Response of Rat Retinal Capillary Pericytes and Endothelial Cells to Glucose

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Abstract

Aim: The purpose of this study was to investigate the effects of hyperglycemia, its fluctuations, and glucose starvation on the expression of glucose-regulated protein 78/binding immunoglobulin protein (GRP78/BiP), one of the most commonly used markers of endoplasmic reticulum stress, in rat capillary pericytes and endothelial cells cultured separately and together.

Methods: Conditionally immortalized rat retinal pericyte and endothelial cell lines were cultured in dishes coated with collagen type I in Dulbecco's modified Eagle's medium containing 5.5 mM glucose. For cocultures, pericytes and endothelial cells were seeded together on rat tail collagen type I-coated cell culture plates. After 24 h of initial culture, the medium was replaced with serum-free medium containing 0–100 mM glucose for periods of up to 72 h. GRP78/BiP, caspase-3, and nuclear factor- κ B expression were investigated using western blots.

Results: No significant increase in GRP78/BiP expression was observed when pericytes, endothelial cells, or cocultures were exposed to either 25, 50, or 100 mM glucose for 48 h compared with the control level of 5.5 mM glucose. Similarly, no change in expression of GRP78/BiP was observed when media glucose levels were reduced from either 5.5 or 25 to 1 mM. GRP78/BiP expression significantly increased when cells were cultured for 24 h in glucose-deprived medium. This was accompanied by a time-dependent increase in the expression of caspase-3 and nuclear factor- κ B.

Conclusion: In diabetic retinopathy, hyperglycemia has been reported to induce apoptosis in retinal capillary vascular cells, but these studies suggest that the apoptosis is not linked to the expression of GRP78/BiP, one of the most commonly used markers of endoplasmic reticulum stress. However, GRP78/BiP-linked apoptosis may play a role in vascular changes associated with retinal ischemia/reperfusion.

Introduction

DIABETES MELLITUS (DM) is a metabolic disease that is characterized by the presence of hyperglycemia that leads to micro- and macrovascular abnormalities resulting in kidney failure, vision loss, neuropathy, and amputations. Diabetic retinopathy (DR) is primarily a vascular disease in which hyperglycemia is the underlying cause in patients with either type 1 or type 2 DM. This has been clinically confirmed by the Diabetes Control and Complications Trials (DCCT) and the United Kingdom Prospective Diabetes Study trials, which concluded that the development and progression of DR is directly related to the tight control of blood glucose levels.^{1,2} Microvascular lesions associated with DR also develop in animals fed a diet rich in galactose, indicating that the metabolism of either excess glucose or galactose results in the development of similar vascular

lesions.^{3–5} Similarly, the onset and progression of these vascular lesions in animals with either DM or galactosemia are also reduced by the tight control of hyperglycemia or the reduction of galactosemia, respectively.^{6–8}

In addition to hyperglycemia, the establishment of tight glycemic control is also associated with deleterious effects because the administration of insulin or hyperglycemic drugs often results in acute glucose fluctuations and hypoglycemia.⁹ For example, a 3-fold higher incidence of severe hypoglycemia was reported for patients receiving tight glycemic control in the DCCT trials compared with those receiving conventional glycemic control.¹⁰ Moreover, the establishment of tight glycemic control was accompanied by an initial worsening of DR.¹¹

Cellular responses to hyperglycemia are numerous and varied. In cells containing the enzyme aldose reductase (AR), hyperglycemia activates this enzyme, increases flux through

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the sorbitol pathway, and results in the intracellular accumulation of sorbitol.^{12,13} High glucose can also induce oxidative and nitrosative stress through the generation of reactive oxygen species (ROS) such as superoxide, nitric oxide, peroxynitrite, and their derivatives.¹⁴ Both ROS and sorbitol accumulation associated with hyperglycemia and hypoglycemia have been linked to functional changes and cell death through the activation of apoptotic signaling.^{13,15–18}

In the development of diabetes and its diabetic complications, cellular responses to hyperglycemia, its fluctuations, and the induction of hypoglycemia have been recently linked to stress of the endoplasmic reticulum (ER).^{19–26} In addition to being the primary intracellular compartment responsible for protein biosynthesis and folding, the ER is a signal-transducing site that responds to various cellular stressors that include hypoxia, oxidative stress, osmotic stress, and glucose levels.^{19,27,28} During ER stress, chaperone proteins within the ER, such as the glucose-regulated protein 78/ binding immunoglobulin protein (GRP78/BiP), are upregulated to promote cell survival by facilitating correct folding and assembly of ER proteins and preventing their aggregation.^{29–31} In an inactive state, GRP78/BiP separately binds to the inositol-requiring kinase 1 α (IRE-1 α), PERK-like ER kinase (PERK), and activating transcription factor 6 (ATF6) in the lumen of the ER. Accumulation of misfolded proteins causes a stressing of the ER and GRP78/BiP to dissociate from IRE-1 α , PERK, and ATF6, subsequently activating these 3 proximal ER stress sensors, which triggers the unfolded protein response (UPR). This UPR response induces genes to increase the capacity to fold proteins, that is, increase the production of chaperones such as GRP78/BiP. However, persistent and severe ER stress leads to an increased UPR resulting in cell apoptosis.³²

A number of studies describe ER stress in diabetic/hyperglycemic ocular and vascular tissues. In human lens epithelial cells and rat lenses, ER stress is activated by osmotic stress resulting from the AR-catalyzed intracellular accumulation of sugar alcohols from excess glucose or galactose. This, in turn, initiates UPR and the subsequent generation of ROS and apoptosis.²⁰ Hyperglycemia, but not oxidative stress, also directly induces ER stress in human umbilical chord vascular endothelial cells.³³ Increased ER stress is present in the retinas of diabetic Akita mice³⁴ and ER stress activates vascular endothelial growth factor levels in cultured human retinal pigment epithelial cells³⁵ and human vascular endothelial cells.^{34,36} Under nondiabetic conditions, ER stress in rat retinas has been also linked to neuronal abnormalities resulting from ischemia-reperfusion injury³⁷ and to retinal ganglion cell death in chronic rat glaucoma models.^{38–40} Moreover, the increased expression of GRP78/BiP can serve as a potential therapeutic agent for reducing ER stress-induced retinal cell death diseases⁴¹ and retinal degenerative diseases.⁴²

DR is characterized by vascular changes of the retinal capillary bed, which include pericyte loss, capillary basement membrane thickening, endothelial cell hypertrophy, and endothelial cell degeneration that leads to capillary non-perfusion and hypoxia. Retinal capillaries are composed of endothelial cells and pericytes, which can influence and communicate with one another through soluble growth factors, gap junctions, and adhesion molecules. These communications are central to vessel assembly, growth control, and normal function.⁴³ The selective loss of pericytes has been

reported to be the hallmark of DR^{44,45} and pericyte loss in dogs has been linked to AR activity^{17,46,47}; however, the selective loss of pericytes versus endothelial cells is controversial.^{48–52} Using rat retinal capillary pericyte (TR-rPCT) and endothelial (TR-iBRB) cells, we have previously reported that short-term hyperglycemia results in pericyte apoptosis that is linked to the AR-catalyzed accumulation of sorbitol and that pericytes exposed to hypoglycemia demonstrate ER stress-linked apoptotic signaling.⁵³ More recently, Leal et al. have reported that long-term hyperglycemia induces apoptosis in retinal endothelial (TR-iBRB) cells independent of caspase activation.⁵⁴ Here, we compare the effects of hyperglycemia and its fluctuations on ER stress in TR-rPCT and TR-iBRB cells cultured either separately or together.

Methods

Chemicals

All reagents and solvents were commercially obtained from Acros Organics and Fisher Chemicals (Fairlawn, NJ) or Sigma-Aldrich Corporation (St. Louis, MO) and utilized without further purification. All solvents were of reagent or high-performance liquid chromatography grade. Antibodies utilized were as follows: GRP78/BiP rabbit mAb, inhibitor of nuclear factor kappa-B kinase (IKK) alpha antibody [nuclear factor- κ B (NF- κ B)] rabbit mAb active + 3 antibody procaspase 3 rabbit mAb, and α -tubulin rabbit mAb were obtained from Abcam (Cambridge, MA); chemiluminescent reagent and peroxide, biotinylated protein ladder, prestained protein marker, and cell lysis buffer were from Cell Signaling Technology (Beverly, MA). Dulbecco's modified Eagle's medium (DMEM) cell culture media were obtained from Invitrogen (Carlsbad, CA). Rat tail collagen type I and endothelial cell growth factor (ECGF) were obtained from Roche Diagnostics (Indianapolis, IN). Polyacrylamide Reddy Gels (4%–15% Tris-HCl) and Trans-Blot[®] Transfer Medium nitrocellulose membrane were obtained from Bio-Rad Laboratories (Hercules, CA). Block Ace blocking solution was obtained from Dainippon Pharmaceutical (Osaka, Japan).

Retinal capillary pericytes

Frozen cell stocks of the TR-rPCT cells in cryotubes were thawed in a 37°C water bath, and following repeated removal of dimethyl sulfoxide with DMEM media, the cells were seeded into 75-cm² culture flasks coated with collagen type I and cultured in DMEM media containing glutamine, NaHCO₃, and streptomycin at 33°C in a humidified atmosphere of 5% CO₂ and 95% air until subconfluence (2–4 days). The cells were then passaged by treatment with trypsin–ethylenediaminetetraacetic acid when the cells reached 80%–90% confluence. The cells were then plated at a density of 2×10^4 in 96-well plates or 1×10^5 in 24-well plates depending on the experiment. After an overnight incubation, the attached pericytes were washed twice and incubated with serum-free DMEM containing specified concentrations of glucose for specific times. Only cells passaged <7 times were utilized.

Retinal capillary endothelial cells

Frozen cell stocks of the TR-iBRB cells in cryotubes were thawed in a 37°C water bath, and following removal of dimethyl sulfoxide with DMEM, the cells were seeded into

75-cm² culture flasks coated with collagen type I. The cells were then cultured in DMEM containing glutamine, ECGF, NaHCO₃, and streptomycin at 33°C in a humidified atmosphere of 5% CO₂ and 95% air until subconfluence (2–4 days). The cells were passaged by treatment with trypsin–ethylenediaminetetraacetic acid when the cells reached 80%–90% confluence. The cells were then plated at a density of 2×10^4 in 96-well plates, 1×10^5 in 24-well plates, or 2×10^6 in 100-mm dishes depending on the experiment. After an overnight incubation, the attached pericytes were washed twice and incubated with serum-free DMEM containing specified concentrations of glucose for specific times. Only cells passaged <7 times were utilized.

Coculture of pericytes and endothelial cells

These studies were conducted by combining TR-iPCT (5×10^4 cells) and TR-iBRB (0.3×10^4 cells) together and then seeding the mixture onto culture dishes coated with collagen type I. The mixture was cocultured using ECGF-containing medium. After 24 h of coculture, the medium was replaced with serum-free media containing varying concentrations of glucose as described earlier. In the rat, the reported ratio of retinal capillary pericytes to endothelial cells range from 1:1.1 to 1:1.8.^{55–57} The difference in seeded cell numbers reflect the faster growth rate of endothelial cells compared with pericytes. The adjustment in cell numbers utilized resulted in a slightly greater number of endothelial cells to pericytes at the time of medium change to the various concentrations of glucose employed.

Western blot analysis

Cells were washed 3 times with phosphate-buffered saline (PBS), harvested, and lysed in RIPA buffer (1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate in PBS) containing protease inhibitor mixture. Cells were disrupted in a syringe fitted with a 21-gauge needle, with cell debris removed by centrifugation at 13,000 rpm for 15 min at 4°C. Protein samples were prepared in sodium dodecyl sulfate–polyacrylamide gel electrophoresis sample buffer and equal amounts of protein were loaded onto polyacrylamide gels. Protein levels were quantified using the colorimetric DC Protein Assay (Bio-Rad Laboratories) and bovine serum albumin protein standards. Following electrophoresis, the separated proteins were blotted onto nitrocellulose membrane.

For ER stress studies, the membrane was blocked with 5% nonfat dry milk in 0.1% Tween-20 in PBS and incubated overnight at 4°C with primary antibody. The filters were washed 3 times with PBS-T and incubated with secondary antibody labeled with horseradish peroxidase for 1 h. The specific protein bands were made visible by incubating the membrane with luminol reagent and exposing the membrane to X-ray film. To ascertain the comparative expression and equal loading of these protein samples, the membrane was reprobed with antibody against α -tubulin as an internal control. The visualized protein levels were quantified using the NIH ImageJ program (<http://rsbweb.nih.gov/ij/>) and Image-Pro Plus software (Media Cybernetics, Silver Spring, MD).

Statistical analyses

The calculations and statistical analyses (analysis of variance) were conducted using OriginPro[®] software version 8.1

(OriginLab, Northampton, MA) and ProStat ver. 5.01 (Pearl River, NY). Differences with a $P < 0.05$ were defined as significant.

Results

Cell lines of TR-rPCT and TR-iBRB established from a transgenic rat harboring the temperature-sensitive simian virus 40 large T-antigen gene (Tg rat) were grown at 33°C on rat tail type I collagen-coated plates with DMEM containing 10% fetal bovine serum as previously described.⁵³ After reaching confluence, the effect of hyperglycemia and its fluctuations was investigated by varying media glucose levels over a 72-h period as summarized in Fig. 1. During the initial 24 h of culture, all cells were cultured in serum-free medium containing 5.5 mM glucose. For the second 24-h period, the cells were cultured in serum-free medium containing 5.5, 25, 50, or 100 mM glucose. Then, for the final 24-h period, serum-free media containing 0, 1, 5.5, 25, 50, or 100 mM glucose were utilized. All cell groups were then evaluated for changes in the expression of the GRP78/BiP, which is one of the most commonly used markers of ER stress because this protein directly interacts with all 3 ER stress sensors, PERK, ATF6, and IRE1, and maintains them in inactive forms in nonstressed cells.

As summarized in Fig. 2A, no significant increase in GRP78/BiP expression was observed when pericytes were cultured in media in which cells were exposed to either 25, 50, or 100 mM glucose for 48 h compared with the control level of 5.5 mM glucose. This indicates that unlike lens epithelial cells, increased hyperglycemia does not induce the expression of the ER stress chaperone GRP78/BiP in cultured pericytes. Moreover, no change in GRP78/BiP expression in pericytes was observed when media glucose levels were reduced from either 5.5 or 25 to 1 mM. However, compared with pericytes cultured continuously in serum-free medium containing 5.5 mM glucose, the expression of GRP78/BiP is only significantly ($P = 0.05$) increased when glucose levels were reduced from 5.5 to 0 mM for the final 24 h of culture and, to a lesser extent, when glucose levels were reduced from 25 to 0 mM glucose. Culture of endothelial cells under identical conditions resulted in similar results, with expression of GRP78/BiP in endothelial cells significantly increased ($P = 0.05$) only

FIG. 1. Outline of glucose concentrations and their variations utilized with pericytes, endothelial cells, and their cocultures. Color images available online at www.liebertonline.com/jop.

FIG. 2. Effect of glucose and its fluctuations on GRP78/BiP expression normalized to α -tubulin in (A) pericytes and (B) endothelial cells cultured for 72 h. Mean $\pm$ SD; $n = 3$; *significant ($P = 0.05$) increases compared with similar cells cultured for 72 h with 5.5 mM glucose. GRP78/BiP, glucose-regulated protein 78/binding immunoglobulin protein; SD, standard deviation. Color images available online at www.liebertonline.com/jop.

when these cells were cultured for the last 24 h in serum-free medium in which glucose was absent.

The failure of hyperglycemia to induce the expression of GRP78/BiP was confirmed by comparing expression changes in pericytes and endothelial cells cultured in 5.5 mM glucose for 24 h to the expression observed when these cells were cultured for 24 h with media containing either 0, 25, 50, or 100 mM glucose (Fig. 3A, B). Similarly, the induction of GRP78/BiP by glucose depletion was confirmed in both pericytes and endothelial cells by culturing these cells in glucose-free media for 24 h (Fig. 4A, B). Moreover, the induction of GRP78/BiP expression indicative of ER stress was mirrored by a similar sequential activation of caspase-3 and NF- κ B in both pericytes and epithelial cells (Fig. 4C–F). This suggests that GRP78/BiP activation that is linked to ER stress is induced by cellular glucose depletion rather than fluctuations of cellular glucose levels.

Communications between vascular pericytes and endothelial cells are central to vessel assembly, growth control, and normal function of capillaries. To investigate whether physi-

cal interactions between pericytes and endothelial cells could alter their response to glucose and its fluctuations, the glucose fluctuation studies as outlined in Fig. 1 were repeated with cocultures of approximately equal numbers of pericytes and endothelial cells. In these cocultures, which more closely mirror physiological interactions, no significant change in GRP78/BiP expression was observed when both cells together were exposed to either 25, 50, or 100 mM glucose for 48 h compared with the control level of 5.5 mM glucose (Fig. 5). Again, coculture of these cells in glucose-deprived media resulted in a time-dependent increase in GRP78/BiP, which was similar to that observed when pericytes and endothelial cells were cultured alone. This suggests that GRP78/BiP expression in cocultured pericytes and endothelial cells is similar to the expression observed in individual cell cultures.

Discussion

Although a number of animal models are available for investigating the onset and progression of DR, a majority of

FIG. 3. Effect of increased glucose concentrations on the expression of GRP78/BiP normalized to α -tubulin in (A) pericytes and (B) endothelial cells cultured for 24 h. Mean $\pm$ SD; $n = 3$; *significant ($P = 0.05$) increases compared with the initial 5.5 mM glucose.

studies have utilized rats. Nevertheless, specific biochemical investigations on rat retinal capillary pericytes and endothelial cells have been minimal. This may be due, in part, to the difficulty of obtaining adequate amounts of retinal vascular tissue from the rat eye for such studies. The recent availability of TR-rPCT and TR-iBRB cell lines now makes such studies possible. The cell lines used here demonstrate the common characteristics of all retinal capillary vascular cells, that is, the pericytes contain smooth muscle actin, whereas the rat

endothelial cells possess von Willebrand factor and incorporate acetylated low-density lipoprotein.⁵³ Both rat pericyte and endothelial cells express AR; however, only the pericytes accumulate sorbitol, suggesting that the levels of AR present in endothelial cells are too low for significant sorbitol production to occur under hyperglycemic conditions. Compared with endothelial cells, culturing pericytes in serum-free media containing ≥ 25 mM glucose for 24 h results in rapid accumulation of sorbitol and apoptosis, both of which are

FIG. 4. Time-dependent effect of glucose deprivation on the expression of GRP78/BiP (A, B), caspase 3 (C, D), and NF-κB (E, F) normalized to α-tubulin in pericytes and endothelial cells, respectively, which were cultured for 24 h in glucose-absent medium. Mean $\pm$ SD; $n = 3$; *significant ($P = 0.05$) increases compared with the initial 0 time point. NF-κB, nuclear factor-κB.

decreased by the AR inhibitor (ARI) AL-1576, which inhibits sorbitol accumulation. Although we failed to observe endothelial cell apoptosis,⁵³ others have reported apoptosis when these endothelial cells are cultured for 7 days in media containing 30 mM glucose.⁵⁴ Pericyte degeneration is considered the hallmark of DR that leads to localized endothelial cell proliferation and the formation of microaneurysms, whereas the subsequent degeneration of endothelial cells results in acellular capillaries, areas of vascular nonperfusion that result in retinal hypoxia and the induction of vascular endothelial growth factor. *In vivo*, these vascular changes in diabetic rats are inhibited by the administration of ARIs.^{5,58,59}

Rats with DM rapidly form cataracts prior to the development of retinal changes. In these rats, sorbitol accumulation in lens cells has been established to initiate cataract formation and the onset and progression of these cataracts can be inhibited when ARIs are administered at the early stages of DM.^{6,60} The actual accumulation of sorbitol in lens cells,

however, does not initiate cataract formation. Instead, the intracellular accumulation of sorbitol within the lens epithelial cells and superficial lens fibers initiates osmotic stress that causes cellular hydration and the subsequent rupture of the lens fibers.^{61,62} Sorbitol-linked osmotic stress also activates GRP78/BiP-associated ER stress in lens cells that results in an UPR and the subsequent generation of ROS and induction of apoptosis.²⁰ Like the lens cells, the TR-rPCT cells also rapidly accumulate sorbitol and undergo apoptosis that is inhibited by ARIs.⁵³ However, the present studies indicate that retinal capillary cells clearly differ from lens cells in that they do not demonstrate sorbitol-linked activation of GRP78/BiP that is associated with ER stress and leads to ROS generation and apoptosis. In the TR-rPCT cells, activation of GRP78/BiP has been reported to occur only when these cells are exposed to hypoglycemic conditions. This suggests that ER stress is activated only when glucose concentrations were reduced from high to low or no glucose.⁶³ As the establishment of strict

FIG. 5. (A) Effect of glucose and its fluctuations on GRP78/BiP expression normalized with α -tubulin in cocultures of pericytes and endothelial cells cultured for 72 h. (B) Effect of glucose deprivation on GRP78/BiP expression normalized with α -tubulin in cocultures of pericytes and endothelial cells cultured for 24 h in glucose-absent media. (C) Effect of increased glucose concentrations on the expression of GRP78/BiP normalized with α -tubulin in cocultures of pericytes and endothelial cells cultured for 24 h. Mean $\pm$ SD; $n = 3$. *In A and C, significant ($P = 0.05$) increases compared with similar cells cultured for 72 h with 5.5 mM glucose; in B, significant ($P = 0.05$) increases compared with the initial 0 time point. Color images available online at www.liebertonline.com/jop.

glycemic control is associated with increased episodes of hypoglycemia,⁶⁴ one could postulate that the initial worsening of DR observed in the DCCT when tight control of hyperglycemia is established may be linked to ER stress.¹⁰ The present results confirm the observation that GRP78/BiP-associated ER stress is not linked to hyperglycemia in rat retinal capillary pericytes, but expand this observation to also include rat retinal capillary endothelial cells, and cocultures of these cells. Further, GRP78/BiP induction was similarly observed in both pericytes and endothelial cells only when the cells were cultured in the absence of glucose rather than by the rapid reduction of glucose levels to 1 mM (Fig. 2). These results indicate that actual cellular glucose deprivation rather than low, hypoglycemic levels are required to induce GRP78/BiP. Therefore, these observations do not support the premise that GRP78/BiP-associated ER stress is linked to an initial worsening of DR observed when tight control of hyperglycemia is established. As retinal vascular injury is linked to both an increase and fluctuations of blood glucose levels, these studies fail to confirm that GRP78/BiP-associated ER stress plays a role in the onset of retinal vascular changes associated with DR. Li et al. similarly failed to demonstrate an upregulation of GRP78/BiP and ATF4, which are indicative of ER stress *in vivo* with 6-month diabetic rats or *in vitro* human retinal endothelial cells cultured in up to 50 mM glucose.⁶⁵ However, additional studies are required to conclusively rule out a role for ER stress in hyperglycemia-induced vascular changes.

Increased expression of GRP78/BiP by glucose depletion has been reported in other cells including astrocytes,⁶⁶ chondrocytes,⁶⁷ hippocampal neuroblasts,⁶⁸ or pheochromocytoma (PC12) cells.⁶⁹ Glucose deprivation leads to oxidative stress and an altered cellular redox state.⁷⁰ As folding of many secretory proteins in the ER depends upon the formation of disulfide bonds,⁷¹ changes in cellular redox resulting from glucose deprivation could contribute to ER stress by interfering with disulfide bonding in the ER lumen. Alternatively, glucose deprivation could interfere with N-linked protein glycosylation in the ER.⁷² The induction of glucose-related proteins such as GRP78/BiP is an important cellular defense system that is focused on the restoration of ER function.³⁰ This family of proteins was originally discovered in chicken embryo fibroblasts when these cells were cultured in glucose-free media.⁷³ Among this family, GRP78/BiP is the most evolutionarily conserved protein. As upregulation of GRP78/BiP to ER stress is pivotal to cell survival, it is not surprising that this protein was expressed in glucose-deprived pericytes and endothelial cells.

ER stress triggered UPR results in 3 types of effector functions.⁷⁴ The first is adaptation, that is, the reestablishment of homeostatic and normal ER function, which involves the expression of genes capable of enhancing protein folding capacity. An example is the time-dependent increased expression of the chaperone GRP78/BiP observed as both pericytes and endothelial cells or their cocultures became glucose deprived (Fig. 4A, B and Fig. 5B). The second is alarm, which refers to signal induction events that include activation of mitogen-activated protein kinases, Jun N-terminal kinase, p38 mitogen-activated protein kinase, and kinases responsible for activation of the transcription factor NF- κ B. NF- κ B acts as a "first responder" to harmful cellular stimuli and is a regulator of cell death or survival. The final step is cell death, which occurs when the adaptive mecha-

nisms of the UPR fail to reduce the protracted or excessive unfolding in the ER. Activation of NF- κ B is widely accepted to play an antiapoptotic role in cellular responses to injury, whereas caspase-3 is a frequently activated death protease that catalyzes the specific cleavage of many key cellular proteins. Activation of both are often observed during apoptosis as, for example, in mouse bEnd.3 cells and primary human brain microvascular endothelial cells where apoptosis is preceded by both NF- κ B and caspase-3 activation.⁷⁵ The present results (Fig. 4C–F) also demonstrate that glucose deprivation of both pericytes and endothelial cells result not only in increased GRP78/BiP expression but also in activation of NF- κ B and caspase-3.

Chronic retinal vascular occlusions and subsequent ischemia of the retina can result in altered blood flow and areas of nonperfusion that result in localized hypoxia and glucose deprivation. Therefore, it is likely that the observed response of both vascular pericytes and endothelial cells to glucose deprivation can also occur as a result of vascular occlusions/ischemia during DR. Moreover, this mechanism should occur as a result of nondiabetic vascular occlusions or retinal ischemia-reperfusion. Further studies are needed to confirm this premise.

Author Disclosure Statement

No competing financial interests exist.

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Received: April 12, 2010

Accepted: September 24, 2010

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