Kallikrein-Binding Protein Levels Are Reduced in the Retinas of Streptozotocin-Induced Diabetic Rats

Heather C. Hatcher,* Jian-Xing Ma,* Julie Chao,† Lee Chao,† and Anna Ottlecz‡

Purpose. To determine the involvement of rat kallikrein-binding protein (RKBP) in the development of diabetic retinopathy.

Methods. Diabetes was induced by streptozotocin (STZ) (55 mg/kg body weight in 0.05 M citrate buffer, pH 4.5) in male Sprague–Dawley rats (150 to 175 g, 6 weeks old) as confirmed by hyperglycemia and reduced body weight. Retinas were dissected from animals at 1, 2, and 4 months of diabetes. The functional activity of RKBP in retinal homogenates was determined by its complex formation with tissue kallikrein. Immunoreactive RKBP levels were measured by enzyme-linked immunosorbent assay. The RKBP messenger RNA (mRNA) levels in the retina were measured by Northern blot analysis using the RKBP complementary DNA probe. The activity of total Na+,K+-ATPase was determined by a radioassay. Total protein concentration was determined by a protein assay.

Results. The kallikrein-binding activity was reduced in the retinas of STZ-diabetic rats at 1 (59%), 2 (50%), and 4 (38%) months of diabetes compared to those of age-matched control subjects. Levels of immunoreactive RKBP were significantly lower in the diabetic animals at each time point examined compared to those of control subjects. At 1 and 2 months of diabetes, RKBP levels (nanogram/milligram protein) were decreased significantly to 6.9 ± 0.7 (n = 8) and 10.6 ± 1.0 (n = 8), respectively, compared to those of age-matched control subjects (14.1 ± 0.7, n = 8, P < 0.001, and 14.1 ± 1.2, n = 8, P < 0.01). At 4 months of diabetes, retinal RKBP levels were lower in both control and diabetic groups, but RKBP levels in diabetic groups were significantly lower (5.8 ± 0.6, n = 8) than those of age-matched control subjects (8.4 ± 0.9, n = 8, P < 0.01). Similarly, Northern blot analysis showed that RKBP mRNA levels were reduced in the retina of each group of STZ-diabetic rats, suggesting that the decrease in RKBP occurred at the level of transcription.

Conclusions. The results show that STZ-induced diabetic rats have decreased retinal RKBP; moreover, this suggests that RKBP may contribute to diabetic retinopathy. Invest Ophthalmol Vis Sci. 1997;38:658–664.

The tissue kallikrein-kinin system consists of tissue kallikrein, kallikrein-binding protein (KBP), kinins, kinin-precursor kininogens, kinin receptors, and kininases.1 Tissue or glandular kallikrein is a serine proteinase that releases kinins from kininogen substrates. Kinins are potent vasoactive peptides that interact with their receptors on the cell surface and mediate vasodilation and blood pressure reduction, and they regulate local blood flow and rate of tissue metabolism. Kinins also induce smooth muscle contraction, cell proliferation, and inflammatory responses.1–4 Tissue kallikrein is regulated at the transcriptional level by multiple hormones5 and at the posttranslational level by its modulator, KBP.6–8 KBP binds covalently with tissue kallikrein and inhibits its activities in vitro.7,8 In addition, KBP has a profound effect on the clearance rate of tissue kallikrein in vivo because it prolongs the half-life of kallikrein.10 These results suggest that KBP is a modulator of tissue kallikrein’s activity and bioavailability.11 The physiological function of KBP is currently unknown; however, several lines of evidence indicate that it may participate in blood pressure regulation, inflammatory response, growth, and development.11–14

Studies in diabetic animal models and patients

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with diabetes show potential involvement of the tissue kallikrein-kinin system in diabetes mellitus.\textsuperscript{15,16} In streptozotocin (STZ)-diabetic rats, tissue kallikrein levels in the submandibular gland, kidney, and plasma are reduced.\textsuperscript{17–19} Urinary active kallikrein was reduced, which resulted in the decreased ratio of active-to-total kallikrein.\textsuperscript{20} Conversely, mice with diabetes and patients with diabetes were found to have increased renal kallikrein levels.\textsuperscript{21–24} In addition, the renal kallikrein activity was increased significantly in genetic C57Bl/KsJ-mdh mice with diabetes. Therefore, the alterations of kallikrein levels may be dependent on the mechanism or type of diabetes.\textsuperscript{22} The mechanism linking the tissue kallikrein-kinin system to diabetic complications remains unclear. Tissue kallikrein is prevalent in vascular tissues.\textsuperscript{25} The high efficiency with which kinins regulate vasomotion implies that they are regulated tightly.\textsuperscript{26} Abnormalities in the tissue kallikrein-kinin system, which involve overproduction of kinin, have been implicated in vasodilation, vascular impairment, and hyperfiltration in diabetes.\textsuperscript{26} Moreover, kinin has been shown to have a proliferation-stimulating effect.\textsuperscript{1} Our recent studies have identified high levels of kallistatin, a human counterpart of rat kallikrein-binding protein (RKBP), in various ocular tissues, including retinal neuronal cells and retinal capillary endothelial cells.\textsuperscript{27} Moreover, vitreous kallistatin concentrations in patients with diabetes were significantly lower than those in nondiabetic vitreous fluids.\textsuperscript{27} To elucidate the role of RKBP in the pathogenesis of diabetic retinopathy, we have identified the expression and quantified RKBP levels in the retina of a diabetic rat model.

METHODS

Materials

Adenosine triphosphate (ATP; Tris-salt, vanadium free), ethylenediaminetetraacetic acid (EDTA), EGTA, Hanks’ balanced salt solution, Hepes, and charcoal-activated and glucose oxidase kits were from Sigma Chemical (St. Louis, MO). [γ-\textsuperscript{32}P]ATP and [α-\textsuperscript{32}P]dCTP were purchased from New England Nuclear (Boston, MA). Protein assay kits were purchased from Bio-Rad Laboratories (Richmond, CA) and bovine serum albumin as a standard according to the protocol provided by the manufacturer.

Animals

Sprague–Dawley rats (male albino, 150 to 175 g, 6 weeks old) were purchased from Harlan Sprague–Dawley (Houston, TX), housed in stainless steel cages, and fed standard rat chow and tap water ad libitum. The animals were in a room on a 12-hour light–dark cycle with an ambient temperature of 22° ± 1°C. All experimental and animal care procedures were performed in compliance with the Guide for the Care and Use of Laboratory Animals (DHHS Publication No. NIH 85-23, revised 1985) and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Animals were injected intraperitoneally with STZ (55 mg/kg body weight in 0.05 M citrate buffer, pH 4.5) or citrate buffer alone after a 20-hour fast. Diabetes was verified using the Elite glucometer from Bayer (Elkhart, IN) 3 days after the injection of STZ. Animals with a blood glucose level ≥300 mg/dl and a body weight reduction >70g compared to those of control subjects were considered diabetic. The diabetic animals did not receive any exogenous insulin. Body weight was measured and animals were killed by decapitation at 1, 2, and 4 months after injection. Blood samples were collected to measure serum glucose levels using a glucose oxidase kit (Sigma), and the animals’ retinas were dissected within 30 seconds of death.

Preparation of Retinal Homogenates

Retinas were rinsed in Hanks’ balanced salt solution (pH 7.4). One retina from each animal was placed into 800 μl of ice cold 0.05 M Hepes buffer (pH 7.4) and homogenized in a glass homogenizer (Teflon; Thomas Scientific, Swedesboro, NJ) using 20 strokes for enzyme-linked immunosorbent assay (ELISA) and adenosinetriphosphatase (ATPase) assay. The other retina from each rat was placed into GIT buffer (4.0 M guanidine isothiocyanate, 0.1 M Tris–HCl, 1% β-mercaptoethanol, pH 7.5) for RKBP messenger RNA (mRNA) studies. Samples were kept frozen at −70°C until use.

Protein Assay

Protein concentration was measured using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond, CA) and bovine serum albumin as a standard according to the protocol provided by the manufacturer.

Na\textsuperscript{+}K\textsuperscript{+}-ATPase Assay

After thawing, cellular debris was removed by centrifugation (1000g, 5 minutes), plasma membranes were permeabilized by freezing and thawing (three cycles), and aliquots of supernatants were used for ATPase determinations. Total ATPase activity was determined in retinal homogenates by measuring inorganic phosphate (P\textsubscript{i}) liberated from [γ-\textsuperscript{32}P]ATP according to Kowluru et al\textsuperscript{28} with slight modifications. Briefly, the assay mixture contained 50 mM Hepes, 100 mM NaCl, 10 mM potassium chloride, 4 mM magnesium chloride, 1 mM EDTA, 0.25 mM EGTA (pH 7.4), and 10 μl retinal homogenate (5 to 7 μg of protein) in either the absence or presence of 10\textsuperscript{–5} M ouabain in a final volume of 50 μl. The reaction was initiated by the addition of ATP [γ-\textsuperscript{32}P]ATP: ~30,000 cpm/nmol, 2
mM final concentration of unlabeled ATP and proceeded for 20 minutes at 37°C.

**Tissue Kallikrein– Rat Kallikrein-Binding Protein Complex Formation**

Retina tissue homogenates were pooled in each group, and protein concentrations were measured. Tissue homogenates (160 μg of protein) were incubated with [125I]-labeled rat tissue kallikrein (10,000 cpm/sample) in 10 mM sodium phosphate buffer, pH 7.0, at 37°C for 1 hour as described previously. The binding was stopped by adding one-third volume of PBS containing 0.1% Tween-20 and 0.5% gelatin, were added to individual wells. The mixture then was resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis under nonreducing conditions and stained with Coomassie blue. The gel was dried and exposed to Kodak X-Omat film (Eastman Kodak, Rochester, NY).

**Enzyme-Linked Immunosorbent Assay**

ELISA was modified from a method described previously. A 96-well microtiter plate was coated with nonlabeled anti-RKBP immunoglobulin G (1 μg/ml in phosphate-buffered saline (PBS), 100 μl/well) overnight at 4°C. The plate then was blocked with 200 μl/well of PBS (10 mM sodium phosphate, pH 7.4, 150 mM NaCl) containing 1% bovine serum albumin at 37°C for 1 hour and washed three times with PBS containing 0.1% Tween-20 (washing solution). Dilutions of purified RKBP (0.4 to 25 ng/ml) and retina homogenates, which were diluted in 100 μl of freshly made dilution buffer (PBS containing 0.05% Tween-20 and 0.5% gelatin), were added to individual wells. The plate was incubated at 37°C for 90 minutes and washed three times with the washing solution. Biotin-labeled anti-RKBP antibody was added into each well at a concentration of 1 μg/ml in a total volume of 100 μl. The plate was incubated at 37°C for 1 hour and washed three times with the washing solution and once with PBS. Freshly prepared substrate solution (10 ml of 0.1 M citrate buffer, pH 4.3, 3 mg of 2,2'-azino-bis[3-ethylbenzthiazoline-sulfonic acid], and 10 μl of 3% hydrogen peroxide) was added into each well (100 μl/well). After 30 minutes, the plate was measured with an ELISA reader at 414 nm for absorbance.

**Extraction of RNA From Rat Retina**

The dissected retinas were homogenized in the GIT buffer with an ultrasonicator, and the resulting homogenate was layered on a 750 μl cushion of 5.7 M cesium chloride in a clear ultracentrifuge tube. The sample was centrifuged at 135,000 g in a TLA-100 rotor at 20°C for 4 hours. After centrifugation, the supernatant was removed carefully, and the RNA pellet was washed three times with cold 70% ethanol. The pellet was dried in a Speed-Vac (Savant, Farmingdale, NY) and resuspended in diethyl pyrocarbonate-treated water (30 μl) and transferred to a microcentrifuge tube.

**Northern Blot Analysis**

The RNA extracted from rat retina was electrophoresed through a 1.2% agarose gel containing formaldehyde. The samples were prepared by mixing the following in a sterile microfuge tube: 4.5 μl (20 μg) RNA, 2.0 μl 5 × MOPS running buffer (1 × MOPS running buffer = 20 mM 3-[N-morpholino] propanesulfonic acid, pH 7.0, 8 mM sodium citrate, 1 mM EDTA), 3.5 μl formaldehyde, and 10.0 μl formamide. The samples were heated for 15 minutes at 65°C and then chilled on ice. After a brief centrifugation, 2 μl of formaldehyde gel-loading buffer (50% glycerol, 1 mM EDTA, 0.25% bromophenol blue, 0.25% xylene cyanole FF) was added to each tube. The gel was run submerged in 1 × MOPS buffer at 3 to 4 V/cm for approximately 3 hours. At the end of the run, the molecular weight marker was cut from the gel, stained with ethidium bromide for 30 minutes, and photographed under ultraviolet light. The RNA from the gel was transferred to a nylon membrane by capillary transfer using 20 × SSC (1 × SSC = 0.15 M sodium chloride, 15 mM sodium citrate, pH 7.0). The RNA was ultraviolet-crosslinked to the nylon membrane. Full-length RKBP complementary DNA was nick-translated with [α-32P]dCTP and hybridized with the membrane in a hybridization solution containing 5 × SSPE (1 × SSPE = 0.18 M NaCl, 10 mM monosodium acid phosphate, 1 mM EDTA, pH 7.4), 5 × Denhardt’s solution (0.1% Ficoll 400, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin), 0.5% SDS, and 100 μg/ml denatured herring sperm DNA at 60°C overnight. The membrane was washed in a final solution of 6 × SSPE, 0.1% SDS twice at 60°C, and exposed to Kodak X-Omat film (Eastman Kodak, Rochester, NY) at −70°C.

**Data Analysis**

Retinal RKBP levels were averaged within a group and presented as mean ± standard error of the mean. The significance of the difference between two groups was determined by Student’s t-test.

**RESULTS**

**Streptozotocin-Induced Diabetes and Alteration of the Total Na⁺,K⁺-ATPase Activity in the Retina**

Body weight and blood glucose levels (Table 1) were used to assess the general condition of STZ-induced diabetic rats. Diabetic rats displayed significant growth retardation that was reflected in their lower body...
Reduced Retinal Kallikrein-Binding Protein in Diabetic Rats

TABLE 1. Body Weight, Blood Glucose Level, and Retinal Na⁺, K⁺-ATPase

<table>
<thead>
<tr>
<th>Group</th>
<th>Month Injection</th>
<th>n</th>
<th>Body Weight* (g)</th>
<th>Blood Glucose* (mg/dl)</th>
<th>Total Na⁺, K⁺-ATPase† (nmol P./minute · mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>8</td>
<td>325 ± 2.8</td>
<td>104 ± 2.8</td>
<td>216 ± 7.69</td>
</tr>
<tr>
<td>Diabetic</td>
<td>1</td>
<td>8</td>
<td>218 ± 10.2</td>
<td>506 ± 21.1</td>
<td>211 ± 8.20</td>
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<tr>
<td>Control</td>
<td>2</td>
<td>8</td>
<td>411 ± 4.8</td>
<td>106 ± 5.1</td>
<td>220 ± 3.86</td>
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<tr>
<td>Diabetic</td>
<td>2</td>
<td>8</td>
<td>314 ± 6.5</td>
<td>527 ± 25.3</td>
<td>188 ± 2.62</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>8</td>
<td>531 ± 7.6</td>
<td>92 ± 1.0</td>
<td>222 ± 3.97</td>
</tr>
<tr>
<td>Diabetic</td>
<td>4</td>
<td>8</td>
<td>388 ± 9.2</td>
<td>486 ± 16.6</td>
<td>190 ± 7.41</td>
</tr>
</tbody>
</table>

n = number of rats per group.
* Values represent mean ± SEM at the time of death.
† The total Na⁺, K⁺-ATPase activity was the difference between the activities recorded in the absence and presence of 10⁻⁴ M ouabain.
‡ P < 0.05 versus age-matched controls.

weight compared to that of age-matched control subjects. Blood glucose levels were fourfold to fivefold higher in diabetic animals than in age-matched control subjects. The total Na⁺, K⁺-ATPase activity was significantly lower in the retinas of diabetic rats after 2 (16%) and 4 months (15%) of the disease compared to that of age-matched control subjects (Table 1).

Analysis of Kallikrein-Binding Activity in the Retina
The RKBP is present in retina tissue homogenates from all groups as it formed an SDS-stable complex with rat tissue kallikrein. The molecular weight of the complex was 92 kDa, which is identical to that of the complex formed from purified RKBP and tissue kallikrein, indicating the existence of a functional RKBP in rat retina. As measured by densitometry, the quantities of the high molecular weight complex were decreased in the retinas of diabetic rats at 1 (59%), 2 (50%), and 4 (38%) months of diabetes compared to their age-matched control subjects, when the same amount of protein was used for the complex formation assay (Fig. 1). These results indicate that the functional RKBP is reduced in retinas of diabetic rats.

Measurements of the Immunoreactive Rat Kallikrein-Binding Protein in the Retina
Immunoreactive RKBP levels in the retina were measured by ELISA specific to RKBP. Retina tissue homogenates were diluted serially and absorbance values at 414 nm (A414) were measured and plotted as the function of dilutions. As shown in Figure 2, serial dilution curves of the retinal homogenates of both control and diabetic rats were parallel to that of the RKBP standard, suggesting their immunologic identity.

The RKBP concentration in the retinal homogenate from each rat was determined by ELISA and normalized by the total protein concentration as measured by the Bio-Rad protein assay. The RKBP levels were averaged in each group, and a comparison was made between diabetics and their correspondent control subjects (Table 2). As listed in Table 2, retinal RKBP levels in STZ-diabetic rats were reduced by 51% (P < 0.001) and by 25% (P < 0.01) at 1 and 2 months of diabetes, respectively, compared to those of the age-matched control subjects. After 4 months of diabetes, RKBP levels were decreased in both control (8.4 ± 0.9 ng/mg protein, n = 8) and diabetic rats (5.8 ± 0.6, n = 8) compared to those of the control animals at 1 and 2 months. Interestingly, the RKBP levels in the diabetic retinas still were significantly lower than those in the control subjects (31%, P < 0.01) at the same time point.

Rat Kallikrein-Binding Protein mRNA Levels in the Retina
Total RNA (20 μg) from each group was subjected to Northern blot analysis using a full-length RKBP com

![Figure 1](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933197/)
FIGURE 2. Enzyme-linked immunosorbent assay of rat retina homogenates and purified rat kallikrein-binding protein. The standard curve of rat kallikrein-binding protein ranges from 0.39 to 25.0 ng/ml. Retina homogenates from control and diabetic rats were diluted serially. A polyclonal antibody to rat kallikrein-binding protein was used in the assay.

FIGURE 3. Northern blot analysis of rat kallikrein-binding protein messenger RNA in the rat retina. The same amounts of total RNA (20 μg) from retinas of control and diabetic rats were hybridized with a 32P-labeled rat kallikrein-binding protein complementary DNA probe. Lane 1 = control, 1 month after injection; lane 2 = diabetic, 1 month after injection; lane 3 = control, 2 months after injection; lane 4 = diabetic, 2 months after injection; lane 5 = control, 4 months after injection; lane 6 = diabetic, 4 months after injection. The rat kallikrein-binding protein messenger RNA (1.8 kb) is indicated at left.

DISCUSSION

In the current study, we have identified high levels of functional RKBP in the retina. Retinal RKBP levels were reduced significantly in STZ-diabetic rats after 1, 2, and 4 months of diabetes compared to those of control subjects (Fig. 3). Densitometry of the specific RKBP mRNA bands showed a 56%, 30%, and 41% reduction in the STZ-diabetic rats after 1, 2, and 4 months of diabetes, respectively (Fig. 3). This result showed that RKBP is synthesized in the retina, and the decrease of RKBP in the retina occurred at the transcription level.

TABLE 2. RKBP Levels in Rat Retina

<table>
<thead>
<tr>
<th>Group</th>
<th>Month After Injection</th>
<th>n</th>
<th>RKBP (ng/mg protein)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>8</td>
<td>14.1 ± 0.7</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Diabetic</td>
<td>1</td>
<td>7</td>
<td>6.9 ± 0.7</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Control</td>
<td>2</td>
<td>8</td>
<td>14.1 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>2</td>
<td>8</td>
<td>10.6 ± 1.0</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>8</td>
<td>8.4 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>4</td>
<td>8</td>
<td>5.8 ± 0.6</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

n = number of rats per group.
* Values represent the mean ± SEM.
RKBP = rat kallikrein-binding protein.

We recently have identified kallistatin, the counterpart of RKBP in humans, by in situ hybridization in multiple ocular tissues, including the retina neuronal and vascular tissues. The RKBP, which is a member of the serine protease inhibitor superfamily, forms a heat- and SDS-resistant complex with tissue kallikrein. In this study, we showed that the retina contains kallikrein-binding activity. In the binding assay, the 68-kDa albumin was visualized with labeled tissue kallikrein alone (Fig. 1, lane 7). Albumin disappears after incubation with the retinal homogenates, which suggests that it was degraded by proteinases present in the retinal homogenates. It is possible that the retina contains other serine proteinase inhibitors that may form high molecular weight complexes with tissue kallikrein; however, retinal RKBP was confirmed to be immunologically identical to purified RKBP from serum by ELISA (Fig. 2). Consistent with the results from human retina, the current study confirms that RKBP mRNA is synthesized endogenously in the rat retina.

Although a typical proliferative diabetic retinopathy does not develop in STZ-diabetic rats, similar to that which develops in humans, these rats do exhibit some retinal vascular abnormalities. The tissue kallikrein-kinin system participates in the regulation of vascular function and has been implicated in the diabetic complications of animal models and patients. Our results show that both functional and immunoreactive RKBP levels are reduced in STZ-diabetic rats as early as 1 month of diabetes and contin-
Reduced Retinal Kallikrein-Binding Protein in Diabetic Rats

Thus, the decrease in retinal RKBP levels at an early stage of diabetes suggests that RKBP may play a role in the pathogenesis of diabetic retinopathy and may not be a secondary consequence of other diabetic abnormalities. These findings are consistent with the results in humans, which showed that vitreous kallistatin is decreased significantly in patients with diabetic retinopathy.27 Interestingly, reduced RKBP levels were observed in both the control and diabetic animals at 4 months after injection (Table 2), whereas reduction was seen only in the diabetic animals at 1 and 2 months after injection. Because RKBP expression is dependent on growth hormone,15,36 age-related changes in growth hormone levels may explain the decrease of RKBP in the older animals.

The role of RKBP in the development of diabetic retinopathy currently is unclear. Previously, we have identified and localized components of the tissue kallikrein-kinin system in the eye, including tissue kallikrein, kininogen, kallistatin, and kinin receptors in photoreceptor cells, bipolar cells and retinal ganglion cells of the human retina, and endothelial cells of blood vessels.27 Furthermore, components of the renin-angiotensin system appear to be synthesized locally in the eye.37 The tissue kallikrein-kinin system, in counterbalance with the renin-angiotensin system, regulates vascular function and affects blood pressure and local blood flow.115 A disturbance in the balance of these two systems has been implicated in hypertension and microvascular complications of diabetes mellitus.1,15,26,37,38 Intramuscular delivery of the RKBP gene increases systemic blood pressure significantly in hypertensive transgenic mice that overexpress kallikrein, suggesting that RKBP inhibits tissue kallikrein’s activity in vivo.14 Thus, the decreased level of retinal RKBP in STZ-diabetic rats may allow accumulation of kinin due to increased kininogenase activity of tissue kallikrein.

Kinin possesses a potent vasorelaxant effect that has been linked to the release of nitric oxide and prostacyclin from endothelial cells.1,99 Excess kinin may contribute to vasodilation and increased retinal blood flow, which are early events in diabetic retinopathy40,41; furthermore, it may stimulate proliferation of endothelial cells through its proliferation-stimulating activities or through the induction of other growth factors8,42,43 and also may inhibit Na, K-ATPase activity through the stimulation of phosphoinositide turnover.44 Future studies may elucidate whether decreased retinal RKBP facilitates vasodilation of retinal capillaries associated with diabetic retinopathy.

Acknowledgments

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References