Acute Hyperglycemia-Induced Endothelial Dysfunction in Retinal Arterioles in Cats

Kenji Sogawa, Taiji Nagaoka, Naohiro Izumi, Seigo Nakabayashi, and Akitoshi Yoshida

Purpose. To investigate the effects of acute hyperglycemia on retinal microcirculation and endothelial function in cats and removal of superoxide to prevent retinal endothelial dysfunction from hyperglycemia.

Methods. Hyperglycemia was induced by intravenous injection of 25% glucose to maintain the plasma glucose concentration at 30 mM. Laser Doppler velocimetry was used to measure the vessel diameter (D) and blood velocity (V) simultaneously and calculated retinal blood flow (RBF) in second-order retinal arterioles in cats. Intravenous, endothelial-dependent vasodilator bradykinin (BK) and endothelium-independent vasodilator sodium nitroprusside (SNP) were administered into the vitreous cavity to evaluate endothelial function in the retinal arterioles. To control osmolality, 25% mannitol was administered the same way. Systemic hyperoxia was induced to noninvasively examine endothelial function during hyperglycemia. To determine the effect of the superoxide on the hyperglycemia-induced changes in the retinal circulation, 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL) was administered in drinking water for 14 days before the experiment.

Results. The D, V, and RBF increased with acute hyperglycemia and mannitol compared with baseline. BK-induced increases in D, V, and RBF significantly declined, whereas SNP-induced increases were unattenuated during acute hyperglycemia. Return of the decreased RBF to baseline after cessation of systemic hyperoxia was significantly (P < 0.05) inhibited by acute hyperglycemia. TEMPOL significantly (P < 0.05) prevented a decrease in the BK-induced increase in RBF during hyperglycemia.

Conclusions. The results suggest that acute hyperglycemia increases RBF via increased osmolality and may cause retinal endothelial dysfunction partially via increased oxidative stress. Systemic hyperoxia can be used to noninvasively examine endothelial function during hyperglycemia. To control osmolality, 25% mannitol was administered the same way. Systemic hyperoxia was induced to noninvasively examine endothelial function during hyperglycemia. To determine the effect of the superoxide on the hyperglycemia-induced changes in the retinal circulation, 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL) was administered in drinking water for 14 days before the experiment.

Diabetic retinopathy is a severe diabetes complication and the leading cause of blindness among adults worldwide. However, the mechanism of the onset of diabetic retinopathy remains unclear. The retinal blood flow (RBF) reportedly is impaired in diabetes, but this effect remains controversial.1-3 The changes in the retinal microcirculation seem to depend on the disease status, making it necessary to identify a parameter to detect abnormal vascular function in the retinal microcirculation before diabetic retinopathy begins.

In tissue other than the retina, endothelium-dependent vasodilation is impaired in diabetic animal models4-6 and patients with diabetes,7-8 suggesting that endothelial dysfunction is important in the pathogenesis of diabetic vascular disease. In addition, hyperglycemia reduced endothelium-dependent vasodilation in a nondiabetic animal model8 and healthy subjects.9,10 Taken together, hyperglycemia, a major independent risk factor for diabetic vascular disease,11,12 may be a fundamental abnormality underlying endothelial dysfunction in diabetes. Because previous studies have focused mainly on the aorta and resistance vessels from the mesenteric circulation in animal studies4-6 and the forearm circulation in human studies7,8 it is unclear whether hyperglycemia causes endothelial dysfunction in the retinal microcirculation. Furthermore, one mechanism underlying hyperglycemia-induced vascular damage is related to increased production of superoxide from endothelial cells, resulting in nitric oxide (NO) inactivation.5,13-16 Although oxidative stress also may contribute to diabetic retinopathy,17-20 no studies have been undertaken to examine the effect of hyperglycemia-induced superoxide on the retinal circulation in vivo.

We investigated the effect of acute hyperglycemia on the retinal microcirculation and endothelial function in the retinal arterioles in nondiabetic cats and the effect of superoxide on the retinal microcirculation during acute hyperglycemia.

Materials and Methods

Animal Preparation

Protocols describing the use of cats (90 healthy adult cats of either sex; weight, 2.1–4.8 kg) were approved by the Animal Care Committee of Asahikawa Medical College and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Each cat was anesthetized with sevoflurane, oxygen, and nitrous oxide in a closed box followed by intraperitoneal injection of atropine (0.04 mg/kg). The animals were tracheostomized and mechanically ventilated with 1.5% to 2.0% sevoflurane and room air. Catheters were placed in the femoral arteries and vein. Pancuronium bromide (0.1 mg/kg/h; Daiichi Sankyo Co., Tokyo, Japan) was infused continuously. Arterial pH, arterial partial carbon dioxide tension (PaCO2), and arterial partial oxygen tension (PaO2) were measured intermittently with a blood gas analyzer (model ABL5; Radiometer, Copenhagen, Denmark). The mean arterial blood pressure (MABP) and heart rate (HR) were monitored continuously. The rectal temperature was maintained between 37°C and 38°C with a heated blanket.

The pupils were dilated with 0.5% tropicamide (Santen Pharmaceutical Co., Osaka, Japan). A 26-gauge butterfly needle was inserted into the anterior chamber and connected to a pressure transducer to monitor the intraocular pressure (IOP), which was maintained at 10 mm Hg.

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RBF Measurement

We measured the RBF with a laser Doppler velocimetry system (Laser Blood Flowmeter, CLBF model 100; Canon, Inc., Tokyo, Japan) customized for feline use21,22 that is designed to measure vessel diameter (D, in micrometers) and blood velocity (V; millimeters per second) simultaneously in retinal vessels and automatically calculates the RBF (microliters per minute). V was measured by bidirectional laser Doppler velocimetry, which provides absolute measurements of the speed of the red blood cells flowing at discrete selected sites in the retinal vessel, assuming Poiseuille flow.23,24 The signals from the two photomultiplier tube detectors undergo computer-controlled spectrum analysis and sequential measurement of the maximum speed (Vmax) at the center of the vessel. In this system, each pair of spectra was recorded, and Vmax was calculated automatically every 5 ms for 1 second during each measurement. V was defined as the averaged Vmax during one cardiac cycle.

The retinal D was determined automatically by computer analysis of the signal produced by the vessel image and defined as the average of the values determined at each time point.

The RBF was calculated from the formula RBF = S × Vmean where S is the cross-sectional area of the retinal artery at the laser Doppler measurement site, assuming a circular cross section, and Vmean is the mean blood velocity calculated as Vmean = Vmax/2.25 The ocular perfusion pressure (OPP) was calculated as 2/3(MABP – IOP).26

Effect of Intravitreal Microinjection of Bradykinin or Sodium Nitroprusside during Normoglycemia

Because we reported that bradykinin (BK) is a dose- and NO-dependent vasodilator in isolated porcine retinal arterioles,27 we injected it into the vitreous cavity as the endothelium-dependent vasodilator and sodium nitroprusside (SNP) as the endothelium-independent vasodilator. The intravitreal microinjection technique was performed with a 30-gauge needle inserted into the vitreous 3 mm posterior to the limbus and positioned over the disc21 by using a 100-µL syringe (Hamilton, Reno, NV) with care taken not to injure the lens or retina. Given that the volume of the feline vitreous cavity is approximately 2.5 mL,21 50 µL of BK (5 × 10−7 M) or SNP (5 × 10−5 M) dissolved in phosphate-buffered saline (PBS) was injected into the vitreous for extracellular concentrations of 1.0 × 10−6 M and 1.0 × 10−4 M, respectively, near the retinal vessels. These concentrations were sufficient for the maximum vasodilation concentrations of BK and SNP, on the basis of our previous in vitro study.27 The vehicle, 50 µL of PBS, was injected into other cats in the same manner.

Experimental Protocols

The plasma glucose concentration increased gradually and remained at 30 mM (540 mg/dL) with a 25% glucose infusion through the femoral vein catheter. The infusion rate was adjusted every 10 to 15 minutes to maintain the plasma glucose level. As an osmolality control, the protocol was repeated with an equimolar 25% mannitol infusion. Serum osmolality was determined by freezing-point depression. All drugs were obtained from Sigma-Aldrich (St. Louis, MO).

Effect of Intravitreal Injections of BK and SNP during Normoglycemia

Because the increase in RBF induced by intravitreal injections of BK and SNP reached the maximum level at 120 minutes and persisted for at least 3 hours in our preliminary study, RBF measurements were performed before and 2 hours after intravitreal microinjection. To evaluate the role of NO and prostanoids in the change in RBF in response to BK, we injected N-nitro-l-arginine-methylester (L-NAME, 100 mM)21 intravitreally and injected indomethacin (5 mg/kg)28 intravenously simultaneously with intravitreal injection of BK. We confirmed that the dose of indomethacin did not change the systemic and retinal circulatory parameters for 3 hours.

Effects of Hyperglycemia and Hyperosmolality on Retinal Circulation

The RBF measurements began 10 minutes before glucose or mannitol infusion. The average of five measurements obtained at 2-minute intervals was defined as baseline before the infusion; measurements were performed again 3 hours after glucose or mannitol infusion. In another group, we adjusted the serum glucose levels to 10, 20, and 30 mM, and the RBF was measured every 30 minutes for 3 hours.

Repeatability of Retinal Circulation Response after Acute Hyperglycemia

To determine whether the changes in retinal circulatory parameters in response to BK during hyperglycemia can be restored after returning to normoglycemia, we compared the changes in endothelial-dependent vasodilator BK-mediated increases in D, V, and RBF after cessation of acute hyperglycemia. The glucose infusion was stopped 5 hours after hyperglycemia began. When the plasma glucose decreased to baseline (5–7 mM), an average of five measurements at 2-minute intervals was defined as the baseline value. After measuring the baseline RBF at the return to the normal glucose level, we injected BK intravitreally and measured the RBF 2 hours after the BK microinjection.

Evaluation of Endothelial Function in Retinal Arterioles after Acute Hyperglycemia

PBS, BK, or SNP was injected into the vitreous cavity 3 hours after glucose or mannitol infusion. RBF measurements were performed before and 2 hours after intravitreal microinjection. To determine the dose-dependent effect of the serum glucose concentration on the retinal microcirculation, BK-induced changes in the RBF were measured in the same manner after the serum glucose concentrations were maintained at 10, 20, or 30 mM in other cats.

Effect of Hyperglycemia on the Reaction of the Retinal Arterioles in Response to Hyperoxia

We used the same protocol as our previous feline study to evaluate noninvasively the endothelial function in the retinal arterioles.29 Briefly, systemic hyperoxia was induced by inhalation of 100% oxygen for 10 minutes, followed by the previously described protocol 5 hours after glucose or mannitol infusion. Before, during, and after systemic hyperoxia, RBF measurements were performed every 2 minutes. At each point, three successive measurements at 20-second intervals were recorded, and the average of the three was recorded. Blood gas analysis was performed before and at the end of hyperoxia.

Role of Superoxide in BK-Induced Increases in Retinal Circulation during Acute Hyperglycemia

To ascertain whether increased superoxide is involved in hyperglycemia-induced retinal endothelium dysfunction, we compared the responses of BK-induced increases in D, V, and RBF, as described previously after treatment with 2 mM of the cell-permeable superoxide scavenger 4-hydroxy-2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL) or distilled water added to drinking water for 14 days before the experiment.

Statistical Analysis

All data are expressed as the mean ± SE. For statistical analysis of changes in systemic parameters and retinal circulation compared with baseline, we used Student’s paired t-test for simple comparison between two values. To compare changes in the retinal circulation, we
used the unpaired t test to compare two groups and one-way ANOVA or two-way repeated-measures ANOVA followed by the Tukey-Kramer procedure to compare more than two groups. Changes in systemic hyperoxia and glucose dose-dependent changes in the retinal circulation were analyzed by repeated-measures ANOVA followed by Dunnett's procedure compared with baseline. *P < 0.05 was considered significant.

RESULTS

Changes in Retinal Circulation by SNP or BK during Normoglycemia

The group-averaged D of the retinal arterioles used in the present study was 82.4 ± 3.4 μm at baseline. The microinjections of PBS, BK, and SNP into the vitreous cavity did not alter the systemic circulatory parameters. Two hours after microinjection of PBS, there were no significant differences in D, V, or RBF compared with before injection. Two hours after intravitreal injection of SNP, D, V, and RBF increased significantly compared with before the injection (P < 0.05). Two hours after intravitreal microinjection of BK, D, V, and RBF increased significantly compared with before the injection (P < 0.05). All retinal circulatory parameters increased significantly in response to SNP and BK compared with PBS, but there were no significant differences in any retinal circulatory parameters between SNP and BK (Fig. 1A). Pretreatment with l-NAME greatly reduced the BK-induced increase in RBF, but pretreatment with indomethacin did not change the RBF after intravitreal injection of BK (Fig. 1B).

Changes in Systemic and Retinal Circulations Resulting from Acute Hyperglycemia

During hyperglycemia, there were no significant differences in the pH, PaCO₂, or PaO₂ (Table 1), whereas the systemic BP and HR increased significantly (P < 0.05). There were no significant differences in any systemic parameters between groups before the administration of glucose or mannitol. The serum osmolality increased significantly (P < 0.05) in both groups compared with the normoglycemic baseline, but there were no significant differences between the hyperglycemia and mannitol groups (Table 1).

Three hours after hyperglycemia was induced, D, V, and RBF increased significantly (P < 0.05) compared with the normoglycemic baseline (Fig. 2). In the mannitol group, D, V, and RBF increased significantly (P < 0.05) compared with baseline. There were no significant differences in the changes in any retinal circulatory parameters between the hyperglycemia and mannitol groups (Fig. 2).

TABLE 1. Changes in Systemic Parameters, Serum Glucose Concentration, and Osmolality in the Hyperglycemia Group and the Mannitol Group

<table>
<thead>
<tr>
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<th>Hyperglycemia Group</th>
<th>Mannitol Group</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
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<tr>
<td>pH</td>
<td>7.39 ± 0.21</td>
<td>7.36 ± 0.19</td>
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<tr>
<td>PaCO₂, mm Hg</td>
<td>31.3 ± 1.6</td>
<td>29.3 ± 2.4</td>
</tr>
<tr>
<td>PaO₂, mm Hg</td>
<td>104.7 ± 5.4</td>
<td>109.1 ± 7.2</td>
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<tr>
<td>MAP, mm Hg</td>
<td>89.4 ± 3.7</td>
<td>101.4 ± 4.7*</td>
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<tr>
<td>OPP, mm Hg</td>
<td>46.2 ± 2.4</td>
<td>54.4 ± 2.7*</td>
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<td>HR, beats/min</td>
<td>152.0 ± 4.3</td>
<td>146.9 ± 5.9*</td>
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<td>Glucose (mg/dL)</td>
<td>91.4 ± 5.7</td>
<td>551.4 ± 19.7*</td>
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<tr>
<td>Osm (mOsm/KgH₂O)</td>
<td>305.2 ± 2.3</td>
<td>324.5 ± 3.2*</td>
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Data are expressed as the mean ± SE, before and 5 hours after injection of glucose or mannitol.

* P < 0.05 versus preinjection values by paired Student’s t test.
Changes in BK-Induced Increases in Retinal Circulation after the Plasma Glucose Concentration Returned to Baseline

After the cessation of intravenous infusion of 25% glucose, the plasma glucose concentration decreased to the baseline value (92.5 ± 4.8 mg/dL) after 7 to 9 hours. All retinal circulatory parameters returned to the baseline values after the plasma glucose returned to the baseline value. After returning to normoglycemia, an intravitreal microinjection of BK increased D, V, and RBF compared with the premicroinjection level. There were no significant differences in the BK-induced increases in D, V, and RBF after returning to normoglycemia compared with the BK-induced response during normoglycemia shown in Figure 1 (Fig. 5).

Changes in Retinal Circulation by Systemic Hyperoxia during Hyperglycemia

Five hours after intravenous administration of glucose or mannitol, 100% oxygen inhalation induced systemic hyperoxia (Table 2). There were no significant changes in systemic parameters other than the PaO2 in both groups after systemic hyperoxia. Ten minutes after the onset of hyperoxia, D, V, and RBF significantly (P < 0.05) decreased compared with baseline (Fig. 6). There were no significant differences in any retinal

Dose-Dependency of the Changes in Retinal Circulation in Response to Hyperglycemia

In response to 10-, 20-, and 30-mM glucose infusions for 3 hours, D did not increase until 2 hours after induction of acute hyperglycemia; V and RBF increased significantly 1 hour after acute hyperglycemia (Fig. 4A). Three hours after hyperglycemia, V and RBF increased in a dose-dependent manner among the groups. There were no differences in the changes in D among the three groups.

After 3 hours of each dose of glucose infusion, RBF was measured 2 hours after microinjection of BK during hyperglycemia (Fig. 4B). The 10-mM glucose concentration did not affect the BK-induced increases in any retinal circulatory parameters compared with the normoglycemia group. However, the 20- and 30-mM glucose concentrations significantly (P < 0.05) impaired the BK-induced response to the retinal circulatory parameters compared with the normoglycemia group.

FIGURE 4. (A) The time course of the changes in retinal circulation in response to normoglycemia and the 10-, 20-, and 30-mM glucose concentrations. *Significant differences with all concentrations compared with baseline at each time point. (B) The concentration-dependent effect of glucose on the retinal circulation in response to microinjection of BK. *Significant differences between the normoglycemia group and the 20- and 30-mM glucose concentrations (P < 0.05). †Significant difference between the 20- and 30-mM glucose concentrations (P < 0.05).
circulatory parameters between the groups 10 minutes after hyperoxia was induced. D, V, and RBF reached baseline 10 minutes after systemic hyperoxia ended in the mannitol group. The recoveries of the decreased D and RBF after systemic hyperoxia were attenuated significantly ($P < 0.05$) in the hyperglycemia group compared with the mannitol group. All parameters returned to the prehyperoxic levels approximately 30 minutes after the hyperoxia ended.

### Changes in BK-Induced Increases in the Retinal Circulation after TEMPO

In the group pretreated with TEMPOL, intravitreal BK microinjections were administered after 3 hours of acute hyperglycemia. D, V, and RBF were compared with the premicroinjection levels ($P < 0.05$). In the group given distilled water, D, V, and RBF increased compared with the pre-BK injection ($P < 0.05$). There were significant ($P < 0.05$) differences in the BK-induced increases in V and RBF but no significant differences in the BK-induced increases in D between the groups with and without TEMPOL pretreatment (Fig. 7).

### DISCUSSION

Endothelial dysfunction plays a key role in the pathogenesis of diabetic vascular disease in various animals, vessels, and organs, except the retina. To our knowledge, only a few animal studies of STZ-induced diabetic rats have been conducted to evaluate the endothelial function of the retinal arterioles in vivo with the administration of acetylcholine.30-32 The lack of any evidence of the importance of endothelial dysfunction in the pathogenesis of diabetic retinopathy may be related to the fact that this direct evaluation of endothelial function in the retinal microcirculation in vivo has not been well established. In the present study, we confirmed for the first time that BK injected into the vitreous cavity can cause vasodilation and increased blood flow in the feline retinal microcirculation in vivo (Fig. 1A). In addition, our current data clearly showed that BK increased the RBF mainly via NO rather than via prostaglandins in cats in vivo (Fig. 1B). Although we27 and another group33 that used isolated porcine arterioles have reported that BK causes vasodilation of the retinal arterioles mainly by production of endothelial-derived NO, a recent study showed that BK also can stimulate the release of endothelium-derived prostaglandins in the retinal arterioles.34 In contrast to these findings from in vitro isolated retinal vessels, our current in vivo data are consistent with our previous in vitro findings.27 Taken together, the current results indicate that BK-induced increases in RBF are mediated mainly by NO production from the endothelium and that the retinal vessel response to BK may be a good way to evaluate retinal endothelial function in vivo, at least in cats.

In the present study, we administered BK and SNP by microinjection into the vitreous cavity to minimize the effects on the systemic circulation (Table 1). In our preliminary study, the BP decreased and the HR increased immediately after intravenous administration of SNP and BK. These systemic circulatory changes may affect the hemodynamics of the retinal microcirculation because of the autoregulatory system in the retinal microcirculation that responds to changes in the systemic BP.35 Therefore, microinjection of BK into the vitreous cavity can cause vasodilation and increased blood flow in the feline retinal microcirculation in vivo (Fig. 1A). In addition, our current data clearly showed that BK increased the RBF mainly via NO rather than via prostaglandins in cats in vivo (Fig. 1B). Although we27 and another group33 that used isolated porcine arterioles have reported that BK causes vasodilation of the retinal arterioles mainly by production of endothelial-derived NO, a recent study showed that BK also can stimulate the release of endothelium-derived prostaglandins in the retinal arterioles.34 In contrast to these findings from in vitro isolated retinal vessels, our current in vivo data are consistent with our previous in vitro findings.27 Taken together, the current results indicate that BK-induced increases in RBF are mediated mainly by NO production from the endothelium and that the retinal vessel response to BK may be a good way to evaluate retinal endothelial function in vivo, at least in cats.

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### Table 2. Changes in Systemic Parameters during Hyperoxia

<table>
<thead>
<tr>
<th>Hyperglycemia Group</th>
<th>Mannitol Group</th>
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<td>Before</td>
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<tr>
<td>pH</td>
<td>7.38 ± 0.23</td>
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<tr>
<td>PaCO₂, mm Hg</td>
<td>32.6 ± 1.4</td>
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<tr>
<td>PaO₂, mm Hg</td>
<td>108.7 ± 5.4</td>
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<td>MABP, mm Hg</td>
<td>89.8 ± 2.9</td>
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<tr>
<td>OPP, mm Hg</td>
<td>48.6 ± 1.9</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>135.7 ± 4.9</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SE, before and induction of hyperoxia and after its conclusion.

* $P < 0.05$ versus prehyperoxic values by paired Student’s $t$-test.
cavity is a good tool for evaluating the retinal circulation and endothelial function without affecting the systemic circulation.

Systemic infusions of high glucose (30 mM) and mannitol significantly increased D, V, and RBF (Fig. 2). Previous studies have reported that acute hyperglycemia increases RBF, but acute infusion of mannitol and urea, used as osmolality controls, did not change RBF in normal cats or minipigs. In those studies, RBF increases during acute hyperglycemia were comparable to those in the present study, but the absence of RBF increases in response to mannitol or osmolality-controlled urea were inconsistent with our results. It is difficult to explain this discrepancy, because no serum osmolality data have been reported in the previous studies. A human study also reported that forearm blood flow significantly increased after hyperglycemia and mannitol infusions, suggesting that increased osmolality may cause hyperglycemia-induced increases in forearm blood flow. Indeed, RBF and serum osmolality also increased significantly to the same degree in the hyperglycemic and mannitol groups (Table 1, Fig. 2). In addition, higher serum glucose concentrations increased the serum osmolality in a dose-dependent manner (data not shown). Taken together, the current findings strongly suggested that hyperosmolality likely causes RBF increases during acute hyperglycemia.

In addition, blood velocity and RBF increased before the vessel diameter in response to acute infusion of glucose (Fig. 4A). We reported previously the similar delayed dilation of the retinal arterioles after the blood velocity and RBF increased in response to the increased RBF caused by systemic hypoxia, probably due to flow-mediated dilation that maintains the wall shear stress on the walls of the retinal arterioles. It is likely that hyperglycemia would dilate the small arterioles and/or capillaries downstream first and then dilate the upstream larger arterioles in which we measured RBF in response to increased RBF and wall shear rate (WSR) by the flow-mediated dilation mechanism as that observed in response to systemic hypoxia.

In the present study, acute hyperglycemia and mannitol significantly increased MABP and OPP (Table 1). Increased MABP and OPP per se may have some effect on the retinal microcirculation, and studies have shown that an acute increase in MABP of approximately 20 mm Hg causes constriction of the retinal arterioles in response to increased blood V to maintain the RBF in humans, which indicates autoregulation of the RBF. The current results indicated that hyperglycemia and mannitol caused an acute increase in the systemic BP of approximately 10 mm Hg, which is smaller than that in our human study. Although it is difficult to explain how such small increases in MABP and OPP affected the retinal microcirculation in the present study, we speculate that the increased MABP and OPP had little effect on the current results because all the D, V, and RBF measurements greatly increased in response to acute administration of glucose and mannitol.

Although it is unknown whether endothelial dysfunction contributes to diabetic retinopathy, studies have reported impaired endothelium-dependent vasodilation in the mesenteric artery and retinal microcirculation in streptozotocin-induced diabetic rats and the forearm vessels of patients with diabetes. Furthermore, acute hyperglycemia attenuates endothelium-dependent vasodilation by measuring forearm blood flow, even in subjects without diabetes. In the present study, BK-induced (endothelium-dependent) increases in the retinal circulation decreased significantly during acute hyperglycemia (Fig. 3B) but were unaffected in the mannitol group, whereas SNP-induced (endothelium-independent) increases in the retinal circulation were unchanged in either group (Fig. 3A). This result is not surprising, because acute hyperglycemia caused markedly reduced endothelium-dependent vasodilation within 15 minutes after initiation of hyperglycemia in the intestinal arterioles in normal rats and within 30 minutes in the femoral artery in normal humans. Of interest, eNOS expression and stimulated increases in NO release decreased significantly after increasing the glucose concentrations in bovine microvascular retinal endothelial cells. Taken together, our data clearly showed that acute hyperglycemia may cause endothelial dysfunction in the retinal arterioles without affecting retinal smooth muscle function, probably via decreased NO release from the retinal endothelial cells.

It is worth noting whether the endothelial dysfunction caused by acute hyperglycemia can be restored by immediate normalization of the increased blood glucose level. In the present study, we found that transiently increased RBF in response to acute hyperglycemia returned to the baseline level and the BK-mediated increases in RBF did not decrease after the plasma glucose returned to the baseline level (5–6.5 mM, Fig. 5), suggesting that retinal endothelial dysfunction may be a temporal phenomenon during acute hyperglycemia that can be restored by normalized serum glucose.

Although the current results suggest that BK microinjection into the vitreous cavity can be used to evaluate endothelial function in the retinal arterioles in animal studies, this technique cannot be used clinically, because intravitreal microinjections may cause intraocular infections, vitreous hemorrhage, or retinal detachment. Another noninvasive technique for evaluating retinal vascular function is needed for clinical research. In a recent feline study, we reported that changes in the retinal circulation in response to systemic hyperoxia, which had been used previously to evaluate the vascular reactivity in patients with diabetes in the retinal microcirculation, could be useful for noninvasive study of endothelial function in retinal arterioles. Our previous results (i.e., that intravitreal injection of L-NAME markedly inhibits return of RBF to baseline after hyperoxia ends despite there being no difference in the RBF decreases during hyperoxia between the PBS and L-NAME groups) indicated that NO production from the endothelium may be associated with the RBF response after cessation of systemic hyperoxia. Because we previously ruled out the role of neuronal NO synthase in the vasoactive response to systemic hyperoxia in the retina using a specific neuronal NOS inhibitor 7-nitroindazole, the current findings that recovery of the decreased RBF to baseline after hyperoxia was inhibited significantly by acute hyperglycemia (Fig. 6) pointed to reduced NO production from the endothelium in response to systemic hyperoxia. Although we could not totally exclude the systemic effect of hyperoxia on the physiologic system in the body, systemic hyperoxia may be a good, noninvasive method for evaluating retinal endothelial function during hyperglycemia. Further clinical study is warranted to examine whether endothelial function can be evaluated non-
invasively by using this technique in patients with diabetes mellitus.

The current results showed that TEMPOL pretreatment prevented reduction of the BK-induced increases in RBF during hyperglycemia (Fig. 7). Studies have shown that oxygen-derived free radicals rapidly combine with NO, resulting in decreased bioavailability of NO and impaired endothelial function.15,14 Recent studies have suggested that hyperglycemia induces overproduction of superoxide by the mitochondrial electron transport chain, and superoxide could be the first key event in the activation of other pathways involved in the pathogenesis of diabetes complications.15,42 A recent in vitro study also reported that 25 mM of glucose reduced release of NO from bovine retinal endothelial cells.17 Considering this, our findings suggest that increased superoxide plays a role in the abnormal retinal endothelial function in response to acute hyperglycemia, probably via decreasing NO bioavailability in the retina.

The present study had some limitations. First, we did not determine whether the increase in insulin, which may be stimulated by acute hyperglycemia,9,10 is associated with changes in the retinal circulation during acute hyperglycemia. An in vitro study reported that insulin has a direct vasodilatory effect on isolated retinal arteries,43 in contrast to a clinical study that reported no RBF changes during hyperinsulinemia in healthy humans.44 However, although we did not measure the serum insulin concentration, we injected glucose with somatostatin to mimic the increased insulin during hyperglycemia45 and confirmed that there were no significant differences in the BK-induced increases in RBF between hyperglycemia in the groups treated with \( n = 4 \) and without \( n = 5 \) somatostatin (32.4% ± 7.6% vs. 28.2% ± 8.9%, \( P = 0.51 \)), suggesting that the observed changes in the retinal circulation may not be associated with increased serum insulin levels in response to acute hyperglycemia. Williams et al.,10 using a somatostatin analogue in patients with diabetes, also reported that the effects of acute hyperglycemia on endothelium-dependent vasodilation were unaffected by blocked insulin release. Their results are consistent with our preliminary data, both suggesting that hyperglycemia plays an important role in hyperglycemia-induced vascular dysfunction independent of the serum insulin level.

Second, despite scavenging of oxygen-derived free radicals, TEMPOL only partly improved the reduction of the increased RBF in response to BK (Fig. 7). The blood V was restored compared with the group without TEMPOL treatment, whereas there was no significant difference in vessel D between the groups with and without TEMPOL pretreatment. The concentration of TEMPOL in the present study seemed sufficient for reducing the free radicals in vivo46–48 and retinal isolated vessel studies,27 because TEMPOL added to drinking water can decrease oxidative stress.49–51 It is possible that smaller arterioles and/or capillaries are more sensitive to oxidative stress, resulting in significantly reduced blood V and RBF and no change in D after TEMPOL had scavenged superoxide. Further study is needed to determine the importance of oxidative stress in the mechanisms of endothelial dysfunction during hyperglycemia in the retinal microcirculation.

Third, we examined the effect only of acute hyperglycemia not of chronic diabetes on the retinal microcirculation in vivo in the present study. It is apparent that acute hyperglycemia and chronic diabetes have different mechanisms that produce vascular dysfunction. The relevance of the current findings to diabetes is speculative.

In conclusion, we showed for the first time that acute hyperglycemia increases the retinal circulation probably via increased serum osmolality and may cause endothelial dysfunction in the retinal microcirculation in healthy cats. In addition, systemic hyperoxia can be used for noninvasive clinical examination to evaluate retinal endothelial function during hyperglycemia because acute hyperglycemia inhibited recovery of the decreased RBF to baseline after hyperoxia stopped. Moreover, the current findings suggest that increased superoxide is probably associated with the retinal endothelial dysfunction caused by acute hyperglycemia.

References

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