tvst

Article

Hyperglycemia Augments Endothelin-1–Induced Constriction of Human Retinal Venules

Yen-Lin Chen[1], Robert H. Rosa, Jr.[1,2], Lih Kuo[1], and Travis W. Hein[1]

[1] Department of Medical Physiology, College of Medicine, Texas A&M University Health Science Center, Bryan, TX, USA
[2] Department of Ophthalmology, Baylor Scott & White Eye Institute, Temple, TX, USA

Correspondence: Travis W. Hein, PhD, Texas A&M HSC, 8447 Riverside Parkway, Bryan, TX, 77807, USA. e-mail: thein@tamu.edu
Lih Kuo, PhD, Texas A&M HSC, 8447 Riverside Parkway, Bryan, TX, 77807, USA. e-mail: lkuo@tamu.edu

Received: March 22, 2020
Accepted: June 17, 2020
Published: August 3, 2020

Keywords: endothelin-1; microvascular complications; retina; vasoconstriction; veins

Citation: Chen Y-L, Rosa RH Jr., Kuo L, Hein TW. Hyperglycemia augments endothelin-1–induced constriction of human retinal venules. Trans Vis Sci Tech. 2020;9(9):1, https://doi.org/10.1167/tvst.9.9.1

Purpose: Endothelin-1 (ET-1) is a potent vasoactive factor implicated in development of diabetic retinopathy, which is commonly associated with retinal edema and hyperglycemia. Although the vasomotor activity of venules contributes to the regulation of tissue fluid homeostasis, responses of human retinal venules to ET-1 under euglycemia and hyperglycemia remain unknown and the ET-1 receptor subtype corresponding to vasomotor function has not been determined. Herein, we addressed these issues by examining the reactivity of isolated human retinal venules to ET-1, and results from porcine retinal venules were compared.

Methods: Retinal tissues were obtained from patients undergoing enucleation. Human and porcine retinal venules were isolated and pressurized to assess diameter changes in response to ET-1 after exposure to 5 mM control glucose or 25 mM high glucose for 2 hours.

Results: Both human and porcine retinal venules exposed to control glucose developed similar basal tone and constricted comparably to ET-1 in a concentration-dependent manner. ET-1–induced constrictions of human and porcine retinal venules were abolished by ET_A receptor antagonist BQ123. During high glucose exposure, basal tone of human and porcine retinal venules was unaltered but ET-1–induced vasoconstrictions were enhanced.

Conclusions: ET-1 elicits comparable constriction of human and porcine retinal venules by activation of ET_A receptors. In vitro hyperglycemia augments human and porcine retinal venular responses to ET-1.

Translational Relevance: Similarities in vasoconstriction to ET-1 between human and porcine retinal venules support the latter as an effective model of the human retinal microcirculation to help identify vascular targets for the treatment of retinal complications in patients with diabetes.

Introduction

Diabetes mellitus is a global epidemic and the associated hyperglycemia can cause a range of macrovascular and microvascular complications, which consequently evoke various diseases. One of the major microvascular complications of diabetes is retinopathy, a leading cause of vision impairment and blindness in working age adults.[1,2] Retinal neovascularization and macular edema, hallmarks of diabetic retinopathy, are associated with decreased retinal perfusion and increased capillary fluid filtration caused by microvascular dysfunction.[3] However, the precise mechanisms involved in circulatory dysregulation in diabetes remain unclear. Accumulating evidence indicates that vitreous humor and plasma levels of endothelin-1 (ET-1) are increased in diabetes, especially in patients with diabetic retinopathy.[4,5] The ET-1 peptide is a potent vasoconstrictor and its overproduction can contribute to the development of cardiovascular disorders, including remodeling, tissue inflammation, cell proliferation, and vasomotor dysfunction.[4,6–11] Although elevated levels of ET-1

have been suggested to contribute to retinal pathophysiology,[12,13] the clinical impact of ET-1 on retinal microvascular function is incompletely understood.

In the retinal microcirculation, the arterioles play an important role in governing retinal blood flow distribution and perfusion through changes in their local vasomotor tone and overall resistance, respectively.[14] In the early stage of diabetes, reduction of arteriolar endothelium-dependent vasodilator function without apparent structural changes in the retina has been demonstrated recently.[15,16] At the segment of retinal venules, changes in their resistance, that is, diameter, influence hydrostatic pressure and fluid homeostasis in upstream capillaries.[17,18] Constriction of retinal venous vessels leads to an increased retinal venous pressure, which could subsequently decrease retinal perfusion and promote capillary fluid filtration (i.e., edema) when compensatory or defense mechanisms are exhausted in the earlier stages of diabetes before the development of retinopathy. However, our understanding of factors directly affecting the vasomotor activity of retinal venules is seemingly limited to three studies in pigs,[19–21] which resemble human retinal morphology and structure.[22,23] Our previous study found that ET-1 causes marked constriction of porcine retinal venules through ET_A receptors (ET_ARs) and extracellular Ca^{2+} influx independent of L-type voltage-operated Ca^{2+} channels.[20] We have also shown that hyperglycemia, a hallmark of diabetes, augments porcine venular constrictions to ET-1, U46619, and norepinephrine.[21] However, there has been no study addressing whether ET-1 affects the vasomotor tone of human retinal venules under euglycemia or hyperglycemia. In the present study, we addressed these issues by examining the vasomotor response to ET-1 in human retinal venules isolated from eyes donated by patients undergoing enucleation. Because hyperglycemia is one of the major risk factors for the development of diabetic retinopathy,[24,25] we also investigated whether short-term exposure of human retinal venules to high glucose in vitro affects the vasoreactivity to ET-1. Furthermore, we compared the ET-1–induced constriction from human vessels with results from the pig, a potential large animal model for the study of retinal microvascular diseases.

Methods

Human Subjects Study

Retinal tissues were obtained, over a period of 4 years, from 1 male (81 years old) and 4 female patients (45, 62, 69, and 69 years old) undergoing enucleation because of ocular melanoma. The study was conducted after informed consent with approval from the Baylor Scott & White Health Institutional Review Board and followed the tenets of the Declaration of Helsinki. The clinical characteristics for the patients in this study are summarized in the Table. No retinal complications were observed on gross examination of the enucleated eyes except for ocular melanoma. Two patients (one male and one female) had type 2 diabetes along with primary hypertension and hyperlipidemia; one patient had primary hypertension; and one patient had hyperlipidemia. One patient had not been diagnosed with cardiovascular risk factors but was taking a nonsteroidal anti-inflammatory drug. Additional medications and supplements that were taken for the noted cardiovascular disease risk factors included nonsteroidal anti-inflammatory drugs ($n = 2$), statin ($n = 1$), krill or fish oil ($n = 2$), Ca^{2+} channel blocker ($n = 1$), centrally acting alpha-2 adrenergic receptor agonist ($n = 1$), alpha-1 adrenergic receptor antagonist ($n = 1$), noninsulin antidiabetic drugs ($n = 2$), angiotensin-converting enzyme inhibitor ($n = 1$), and angiotensin II type 1 receptor antagonist ($n = 2$). Immediately after enucleation, a large part of the posterior eye wall segment associated with the tumor was removed for histopathologic examination. The remaining posterior eye wall segment (including the attached retina) was transferred to a moist chamber on ice for microvessel isolation. Depending on the amount of available tissue, we were able to isolate one to four retinal vessels per eye specimen.

Animal Preparation

All animal procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Baylor Scott & White Institutional Animal Care and Use Committee. Domestic (Yorkshire) pigs (8–12 weeks old, 10–15 kg, 3 females and 17 males) purchased from Real Farms (San Antonio, TX) were sedated with Telazol (4–8 mg/kg, intramuscularly), anesthetized with 2% to 5% isoflurane, and intubated for eye harvesting as described previously.[26,27] The eyes were enucleated and immediately placed in a moist chamber on ice.

Isolation and Cannulation of Microvessels

Techniques for identifying and isolating human and porcine retinal microvessels were similar to those used in our previous studies.[20,21,28] Briefly, retinal venules (1.0–1.5 mm in length without side branches) were identified based on the dark red deoxygenated blood in

Table. Clinical Characteristics of Patients

Patient	Sex	Cardiovascular Diseases	Medications and Supplements
1	Female	Hyperlipidemia	Krill oil
2	Female	Primary hypertension	Ca^{2+} channel blocker Central alpha-2 adrenergic receptor agonist
3	Female	None	Nonsteroidal anti-inflammatory drug
4	Female	Hyperlipidemia Type 2 diabetes Primary hypertension	Noninsulin antidiabetic drug Angiotensin-converting enzyme inhibitor Angiotensin II type 1 receptor antagonist
5	Male	Hyperlipidemia Type 2 diabetes Primary hypertension	Fish oil, statin, noninsulin antidiabetic drug Nonsteroidal anti-inflammatory drug Alpha-1 adrenergic receptor antagonist Angiotensin II type 1 receptor antagonist

The sex, cardiovascular diseases, and medications and supplements to treat these diseases are provided for the five patients who donated retinal tissue for the vascular studies.

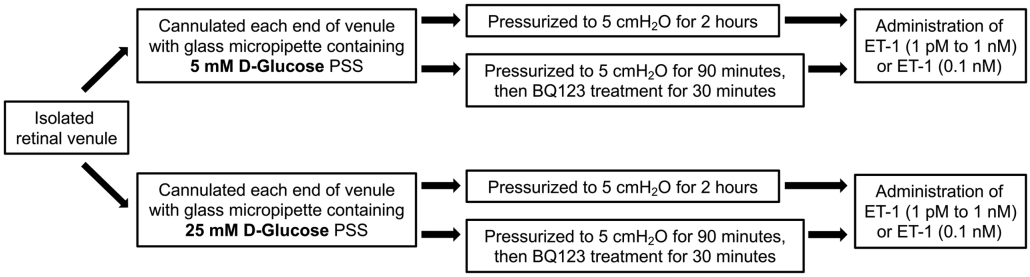

Figure 1. Experimental protocol for the study of isolated and pressurized retinal venules.

the lumen and their thin vascular wall compared with the parallel arterioles containing bright red oxygenated blood and a thick vascular wall.[20] Single venules were dissected from surrounding neural and connective tissues and then cannulated on each end with glass micropipettes containing physiologic saline solution (PSS) with 5 mM (approximately 90 mg/dL; control) D-glucose and 1% albumin.[21] The hyperglycemic solution contained 25 mM (approximately 450 mg/dL; high glucose) D-glucose in PSS.[15,21] Osmolarity in the 25 mM D-glucose solution was balanced to 290 mOsm by reducing the NaCl concentration to avoid a hyperosmolarity effect.[29] Vessels were pressurized to 5 cm H_2O (4 mm Hg) intraluminal pressure without flow by two independent pressure reservoirs and the inner diameter was recorded using videomicroscopic techniques throughout the experiments.[20]

Experimental Protocols

Cannulated venules were bathed in control solution at 36° to 37°C to allow development (2 hours) of basal tone (Fig. 1). The vasomotor response to cumulative administration of ET-1 (1 pM to 1 nM; Bachem, Torrance, CA) was then evaluated.[20,21,30] Retinal venules were exposed to each concentration of ET-1 until a stable diameter was established (about 10 minutes). Because of the possible tachyphylaxis effect of ET-1 as noted in retinal arterioles[31] and the sustained vasoconstriction to ET-1 after washing out, only one concentration–response curve was evaluated in each vessel. In another series of studies, the role of ET_ARs in the retinal venular responses to ET-1 was evaluated after a 30-minute treatment of the vessel with ET_AR antagonist BQ123 (1 µM).[20,30] An additional group of retinal venules from pigs and human patients without diabetes was exposed to a high glucose solution for 2 hours and its impact on vasomotor responses to ET-1 was examined. The effect of hyperglycemia and ET_AR activation on venular constriction to ET-1 (0.1 nM) was evaluated after co-incubation of control or high glucose solution with BQ123 (1 µM) for 30 minutes.

Chemicals

All drugs were obtained from MilliporeSigma (St. Louis, MO). ET-1 was dissolved in water and BQ123

was dissolved in ethanol.[20,30] Subsequent concentrations of these drugs were diluted in PSS. The final concentration of ethanol in the vessel bath was less than 0.1% by volume, which had no effect on vasoconstrictor responses or basal tone in vehicle control studies.

Data Analysis

At the end of each experiment, the vessel was relaxed with 0.1 mM sodium nitroprusside in EDTA (1 mM) Ca^{2+}-free PSS to obtain its maximum diameter at 5 cm H_2O intraluminal pressure. Diameter changes in response to ET-1 were normalized to the resting diameter and expressed as percentage changes in diameter. Data are reported as mean ± standard error of the mean, and *n* represents the number of vessels. The repeated measures two-way analysis of variance followed by Bonferroni multiple-range test was used to determine the significance of experimental interventions, as appropriate (GraphPad Prism, Version 6.0, GraphPad Software, La Jolla, CA). A *P* value of less than 0.05 was considered significant.

Results

For the present study, a total of 14 human retinal venules from five patients were isolated with average maximum diameter of 68 ± 4 µm (range, 47–79 µm) at 5 cm H_2O lumenal pressure. In the presence of normal (5 mM) glucose, the human vessels (seven vessels, one to two per patient) developed stable basal tone by constricting to about 88% of maximum diameter (resting and maximum diameters are provided in Supplementary Fig. S1) within 2 hours at 36°C to 37°C. An image of a cannulated and pressurized human retinal venule is shown in Figure 2A. In the pig study, the average maximum diameters of retinal venules (six vessels, one per pig) in the presence of normal glucose was 106 ± 4 µm, and these vessels constricted to about 92% of maximum diameter (resting and maximum diameters are provided in Supplementary Fig. S1). Administration of ET-1 caused constriction of both human and porcine retinal venules in a concentration-dependent manner (Fig. 2B). The threshold concentration of ET-1 for venular constriction was about 1 pM, and the vessels constricted to about 40% to 50% of their resting diameters at 1 nM for both species (see Supplementary Fig. S2 for individual vessel responses).

Incubation of both human (Fig. 3A) and porcine (Fig. 3B) retinal venules with ET_AR antagonist BQ123 in the presence of normal glucose did not alter the resting vascular diameter but ET-1–induced vasoconstrictions were abolished (see Supplementary Fig. S3 for individual vessel responses). The individual responses of vessels from three human patients (without diabetes) to ET-1 after normal or high glucose (25 mM) exposure are shown in Figures 4A to 4C. In each patient, ET-1 produced concentration-dependent venular constrictions under normal level of glucose.

Under high glucose exposure for 2 hours, the resting vascular tone was not noticeably altered, but the constriction of retinal venules to ET-1 was consistently augmented in each patient (Figs. 4A–4C). The averaged responses to ET-1, in the presence of normal and high glucose, are shown in Figure 4D. Exposure of porcine retinal venules to high glucose enhanced vasoconstrictions to ET-1 (Fig. 5A) in a manner comparable with those of human vessels (see Supplementary

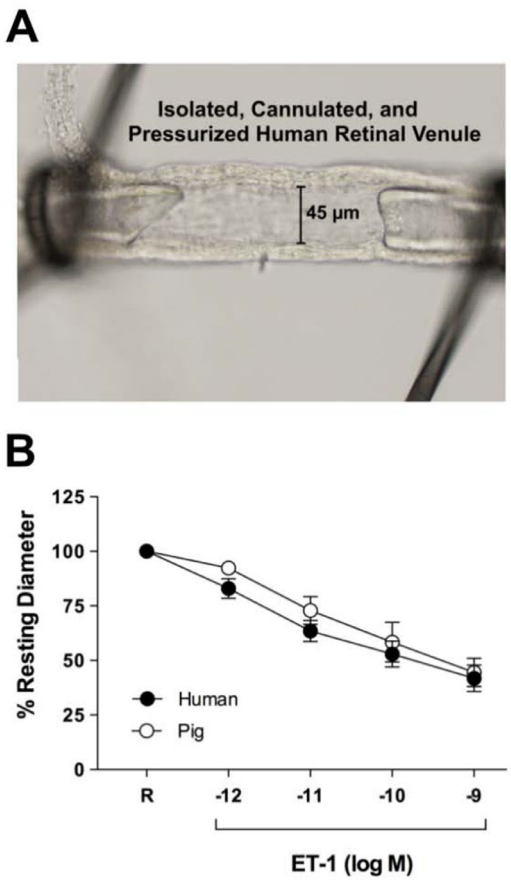

Figure 2. Vasomotor response of isolated and pressurized human and porcine retinal venules to ET-1. (A) Representative image of an isolated human retinal venule cannulated with glass micropipettes and secured with ophthalmic sutures. The vessel was transferred to the stage of an inverted microscope and was allowed to develop basal tone (45 µm internal diameter) at 5 cm H_2O intraluminal pressure. (B) Human ($n = 7$) and porcine ($n = 6$) venular diameters were recorded before (R, resting diameter) and after administration of ET-1.

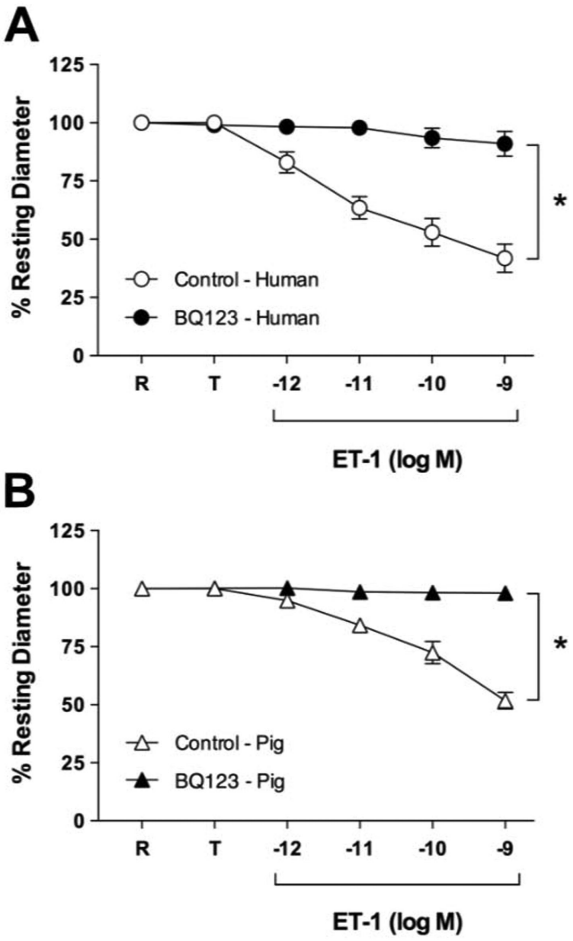

Figure 3. Contribution of ET_ARs to ET-1–induced constriction of isolated and pressurized human and porcine retinal venules. (A) Human and (B) porcine venular diameters were recorded before (R, resting diameter) and after 30-minute treatment (T) with ET_AR antagonist BQ123 (1 µM). In the absence of BQ123, retinal venules constricted in response to ET-1 in a concentration-dependent manner (human control, $n = 3$; porcine control, $n = 4$). Treatment with BQ123 (1 µM; BQ123 - human, $n = 3$; BQ123 - pig, $n = 4$) abolished venular constrictions to ET-1. *$P < 0.05$ vs. control.

Fig. S4 for individual vessel responses). The constriction of porcine retinal venules to 0.1 nM ET-1 during exposure to control normal glucose or high glucose was prevented in the presence of BQ123 (Fig. 5B).

Discussion

It is generally accepted that the vasomotor activity of venules in the microcirculation plays an important role in the regulation of tissue perfusion and fluid homeostasis by influencing the longitudinal pressure gradient and postcapillary pressure, respectively.[17] Alterations in this regulatory process may link to retinal pathophysiology, including retinal edema and diminished retinal perfusion. However, there is limited information regarding the vasomotor function of individual retinal venules, especially the responses of human retinal venules to the pathogenic vasoconstrictor ET-1. The salient findings of the present study show that ET-1 elicits mostly ET_AR-dependent constriction of human retinal venules, with enhanced vasoconstriction during acute exposure to a diabetic level of high glucose.

An earlier study has shown that the intravenous administration of ET-1 in healthy human subjects decreases blood velocity and flow in large retinal veins without altering their diameter,[32] but it could not rule out whether ET-1 directly influenced the diameter of the small retinal venules. Based on a previous evaluation of porcine retinal venular reactivity in vitro to various vasoconstrictors, ET-1 elicits the most potent response compared with the thromboxane analog U46619 and norepinephrine.[21] In the present study, we evaluated for the first time the direct vasomotor response of isolated and pressurized human retinal venules to ET-1. These vessels had an average maximum diameter of 68 µm, which was slightly smaller than the porcine retinal venules (about 100 µm) examined in the current study. Both human and porcine retinal venules developed comparable basal tone (constricted to about 88%–92% of the maximum diameter). Furthermore, human and porcine retinal venules displayed comparable vasoconstrictor responsiveness to ET-1 (Fig. 2B), with a threshold concentration of about 1 pM and a maximum constriction to about 50% of their resting diameters at the highest concentration tested (1 nM). These concentrations are within the range reported for vitreous fluid (picomolar range) in both experimental and clinical models[5,21,33] and the estimated level at the local microvasculature (nanomolar range),[34] which support the pathophysiologic relevance of these ET-1–induced vasoconstrictor responses. Although a low number of male human vessels was available for the present study, we did not find statistical significance in ET-1–induced constriction of retinal venules between male ($n = 3$) and female ($n = 3$) pigs.

The retinal arteriolar microcirculation contains two major receptor subtypes, ET_AR and ET_BR, for ET-1 binding and alteration of vasomotor tone.[7,30] In the present study, the ET-1–elicited constrictions of small human and porcine retinal venules appeared to be mediated solely by ET_AR, because selective ET_AR antagonist BQ123 (1 µM) eliminated the vasoconstriction (Fig. 3). This finding is consistent with our previous report that ET_BRs do not play a role in the constriction of large porcine retinal venules (averaged maximum diameter of about 133 µm) to ET-1.[20]

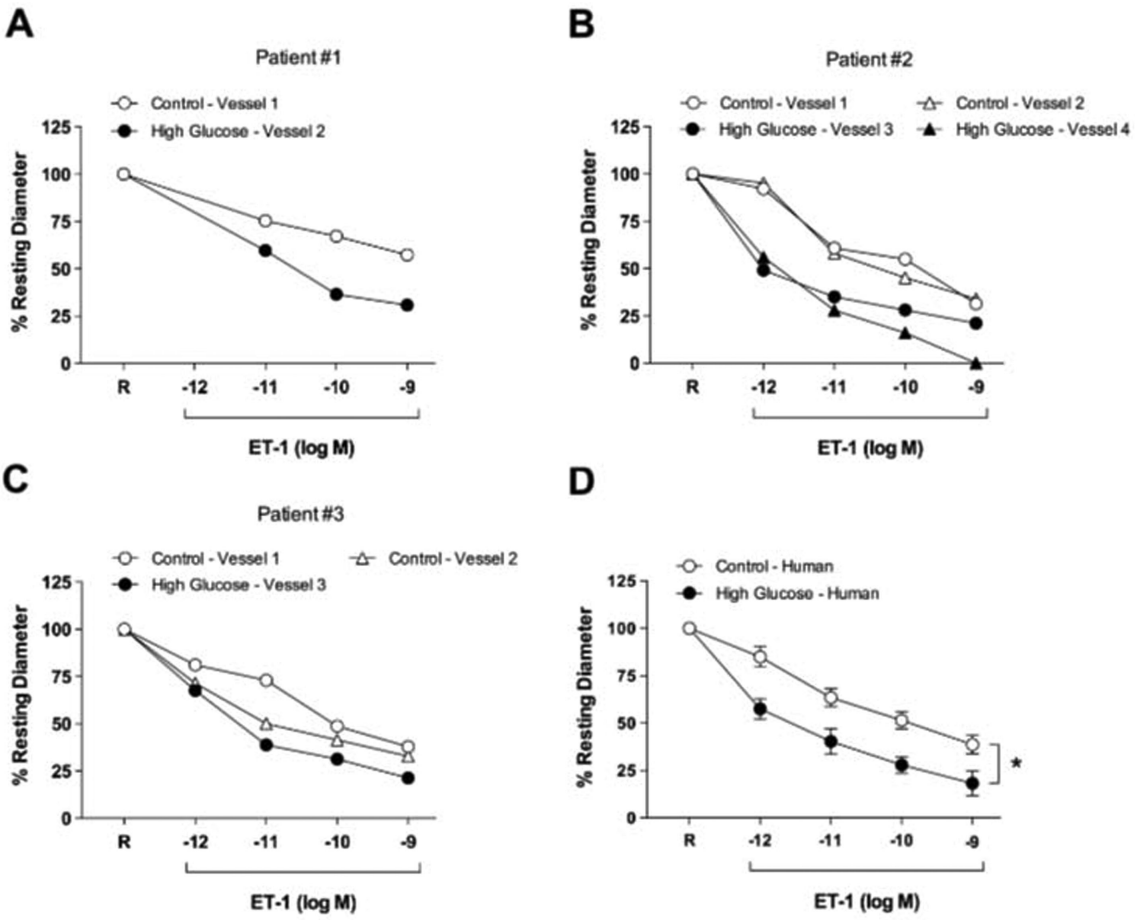

Figure 4. Impact of hyperglycemia on ET-1–induced constriction of isolated and pressurized human retinal venules. Venular diameters were recorded before (R, resting diameter) and after administration of ET-1 in the presence of control normal glucose (5 mM) or high glucose (25 mM). (A–C) Individual responses of human retinal venules isolated from three patients (A, 1 control vessel and 1 high glucose vessel; B, 2 control vessels and 2 high glucose vessels; C, 2 control vessels and 1 high glucose vessel). (D) Vasoconstrictions to ET-1 were significantly greater after 2 hours of intraluminal exposure to high glucose (control, $n = 5$; high glucose, $n = 4$). *$P < 0.05$ vs. control.

The nominal vasomotor influence of ET_BR in retinal venules was further supported by the inability of ET_BR agonist sarafotoxin to alter resting diameter of porcine retinal venules.[20] By contrast, porcine retinal arterioles constricted in response to sarafotoxin, which was abolished by selective ET_BR antagonist BQ788 (0.1 μM)[35] and unaffected by BQ123 (1 μM).[30] The 1 μM concentration of BQ123 used in the present study is in the range that has been shown to selectively prevent ET-1 binding to ET_ARs.[36] The median inhibitory concentration for BQ123 to inhibit the binding of ET-1 to ET_ARs in porcine aortic vascular smooth muscle cells and to ET_BRs in human glioblastoma cells and porcine cerebellar membranes was 7.3 nM and 9.7 to 18 μM, respectively.[36] Therefore, our current findings demonstrate for the first time in small human retinal venules that ET_AR is the dominant receptor subtype responsible for the vasoconstriction evoked by the physiologic and pathologic levels of ET-1. Taken together, the ability of human and porcine retinal venules to develop significant basal tone and to respond to ET-1 via activation of ET_AR with robust vasoconstriction supports the potential role of these vessels in the regulation of flow resistance, local pressure, and fluid exchange in the retinal microcirculation.

The retina is capable of synthesis and release of ET-1 via endothelin-converting enzyme-1, which was recently found to be functionally expressed in the porcine retinal microcirculation[30,37] and in bovine[38] and human[39] retinal tissues. It is reasonable to surmise that ET-1 may contribute to the retinal complications of diabetes, because multiple clinical studies have reported increased plasma[40] and vitreous[12] levels of ET-1 and decreased retinal blood flow[41] in patients with diabetes. Furthermore, we recently detected elevated ET-1 protein levels in the vitreous humor of

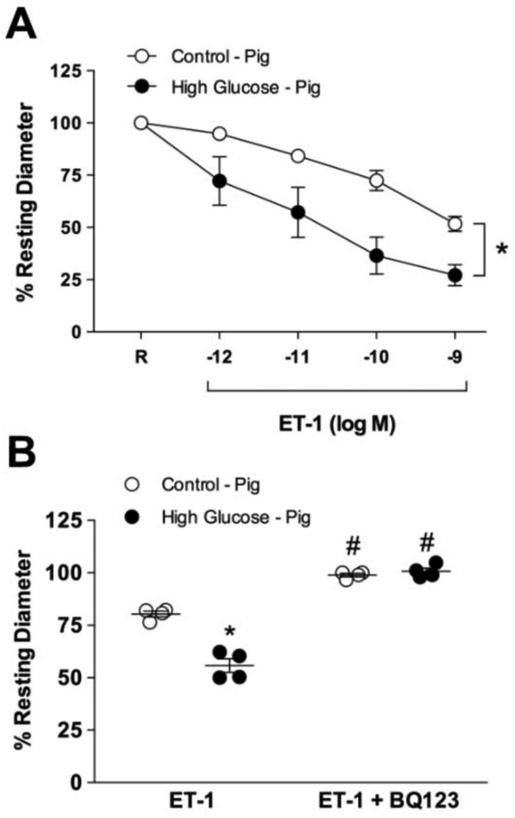

Figure 5. Impact of hyperglycemia and ET_AR activation on ET-1–induced constriction of isolated and pressurized porcine retinal venules. Venular diameters were recorded before (R, resting diameter) and after administration of ET-1 in the presence of control normal glucose (5 mM) or high glucose (25 mM). (A) Vasoconstrictions to ET-1 were significantly greater after 2 hours of intraluminal exposure to high glucose (control, $n = 4$; high glucose, $n = 4$). *$P < 0.05$ vs. control. (B) The vasoconstriction to 0.1 nM ET-1 during exposure to control ($n = 4$) or high glucose ($n = 4$) was abolished in the presence of ET_AR antagonist BQ123 (1 µM). *$P < 0.05$ vs. control; #$P < 0.05$ vs. ET-1.

pigs after 2 weeks of hyperglycemia.[21] Interestingly, hyperglycemia, either in vitro or in vivo, appears to enhance the constriction of large retinal venules to general vasoconstrictors, such as norepinephrine and thromboxane analog, with the most pronounced effect on ET-1.[21] It is conceivable that elevated levels of ET-1 in the vitreous in diabetes may exert a local influence not only on venules, but also on arterioles and pericyte-containing capillaries in the retinal microcirculation. However, previous studies have shown that hyperglycemia decreases the ET-1–induced contraction of bovine retinal pericytes in vitro.[42] Although we have shown that both in vitro and in vivo hyperglycemia impair endothelium-dependent nitric oxide-mediated dilation of isolated porcine retinal arterioles,[15,16] up to 12 weeks of in vivo hyperglycemia does not alter the vasoconstrictor responsiveness of these vessels to ET-1.[16] These earlier findings in retinal arterioles and our current results with retinal venules suggest a differential impact of hyperglycemia/diabetes on the vasoconstrictor function at different segments of vessels in the retinal microcirculation. Moreover, hyperglycemia during diabetes may promote the increased local retinal production of ET-1[21] that could preferentially enhance retinal venular constriction. To support the clinical relevance of this idea to the human retinal microcirculation, the present investigation showed for the first time that ET-1–induced constriction of retinal venules from patients without diabetes was augmented by a high level of glucose in the lumen (Fig. 4). Similar results were observed for the porcine retinal venules exposed to high glucose and the enhanced constriction of these vessels to a pathophysiologic level of 0.1 nM ET-1 was abolished in the presence of ET_AR blockade (Fig. 5). Future studies will assess the influence of high glucose on the ET-1–induced activation of ET_AR in human retinal venules. The current findings also suggest the potential promotion of retinal complications by altering reactivity of these microvessels with decreased retinal perfusion and evoked tissue edema if the compensatory or defense mechanisms against microvascular disturbance (e.g., blood flow dysregulation and disarray of tissue fluid homeostasis) are compromised in diabetes before the morphologic changes of diabetic retinopathy. The progression to severe diabetic retinopathy is associated with widening of large retinal venules (>120 µm),[43,44] possibly related to development of retinal arteriole to venule shunts that bypass the diminished perfusion of capillaries.[45,46] The impact of different stages of diabetes on the diameter of small retinal venules in humans, as evaluated in the present study, remain to be determined. Nonetheless, our findings herein provide new insight that early exposure to hyperglycemia in diabetes may influence vasomotor function of human retinal venules. Additional investigations are needed to address the potential alteration of signaling mechanisms, such as the reverse-mode sodium–calcium exchanger, contributing to the augmented ET-1–induced constriction of human retinal venules during hyperglycemia, as we have observed in porcine retinal venules.[21]

A potential limitation of the present study is the inability to determine whether melanoma or cardiovascular disease influences the results. Four of the five patients had documented risk factors for cardiovascular disease (i.e., diabetes, hypertension, and hyperlipidemia) and were being treated with appropriate medications. A small sample size for the different combinations of cardiovascular disease risk and

pharmacotherapy precludes the discovery of statistically significant differences based on these factors. It is important to note that access to human retinal tissue after enucleation is rare with only about one or two samples per year from our institution. Furthermore, the ability to obtain the retinal tissue within about 1 to 2 hours after enucleation has been critical to maintain venule viability for vasoreactivity studies. We did not have sufficient retinal tissue and vessels from patients with diabetes to investigate the effect of high glucose on the responsiveness to ET-1. However, their vessels seemed to constrict to ET-1 comparably with the patients without diabetes during normal glucose exposure. This finding is not surprising, because these patients did not have retinal complications (i.e., clinically apparent diabetic retinopathy) and the glucose level was controlled by the noninsulin antidiabetic drugs. Moreover, only one ET-1 response curve could be generated in an individual vessel because the constriction is sustained after washing out ET-1, so the control and treatment (BQ123 or high glucose) cannot be performed and compared in the same vessel. Nonetheless, our current findings unequivocally show that ET-1 causes constriction of retinal venules from patients without type 1 or type 2 diabetes, with an augmented response after exposure to high glucose in vitro.

In summary, we found that isolated human retinal venules develop stable basal tone and constrict to ET-1 by activation of ET_ARs. Our present studies revealed similarities in the ET-1–induced constriction of human and porcine retinal venules, which corroborates the pig as a relevant animal model for the study of vasomotor function in the retinal microcirculation. We also demonstrated that hyperglycemia does not influence basal tone, but rather enhances ET-1–induced constriction of small human and porcine retinal venules. Our findings provide the initial insight into the direct impact of hyperglycemia on the reactivity of small human retinal venules to ET-1, which may suggest a key therapeutic vascular target for retinal complications, especially in patients with diabetes or other retinal vascular diseases[47,48] related to elevated ET-1.

Acknowledgments

The authors thank Jonathan H. Tsai for his contribution to patient enrollment in the present studies.

Supported by NIH NEI R01EY023335 and R01EY024624 (T.W. Hein), the Retina Research Foundation (T.W. Hein and L. Kuo), and Liles Macular Degeneration Research Fund (R.H. Rosa, Jr.).

Disclosure: **Y.-L. Chen,** None; **R.H. Rosa Jr.,** None; **L. Kuo,** None; **T.W. Hein,** None

References

1. Lutty GA. Effects of diabetes on the eye. *Invest Ophthalmol Vis Sci*. 2013;54:ORSF81–ORSF87.
2. Wong TY, Cheung CM, Larsen M, Sharma S, Simo R. Diabetic retinopathy. *Nat Rev Dis Primers*. 2016;2:16012.
3. Bandello F, Battaglia Parodi M, Lanzetta P, et al. Diabetic macular edema. *Dev Ophthalmol*. 2017;58:102–138.
4. Salvatore S, Vingolo EM. Endothelin-1 role in human eye: a review. *J Ophthalmol*. 2010;2010:354645.
5. Roldan-Pallares M, Rollin R, Mediero A, et al. Immunoreactive ET-1 in the vitreous humor and epiretinal membranes of patients with proliferative vitreoretinopathy. *Mol Vis*. 2005;11:461–471.
6. Brunner F, Bras-Silva C, Cerdeira AS, Leite-Moreira AF. Cardiovascular endothelins: essential regulators of cardiovascular homeostasis. *Pharmacol Ther*. 2006;111:508–531.
7. Horinouchi T, Terada K, Higashi T, Miwa S. Endothelin receptor signaling: new insight into its regulatory mechanisms. *J Pharmacol Sci*. 2013;123:85–101.
8. Houde M, Desbiens L, D'Orleans-Juste P. Endothelin-1: biosynthesis, signaling and vasoreactivity. *Adv Pharmacol*. 2016;77:143–175.
9. Iglarz M, Clozel M. At the heart of tissue: endothelin system and end-organ damage. *Clin Sci (Lond)*. 2010;119:453–463.
10. Thengchaisri N, Hein TW, Ren Y, Kuo L. Endothelin-1 impairs coronary arteriolar dilation: role of p38 kinase-mediated superoxide production from NADPH oxidase. *J Mol Cell Cardiol*. 2015;86:75–84.
11. Tsai SH, Lu G, Xu X, Ren Y, Hein TW, Kuo L. Enhanced endothelin-1/Rho-kinase signalling and coronary microvascular dysfunction in hypertensive myocardial hypertrophy. *Cardiovasc Res*. 2017;113:1329–1337.
12. Oku H, Kida T, Sugiyama T, Hamada J, Sato B, Ikeda T. Possible involvement of endothelin-1 and nitric oxide in the pathogenesis of proliferative diabetic retinopathy. *Retina*. 2001;21:647–651.

13. Khuu LA, Tayyari F, Sivak JM, et al. Aqueous humor endothelin-1 and total retinal blood flow in patients with non-proliferative diabetic retinopathy. *Eye (Lond)*. 2017;31:1443–1450.
14. Needham M, McGahon MK, Bankhead P, et al. The role of K^+ and Cl^- channels in the regulation of retinal arteriolar tone and blood flow. *Invest Ophthalmol Vis Sci*. 2014;55:2157–2165.
15. Hein TW, Xu W, Xu X, Kuo L. Acute and chronic hyperglycemia elicit JIP1/JNK-mediated endothelial vasodilator dysfunction of retinal arterioles. *Invest Ophthalmol Vis Sci*. 2016;57:4333–4340.
16. Hein TW, Potts LB, Xu W, Yuen JZ, Kuo L. Temporal development of retinal arteriolar endothelial dysfunction in porcine type 1 diabetes. *Invest Ophthalmol Vis Sci*. 2012;53:7943–7949.
17. Davis MJ, Hill MA, Kuo L. Local regulation of microvascular perfusion. In: Tuma RF, Duran WN, Ley K (Eds.), *Handbook of physiology: the cardiovascular system, microcirculation (section 2)*. San Diego, CA: Academic Press; 2008:161–284.
18. Johnson PC. Overview of the microcirculation. In: Tuma RF, Duran WN, Ley K (Eds.), *Handbook of physiology: the cardiovascular system, microcirculation*. San Diego, CA: Academic Press; 2008:xi–xxiv.
19. Yu DY, Su EN, Cringle SJ, Morgan WH, McAllister IL, Yu PK. Local modulation of retinal vein tone. *Invest Ophthalmol Vis Sci*. 2016;57:412–419.
20. Chen YL, Ren Y, Xu W, Rosa RH, Jr, Kuo L, Hein TW. Constriction of retinal venules to endothelin-1: obligatory roles of ET_A receptors, extracellular calcium entry, and Rho kinase. *Invest Ophthalmol Vis Sci*. 2018;59:5167–5175.
21. Chen YL, Xu W, Rosa RH, Jr., Kuo L, Hein TW. Hyperglycemia enhances constriction of retinal venules via activation of the reverse-mode sodium-calcium exchanger. *Diabetes*. 2019;68:1624–1634.
22. Xie W, Zhao M, Tsai SH, et al. Correlation of spectral domain optical coherence tomography with histology and electron microscopy in the porcine retina. *Exp Eye Res*. 2018;177:181–190.
23. Xie W, Zhao M, Tsai SH, et al. Data on SD-OCT image acquisition, ultrastructural features, and horizontal tissue shrinkage in the porcine retina. *Data Brief*. 2018;21:1019–1025.
24. Sasaki A, Horiuchi N, Hasewgawa K, Uehara M. Development of diabetic retinopathy and its associated risk factors in type 2 diabetic patients in Osaka district, Japan: a long-term prospective study. *Diabetes Res Clin Pract*. 1990;10:257–263.
25. Xu J, Wei WB, Yuan MX, et al. Prevalence and risk factors for diabetic retinopathy: the Beijing communities diabetes study 6. *Retina*. 2012;32:322–329.
26. Hein TW, Yuan Z, Rosa RH, Jr, Kuo L. Requisite roles of A_{2A} receptors, nitric oxide, and K_{ATP} channels in retinal arteriolar dilation in response to adenosine. *Invest Ophthalmol Vis Sci*. 2005;46:2113–2119.
27. Hein TW, Xu W, Kuo L. Dilation of retinal arterioles in response to lactate: role of nitric oxide, guanylyl cyclase, and ATP-sensitive potassium channels. *Invest Ophthalmol Vis Sci*. 2006;47:693–699.
28. Hein TW, Rosa RH, Jr, Yuan Z, Roberts E, Kuo L. Divergent roles of nitric oxide and Rho kinase in vasomotor regulation of human retinal arterioles. *Invest Ophthalmol Vis Sci*. 2010;51:1583–1590.
29. Ishizaka H, Kuo L. Endothelial ATP-sensitive potassium channels mediate coronary microvascular dilation to hyperosmolarity. *Am J Physiol*. 1997;273:H104–H112.
30. Hein TW, Ren Y, Yuan Z, et al. Functional and molecular characterization of the endothelin system in retinal arterioles. *Invest Ophthalmol Vis Sci*. 2009;50:3329–3336.
31. Torring MS, Aalkjaer C, Bek T. Constriction of porcine retinal arterioles induced by endothelin-1 and the thromboxane analogue U46619 in vitro decreases with increasing vascular branching level. *Acta Ophthalmol*. 2014;92:232–237.
32. Polak K, Luksch A, Frank B, Jandrasits K, Polska E, Schmetterer L. Regulation of human retinal blood flow by endothelin-1. *Exp Eye Res*. 2003;76:633–640.
33. Adamiec-Mroczek J, Oficjalska-Mlynczak J, Misiuk-Hojlo M. Roles of endothelin-1 and selected proinflammatory cytokines in the pathogenesis of proliferative diabetic retinopathy: analysis of vitreous samples. *Cytokine*. 2010;49:269–274.
34. Masaki T, Yanagisawa M, Goto K. Physiology and pharmacology of endothelins. *Med Res Rev*. 1992;12:391–421.
35. Ishikawa K, Ihara M, Noguchi K, et al. Biochemical and pharmacological profile of a potent and selective endothelin B-receptor antagonist, BQ-788. *Proc Natl Acad Sci U S A*. 1994;91:4892–4896.
36. Ihara M, Ishikawa K, Fukuroda T, et al. In vitro biological profile of a highly potent novel endothelin (ET) antagonist BQ-123 selective for the ET_A receptor. *J Cardiovasc Pharmacol*. 1992;20(Suppl 12):S11–S14.
37. Potts LB, Bradley PD, Xu W, Kuo L, Hein TW. Role of endothelium in vasomotor responses to endothelin system and protein kinase C activation

in porcine retinal arterioles. *Invest Ophthalmol Vis Sci*. 2013;54:7587–7594.
38. Dibas A, Prasanna G, Yorio T. Localization of endothelin-converting enzyme in bovine optic nerve and retina. *J Ocul Pharmacol Ther*. 2005;21:288–297.
39. Wollensak G, Loffler B, Beyermann B, Ihling C. An immunohistochemical study of endothelin-1 converting enzyme in the human eye. *Curr Eye Res*. 2002;24:6–11.
40. Schneider JG, Tilly N, Hierl T, et al. Elevated plasma endothelin-1 levels in diabetes mellitus. *Am J Hypertens*. 2002;15:967–972.
41. Bursell SE, Clermont AC, Kinsley BT, Simonson DC, Aiello LM, Wolpert HA. Retinal blood flow changes in patients with insulin-dependent diabetes mellitus and no diabetic retinopathy. *Invest Ophthalmol Vis Sci*. 1996;37:886–897.
42. Chakravarthy U, McGinty A, McKillop J, Anderson P, Archer DB, Trimble ER. Altered endothelin-1 induced contraction and second messenger generation in bovine retinal microvascular pericytes cultured in high glucose medium. *Diabetologia*. 1994;37:36–42.
43. Kifley A, Wang JJ, Cugati S, Wong TY, Mitchell P. Retinal vascular caliber, diabetes, and retinopathy. *Am J Ophthalmol*. 2007;143:1024–1026.
44. Shao Q, Heussen FM, Ouyang Y, Hager A. Retinal vessel diameter changes in different severities of diabetic retinopathy by SD-OCT. *Eur J Ophthalmol*. 2016;26:342–346.
45. Bek T. Arterial oxygen saturation in neovascularizations in proliferative diabetic retinopathy. *Retina*. 2018;38:2301–2308.
46. Petersen L, Bek T. The oxygen saturation in vascular abnormalities depends on the extent of arteriovenous shunting in diabetic retinopathy. *Invest Ophthalmol Vis Sci*. 2019;60:3762–3767.
47. Iannaccone A, Letizia C, Pazzaglia S, Vingolo EM, Clemente G, Pannarale MR. Plasma endothelin-1 concentrations in patients with retinal vein occlusions. *Br J Ophthalmol*. 1998;82:498–503.
48. Zhang Y, Zhao L, Li H, Wang Y. Risk factors for hypertensive retinopathy in a Chinese population with hypertension: the Beijing eye study. *Exp Ther Med*. 2019;17:453–458.