormalities in blood flow and vascular permeability. Under healthy conditions, pressure, metabolic and hormonal autoregulatory control of the ocular vasculature, maintains tight hemodynamic regulation of the ophthalmic circulation, whereas in diabetes this autoregulatory control is lost.2

Resistance arteries of the ophthalmic vascular bed, by virtue of their myogenic tone, protect retinal and choroidal arterioles from being exposed to higher systemic arterial pressures and blood flow. In the Sprague-Dawley (SD) rat, we recently showed evidence of pressure-mediated autoregulation of diameter. The ophthalmic artery can maintain myogenic constriction up to intraluminal pressures of at least 200 mm Hg, suggesting strong myogenic regulation of arterial caliber in the vascular bed.3 The unique anatomy of the vascular bed4 and sudden transition of the ophthalmic artery to several small retinal and choroidal arterioles requires physiologically efficient autoregulation of blood flow. In diabetes, pressure-mediated autoregulation of blood flow may fail, resulting in exposure of the retinal vasculature to higher pressures and blood flow.

Using either isolated artery preparations or cultured endothelial cells, investigators have shown that acute exposure to elevated glucose alters signaling mechanisms, resulting in vascular dysfunction involving either increased or decreased endothelial dilatory mechanisms.5 In ocular vasculature, no functional studies have been performed that focus on the effect of exposure of elevated glucose on the autoregulatory properties in pressurized artery preparations. In this study, we investigated the acute effect of high glucose on pressure-mediated autoregulation of the rat ophthalmic artery.

Furthermore, we studied pressure-induced myogenic tone in excised ophthalmic arteries from a diabetic rat for comparison with our observations from in vitro studies involving acute exposure to high glucose. We used a relatively new model of type II diabetes, the BBZDR/Wor rat,6 produced by crossing the Zucker fatty rat and the nondiabetic BB/Wor rat (also known as the BBDR/Wor rat, which serves as control for the BBDP/Wor type I diabetic rat7). Lean nondiabetic heterozygous littermates serve as a control for the obese diabetic BBZDR/Wor rats. Clinical characteristics of diabetic syndrome in BBZDR/Wor rats include hyperglycemia, hyperinsulinemia, and hyperlipidemia, and they tend to have moderate hypertension, polyneuropathy, retinopathy, and erectile dysfunction.8-10 These characteristics make this model closely resemble the clinical features of type II diabetes in humans.
METHODS
Preparation of Ophthalmic Arteries and Measurement of Luminal Diameter

Animal procedures have been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Florida and are in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Male SD rats (250–300 g) were anesthetized with an intraperitoneal injection of pentobarbital sodium (160 mg/kg) and killed by decapitation. The brain was removed, and the skull with intact eyes was placed in an ice-cold oxygenated physiological saline solution (PSS; composition provided later). The main ophthalmic artery was dissected, cannulated with glass pipettes in an arteriograph, and the outer diameter visualized as described earlier.5 Arteries were slowly pressurized to 70 mm Hg under no-flow conditions, with a pressure servo-null system (Living Systems Inc., Burlington, VT), and warmed to 37°C while being continuously superfused (3 mL/min) with PSS bubbled with 21% O2, 5% CO2, and 74% N2 (pH 7.3–7.4 in the bath). A working pressure of 70 mm Hg was chosen based on the earlier observations by Riva et al.11 in human ocular circulation and McCarron et al.12 in rat cerebral circulation.

Male BBZDR/Wor and age-matched, lean control rats were obtained from Biomedical Research Models, Inc. (Worcester, MA). Rats obtained were of prediabetic age (~10 weeks) or 3 months after the onset of diabetes (22–23 weeks of age). All rats were acclimatized before use. Blood glucose was analyzed by a glucometer (OneTouch Ultra; LifeScan, Johnson & Johnson, Miltipas, CA). Ophthalmic arteries were isolated and studied in vitro, as mentioned earlier.

Experimental Protocol

After an equilibration period of ~20 minutes, arteries showed stable myogenic tone at 70 mm Hg. Afterwards, the effect of elevated glucose or mannitol in PSS on myogenic tone was evaluated. Concentration response curves (CRCs) to different pharmacological agents were obtained by cumulative addition to the superfusate. CRCs were generated in concentrations on the order of half log, and the arteries were exposed to different concentrations for at least 8 minutes or until the observed effect reached steady state. In some experiments, endothelium was denuded by introducing human hair into the lumen followed by perfusion with PSS. Presence of functional endothelium was tested by applying 1 μM carbachol. Endothelium-intact arteries showed almost complete relaxation or loss of myogenic tone, whereas in endothelium-denuded arteries no relaxation was observed. Obtaining the maximum possible diameter or passive diameter in calcium-free PSS concluded all experiments. Pressure-dependent changes in the diameter were evaluated by increasing the intraluminal pressure in 10 mm Hg steps from 1 to 199 mm Hg in PSS. Pressure-diameter curves were repeated in the presence of elevated glucose with or without different pharmacological agents and calcium-free PSS as superfusate. The myogenic tone was calculated according to the following equation:

\[ \text{Myogenic tone} = \frac{(D_p - D_a)}{D_p} \times 100, \]

where \( D_p \) is the internal diameter of the arterial segment with active myogenic tone in the presence of PSS at a particular intraluminal pressure and \( D_a \) is the passive diameter obtained at the same pressure in the presence of calcium-free PSS.

Acute exposure to glucose was evaluated at two concentrations: 25 and 40 mM. A wide range of glucose concentrations (15–60 mM) were used in the different studies to evaluate the effect of acute high glucose in isolated vascular preparations and cultured cells (few examples: Haselton et al.13 (25 mM), Gilles et al.14 (30 mM), Gupta et al.15 (44 mM), Taylor and Poston16 (45 mM), and Hoshiyama et al.17 (15, 30, and 60 mM). Most of them showed increased constriction with 25 mM glucose in different vascular preparations, whereas concentrations of glucose <15 mM showed no effect.16,18–20 Studies in rat posterior cerebral arteries and coronary blood flow reported dilation and increased blood flow with 40 mM glucose, whereas in rat pial arteries 44 mM glucose increased arterial tone.20–22 We have chosen these two concentrations that showed contrasting results so that we can compare our findings with those in the published reports.

An artery was used for only one concentration of glucose or mannitol. Reactivity to agonists and pressure-diameter experiments were evaluated in different groups of arteries so that the reactivity was not affected by exposure to a range of pressures and vice versa. All experiments using arteries from diabetic and control rats were performed in PSS.

Data Analysis and Statistics

Results are expressed as the mean ± SEM, \( n \) indicates the number of independent experiments, which equals the number of animals used. Results were compared by either ANOVA or Student’s t-test, as applicable (Prism 3.0; GraphPad Software, San Diego, CA), and \( P < 0.05 \) was considered statistically significant.

The efficacy of different agonists was expressed as the maximum response, constriction or dilation, produced. The potency was expressed as pEC50 (negative logarithm of the concentration of the agonist that produces 50% of the maximum effect). pEC50 was calculated by analyzing CRCs (Prism 3.0; GraphPad) that fit the data to the four-parameter logistic equation

\[
Y = \text{minimum} + \frac{(\text{maximum} - \text{minimum})}{1 + 10^{\log EC_{50} - X/P}},
\]

where minimum and maximum indicate the smallest and the highest responses produced by an agonist, \( X \) is the logarithm of the molar concentration of an agonist, \( Y \) is the response at a concentration of \( X \), and \( P \) is the Hill slope.

Drugs, Chemicals, and Solutions

All the drugs used in the study were obtained from Sigma-Aldrich (St. Louis, MO). Stock solutions (10 mM) of phenylephrine, carbachol, N-nitro-L-arginine (L-NAME), L-NAME, L-arginine, and dihydro-ouabain were prepared in distilled water, whereas that of miconazole was prepared in dimethyl sulfoxide.

The composition of PSS (mM): NaCl (120), KCl (3), NaHCO3 (24), NaH2PO4.2H2O (1.2), CaCl2 (1.25), MgSO4.7H2O (1.2), and glucose (4) in the bath. A working pressure of 70 mm Hg was chosen based on the earlier observations by Riva et al.11 in human ocular circulation and McCarron et al.12 in rat cerebral circulation.

The composition of PSS (mM): NaCl (120), KCl (3), NaHCO3 (24), NaH2PO4.2H2O (1.2), CaCl2 (2.5), MgSO4.7H2O (1.2), and glucose (4) in the bath. A working pressure of 70 mm Hg was chosen based on the earlier observations by Riva et al.11 in human ocular circulation and McCarron et al.12 in rat cerebral circulation.

RESULTS

The outer diameter of ophthalmic arteries from SD rats used in the study was 308 ± 7 μm (n = 60), as obtained in the presence of calcium-free PSS at an intraluminal pressure of 70 mm Hg.

Effect of Acute Exposure to High Glucose on Myogenic Tone of Rat Ophthalmic Artery

Pressure-dependent changes in the arterial diameter were monitored at intraluminal pressures ranging from 1 to 199 mm Hg in the absence and presence of 25 and 40 mM glucose. Figure 1 shows representative tracings of diameter recordings with different experimental protocols. Arteries were superfused extraluminally (abuminally) with PSS containing high glucose for 1 hour before a pressure-diameter relationship was evaluated. In the presence of PSS (control conditions), an increase in pressure resulted in decreased diameter (increased myogenic tone), compared with that in the presence of calcium free PSS.
As reported earlier, constant myogenic tone was maintained over pressures of 70 to 199 mm Hg. In the presence of 25 mM glucose, arteries showed higher sensitivity to pressure and the observed myogenic tone was higher than the control throughout the range of intraluminal pressures (Fig. 2a). Pressure–myogenic tone curves were significantly greater than that in the control (t = 5; P < 0.05, one-way ANOVA). In the presence of 40 mM glucose, the myogenic tone developed over pressures of 30 to 199 mm Hg was lower than that of the control, and arteries tended to lose tone and dilate at pressures ≥180 mm Hg. Pressure–myogenic tone curves were significantly lower than those observed in control conditions (t = 6; P < 0.01, one-way ANOVA). In the presence of 25 and 40 mM mannitol, an osmotic control, no differences in pressure-myogenic tone curves were observed (Figs. 2b, 2c).

Effect of high glucose on myogenic tone was evaluated in arteries denuded of endothelium. The removal of endothelium resulted in increased basal tone of the arterial segments and higher sensitivity to intraluminal pressures. In the absence of endothelium, pressure-myogenic tone curves obtained in the presence of high glucose were similar to that in the control (t = 6, Figs. 3a, 3b). Both potentiating and attenuating effects of 25 and 40 mM glucose, respectively, on myogenic tone were not observed in the absence of endothelium.

Attenuating effect of 40 mM glucose on myogenic tone was compared by exposing the artery to high glucose intralumino-
Finally and both extra- and intraluminally at the intraluminal pressure of 70 mm Hg for 1 hour. The effect of glucose was observed after 15 to 20 minutes of exposure. The myogenic tone observed in control conditions (24% ± 0.5%) was reduced by 40 mM glucose to a similar extent when applied in three different ways according to one-way ANOVA (myogenic tone [%] extraluminal 11 ± 1.4, intraluminal 12 ± 3.3, both 12 ± 3.4; n = 5).

**Responses to Carbachol and Phenylephrine in the Presence of High Glucose**

Vasodilatory responses ($E_{max}$, expressed as loss of myogenic tone) to carbachol, a stable analogue of acetylcholine (a muscarinic receptor agonist producing endothelium-dependent dilation), were not modified when arteries pressurized at 70 mm Hg were acutely exposed to 25 ($n = 5$) and 40 ($n = 6$) mM glucose (Fig. 4a; Table 1). Potency of carbachol (pEC$_{50}$) was not affected by 40 mM glucose but was significantly decreased by 25 mM glucose ($n = 5, P < 0.001$, paired Student’s t-test; Table 1).

Vasoconstrictory responses to phenylephrine, an $\alpha_1$-adrenoceptor agonist, were expressed as the percentage of KCl (60 mM) response, which was unaltered by high glucose (percentage decrease in diameter): control, −31 ± 1.3; glucose: 25 mM, −28 ± 1.0; 40 mM, −27 ± 1.4 ($n = 10$). Constriction in response to phenylephrine was not altered by 25 mM glucose, but was significantly attenuated by 40 mM (Fig. 4b). Efficacy was significantly reduced ($n = 5, P < 0.01$, paired Student’s $t$-test) with no change in potency (Table 1). In the absence of endothelium, responses to phenylephrine were unaffected in the presence of high glucose at both concentrations ($n = 5$, Fig. 4c; Table 1).

![Figure 2](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933234/) (a) Effect of 25 and 40 mM glucose on the myogenic tone of the rat ophthalmic artery at intraluminal pressures ranging from 1 to 199 mm Hg. Pressure–myogenic tone curves were significantly altered by high glucose according to one-way ANOVA ($P < 0.0001$, df = 2, F = 12.44). Newman-Keuls post hoc test for multiple comparisons revealed a significance of *$P < 0.05$ between the control and 25-mM glucose effect and **$P < 0.01$ between the control and 40 mM glucose. Mannitol did not alter the myogenic tone of the artery over the range of intraluminal pressures used (b, c).

![Figure 3](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933234/) (a) Effect of (a) 25 and (b) 40 mM glucose on the myogenic tone of the rat ophthalmic artery denuded of endothelium at intraluminal pressures ranging from 1 to 199 mm Hg. Pressure–myogenic tone curves were not significantly different according to one-way ANOVA.
Effect of Pharmacological Agents on High-Glucose–Mediated Increase or Decrease in Myogenic Tone

To understand mechanisms involved in the endothelium-dependent attenuating effect of 40 mM on myogenic tone, different pharmacological agents were used to block different endothelial factors. The following pharmacological tools were used: L-NAME, an inhibitor of constitutive nitric oxide synthase (cNOS); 4-aminoguanidine, an inhibitor of inducible nitric oxide synthase (iNOS); 30 mM KCl, a partial depolarizer of smooth muscle cells and therefore an antagonist of the action of endothelium-derived hyperpolarizing factor (EDHF); miconazole, a nonselective inhibitor of cytochrome P450 (Cyt-P450); enzymes and dihydro-ouabain, an inhibitor of Na⁺/K⁺-ATPase.

Treatment with L-NAME, 30 mM KCl, and dihydro-ouabain increased myogenic tone of the ophthalmic artery (Table 2). The presence of 100 μM L-NAME (Table 2) or 30 mM KCl (Table 2) or their combination (data not shown) did not influence the 40-mM glucose-induced decrease in myogenic tone of ophthalmic artery pressurized at the intraluminal pressure of 70 mm Hg. Superfusion with 10 μM 4-aminoguanidine also did not prevent the decrease in myogenic tone produced by high glucose (Table 2). Superfusion with 1 μM miconazole prevented the attenuating effect of 40 mM glucose on myogenic tone in the presence of 40 mM glucose was compared with that before exposure to high glucose in the absence (no treatment) or presence of different pharmacological agents. Mean data were compared by paired Student’s t-test.

### Table 1. Responses to Carbachol and Phenylephrine in the Presence of High Glucose in Rat Ophthalmic Artery Pressurized at 70 mm Hg

<table>
<thead>
<tr>
<th>Carbachol</th>
<th>Phenylephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E_max</strong></td>
<td><strong>pEC_{50}</strong></td>
</tr>
<tr>
<td>PSS</td>
<td>100 6.6 ± 0.03</td>
</tr>
<tr>
<td>High glucose 25 mM</td>
<td>100 6.0 ± 0.07*</td>
</tr>
<tr>
<td>40 mM</td>
<td>100 6.4 ± 0.20</td>
</tr>
</tbody>
</table>

*Expressed as percent loss of myogenic tone (dilation).
†Expressed as percent KCl (60 mM) response.
‡P < 0.01 and §P < 0.001, significantly different from the corresponding value in the presence of PSS (paired Student’s t-test).

### Table 2. Effect of Pharmacological Treatments on 40 mM Glucose–Mediated Decrease in Myogenic Tone of Ophthalmic Artery Pressurized at 70 mm Hg

<table>
<thead>
<tr>
<th>Myogenic Tone (%)</th>
<th>n</th>
<th>No treatment</th>
<th>100 μM t-NAME</th>
<th>30 mM KCl</th>
<th>10 μM 4-Aminoguanidine</th>
<th>1 μM Miconazole</th>
<th>10 μM Dihydro-ouabain</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSS</td>
<td>34</td>
<td>25 ± 0.5</td>
<td>17 ± 1.2*</td>
<td>25 ± 1.4†</td>
<td>25 ± 1.8‡</td>
<td>25 ± 0.9†</td>
<td>29 ± 1.0</td>
</tr>
<tr>
<td>40 mM Glucose</td>
<td>34</td>
<td>25 ± 0.5</td>
<td>17 ± 1.2*</td>
<td>25 ± 1.4†</td>
<td>25 ± 1.8‡</td>
<td>25 ± 0.9†</td>
<td>29 ± 1.0</td>
</tr>
</tbody>
</table>

* P < 0.04.
† P < 0.03.
‡ P < 0.001.

Effect of Pharmacological Agents on High-Glucose–Mediated Increase or Decrease in Myogenic Tone

To understand mechanisms involved in the endothelium-dependent attenuating effect of 40 mM on myogenic tone, different pharmacological agents were used to block different endothelial factors. The following pharmacological tools were used: L-NAME, an inhibitor of constitutive nitric oxide synthase (cNOS); 4-aminoguanidine, an inhibitor of inducible nitric oxide synthase (iNOS); 30 mM KCl, a partial depolarizer of smooth muscle cells and therefore an antagonist of the action of endothelium-derived hyperpolarizing factor (EDHF); miconazole, a nonselective inhibitor of cytochrome P450 (Cyt-P450); enzymes and dihydro-ouabain, an inhibitor of Na⁺/K⁺-ATPase.

Treatment with L-NAME, 30 mM KCl, and dihydro-ouabain increased myogenic tone of the ophthalmic artery (Table 2). The presence of 100 μM L-NAME (Table 2) or 30 mM KCl (Table 2) or their combination (data not shown) did not influence the 40-mM glucose-induced decrease in myogenic tone of ophthalmic artery pressurized at the intraluminal pressure of 70 mm Hg. Superfusion with 10 μM 4-aminoguanidine also did not prevent the decrease in myogenic tone produced by high glucose (Table 2). Superfusion with 1 μM miconazole prevented the attenuating effect of 40 mM glucose on myogenic tone.

**Table 2. Effect of Pharmacological Treatments on 40 mM Glucose–Mediated Decrease in Myogenic Tone of Ophthalmic Artery Pressurized at 70 mm Hg**

<table>
<thead>
<tr>
<th>Myogenic Tone (%)</th>
<th>n</th>
<th>PSS</th>
<th>40 mM Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>34</td>
<td>25 ± 0.5</td>
<td>17 ± 1.2*</td>
</tr>
<tr>
<td>100 μM t-NAME</td>
<td>6</td>
<td>51 ± 1.5</td>
<td>25 ± 1.4†</td>
</tr>
<tr>
<td>30 mM KCl</td>
<td>5</td>
<td>31 ± 1.3</td>
<td>25 ± 1.8‡</td>
</tr>
<tr>
<td>10 μM 4-Aminoguanidine</td>
<td>3</td>
<td>28 ± 1.5</td>
<td>22 ± 0.9†‡</td>
</tr>
<tr>
<td>1 μM Miconazole</td>
<td>5</td>
<td>26 ± 1.1</td>
<td>25 ± 0.9</td>
</tr>
<tr>
<td>10 μM Dihydro-ouabain</td>
<td>6</td>
<td>31 ± 1.0</td>
<td>29 ± 1.0</td>
</tr>
</tbody>
</table>

Myogenic tone in the presence of 40 mM glucose was compared with that before exposure to high glucose in the absence (no treatment) or presence of different pharmacological agents. Mean data were compared by paired Student’s t-test.

* P < 0.04.
† P < 0.03.
‡ P < 0.001.
A similar blocking effect was observed with 10 μM dihydro-ouabain (DHO). The potentiating effect on myogenic tone by 25 mM glucose was also studied in the presence of 1 mM miconazole. The pressure-dependent increase in myogenic tone was significantly higher in the presence of 25 mM glucose over the range of pressures used (1–160 mm Hg) and this potentiation was not altered by the presence of miconazole (n = 6, P < 0.01, one-way ANOVA; Fig. 6).

Studies in Arteries from Type II Diabetic BBZDR/Wor Rat
At the prediabetic age of 10 weeks BBZDR/Wor rats and their age-matched control rats have similar body weights (BBZDR/Wor, 234 ± 6 g; control: 215 ± 5 g) and blood glucose levels (BBZDR/Wor, 10.7 ± 0.7 mM; control, 9.6 ± 0.3). Three months after the development of diabetes and obesity, the body weights and blood glucose levels of BBZDR/Wor rats were significantly higher than those of the age-matched, lean control animals (body weights: BBZDR/Wor, 586 ± 11 g, and control, 278 ± 12 g; P < 0.0001, n = 6; blood glucose, 32.6 ± 1.1 mM, and control, 10.1 ± 0.5 mM; P < 0.0001, n = 6).

Ophthalmic arteries from both BBZDR/Wor and age-matched lean control rats showed pressure-induced constriction that was maintained over the range of intraluminal pressures from 50 to 199 mm Hg. At the prediabetic age of 10 weeks, pressure-myogenic tone curves were not significantly different in arteries from BBZDR/Wor and age-matched, lean control rats (Fig. 7a). Arteries from BBZDR/Wor rats with 3 months of diabetes showed significantly decreased myogenic tone compared with that in the arteries from age-matched, lean control rats over the range of intraluminal pressures 50 to 190 mm Hg (P < 0.01, n = 6, one-way ANOVA).

In some experiments, endothelium was denuded as explained before, and the absence of functional endothelium was confirmed by the lack of dilatory response to 1 μM carbachol. In the absence of endothelium, myogenic tone developed in arteries of diabetic rats was similar to that observed in arteries of lean control rats (Fig. 7b).

Dilatory and constrictor responses to carbachol and phenylephrine, respectively, were also assessed in ophthalmic arteries of diabetic and their lean control rats. Carbachol produced similar dilatory responses resulting in complete reversal of myogenic tone in arteries from both diabetic and control rats with similar potency (pEC50: control, 6.4 ± 0.04, n = 5; diabetic 6.5 ± 0.03, n = 4). Phenylephrine-mediated constriction was expressed as percent of 60 mM KCl response, which was similar in arteries from diabetic and control rats (percent...
similar potency (pEC 50: control 6.1 ± 0.07).

diabetes, the BBZDR/Wor rat.
reactivity of the ophthalmic artery in a genetic model of type II
arteries of SD rats and to characterize myogenic tone and
acute exposure to high glucose in pressurized ophthalmic

DISCUSSION

This is the first functional study to characterize the effect of
acute exposure to high glucose in pressurized ophthalmic
arteries of SD rats and to characterize myogenic tone and
reactivity of the ophthalmic artery in a genetic model of type II
diabetes, the BBZDR/Wor rat.

Acute exposure to high glucose showed a concentration-
dependent effect on pressure-mediated myogenic tone in rat
ophthalmic artery: increase in pressure-dependent tone of the
artery in the presence of 25 and a decrease in pressure-depen-
dent tone in the presence of 40 mM glucose that was not

observed in the presence of mannitol (25 and 40 mM), an
osmotic control. The experimental procedure we followed
involves superfusion of pressurized artery extraluminally with
PSS, with or without high glucose. To make sure that the
endothelial layer was equally exposed to high glucose in this
procedure, we compared the effects of 40 mM glucose per-
used, and the results obtained in isolated cells cannot neces-
arily be indicative of that observed in isolated intact artery
preparations.

According to the present study, high glucose (25 mM)
increased constriction in response to pressure without affect-

Potentiating Effect of Acute Exposure to High
Glucose on Myogenic Tone

An increase in pressure-mediated constriction in the presence
of 25 mM glucose indicates pressure-mediated autoregulation
during this degree of hyperglycemia, operating at higher per-
fusion pressures and decreasing blood flow to retina,23–24 po-
tentially resulting in ischemic damage. Although myogenic
tone, a response that originates from arterial smooth muscle,
was increased by this concentration of high glucose, constric-
tion in response to phenylephrine, the response mediated by
α1-adrenergic receptors in the smooth muscle, was not affected.
These observations suggest that increased glucose has no di-
rect effect on smooth muscle, and the observed decrease in
myogenic tone may be secondary to its effect on endothelium.
Consistent with this supposition, in the absence of endothe-
lum the potentiating effect of 25 mM glucose on pressure-
mediated myogenic tone was not observed.

Endothelium modulates arterial tone by releasing constrict-
ing as well as dilating factors and radicals. In the present study,
maximum dilation to carbachol was unaffected in the presence
of 25 mM glucose. However, the potency of carbachol was
significantly decreased, with a rightward shift in the CRCs,
suggesting altered kinetics of the signaling cascade involved in
the release of endothelial mediators of vasodilation. This effect
may make a significant direct contribution to the increased
myogenic tone observed with 25 mM. Furthermore, we inves-
tigated the role of Cyt-P450 metabolites of arachidonic acid
that are known to be produced by endothelium as well as
smooth muscle and have been shown to be potent vasocon-
strictors, such as 20-hydroxyeicosatetraenoic acid (20-
HETE).25,26 However, miconazole, a nonselective inhibitor of
Cyt-P450 enzymes, did not alter the potentiating effects of 25
mM glucose, ruling out the involvement of Cyt-P450 metabo-
lites in this response.

Earlier studies in intact arteries have shown evidence of the
endothelial dysfunction leading to increased arterial constric-
tion with simultaneous endothelial dilation, and it has been
attributed to altered release of endothelium-derived dilatory or
constrictive factors. It was shown that the increased constric-
tion and decreased dilation by 25 mM glucose was due to

elevated PKC,8 and increased noradrenaline-mediated constric-
tion by 20 mM glucose has been shown to be due to increased
endothelial eicosanoids.8 Decreased acetylcholine-mediated di-
lation in mesenteric arteries by 20 mM glucose was due to
increased endothelial eicosanoids.8 Decreased acetylcholine-mediated
dilation in mesenteric arteries by 20 mM glucose was due to
to an eico-
sanoid-free radical pathway.27 Studies in isolated endothelial
cells showed either decreased NO release by bradykinin stimu-
lation28 or increased NO release due to higher calcium mo-

bulation.29 These reports show that mechanisms involved in
high-glucose-mediated constriction vary with the vascular bed
used, and the results obtained in isolated cells cannot neces-
ecessarily be indicative of that observed in isolated intact artery
preparations.

According to the present study, high glucose (25 mM)
increased constriction in response to pressure without affect-
ing contractile responses to agonists, while decreasing the sensitivity to endothelium-dependent dilation. Mechanisms of myogenic tone in rat ophthalmic artery are not known and could be different from that involved in \( \alpha_1 \)-adrenoceptor-mediated constriction. High glucose may selectively activate mechanisms involved in myogenic tone while not affecting the \( \alpha_1 \)-adrenoceptor-Gq-protein-PLC pathway. The role of endothelium in the potentiating effect of 25 mM glucose is likely the decreased release of a factor or a free radical that could activate pressure-mediated signaling mechanisms.

**Attenuating Effect of Acute Exposure to High Glucose on Myogenic Tone**

Acute exposure to 40 mM glucose, but not to 40 mM mannitol, resulted in decreased myogenic tone. A decrease in pressure-mediated constriction could be the underlying mechanism of reduced arterial resistance in ophthalmic vascular bed that would explain the increased ocular blood flow and the increased risk of retinal hemorrhage associated with diabetes. This finding is consistent with that observed in cerebral arteries acutely exposed to 40 mM glucose, and the resultant decrease in myogenic tone could contribute in part to the increased risk of cerebral hemorrhage and stroke in diabetic patients. Besides myogenic tone, agonist-mediated constriction was also attenuated. These effects were abolished by the removal of endothelium, suggesting the involvement of endothelium-derived factors. Dilatory effects of carbachol were not altered by 40 mM glucose, ruling out an increased release of nitric oxide that would attenuate myogenic tone.

**Mechanisms of High-Glucose–Mediated Decrease in Myogenic Tone in Rat Ophthalmic Artery**

Endothelium-dependent dilation can be achieved by the release of nitric oxide produced by calcium-dependent \( \text{L-arginine-eNOS} \) pathway as well as by cyclooxygenase-dependent production and release of prostaglandin \( \text{I}_2 \) (PG\( \text{I}_2 \)). \( \text{NO} \) and \( \text{PGI}_1 \) act via the protein kinases \( \text{PKA} \) and \( \text{PKG} \), respectively, which decrease the sensitivity of MLCK for calcium, resulting in smooth muscle relaxation. Besides \( \text{NO} \) and \( \text{PGI}_2 \), eicosatetraenoic acid (EET) a \( \text{Cyt-P450 metabolite of arachidonic acid} \) was also identified as an endothelium-dependent relaxing factor released in response to stimulation by certain agonists. EET produces smooth muscle relaxation via a novel mechanism of action involving the activation of large-conductance, \( \text{K}^+ \)-selective \( \text{K}^+ \)-ATPase on smooth muscle membrane. This description well fits to the actions of EET. Future investigations involving biochemical and analytical methods are mandatory to support the findings from the present functional study. There is no indication in the literature of the involvement of an \( \text{EET-Na}^+ \text{-K}^+ \)-ATPase pathway in the dilatory effects of high glucose. Instead, contrasting observations were made by Xia et al. in rat mesenteric smooth muscle cells and Gupta et al. in rabbit aorta proposing the inhibition of \( \text{Na}^+ \text{-K}^+ \)-ATPase as underlying mechanism of high-glucose–mediated constriction. These observations show either vascular-bed–dependent variations or variations with the experimental system being used.

**Myogenic Tone of Ophthalmic Artery in the Type II Diabetic BBZDR/Wor Rat**

This is the first study to evaluate pressure-mediated autoregulation of blood flow in the ophthalmic artery in a diabetic model. Arteries from BBZDR/Wor rats that were diabetic for 3 months had chronic exposure to high glucose and were studied in vitro in PSS containing normal levels of glucose. Diabetic arteries showed decreased pressure-dependent myogenic tone over the range of intraluminal pressures used in the study. This decrease was due to diabetic condition and not to a strain difference, as in the prediabetic age group, BBZDR/Wor arteries (normoglycemic) and their age-matched controls have similar pressure–myogenic tone responses. We further observed that the decreased myogenic tone in diabetic arteries was endothelium dependent, suggesting that the endothelial release of a factor/radical mediator is involved in the decreased reactivity of ophthalmic artery to pressure in diabetes. Furthermore, dilation and constriction to agonists was unaltered, suggesting that the attenuating effect of diabetic endothelium is selective for pressure-mediated constriction. These findings show that in diabetes, pressure-mediated autoregulation in the ophthalmic artery operates at low resistance, causing retinal vasculature exposed to increased blood flow with a risk of hemorrhage.

For in vitro experiments, we chose 25 and 40 mM for acute exposure, and, in the rat model, the blood glucose levels were found to be 25 to 35 mM. In vitro observations at 40 mM are in agreement with those observed in experimental diabetes. However, it is likely that chronic exposure to glucose levels of 25 mM, which increased myogenic tone in vitro, may cause attenuation of tone. Results from acute and chronic experiments in arteries clearly show that the chronic diabetic effect could be demonstrated in in vitro conditions by using the relatively higher concentration of 40 mM.

In conclusion, the effect of in vitro acute exposure to high glucose on myogenic tone of rat ophthalmic artery was concentration and endothelium dependent, producing either enhancement or attenuation. Attenuation of myogenic tone by 40 mM was due to the endothelium-dependent activation of \( \text{Na}^+ \text{-K}^+ \)-ATPase in the smooth muscle, most likely by the release of EETs. In experimental diabetes, the pressure-mediated autoregulation in ophthalmic artery operates at lower perfusion pressures, an effect mediated by endothelium.

**Limitations of the Study**

The autoregulation of blood flow in any organ is achieved by myogenic response of smooth muscle to pressure (myogenic, or pressure-mediated, autoregulation), by tissue needs (metabolic autoregulation) and neurohumoral factors. The effect of

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diabetic syndrome on these different kinds of autoregulation varies with the organ or vasculature under study. The present study was specifically focused at the effect of acute high-glucose exposure on pressure-induced autoregulation, and the findings were compared with those from experimental diabetes. The impact of decreased tone in this artery in experimental diabetes on overall ocular autoregulation needs further investigation.

Our results are limited to the ophthalmic artery and cannot be directly extrapolated to changes in retinal blood flow, though ophthalmic blood flow determines the blood supply to the retina, changes in the ophthalmic artery may not be reflected in the retinal circulation. The distal retinal and choroidal arteries of the rat are smaller and may respond differently to pressure changes. However, based on the earlier clinical reports that used hemodynamics in the ophthalmic artery as a measure of overall function of the ophthalmic circulation, we presume this artery could be a valid preparation for the study and understanding of functional alterations in the dynamics of ocular circulation in disease. However, earlier clinical studies have shown evidence of pressure-mediated autoregulation of retinal blood flow and impaired autoregulation of retinal blood flow in diabetes. These findings support our observations in ophthalmic artery acutely exposed to 40 mM glucose, and the present study will serve as a reference for future mechanistic studies in rat models of diabetes.

References


