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CHAPTER ONE

C0005

Neuropeptides, Trophic Factors, and Other Substances Providing Morphofunctional and Metabolic Protection in Experimental Models of Diabetic Retinopathy

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Abstract

Vision is the most important sensory modality for many species, including humans. Damage to the retina results in vision loss or even blindness. One of the most serious complications of diabetes, a disease that has seen a worldwide increase in prevalence, is diabetic retinopathy. This condition stems from consequences of pathological metabolism and develops in 75% of patients with type 1 and 50% with type 2 diabetes. The development of novel protective drugs is essential. In this review we provide a description of the disease and conclude that type 1 diabetes and type 2 diabetes lead to the same retinopathy. We evaluate existing experimental models and recent developments in finding effective compounds against this disorder. In our opinion, the best models are the long-term streptozotocin-induced diabetes and Otsuka Long–Evans Tokushima Fatty and spontaneously diabetic Torii rats, while the most promising substances are topically administered somatostatin and pigment epithelium-derived factor analogs, antivasculogenic substances, and systemic antioxidants. Future drug development should focus on these.

st0015 ABBREVIATIONS

dt0005 **ACE** angiotensin-converting enzyme
dt0010 **ACEI** angiotensin-converting enzyme inhibitor
dt0015 **AG** aminoguanidine
dt0020 **AGE** advanced glycosylated end product
dt0025 **AIF** apoptosis-inducing factor
dt0030 **ALE** advanced lipoxidation end product
dt0035 **AQP** aquaporin
dt0040 **BB/W** Bio-Breeding/Worcester
dt0045 **BDNF** brain-derived neurotrophic factor
dt0050 **BRB** blood–retina barrier
dt0055 **BSA** bovine serum albumin
dt0060 **CAV-1** caveolin 1
dt0065 **CBD** cannabidiol
dt0070 **CGA** chlorogenic acid
dt0075 **CNTF** ciliary neurotrophic factor
dt0080 **CTGF** connective tissue growth factor
dt0085 **DAG** diacylglycerol
dt0090 **DR** diabetic retinopathy
dt0095 **E4** exendin-4
dt0100 **EPO** erythropoietin
dt0105 **ERK** extracellular signal-regulated kinases
dt0110 **ERG** electroretinogram
dt0115 **FDP-lysine** Nε-(3-formyl-3,4-dehydropiperidino)lysine
dt0120 **GABA** γ-aminobutyric acid
dt0125 **GBE** *Ginkgo biloba* leaf extract
dt0130 **GCL** ganglion cell layer
dt0135 **GDNF** glial cell line-derived neurotrophic factor

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dt0140 **GFAP** glial fibrillary acidic protein
dt0145 **GK** Goto–Kakizaki
dt0150 **GLAST** glutamate aspartate transporter
dt0155 **GLP-1R** cognate receptor
dt0160 **GLUT1** glucose transporter 1
dt0165 **Grx1** glutaredoxin 1
dt0170 **GS** glutamine synthase
dt0175 **GSPE** grape seed proanthocyanidin extracts
dt0180 **GT** green tea
dt0185 **HIF1α** hypoxia-inducible factor-1α
dt0190 **H_2S** hydrogen sulfide
dt0195 **IBA-1** ionized calcium-binding adaptor molecule 1 Au5
dt0200 **ICAM-1** interstitial cell adhesion molecule 1 Au6
dt0205 **IGF-1** insulin-like growth factor 1
dt0210 **IL-1** interleukin-1
dt0215 **ILM** inner limiting membrane
dt0220 **INL** inner nuclear layer
dt0225 **IOP** intraocular pressure
dt0230 **IPL** inner plexiform layer
dt0235 **JNK** c-Jun N-terminal kinase
dt0240 **LDL** low-density lipoprotein
dt0245 **LETO** Long–Evans Tokushima Otsuka
dt0250 **LPA** lysophosphatidic acid
dt0255 **MAPK** mitogen-activated protein kinase
dt0260 **mtDNA** mitochondrial DNA
dt0265 **NF-κB** nuclear factor κB
dt0270 **NFL** nerve fiber layer
dt0275 **NGF** nerve growth factor
dt0280 **NMDA** *N*-methyl-D-aspartate
dt0285 **nNOS** neuronal nitric oxide synthase Au7
dt0290 **OLETF** Otsuka Long–Evans Tokushima Fatty
dt0295 **OLM** outer limiting membrane
dt0300 **ONL** outer nuclear layer
dt0305 **OP** oscillatory potential
dt0310 **OPL** outer plexiform layer
dt0315 **OS** outer segment
dt0320 **PACAP** pituitary adenylate cyclase-activating polypeptide
dt0325 **PARP** poly-(ADP)-ribose polymerase
dt0330 **PEDF** pigment epithelium-derived factor
dt0335 **PI3K** phosphatidylinositide 3-kinase
dt0340 **PKC** protein kinase C
dt0345 **PKCα** protein kinase Cα Au8
dt0350 **PKCζ** protein kinase Cζ
dt0355 **PPAR** peroxisome proliferator-activated receptor
dt0360 **PRL** photoreceptor layer
dt0365 **RA** retinoic acid
dt0370 **RAGE** receptor of AGEs

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dt0375 **RAS** renin–angiotensin system
dt0380 **RGC** retinal ganglion cell
dt0385 **ROS** reactive oxygen species
dt0390 **RPE** retinal pigment epithelium
dt0395 **SDT** spontaneously diabetic Torii
dt0400 **SST** somatostatin
dt0405 **STZ** streptozotocin
dt0410 **TC** *Tinospora cordifolia*
dt0415 **TNFα** tumor necrosis factor-α
dt0420 **TH** tyrosine hydroxylase
dt0425 **TUNEL** terminal dUTP nick-end labeling
dt0430 **UCP-2** uncoupling protein 2
dt0435 **VEGF** vascular endothelial growth factor
dt0440 **VEP** visually evoked potential
dt0445 **VIP** vasoactive intestinal peptide
dt0450 **ZDF** Zucker diabetic fatty
dt0455 **ZF** Zucker fatty
dt0460 **ZFDM** Zucker fatty diabetes mellitus

s0005 # 1. PREVALENCE AND CAUSES OF DIABETIC RETINOPATHY

s0010 ## 1.1. Why experimental models are needed

p0465 The visual world is the most important environmental information source for many species, including humans. None of the other sensory signals reaches the brain in such variety and none is processed by as many cortical areas as the visual cues. The first steps of visual processing are performed by a thin sheath of neural tissue at the back of the eye, called retina. After phototransduction, light information is translated into neural signals and shaped by the retinal interneurons. Bipolar cells transmit the processed signal to ganglion cells that project to the brain and this is the sole source of visual signals arriving there. Therefore, any damage to the retinal tissue immediately results in vision loss and, in the worst case, causes total blindness.

p0470 Many retinodegenerative disorders such as glaucoma, ischemia, and diabetic retinopathy (DR) are thought to be consequences of pathological metabolic processes (Osborne et al., 2004). Metabolic insults vary and include exposure to extremely strong light and changes in hormone/metabolite levels or in blood/aqueous humor pressure. These processes lead to elevated extracellular glutamate levels and can provoke excitotoxic insults (Atlasz et al., 2008). The balance between the neurotoxic and neuroprotective

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factors is crucial in determining the survival of retinal neurons (Hernandez and Simo, 2012). DR, a common complication of diabetes, develops in 75% of patients with type 1 and 50% with type 2 diabetes, progressing to legal blindness in about 5% (Engerman and Kern, 1995). Type 1 diabetes occurs most commonly in children and young adults and constitutes 5–10% of the diagnosed diabetics (Maahs et al., 2010). Type 2 diabetes accounts for 90–95% of diagnosed diabetes cases globally and typically develops in middle-aged adults. Both type 1 and type 2 diabetic patients may develop retinopathy; in particular, almost everyone with type 1 diabetes will develop it over a 15–20-year period and greater than 60% of type 2 diabetes patients will have retinopathy after 20 years (Hazin et al., 2011).

p0475 The aforementioned data call for increased efforts to learn more from the pathogenesis of this disease and search for possible treatments/cures. Apart from the increased diagnostic opportunities (e.g., the use of optical coherence tomography) and the carefully analyzed results of clinical trials regarding invasive and noninvasive treatment options (Gabriel, 2013; Hammes, 2013), experimental models are also necessary to utilize. However, one has to be careful when translating results obtained in commonly used animal models, because, besides similarities in the pathogenesis, profound differences can also be found (vascular symptoms and proliferativity) between animal models and human disease. Despite these differences, several animal models have been developed and used successfully in revealing basic mechanisms both in the pathogenesis of DR and in studying possible protective treatments. This is because the underlying physiological and biochemical processes in humans are identical or very similar to those of experimental animals.

p0480 The same statement applies also for the causes, as hyperglycemia is a major risk factor for the development and progression of DR. It plays an important role in the pathogenesis of diabetic complications by increasing the levels of advanced glycosylated end products (AGEs), which are late products of nonenzymatic glycation. The accumulation of AGEs appears to be a key factor in the development of DR. AGEs are thought to promote many diabetic complications, including retinopathy, nephropathy, neuropathy, and cardiovascular disease (Brownlee, 2005; Huebschmann et al., 2006).

p0485 Capillary occlusions are also characteristic features of early DR and are presumed to initiate neovascularization. Diabetic rats (2–9 months into the disease) showed capillary occlusions by leukocytes (especially monocytes), endothelial cell damage, extravascular macrophage accumulation, and tissue disintegration. In both induced diabetes and genetically diabetic mice, the

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development of diabetes is macrophage-dependent. The presence of numerous interstitial macrophages is characteristic to DR. Leukocytes in general and monocytes/macrophages in particular may not only be involved in the pathogenesis of early DR but may also, at least in part, initiate the microvascular pathology observed at later stages. Occluding monocytes or granulocytes were found only in diabetic retinas. In rat models, areas of capillary loss and neovascularization were associated with the presence of monocytes or macrophages. Phagocytes are known to be necessary for the removal of products of pathological processes. Capillary disintegration is also a major factor in disease development. Accordingly, pericytes are absent from the postmortem diabetic retinas. Some blood vessels that still contained intact endothelial cells and vessels with microaneurysms tended not to contain pericytes, suggesting that their loss permits uncontrolled proliferation of endothelial cells. Pericyte dropout has been used as an index of DR (Kern and Engerman, 1994). Permeability increase occurs in many vessels simultaneously, first occurring in the larger superficial vessels and then progressing to the capillaries of the outer retina within 2 months from the onset of diabetes. The vascular permeability increase is a consequence of regulatory changes in tight junction proteins within a broad population of endothelial cells rather than the apoptosis of a small number of endothelial cells. Increased permeability causes edema in the nearby tissue. Edema is the main reason of impaired vision in nonproliferative DR. Water transport through aquaporins (AQPs) facilitates the development of ischemic edema in the retina. Experimental diabetes is associated with the altered regulation of AQPs in the pigment epithelium and the outer retinal layers. These alterations might be involved in the adaptation of retinal cells to hyperglycemic conditions and the development and/or resolution of retinal edema (Hollborn et al., 2011).

s0015 1.2. Aims

p0490 As it can clearly be seen from the aforementioned facts, there are several areas of research that have led and may further lead to breakthroughs in either the diagnosis or treatment of DR. This review focuses on the recent advances and current hopes in the area of experimental DR from the following aspects: (i) What are the primary mechanisms driving the pathogenesis? How do different neuronal cell types change during DR and what may help to possibly rescue them? (ii) Which molecular pathways lead to cell death? What is the contribution of nonneuronal cell types of the retina to these

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signaling pathways? (iii) Are there any differences between type 1 diabetes and type 2 diabetes in the course of pathogenesis in developing DR? Can animal models be established to study those? (iv) Which animal models are the best for translational research? Finally, we will also give an overview of a number of endogenous compounds and list several other (synthetic or natural) substances that showed discernible protection against DR. In some cases, their mode of action will also be discussed.

2. DIABETIC RETINOPATHY: EARLY SIGNS AND LATE-DEVELOPING SYMPTOMS

p0495 As the worldwide prevalence of diabetes continues to increase, DR is a leading cause of vision loss in developed countries (Fong et al., 2004). The inability of the retina to adapt to metabolic stress leads to a glucose-mediated microvascular disease along with chronic inflammation, which finally causes neurodegeneration and dysfunction in the retina. The retina is one of the most metabolically demanding tissues in the body, and therefore, it is highly vulnerable. The interplay between the neuroretina and the vasculature is critical in developing neurological symptoms of disease. In DR, the rate of neuronal loss in the retina is slow, leading to a gradual, cumulative reduction mostly in amacrine and ganglion cells (Liu et al., 2008). Two forms of DR can be clearly distinguished: an early, nonproliferative DR, when neovascularization of the macula is not evident, and proliferative DR where the symptoms include macular neovascularization. Other vascular symptoms involve microangiopathy, formation of microaneurysms, flame hemorrhages, leukocyte adherence to the vascular wall, and formation of exudates in the extravascular space. These observations have led to the theory that DR is primarily a vascular disorder whose degrading effects are due to the consequences of vascular failure: ischemia followed by increased reactive oxygen species (ROS) production, as in the case of the retinal ischemic diseases (Fulton et al., 2009).

p0500 However, it has also been described that neuronal damage may precede any detectable microvascular change (Barber et al., 1998). The deterioration of the intrinsic time and also of oscillatory potentials (OPs) in the electroretinogram (ERG) starts early, in some cases as early as 2 days after induction of diabetes in experimental animals (Li et al., 2002). These observations led to the formulation of the "neurodegeneration-first" hypothesis (Villaroel et al., 2010). Patients suffering from DR experience gradual vision loss; after ERG deterioration, the visually evoked potentials (VEPs) also start to

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decrease (Wolff et al., 2010), which indicates cortical dysfunction. However, recent observations led to the conclusion that inflammation may precede or at least runs parallel with the vascular and neural events (Joussen et al., 2004; Liu et al., 2008; Tang and Kern, 2011).

p0505 Inflammatory molecules in the retina can be produced not only by leukocytes but also by glial cells; many of them are produced by the Müller glia (Bringmann and Wiedemann, 2012). Müller cells are primarily responsible for ion and volume regulation of the retina and also control extracellular glutamate levels through their excitatory amino acid transporters. They also participate in protection against free radicals and hypoxic damage through glutathione synthesis. Usually the first sign of the Müller cell stress is upregulation of glial fibrillary acidic protein (GFAP) content, which may be accompanied by hypertrophy and proliferation under certain damaging conditions. Diabetes itself is able to upregulate GFAP in Müller cells without the presence of other symptoms of DR in humans (Villaroel et al., 2010), and this goes along well with what we see in experimental models where after 2 days of diabetes induction, GFAP upregulation is already apparent (K. Szabadfi, unpublished observation). All the aforementioned observations may lead to the formation of a fourth hypothesis of the initiation of DR, the "glial cells-first" scenario. In this case, Müller cells, by sensing the elevated glucose level, activate their volume and ion-regulating machinery and release vasoproliferative vascular endothelial growth factor (VEGF; Amandio et al., 2010; Eichler et al., 2000) and inflammatory substances (prostaglandins, tumor necrosis factor (TNF), and interleukins; Behl et al., 2008; Joussen et al., 2009; Tang and Kern, 2011). These would in turn initiate neurodegeneration, inflammation, and vascular growth. Although the exact order of events is unknown, all the aforementioned evidences suggest that low oxygen supply and high blood glucose level are important pro-DR parameters and all signaling pathways converge to activate VEGF production.

p0510 The intraocular concentration of VEGF is closely correlated with active neovascularization in diabetes. Hypoxia increases VEGF expression in retinal cells, which promotes retinal endothelial cell proliferation, suggesting that VEGF plays a major role in mediating intraocular neovascularization resulting from ischemic retinal diseases. An increased expression of VEGF in the retina is involved in generating vascular leakage and angiogenesis in DR. In addition to VEGF, pigment epithelium-derived factor (PEDF), a potent inhibitor of angiogenesis, is also involved in the pathogenesis of DR (Patel et al., 2006). The time course of the VEGF-to-PEDF ratio

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change is correlated with the development and progression of retinal neovascularization. The VEGF-to-PEDF ratio represents a dynamic balance between the angiogenic stimulators and inhibitors; a disturbance in the balance plays a key role in the pathogenesis of DR (Chen et al., 2008; Gao et al., 2001, 2002). Vascular changes especially regarding permeability have been reported to occur as early as 8 days after onset of diabetes in rats (Do Carmo et al., 1998). Capillary dilation and increased blood flow are some of the earliest signs of diabetes both in rats (Cringle et al., 1993) and in humans (Grunwald et al., 1994), supporting a "capillaries first" hypothesis.

s0025 2.1. Histological alterations

p0515 The neural retina is composed of diverse neurons characterized by morphological and biochemical criteria and numerous neural networks formed through chemical and electrical synapses among the processes of these neurons. These neuronal components are arranged into layers. Retinal ganglion and displaced amacrine cells form the ganglion cell layer (GCL); bipolar, horizontal, and amacrine cells occupy the inner nuclear layer (INL), while the processes (and synapses) of the bipolar and amacrine cells and the ganglion cells contribute to assemble the inner plexiform layer (IPL). The somata of photoreceptors (rods and cones) gather in the outer nuclear layer (ONL), and their light-sensitive processes form the layer of the outer segments (PRL). The photoreceptors synapse with the horizontal and bipolar cells in the outer plexiform layer (OPL).

p0520 The limits of the retina from the intravitreal side are the inner limiting membrane (ILM), which is a close association of Müller glial cells end feet and the pigment epithelial cells on the opposite side. Müller cells are the major glial elements of the retina; their somata are positioned in the INL and extend their processes toward the photoreceptors and retinal ganglion cells (RGCs). Au9

p0525 The retinal pigment epithelium (RPE) is composed of a single layer of hexagonal cells that are densely packed with pigment granules. The RPE shields the retina from excess incoming light. It supplies omega-3 fatty acids and glucose, the former for building photoreceptive membranes and the latter for energy. It also serves as a layer limiting transport, thus maintaining and protecting the retinal environment.

p0530 The diabetic damage inflicted on all retinal (neuronal and nonneuronal) cells by high glucose initiates a number of metabolic processes that altogether closely approximate the molecular basis for the loss of vision associated with

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this disease. Here, we give a point-to-point overview about the retinal compartments that are altered by (experimental) diabetic retinal degeneration.

s0030 ### 2.1.1 RGCs and the optic nerve

p0535 RGCs are the output elements of the retina. Their death immediately and directly leads to loss of visual information. They have high sensitivity to cellular damage and neurotoxicity; thus, RGCs are prone to degeneration in diabetic retinas. All studied rat strains have shown RGC loss or damage in diabetes starting from 4 weeks after the onset of diabetes up until 12 months (Kern and Barber, 2008). Reduced number of RGCs was demonstrated in the retina of 15-week diabetic rats (Hammes et al., 1995b) and also in short-term (4 weeks) diabetes (Zeng et al., 2000).

p0540 In ocular conditions, RGC loss is often associated with elevated intraocular pressure (IOP). The levels of IOP in 6- and 12-week diabetic mice (C57BL/6) were significantly higher than their controls. In parallel with these phenomena, the number of RGCs was significantly decreased after only 6 weeks of diabetes. During a 12-week examination period, IOP remained constantly high. However, it has to be noted that elevated IOP-induced cell loss is not specific to induced diabetes, but rather is an accompanied symptom of several ocular diseases (Yang et al., 2012).

p0545 In diabetic retina, structural alterations in RGCs can be easily recognized. Their features include swellings on axons, often associated with constriction close to the cell body. There is structural remodeling of dendrites, including an increase in the total length, density, and number of branches. These changes surprisingly were found limited to the large ON-RGCs in short-term diabetes and did not occur in any class of OFF-RGCs (Kern and Barber, 2008). Similar changes in RGC morphology have been observed in human retinas (Meyer-Rusenberg et al., 2007). This alteration in a subset of RGCs could alter the functional output of the certain subtypes of RGCs (Kern and Barber, 2008), leading to changes in VEPs (see later text). While neurons in the INL were resistant to stable and long-lasting moderate hyperglycemia during a 2-months' period, the GCL underwent both reactive and destructive changes. Chromatolytic changes to the RGCs were in the foreground, and there was a somewhat less marked increase in the number of pyknomorphic RGCs (Logvinov et al., 2010). Several molecular and cellular mechanisms may be involved in RGC loss, which will be discussed in the later text.

p0550 The number or density of axons in the rat optic nerve was found reduced in induced diabetes in some, but not in all, rodent studies (Kamijo et al.,

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1993). The cross-sectional area of the optic nerves was not changed at 8 or 12 weeks of diabetes, but the percentage of area occupied by glial cells increased and the axonal component decreased significantly. These alterations showed highly significant correlations with the associated prolongation of the latencies of the VEPs, suggesting that axoglial disjunction and axonal atrophy are major determinants for impaired optic nerve function (Kamijo et al., 1993).

s0035 ### 2.1.2 Amacrine, horizontal, and bipolar cells

p0555 These classes of cells represent the interneurons of the retina. Bipolar cells transmit the signals from the photoreceptors to the RGCs, while horizontal and amacrine cells are the constituents of the lateral signaling pathways in the OPL and IPL, respectively. Despite the fact that bipolar cells are thought to dominantly contribute to the b wave of the ERG, which is a major functional marker of retinal health, there are currently no data available elucidating the bipolar cell reaction to diabetic conditions. The same also applies to horizontal cells.

p0560 The most prominent change in the ERG seen in the early stages of diabetes involves the loss of OPs that are thought to derive from dopaminergic amacrine cells (Shirao and Kawasaki, 1998). Therefore, the alterations in the dopaminergic system seem to be among the first significant events in the development of DR. Seki et al. (2004) named several possible mechanisms underlying the degeneration of dopaminergic amacrine cells in diabetic animals: (i) severe insulin deprivation, (ii) hyperglycemia, and (iii) dysfunction of Müller glial cells. The retina of the insulin-deficient $Ins2^{Akita}$ mice contained 16% less dopaminergic amacrine cells than nondiabetic age-matched control littermates (Gastinger et al., 2006). An extensive loss of cells, dendritic varicosities, and tyrosine hydroxylase (TH) content was seen in acute (3 weeks) diabetes in rats (Szabadfi et al., 2012; see also Fig. 1.1).

p0565 The total number of cholinergic amacrine cells in the diabetic mice was 20% less than that in nondiabetic animals, but they were lost in a random pattern. Diabetes leads to a greater reduction of cholinergic amacrine cell density in the peripheral retina than in the central regions (Gastinger et al., 2006). In the rat retina, most of the neurons containing neuronal nitric oxide synthase (nNOS) appear to be amacrine cells, residing in the INL or displaced to the GCL and closely related to vessels. The number of these nNOS-containing cells was found to decrease by 32% as early as 1 week after the streptozotocin (STZ)-induced diabetes and remained reduced for up to 8 months (Goto et al., 2005). These results suggest that some amacrine cell

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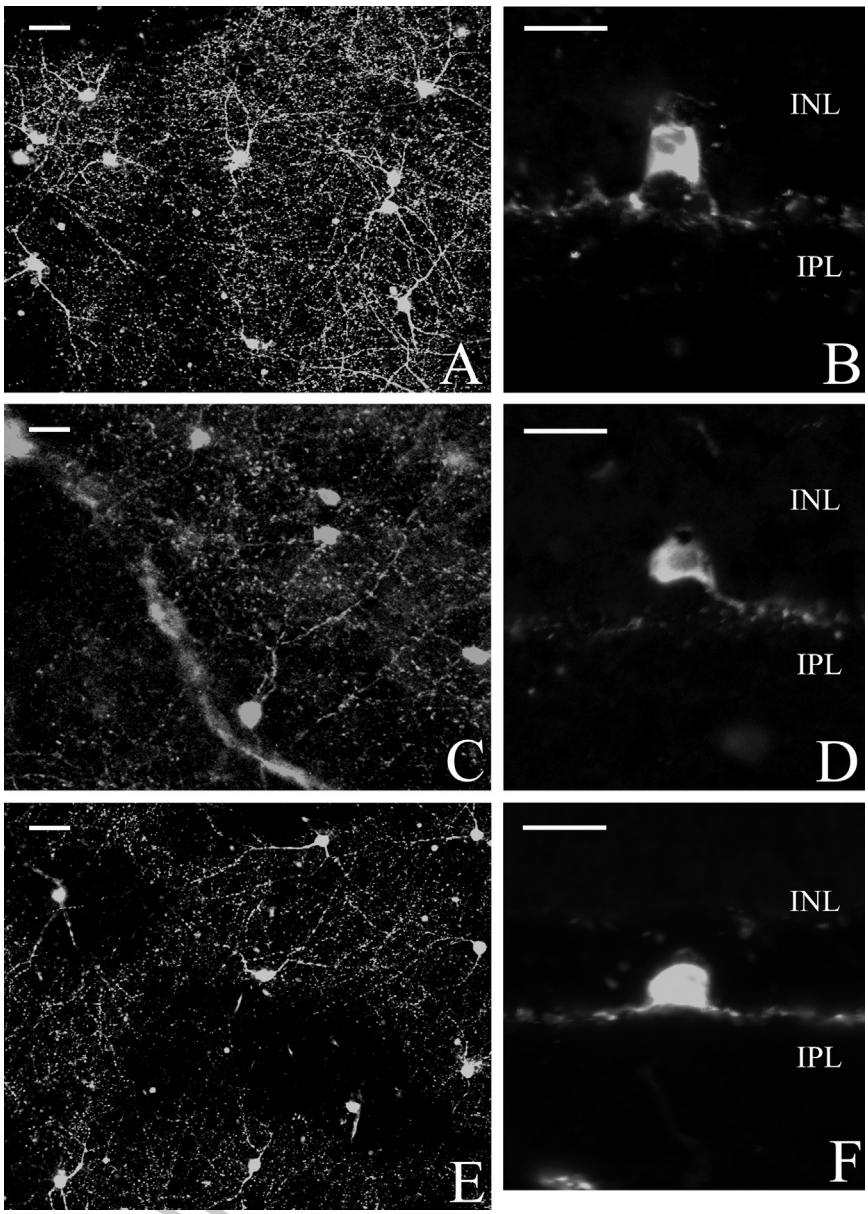

f0005 **Figure 1.1** Tyrosine hydroxylase (TH) immunoreactivity in healthy and diabetic rat retinas. The dopaminergic amacrine cells are situated in the inner retinal layers, at the border of INL and IPL of the retina as were shown in the control vertical retinal section (B). Representative fields of whole-mount retinal preparations of control (A), 3-week diabetic (C), and insulin-treated chronic diabetic animals (4 months of diabetes; E) and retinal cross sections from the same three groups (B, D, F). TH immunoreactivity was in higher density in the control retinas (A) compared with the two diabetic groups (C, D and E, F, respectively), where the arborization of the TH-positive cells was also reduced. Scale bars are 200 μm in pictures A, C, and E and 20 μm in pictures B, D, and F. Abbreviations: INL, inner nuclear layer; IPL, inner plexiform layer.

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types may play pivotal roles in maintaining retinal integrity and if these cells are damaged, retinal circuitry suffers nonreparable consequences. Also, these Au10
cells may not be the most numerous—dopaminergic and nitrergic cells both represent less than 1% of amacrine cells but may confer widespread regulatory functions.

s0040 ### 2.1.3 Photoreceptors

p0570 Various aspects of visual information are transmitted in parallel from photoreceptors to the output neurons of the retina, which code for many different signals, for example, brightness, darkness, contrast, color, and motion. Photoreceptors are one of the most metabolically active cell types of the retina (Wassle, 2004). There are several factors such as metabolic changes associated with diabetes that may lead to outer retinal dysfunction. Aizu and coworkers (2002) provided the first evidence showing changes of photoreceptor layer (PRL) during DR; the shortening of the photoreceptor outer segment (OSs) and the increase in the number of fragmented cone photoreceptors and a disrupted photoreceptor–RPE cell complex were shown by electron microscopy in rats with very early stage of diabetes (1 month after the onset of diabetes). Since RPE cells produce a variety of trophic factors and have an important role in renewing the photoreceptor OSs, their damage will also influence the phototransduction process. A 2-month diabetic period induced destructive changes in the neurosensory cells. Fragmentation and vacuolar degeneration of the OSs are characteristic, as well as nuclear pyknosis, edema of the perikaryon, and enlargement of the scleral processes of Müller cells, which actively phagocytose degraded OSs. Quantitative analysis demonstrated a threefold increase in the proportion of photoreceptors with pyknotic nuclei (Logvinov et al., 2010).

p0575 As a consequence of the aforementioned structural damage after diabetes-induced dysfunction, the retinal phototransduction is apparently hindered even in early-stage diabetes, including deterioration of the flash response and light adaptation (Kim et al., 2005).

s0045 ### 2.1.4 Synaptic layers

p0580 The signal transmission of various classes of retinal neurons is the physiological bases for visual processing and requires a range of synapses with different kinetics, such as electrical synapses, conventional chemical synapses, and ribbon synapses. Diabetes impacts a broad population of retinal synapses, which may explain significant thinning of the IPL, after a longer duration of diabetes (Barber et al., 1998, 2005). Ribbon synapses in both plexiform layers

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are especially strongly affected (Fig. 1.2). The thickness of the IPL in the 6-month diabetic rats was markedly reduced (Aizu et al., 2002). There was a 22% decrease in the thickness of the IPL in rats after 7.5 months of STZ-induced diabetes, suggesting a cumulative loss of neural dendrites and synapses in the inner retina (Barber et al., 1998).

p0585 After 1 month of STZ-induced diabetes, synaptophysin content was found reduced in the whole retina and also in isolated retinal synaptosomes. The size and density of synaptophysin-immunoreactive puncta were reduced in the plexiform layers. The loss of synaptic proteins in retinal synaptosomes was accompanied by decreased mRNA content after 1 month of diabetes; therefore, diabetes may increase local degradation rate of presynaptic proteins at retinal synapses and also that of their mRNA (VanGuilder et al., 2008). Indeed, synaptophysin mRNA translation continues at a higher rate after the induction of diabetes as a compensatory mechanism of the reduction of the mature protein. Nevertheless, there is also an increase in its degradation during an early stage of its maturation, possibly during its posttranslational processing (D'Cruz et al., 2012). These findings suggest multiple potential regulatory mechanisms for synapse disintegration.

s0050 *2.1.5 Müller glial and retinal pigment epithelial cells*

p0590 Although often considered as nonprincipal components, these cell types are integral constituents of the retinal metabolism.

s0055 2.1.5.1 Müller glial cells

p0595 Müller glial cell is the principal glia of the retina, which expresses a diversity of ion channels and transporters, releases a variety of cytokines and survival factors, and has receptors for numerous neurotransmitters and growth factors. Müller cells play an active, dynamic role in the retina. They are radially oriented and span the depth of the retina from the vitreal border to the interphotoreceptor matrix of the subretinal space. Their processes are in close apposition to neuronal cell bodies, neurites, and synapses, and blood vessels and their processes make contact with most neural cells (Sarthy and Harris, 2001). They also form end feet on both large vessels and capillaries in the inner and outer retinal vessel beds. Müller glial cells are vital for maintaining normal neuronal and vascular function in the retina. Their mechanical role is stabilizing the retinal architecture, and they are involved in regulating retinal glucose metabolism, modulating the ionic and molecular composition of the retinal microenvironment, altering blood flow to match the local metabolic

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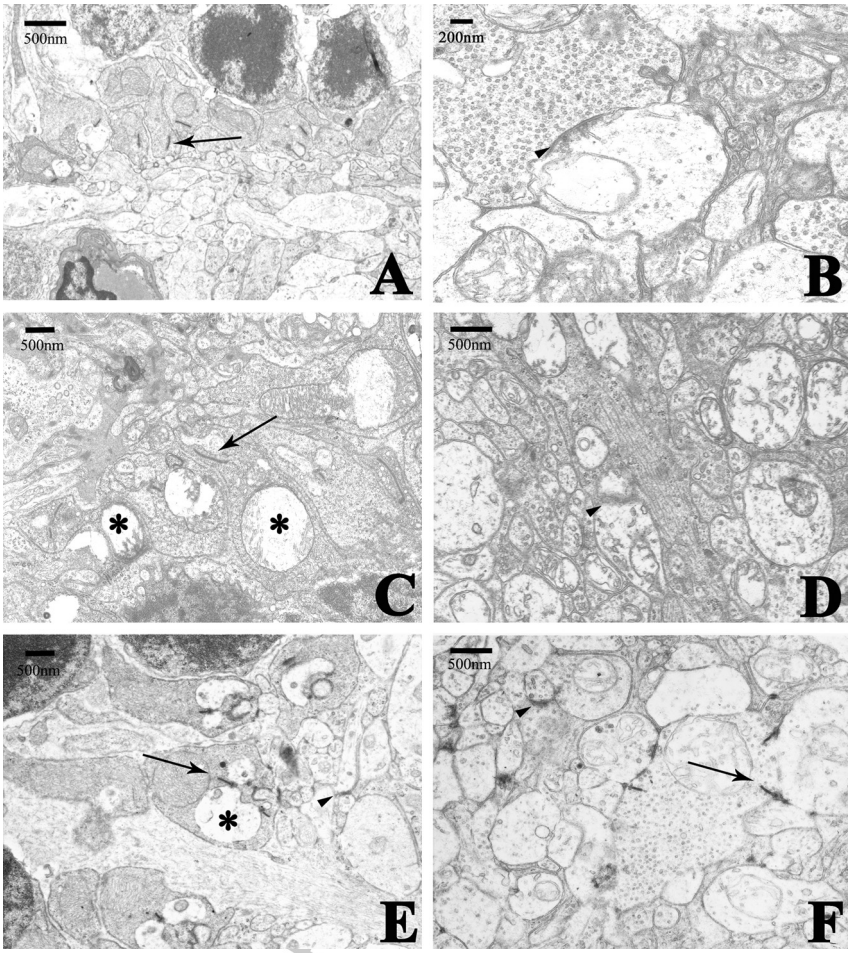

f0010 **Figure 1.2** Ultrastructure of the outer and inner plexiform layers (OPL and IPL) of control, early diabetic (3 weeks) and chronic diabetic (4 months) rat retinas. Although ribbons of the photoreceptors are still present in the OPL of both diabetic retinas (arrows: C, E), they are less densely packed and shorter than those of the controls (A). Some degenerative profiles appear (asterisks: C, E). The processes are often devoid of synaptic vesicles. In the IPL, the ribbon and conventional synapses are (arrowheads) healthy in the control retinas (B) if compared with the same structures of the diabetic retinas (D, F). The shape of the ribbons was distorted in diabetic animals; they did not have sharp contours as found in control retinas. Almost no synapses were present in the IPL compared with the control (B); some unusual ribbons and degenerating synaptic profiles were seen in the IPL (arrowheads: D, F). Scale bars: indicated in the images.

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needs, and contributing to the maintenance of the inner blood–retina barrier (BRB; Bringmann et al., 2006).

p0600 In normal conditions, the end feet of the glia express GFAP. Increased GFAP expression has been widely used as a cellular marker for several retinal pathologies. It has been suggested that elevated expression of GFAP in the cell body and processes is an aspecific metabolic stress signal (Lundkvist et al., 2004). Müller glial cells regulate the extracellular environment of neurons in the retina by clearing glutamate from synaptic clefts and by providing glutamine to neurons for the resynthesis of glutamate to be used in neurotransmission. This ability of glia in diabetic rat retina is reduced to only 65%, which results in an overall increase of total extracellular glutamate levels that is toxic to retinal neurons. Müller glial cells in diabetes showed reduced glutamate aspartate transporter (GLAST) function (Li and Puro, 2002) and impaired glutamine synthesis. The adverse effects of diabetes on the function of Müller cells in transporting glutamate by GLAST or in metabolizing glutamate by glutamine synthase (GS) have been widely studied (Kowluru et al., 2001; Li and Puro, 2002; Lieth et al., 2000). Although alterations in GLAST activity during diabetes remain controversial, impairment of GS activity is convincingly evident (Mysona et al., 2009; Ward et al., 2005; Zeng et al., 2009). A positive-feedback loop involving oxidative stress and dysfunction of GLAST may be important in the progression of DR (Puro, 2002). These dysfunctions also participate in creating elevated glutamate levels in the microenvironment of diabetic retinas, which might induce excitotoxicity in amacrine cells and RGCs (Lieth et al., 1998).

p0605 Glial cells not only send signals to other components of the retina but also change their own makeup. Changes in glia are accompanied by several dysfunctional responses, including altered potassium and glutamate regulation (Li and Puro, 2002; Lieth et al., 1998) and γ-aminobutyric acid (GABA) accumulation (Ishikawa et al., 1996). One of the early histological alterations in the retina of the diabetic rats was the increase in GFAP expression of the Müller cell processes as early as few days after the onset of diabetes, which is a nonspecific response to pathophysiological conditions (Fig. 1.3). Müller glia is adversely affected early in the course of diabetes, the reactive "phenotype" characterized by hyperplasia and upregulation of GFAP (Lieth et al., 1998). Apart from the aspecific glial reactions, hyperglycemia leads to overexpression of several biologically active factors, like proinflammatory cytokines (Gerhardinger et al., 2005) and insulin-like growth factor 1 (IGF-1; Inokuchi et al., 2001).

p0610 Besides excitotoxicity-induced changes, products of carbohydrate metabolism may also cause alterations in Müller glia functions. During early

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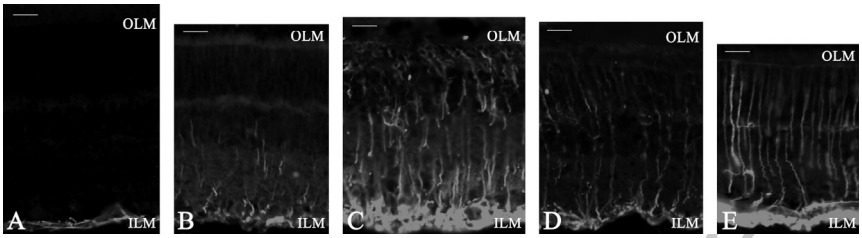

f0015 **Figure 1.3** Immunofluorescent labeling of glial fibrillary acidic protein (GFAP) in the rat retinas. In normal conditions, only the end feet of the Müller glia (ILM) express GFAP (A). Damaging conditions such as diabetes are able to initiate the upregulation of GFAP in the whole extent of the cells. In STZ-induced hyperglycemia, GFAP upregulation is readily observed (B), as well as after 3 weeks of diabetes (C). Upregulation may be present even if insulin treatment is applied in chronic diabetes (4 months; E). Reduced GFAP expression is detected in PACAP-treated 3-week acute diabetic retinas (D). Scale bar: 20 μm. Abbreviations: OLM, outer limiting membrane; ILM, inner limiting membrane.

experimental diabetes, there is a selective accumulation of the acrolein-derived advanced lipoxidation end products (ALEs), one of which is Nε-(3-formyl-3,4-dehydropiperidino)lysine (FDP-lysine), originally restricted to the end feet at the ILM, but as the disease progresses, this adduct appears in the radial fibers. This agent is pathogenic for the Müller glia itself; changes Au11
in polyamine catabolism could underlie the diabetes-induced accumulation of FDP-lysine. The observed accumulation of these adducts could potentially contribute to Müller cell dysfunction and death during long-term diabetes (Yong et al., 2010). Despite all these unfavorable processes, Müller cells are not among the retinal cell populations undergoing apoptosis early in diabetes (Ali et al., 2008).

p0615 During diabetes, hyperglycemia and oxidative stress upregulate VEGF that induces retinal neovascularization, vascular leakage, and macular edema. VEGF may also be expressed in, to a lesser extent, endothelial cells, astrocytes, RPE, and RGCs. However, Müller cells are the major source of VEGF in DR (Arjamaa and Nikinmaa, 2006; Kim et al., 2009b). VEGF plays a leading role in inducing retinal inflammation and vascular leakage that occurs as a consequence of diabetes. The loss of Müller cell-derived VEGF significantly inhibits diabetes-induced vascular leakage and attenuates capillary acellularity. It is possible that VEGF derived from other endothelial and retinal cells contributes to diabetes-induced retinal inflammation and vascular leakage in the presence of Müller cell-derived VEGF, which together may serve as a key to the "pathological threshold" (Wang et al., 2010a). While the endothelial cell-derived VEGF plays a role in the

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pathogenesis of DR (Huang and Sheibani, 2008), the RPE-derived VEGF rather contributes to the RPE–choriocapillaris interaction and regulates outer BRB (Hartnett et al., 2003), which may influence the osmotic balance and the nutrient supply of the outer retina.

p0620 Diabetes also alters osmotic swelling characteristics of glial cells. Glial cells in diabetic retinas are more sensitive to osmotic stress. The functional K^+ channels in retinal glial cells undergo alterations in the course of experimental diabetic conditions, and these are associated with the changed swelling characteristics of retinal glial cells, which contribute to the development of edema. Retinal edema also develops under ischemic–hypoxic and/or inflammatory conditions. Osmotic glial cell swelling has been linked to the decrease of the main K^+ conductance of the cells and to endogenous formation of arachidonic acid in response to osmotic stress (Pannicke et al., 2006), which may in turn regulate the inflammatory responses. This aspect is critical to retinal integrity, since inflammatory cells to the retina mostly arrive through the ILM, which is formed by the end feet of Müller cells. Indeed, Müller cells form two discreet barriers at two strategic locations. Apart from the aforementioned ILM, the second barrier is formed at the top of the ONL, which is a discontinuous, lace-like structure surrounding the photoreceptor cell bodies. It can be disrupted during diseases such as DR (Omri et al., 2010). The apical processes of Müller cells are attached to each other and to inner segments of the photoreceptor cells that collectively form the outer limiting membrane (OLM). The junction proteins are located in the OLM. Adherens junctions, occludins, and desmosomes have been identified there. OLM could serve as a gate for macromolecule transport into the retina and act as a semipermeable barrier. During DR, the Müller cells are not only swollen, but they lose their occluding power at the OLM level, which leads to cyst formation (Omri et al., 2010). Au12

p0625 Since astrocytes are also present in the nerve fiber layer (NFL) and around the capillaries, here, early changes in astrocytes are coincident with inner retinal hypoxia and RGC functional deficits. Therefore, astrocytes may contribute to changes in retinal vasculature and inner retinal dysfunction in diabetes (Ly et al., 2011).

s0060 #### 2.1.5.2 Pigment epithelial cells

p0630 The inner BRB is formed by tight junctions between vascular endothelial cells, whereas the outer retinal barrier is specific to the eye and consists of the tight junction present in a monolayer epithelium, the RPE cells. The

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apical side of RPE faces the photoreceptor OSs of the neuroretina, and the basolateral side lies on Bruch's membrane, which separates the RPE from the fenestrated endothelium of the choriocapillaris (Simo et al., 2010). The RPE is a selective exchange platform between the systemic circulation and the retina, and the subretinal space between RPE and the neuroretina is considered as an immune-privileged site (Xu et al., 2009). Glucose entry into the retina occurs at two major anatomical interfaces: the retinal capillaries and the RPE. These cells form part of the BRB. Glucose entry into the retina through the BRB is mediated by a saturable, facilitated transport process involving glucose transporter 1 (GLUT1), a member of a family of sodium-independent glucose transporter proteins (Kumagai, 1999). It has already been demonstrated that GLUT1 has asymmetrical distribution between the luminal and abluminal membranes (with a 4-to-1 ratio) of the inner BRB in normal rats (Fernandes et al., 2003). Glucose transport in the retinal endothelial cells is mediated by a number of different factors, including hypoxia, hyperglycemia, and cytokines, through a variety of different signaling pathways (DeBosch et al., 2002). Several factors associated with the development and progression of DR, such as hypoxia, VEGF, and IGF-1, have been demonstrated to increase GLUT1 expression and/or glucose transport (DeBosch et al., 2002; Sone et al., 1997).

p0635 High blood glucose results in high glucose levels in the retina by transporting more glucose with the GLUT1 between the blood and retina. Changes in retinal endothelial cell GLUT1 expression and glucose transport may have a major impact in providing substrate to the various pathogenic processes. It has also been thought to underlie the development of DR and be the ultimate cause of it (Kumagai, 1999). To protect the intracellular environment from excessive glucose flux and/or diabetes, most tissues downregulate glucose transport in response to elevated extracellular glucose concentration. In STZ-induced diabetic rats, Badr et al. (2000) reported a 50% decrease in total retinal GLUT1 and retinal microvascular GLUT1 after 8 weeks of diabetes. Persistently high glucose levels may induce the proliferation of RPE cells. RPE cells also secrete VEGF, which is believed to play an important role in the neovascularization of the retina. RPE showed deepened hollows in the basal infoldings 1 month after the onset of diabetes. After 6 months, the RPE contained large concavities, which were created by depressions of basal infoldings. Both the microvilli and the basal infoldings were degenerated, which suggests the destruction of the transport pathway between the choroid and the outer retina suggesting a possible breakdown of the BRB (Aizu et al., 2002; Fig. 1.4).

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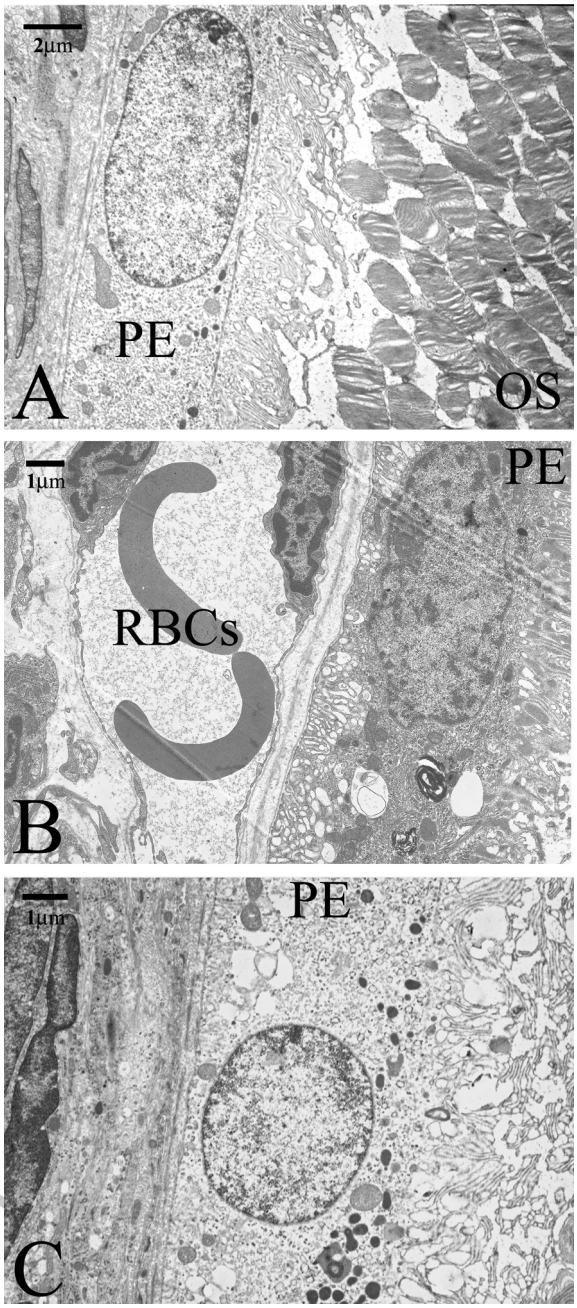

f0020 **Figure 1.4** Electron micrographs of pigment epithelial cells (RPE) and photoreceptor outer segments (OS) in control, early diabetic (3 weeks) and chronic diabetic (4 months)

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s0065 ### 2.1.6 Cells migrating to the retina in response to diabetes

p0640 DR is a chronic inflammatory disorder; low-grade inflammation has been observed in the retinas of both diabetic animals and human patients (Kern, 2007; Krady et al., 2005; Zeng et al., 2008).

p0645 Microglial cells, the main resident sentinel immune cells in the healthy retina, are located in the inner retina, around blood vessels. These cells become activated and migrate in the subretinal space in several retinopathies, including DR (Zeng et al., 2000, 2008). The activation of microglia induced by hyperglycemia has been associated with the early development of DR and occurs as early as electroretinographic modifications (Gaucher et al., 2007; Kern, 2007). Cytokines, among other cells released by activated microglia, were shown to contribute to neuronal cell death (Krady et al., 2005). Activated displaced microglial cells have a pathogenic role in the time course of DR. Indeed, activated microglia produce cytotoxic substances, such as TNFα, ROS, proteases, and excitatory amino acids, which may induce neuronal degeneration. *In vivo*, the time course of diabetes in noninsulin-dependent Goto–Kakizaki (GK) rats is modified by microglia/macrophage trafficking, leading to subretinal accumulation and potential toxicity mediated by these cells. "Pores" in RPE cells are formed at the early stages of DR, when tight junctions between RPE cells are still intact and serve as a migratory pathway for inflammatory cells (microglia/macrophages). This "transcellular" route is also used by leukocytes or lymphocytes for migration through the endothelial cell bodies. The transcellular pathway is established in RPE cells in the following steps: (i) the identification of "tunnel structures" crossing the whole thickness of the cell with preserved tight junctions, (ii) the recruitment of interstitial cell adhesion molecule 1 (ICAM-1) and caveolin 1 (CAV-1) around the pores and getting protein kinase Cζ (PKCζ) involved in the pore formation, and (iii) the identification of ionized calcium-binding adaptor molecule 1-positive cells (resident microglial cells) crossing the RPE through the pore. The presence of microglia/macrophages in the retina of diabetic GK rat could be related to the expression of ICAM-1, which is

rat retinas. In normal retinas, intact structures of the RPE and the OSs were seen (A). Early diabetic retinas contained abnormal blood vessels with red blood cells inside the retina, nearby the RPE. The somata of RPE were pycnotic; the membranes between the RPE and choroid have swollen (B). RPE had intact appearance in chronic diabetic retinas, and it seemed like the control retinas; however, their microvilli were missing and several degenerative structures were seen at the junction of the RPE and OS (C). Scale bars: indicated in the images. Abbreviations: OS, outer segment; RPE, pigment epithelium; RBCs, red blood cells.

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essential for inflammatory cell migration. At the early stages of diabetes (5 months of hyperglycemia), the RPE transcellular pathway is functional. Later in the course of diabetes (12 months), ICAM-1 and CAV-1 protein expressions in RPE cells decrease and the accumulation of subretinal microglia/macrophages is observed, together with significant decrease in the number of RPE pores. Morphological abnormalities are observed in the outer retina, with RPE vacuolization and loss of junction integrity as well as disorganization of photoreceptor OSs. A migration of cells from the retina toward the choroid could occur through RPE; PKCζ is a new actor participating in this process (Omri et al., 2011). Via the inhibition of PKCζ signaling pathway, macrophage survival and deactivation are promoted possibly by changing the intraocular immune environment (Mantovani et al., 2004). PKCζ is induced by TNFα, suggesting its possible implication in diabetes-induced BRB breakdown (Aveleira et al., 2010).

p0650 Leukocyte-mediated retinal cell apoptosis is among the earliest pathological manifestations of DR and results in the formation of acellular-occluded capillaries, microaneurysms, and vascular basement membrane thickening (Engerman and Kern, 1995). The role of VEGF in the development of these diabetic complications in the eye is well established. In human DR, all types of macrophages could be detected regardless of clinical history and duration of the disease. In the eye of a 47-year-old man with simple DR, numerous spherical cells with pseudopodal protrusions were observed throughout the retina. They were identified as macrophages in light, scanning electron and transmission electron microscopic studies. The macrophages were particularly dense and were arranged in a circinate pattern corresponding to circinate lesions within the retina. The macrophages also densely populated retinal areas with hard exudates. In areas adjacent to the macrophages, the inner retinal surface lost its smooth and continuous structure. The matrix in the ILM disappeared in such areas so that a coarse network of filaments lay bare on the retinal surface (Kishi et al., 1982). In the sclera of alloxan-induced diabetic mice, the number of mast cells was significantly increased. No increase in the mast cell number could be observed in the sclera of the obese hyperglycemic mice. The lack of insulin may be of importance for the accumulation of mast cells in the sclera of mice with hyperglycemia (Jansson and Naeser, 1987).

s0070 2.2. Molecular alterations

p0655 As we have shown in the preceding text, DR is a multifactorial progressive disease where its pathogenesis is extremely complex involving many

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different cells, molecules, and factors. Diabetes-induced metabolic changes in the retina induce a range of molecules and pathways involved early in the pathophysiology of DR.

s0075 ### 2.2.1 Apoptotic markers

p0660 Many kinds of retinal cells undergo accelerated apoptosis before other histopathologic changes are detectable in diabetes. One of the most perplexing biochemical changes in DR is the persistent, slow apoptosis of vascular and neural cells, which has been observed in retinal tissues from humans with diabetes and diabetic animal models. The relationship between apoptoses Au13 of vascular and neural cells is unclear, with the possibility that loss of these different classes of the cells occurs over different time frames and by unrelated mechanisms (Barber et al., 2011). Significant neural apoptosis is an earlier event than vascular apoptosis, and the rate of neural apoptosis remains constant throughout the duration of diabetes. Since neurons are unable to proliferate, apoptosis of these cells will result in cumulative loss leading to chronic retinal degeneration (Barber et al., 2011).

p0665 In rats, a 10-fold increase in the frequency of the apoptosis was observed compared with control animals after only 1 month of STZ-induced diabetes, continuing with the same frequency for at least 12 months. The vast majority of apoptotic cells appeared to be RGCs (Barber et al., 1998; Lorenzi and Gerhardinger, 2001). Apoptotic events can be revealed by *in situ* DNA terminal dUTP nick-end labeling (TUNEL) or measuring active caspase 3. After only 2 weeks, STZ-induced diabetic rat retinas, besides 2.5-fold more TUNEL-positive nuclei, had the highest density of caspase 3-immunopositive cells compared to the control (Gastinger et al., 2006). Labeling could be found in photoreceptors and dopaminergic amacrine cells as well. Both methods showed significantly more apoptotic cells in the retinal layer in 6-week diabetic mouse retinas, and in addition, electron microscopic analysis revealed cells in the GCL that had the classical morphological characteristics of apoptosis (i.e., disintegrating nuclei) (Martin et al., 2004).

p0670 Several studies demonstrated that oxidative stress induced by hyperglycemia is closely linked to apoptosis in a variety of retinal cell types (Abu El-Asrar et al., 2007). This may partly be linked with the changes of capillary structure and distribution. Engerman and Kern (1995) had found a small but significant increase in the number of TUNEL-positive apoptotic cells in the capillaries of postmortem human diabetic retinas (who had diabetes for an average of 10 years) compared with normal retinas. A similarly modest increase in vascular cell death was found in rats after 31 weeks of STZ-induced diabetes. The increase in vascular cell apoptosis suggests a potential

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mechanism for the appearance of acellular capillaries given that the endothelial cell bodies and nuclei were thought to disappear while leaving their intact basement membranes behind (Mizutani et al., 1996). Vascular cell dropout can be a response to reduced metabolic demands from the surrounding neurosensory retina, as in photoreceptor degeneration; hence, it is conceivable that vascular apoptosis represents a final response to localized cell death in the surrounding neural tissue (Barber et al., 2011).

s0080 ### 2.2.2 Glutamate excitotoxicity-induced apoptosis in diabetic retina

p0675 Diabetes may impair glutamate uptake and metabolism resulting in accumulation of extracellular glutamate, leading to excitotoxicity in which excess glutamate stimulation causes an uncontrolled intracellular calcium response in postsynaptic neurons (Laabich and Cooper, 2000). This is primarily true for ganglion cells, which are known to possess NMDA receptors (Thoreson and Witkovsky, 1999). The elevated aspartate immunoreactivity of Müller cells in diabetic rats suggests slower clearance of glutamate, whereas excised Müller cells from STZ-treated rats after 1 month of diabetes demonstrated reduced glutamate transporter activity (Li and Puro, 2002; Puro, 2002; Ward et al., 2005). Diabetes may also alter the regulation of glutamate receptor expression. The combined changes in the glutamate signaling system may ultimately increase apoptosis despite compensatory mechanisms designed to protect neurons, and the resultant imbalance in neurotransmission could also lead to dysfunction in visual signal processing in the retina. Thus, glutamate excitotoxicity suggests a mechanism for not only the increases in cell death but also the altered ERG response and the loss of visual function in diabetes (Barber et al., 2011).

s0085 ### 2.2.3 Pathways

p0680 Hyperglycemia has been considered the key initiator of retinal damage associated with DR by activation or dysregulation of several (glycolytic, protein kinase C (PKC), polyol, poly-(ADP)-ribose polymerase (PARP), and hexosamine) pathways. All these converge to increase the production of ROS, which induces apoptosis and inflammatory responses and promotes angiogenesis (Ola et al., 2012). It also induces the diacylglycerol (DAG) pathway *de novo*, initiating its synthesis through actions of phospholipase C. The elevated level of DAG has been linked to vascular dysfunctions and pathogenesis of DR (Geraldes and King, 2010). The activity of PKCs is greatly enhanced by DAG, which has been implicated in several cellular

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and structural abnormalities occurring in DR (Curtis and Scholfield, 2004; Das Evcimen and King, 2007).

p0685 The overexpression of the pro nerve growth factor (NGF) mimics diabetes action resulting in retinal neurodegeneration *in vivo* and *in vitro*. RGCs are more sensitive to death signals in response to proNGF. The direct apoptotic effect of proNGF on RGCs delineates the apoptotic role of RhoA activation in retinal neurodegeneration. The significant activation of RhoA/p38 mitogen-activated protein kinase (MAPK) pathway causes neuronal death with increasing phosphorylation of c-Jun N-terminal kinase (JNK) and p38 MAPK in response to overexpression of proNGF in the diabetic rat retina (Al-Gayyar et al., 2013).

p0690 The polyol pathway of glucose metabolism becomes active when intracellular glucose levels are elevated. The polyol pathway is the only mechanism of glucose toxicity currently proved to be responsible for the spectrum of neural, glial, and vascular abnormalities detectable during the development of DR (Dagher et al., 2004). Aldose reductase is the key and rate-limiting enzyme in polyol pathway, and galactose and glucose are substrates to this enzyme. Under diabetic conditions, the increased level of glucose activates this pathway, which causes reduced NADPH level in the cytoplasm. The resulted decrease in glutathione and increased oxidative stress are the major factors of the retinal damage (Barba et al., 2010). The polyol pathway is therefore a rational candidate mechanism for the ganglion cell apoptosis and Müller glial cell activation that occur early in both human and rat diabetes (Lorenzi and Gerhardinger, 2001). Further, the polyol pathway initiates and multiplies several mechanisms of cellular damage by activation of aldose reductase and other pathogenetic factors such as advanced glycosylated end product (AGE), activation of oxidative–nitrosative stress, and PKC pathway that may lead to initiation of inflammation and growth factor imbalances (Obrosova and Kador, 2011). PARP activation in diabetic retinas causes DNA breaks, thus exacerbating oxidative and nitrosative stress (Drel et al., 2009). The progression of axonal atrophy and axoglial disjunction in the optic nerve in 12-month diabetic Bio-Breeding/Worcester (BB/W) rats is a polyol pathway-related mechanism activated by hyperglycemia and galactosemia. Hexosamine has been shown to impair insulin signaling in retina (Nakamura et al., 2001), therefore being also considered as a potential pathway implicated in DR (Ola et al., 2012).

p0695 The high glucose and the diabetic state stimulate different pathways to produce excess levels of ROS. It is still unclear whether oxidative stress has a primary role in the pathogenesis of diabetic complication occurring

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at an early stage in diabetes or whether it is a consequence of the tissue damage (Fernandes et al., 2011; Izuta et al., 2010). Mitochondria play a key role in the control of apoptotic cell death. Bad is a proapoptotic member of Bcl-2 family that promotes apoptosis by binding to and inhibiting functions of antiapoptotic proteins Bcl-2 and Bcl-xL. Early during the apoptotic process, mitochondria can release several apoptogenic proteins, such as cytochrome *c* and apoptosis-inducing factor (AIF), into the cytosol (van Gurp et al., 2003). Increased ratio of Bax to Bcl-2 occurs early in the DR and plays a role in the apoptosis of retinal cells (Gao et al., 2009b). Retinal mitochondria become leaky during diabetes (8 months). When capillary cell apoptosis is observed, cytochrome *c* starts to accumulate in the cytosol and Bax is secreted into the mitochondria. The inhibition of superoxides in turn inhibits the glucose-induced release of cytochrome *c* and Bax and inhibits apoptosis in both endothelial cells and pericytes (Kowluru and Abbas, 2003). Au14

p0700 Mitochondria are also the major endogenous source of superoxides and hydroxyl radicals. Reactive oxidant intermediates can trigger mitochondria to release cytochrome *c*, resulting in activation of caspase 3 (Sandbach et al., 2001). Overproduction of superoxides by mitochondria is considered a causal link between elevated glucose and the major biochemical pathways postulated to be involved in the development of diabetic complications (Brownlee, 2001). According to a recent report, mitochondrial structure, mitochondrial function, and mitochondrial DNA (mtDNA) are damaged in the diabetic retina and its vasculature, and the mtDNA repair machinery is also compromised. Diabetes also facilitates epigenetic modifications, which contribute to the mitochondria damage. Diabetes-induced abnormalities in mitochondria continue even when the hyperglycemic insult is terminated and are implicated in the metabolic memory phenomenon associated with the continued progression of DR (Kowluru, 2013).

p0705 An increase in peroxynitrite as indicated by tyrosine nitration correlates with accelerated retinal endothelial cell death, breakdown of the BRB, and accelerated neuronal cell death in experimental models of diabetes, inflammation, and neurotoxicity (Ali et al., 2008; Du et al., 2002; El-Remessy et al., 2003a,b, 2005). Peroxynitrite plays a key role in mediating different aspects of DR. In response to hyperglycemia-induced oxidative stress, both microglial and macroglial cells are activated, and the function of macroglia in transporting glutamate by glutamate transporters and in metabolizing glutamate by GS may be impaired (Li and Puro, 2002; Lieth et al., 1998, 2000). This may lead to glutamate accumulation, such as that reported in the vitreous humor of diabetic patients (Ambati et al., 1997) and in the retina of

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diabetic animals (Kowluru et al., 2001; Lieth et al., 1998). GS is susceptible to tyrosine nitration, which subsequently can impair the enzyme activity (Gorg et al., 2005, 2007). This vicious cycle of glial dysfunction will result in cell death and the injury of adjacent retinal neurons.

s0090 ### 2.2.4 Metabolic end products

p0710 Glycation is an important event that occurs in physiological and pathophysiological circumstances. Acute hyperglycemia and chronic hyperglycemia are known to enhance early, intermediate, and advanced glycation. The deposition of hyperglycemia-induced AGEs in retinal blood vessels plays an important role in the onset and development of DR *in vivo*. AGEs are late products of nonenzymatic glycation. The pathogenic significance of ALEs in DR is less well known (Shanmugam et al., 2008; Stitt et al., 2004) than those of AGEs (Denis et al., 2002). AGEs/ALEs can form on the amino groups of proteins, lipids, and DNA through a number of complex pathways including nonenzymatic glycation by glucose and reaction with metabolic intermediates and reactive dicarbonyl intermediates. AGEs/ALEs are known to accumulate in the diabetic retina where they are thought to influence retinal vascular cell function (Stitt et al., 2004). A possible pathway of oxidative stress in the development of DR that may contribute to retinal cell dysfunction and degenerative changes is the formation of ALEs. ALEs may induce a variety of cytopathologic effects, including cross-linking of cell surface proteins, inactivation of enzymes, and stimulation of proinflammatory and proapoptotic signaling pathways.

p0715 During the pathological action, AGEs promote the upregulation of SLIT-ROBO signaling, which acts as a cue in neuronal guidance during development and plays a role in vasculogenesis and angiogenesis throughout life. Hereby, it may actively participate in the progression of DR (Zhou et al., 2011). The SLIT-ROBO signaling cascade includes Slit family of secreted proteins (Slit1, 2, and 3) and their corresponding receptors (Robo1, 2, 3, and 4). Robo1 and 4 are involved in retinal vasculogenesis and angiogenesis (Huang et al., 2009a,b, 2010) and are expressed in the choroid and the retinal endothelial and RPE cells. RPE cells autosecrete Slit2, which then binds the single-pass transmembrane receptor, Robo1, under conditions of DR. AGEs promote the upregulation in Robo1 and Slit2 expressions, which may be involved in the pathological action of AGE. N-Slit2 (the recombinant protein) results in increased proliferation, attachment, and migration of RPE cells, which increases VEGF mRNA expression and VEGF secretion (Zhou et al., 2011).

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p0720 AGEs initiate the onset of DR through both receptor- and nonreceptor-mediated pathways. AGE cross-linking causes proteins that are normally flexible to become rigid, resulting in their dysfunction. The binding of AGEs with receptors in macrophages and endothelial cells initiates a release of a large number of cytokines, inducing endothelial cell proliferation and damaging retinal blood vessels (Qazi et al., 2009). Glucose irreversibly modifies long-lived macromolecules by forming AGEs during periods of normal glucose homeostasis, which may explain the phenomenon of hyperglycemic memory. AGEs cause qualitative and quantitative changes in extracellular matrix components such as collagen and laminin and can affect cell adhesion and growth and matrix accumulation, thus altering cell function. AGEs can attack the nucleus directly and block DNA synthesis, thus interfering with the proliferation of pericytes. AGEs disturb microvascular homeostasis through interaction with the receptor of AGEs (RAGE). This AGE–RAGE axis plays a central role in the inflammation, neurodegeneration, and microvascular dysfunction in DR (Zong et al., 2011).

p0725 Increased AGEs formation and accumulation have been found in retinal blood vessels and vitreous of diabetic patients and animals, which correlate with a degree of DR (Goh and Cooper, 2008). Retinal pericytes accumulate AGEs during diabetes, which are implicated in endothelial cell injury and BRB dysfunction (Stitt et al., 2000). AGEs increase VEGF, monocyte chemoattractant protein 1, and ICAM-1 expressions in microvascular endothelial cells through increased intracellular ROS generation causing apoptosis of pericytes and other retinal cells (Ibrahim et al., 2011; Yamagishi et al., 2008a,b). Luo et al. (2012) found no significant differences in the amount of AGEs between 3- and 6-month STZ-induced diabetic rats, suggesting that AGE deposition in the retinal blood vessels occurs prior to obvious morphological changes in DR; thus, the damage to blood vessels by AGEs is a chronic process. The degree of AGE deposition was negatively correlated with pericyte number, but positively correlated with endothelial cell number, suggesting that AGEs damage pericytes in a cumulative manner. Without pericyte support, retinal capillaries could expand to form microaneurysms and acellular capillaries, which are closely linked with nonperfusion areas and could potentially lead to the onset and development of proliferative DR (Luo et al., 2012).

s0095 2.3. Electrophysiological changes

p0730 Tissue injuries and neurochemical changes of the retina are reflected in electrophysiological changes at all levels of visual information processing. Two

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such electrophysiological recordings are practiced routinely: ERG that reflects retinal functions and VEP that shows cortical processes.

p0735 The ERG recordings had long been believed to be useless for the detection of early DR, because the classical ERG components at that time, such as the a and b waves, had been proven to be normal at early stages of DR. Only after introducing better recording techniques (Galloway et al., 1971) did it become possible to detect fine differences between signals derived from healthy and diseased tissues. The a wave arises principally from photoreceptors, whereas the b wave originates mainly from ON bipolar cells. OPs represent the contribution of certain amacrine cells. Abnormal ERG in diabetic rats suggests the dysfunction of the retinal neuronal processing and reflects deterioration of function (Li et al., 2002). ERG deficit was found as early as 2 weeks after induction of diabetes indicating that the functional integrity of the diabetic retina had been compromised (Li et al., 2002).

p0740 After the first clinically validated change of the OPs, they were widely accepted as one of the earliest manifestations of DR (Galloway et al., 1971). The OP generator is presumed to lie in the intraretinal feedback neuronal circuity, which consist of the interneurons. The disturbance of dopamine metabolism in the retina may play a role in the OP abnormality in diabetes. Morphological changes of the neural cells are seen 10 weeks after the induction of diabetes in mice and rats. This suggested that the alterations of the OPs probably represent metabolic disturbances affecting inner retinal function at the early stages of DR: the depletion of dopamine and abnormal dopamine metabolism (Gibson, 1988). In STZ-induced diabetic rats, the OPs have significantly prolonged implicit times and reduced amplitudes as early as 2 weeks following the induction of hyperglycemia (Hancock and Kraft, 2004). These abnormalities are restored by the administration of insulin, suggesting that the abnormal OPs seen in early DR represent only metabolic and/or functional changes of the neurons. During 6 weeks of diabetes, the peak latency of the OPs was prolonged (Shirao and Kawasaki, 1998).

p0745 The amplitudes of a and b waves and OP1, OP2 and OP3 of ERG were reduced in rats 1 month after the onset of diabetes. The peak latencies of a and b waves were not delayed, but the OP2 and OP3 were (Aizu et al., 2002). The RGC-dominated positive scotopic threshold response was reduced following a single episode of acute IOP elevation in STZ-induced diabetic rats, but not in control rats after 11 weeks of diabetes. These data indicate that hyperglycemia renders the inner retina more susceptible (Kohzaki et al., 2012). The peak latency of the OPs is one of the most sensitive markers for the early phase of DR, while in later phases, the intensity

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response curves of the ERG b wave of diabetic rats during the 25 weeks' diabetic period were shifted to higher intensities. This, along with significant reductions in the maximal amplitude of the b wave, means reduced sensitivity. The maximal amplitude of the b wave was more reduced than the corresponding parameter of the a wave. These observations suggest that the photoreceptors are relatively resistant to the pathological processes in early diabetes (Li et al., 2002; Samuels et al., 2012).

p0750 The loss of amacrine cells may play a role in the reduction of the OPs in animals and humans with diabetes (Dong et al., 2004). The source of abnormal or missing OPs may be from the altered synaptic activity between amacrine and bipolar cells or RGCs. These deficits could be explained by the degeneration in synaptic neurotransmission and/or combined loss of amacrine neurons and RGCs (Kern and Barber, 2008). The function of RGCs is compromised when there is a loss of dopaminergic or cholinergic signaling in the retina (Amthor et al., 2002). Loss of dopaminergic and cholinergic neurons may cause changes to visual processing that play a role in the vision loss associated with diabetes (Gastinger et al., 2006). The significantly prolonged peak latency of the OPs in the ERG and the increased VEGF level are correlated with the increased serum AGE concentration (Segawa et al., 1998).

p0755 In diabetic patients, a functional loss of the inner retina has been detected by ERG and VEP recordings before vasculopathy (Moreo et al., 1995). Spontaneously, diabetic BB/W rats develop central sensory neuropathy, characterized functionally by modification of VEP and structurally by dystrophy in the RGCs and optic nerve fibers without visible vascular changes. The amplitude alterations of ERG and VEP begin 1 week after STZ injection in rats and progress week by week reaching a stable reduction by the end of the first month of diabetes. The b wave and VEP latency changes, however, were significant only after 1 month of diabetes (Biro et al., 1998). Similar VEP latency changes appeared to be parallel with RGC dystrophy and optic nerve axonopathy in 6-month diabetic BB/W rats (Kamijo et al., 1993).

s0100 3. EXPERIMENTAL MODELS OF DIABETIC RETINOPATHY

p0760 This field became a major focus area in the recent years. Journals and academic departments devoted to target this area of research exclusively. Experimental models of any kind of human disorder undergo vigorous evaluation from time to time. DR is no exception from the rule. In this section,

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besides reviewing scientific achievements in the field, this aspect is put to the forefront. To understand the pathophysiological mechanisms of diseases and to test the pharmacological effects of new drugs, animal models for the diseases have been developed and widely utilized. These efforts called to life a new scientific discipline, translational medicine.

s0105 3.1. Type 1 diabetes

p0765 Due to the early discovery of easy inducibility of this disorder (Szkudelski, 2001), an enormous body of data has accumulated over the years. Therefore, most of the solid knowledge on diabetes has been gained through this approach.

s0110 *3.1.1 Chemical induction*

p0770 Alloxan and STZ are widely used as diabetogenic agents in experimental animal models that include rodents, dogs, and primates (Szkudelski, 2001). This approach replicates some of the early symptoms of DR and has the advantage that the onset of diabetes can be defined as the time of injection of the toxin. However, toxin-induced diabetes in mice has been less successful because of strain-dependent resistance to STZ (Rossini et al., 1977).

s0115 3.1.1.1 Alloxan

p0775 Alloxan is synthesized by uric acid oxidation and exerts its diabetogenic action by intravenous, intraperitoneal, or subcutaneous administration. Alloxan induces insulin release for a short duration inducing complete suppression of the islet response to glucose. The mechanism of alloxan action is through the formation of ROS, and in the pancreas, it is preceded by rapid uptake by the β cells (Weaver et al., 1978). Alloxan elevates cytosolic free Ca^{2+} concentrations in pancreatic β cells (Park et al., 1995) and also targets DNA; its fragmentation takes place in β cells exposed to alloxan (Sakurai and Ogiso, 1995).

s0120 3.1.1.2 Streptozotocin

p0780 STZ is synthesized by *Streptomyces achromogenes* and is used to induce insulin-dependent diabetes mellitus (Szkudelski, 2001). STZ-induced diabetic rats are useful models of human type 1 (insulin-dependent) diabetes mellitus with hyperglycemia. STZ action in β cells is accompanied by characteristic alterations in blood insulin and glucose concentrations. This is reflected in the abnormalities in β cell function. STZ is taken up by pancreatic β cells via the glucose transporter GLUT2. It impairs glucose oxidation and decreases

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insulin biosynthesis and secretion. At first, it abolishes the β cell response to glucose. Intracellular action results in changes in DNA in pancreatic β cells comprising its fragmentation and alkylation (Elsner et al., 2000). Some ancillary factors are involved in the process of the STZ effects: nitric oxide, ROS, and superoxide anions. Inhibition of the Krebs cycle and limited mitochondrial ATP production activate poly-ADP ribosylation (Sandler and Swenne, 1983) and are also characteristic of this condition.

p0785 Although STZ-injected rats provide an intensively studied model of diabetes, this is not identical to the clinical situation. One reason for this is that uncontrolled hyperglycemia is not typical in patients. Also, of course, rats may respond differentially to hyperglycemia and hypoinsulinemia than humans. Additionally, a chemically induced loss of β cell function is very rare in humans (Puro, 2002). Still, the most widely accepted animal model for the evaluation of retinal complication of diabetes is the STZ-induced diabetic rats. It has been shown that in STZ-treated rats, significantly more cells undergo apoptosis, resulting in cell loss in the retinas of diabetic rats and not in control animals (Asnaghi et al., 2003; Barber et al., 1998). However, some typical clinical symptoms (neovascularization and macular edema) could not be detected in rats.

p0790 During the preclinical phase of diabetes drug research, it is indispensable to perform experiments on a reliable animal model to mimic diabetes-related complications. Recently, several reviews have been published summarizing the existing methods for the investigation of diabetic eye disorders in rodents (Islam, 2013; Jo et al., 2013; Kong et al., 2013; Robinson et al., 2012). Nowadays, a number of diabetes models are well known and widely used, but only a few of them offer a thorough examination of the diabetic manifestations in their complexity. Such diabetic state should be extended for a time interval, long enough to allow the formation of accurately detectable functional and morphological diabetes-related complications in various severities. However, the main limitation of the currently available diabetes models is that the experimental subjects cannot tolerate the severe general conditions in the absence of any antidiabetic therapy (Fox et al., 1999). Therefore, it is essential to apply an experimental design enabling tolerable general states of the animals. Maintenance of a suboptimal glycemic control mimics the diabetic conditions and permits the development of chronic complications. Our group has recently developed a novel chronic, insulin-controlled method for the investigation of diabetic ocular changes. Diabetes was induced by single-dose STZ injection and maintained for 12 or 16 weeks in male Sprague–Dawley rats. Diabetic rats were divided into four

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groups according to the different doses of insulin replacement with subcutaneously applied implants. In groups where blood glucose levels were between 5–10 mmol/l and around 20 mmol/l, resulting in mild and medium severe forms of diabetes, we found only minimal functional and histopathologic changes in the eye. When the blood glucose concentration was over 30 mmol/l, severe pathophysiological signs developed. Marked corneal neovascularization, retinal degeneration, and cataract formation were observed. This chronic, insulin-controlled animal model is available for the preclinical studies of promising drug candidates against diabetic complications (Hajna et al., 2013).

s0125 ### *3.1.2 Genetic defects*

p0795 Using the advantages of the widespread controlled breeding of laboratory animals (primarily rats and mice), a number of inbred strains were selected with metabolic deficiencies, several of those bearing the hallmarks of diabetes.

s0130 #### 3.1.2.1 BB/W rats

p0800 The BB/W Wistar rat develops autoimmune diabetes similar to type 1 human diabetes mellitus. The syndrome is characterized by sudden onset of hyperglycemia, ketonemia, and pancreatic β cell destruction; therefore, BB/W rat strain is a model of type 1 diabetes. In these animals, diabetes develops spontaneously. It is an excellent laboratory model of type 1 juvenile-onset diabetes mellitus from both a metabolic point of view and an immunological point of view. The diabetic syndrome of these rats spans a spectrum of increasing severity from insulitis without glucoregulatory changes to insulin-dependent diabetes with massive β cell destruction. Obesity is absent, both sexes are affected, and peak incidence of diabetes occurs around the age of sexual maturation (80–100 days). Sublines with expected Au15 zero incidence and high diabetes incidence (40–100% overt type 1 diabetes) have been developed. However, these animals have never been examined extensively for DR syndromes. It remains to be shown that these animals develop DR at all (Yale and Marliss, 1984).

s0135 ## 3.2. Type 2 diabetes

p0805 Type 2 diabetes is a complex, heterogeneous, polygenic disease characterized mainly by insulin resistance and pancreatic β cell dysfunction. Appropriate experimental models are essential tools for understanding the molecular basis, pathogenesis of the vascular and neural lesions, actions of therapeutic agents, and genetic or environmental influences that increase

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the risks of type 2 diabetes. In contrast to type 1 diabetes, where rats are used most often, there are several mouse models of type 2 diabetes too.

s0140 ### 3.2.1 OLETF rats

p0810 A spontaneously diabetic rat with polyuria, polydipsia, and mild obesity was discovered in 1984 in an outbred colony of Long–Evans rats. A strain of rats developed from this rat by selective breeding has since been maintained at the Tokushima Research Institute and named Otsuka Long–Evans Tokushima Fatty (OLETF) strain. They became a widely used model of noninsulin-dependent diabetes. The characteristic features of OLETF rats are late onset of hyperglycemia (after 18 weeks of age), a chronic course of disease, mild obesity, inheritance by males, hyperplastic foci of pancreatic islets, and renal complication (nodular lesions). Histologically, the changes in pancreatic islets can be classified into three stages: (1) an early stage (6–20 weeks of age) of cellular infiltration and degeneration, (2) a hyperplastic stage (20–40 weeks of age), and (3) a final stage (at >40 weeks of age). These clinical and pathological features of disease in OLETF rats resemble those of human noninsulin-dependent diabetes mellitus. Architectural defects of the islet capillaries can cause impaired β cell function in aged OLETF rats (Mizuno et al., 1999).

p0815 OLETF rats exhibited a significantly reduced total retina thickness, especially that of the NFL at 28, 36, and 40 weeks of age. Histological examination revealed increased apoptosis (active caspase 3-positive and TUNEL-positive cells in NFL) and a decrease in the number of RGCs (Yang et al., 2013a). GFAP immunofluorescence staining was upregulated in vertical sections and showed a more ramified pattern in whole-mount retinas. VEGF expression extended into the OPL (Jung et al., 2013). The INL decreased from 3–4 rows to 2 rows, whereas the photoreceptor cell nuclei decreased from 8 rows to 3–6 rows. These results suggest that retinal neurodegeneration occurs in type 2 diabetic OLETF rats. RPE decreased in height, and basal infoldings were poorly developed. Retinal capillary basement membranes were significantly thicker in the OLETF rats than in the Long–Evans Tokushima Otsuka (LETO) rats, and endothelial cell damage was observed (Lu et al., 2003). In the 64-week-old OLETF rats without treatment, corrosion cast revealed diabetic retinal and choroidal vascular changes: tortuosity of the vessels, variations in caliber, narrowing of arteries, arterio-arterial anastomoses and hairpin loop formation in precapillary arterioles, sparse collecting venules in the choroid, and marked capillary changes such as caliber irregularity, narrowing, tortuosity, loop formation and

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decreased capillaries, outpouching, and microaneurysms (Bhutto et al., 2002). However, they are not the most suitable animal model for the study of angiopathic DR because retinal capillaries of OLETF rats were found to remain morphologically normal and pericyte ghosts were barely detectable. There was no difference in the number of acellular capillaries in the retinas between OLETF and control LETO rats. Formation of acellular capillaries and pericyte ghosts, the characteristic morphological changes in early DR, is not accelerated in OLETF rats (Matsuura et al., 2005).

s0145 ### 3.2.2 Zucker diabetic fatty rats

p0820 The Zucker diabetic fatty (ZDF) rat is most widely used for studying type 2 diabetes associated with obesity. The initial characterization of a novel type 2 diabetes model derived from Zucker fatty (ZF) rats. Further inbreeding resulted in new phenotypes.

p0825 Zucker fatty diabetes mellitus rats develop diabetes as early as 10 weeks of age, which reaches 100% incidence at around 20 weeks of age. This rat strain possesses high reproductive efficiency and therefore should serve as a useful model of young- to middle-aged adult-onset type 2 diabetes in the studies of the pathophysiology, therapeutic interventions, and complications of the disease (Yokoi et al., 2013).

p0830 Retinal VEGF mRNA and protein expression increased in ZDF rats after 2 months of disease, but not in ZF rats. Some oxidative stress and inflammatory markers (TNFα, IL-6, ICAM-1, and IL-1β) were upregulated in the retina of ZDF rats after 4 months of the disease. In contrast, activation of nuclear factor κB (NF-κB) in the retina was observed in ZF and ZDF rats (Mima et al., 2012). ZDF rats had thicker basement membranes and more cells per unit capillary length (Yang et al., 2000). Diabetes-increased activated caspase 3- and TUNEL-positive microvascular cell numbers and acellular capillary formation were reduced in ZDF rats (Behl et al., 2008). Acute hyperglycemia did not have an effect on control rats, while chronic hyperglycemia in ZDF rats was associated with enhanced scotopic ERG amplitudes, which were up to 20% higher than those of age-matched controls (8–22 weeks of age), and a reduction in a wave amplitudes and maximum slopes of about 30%. The electrophysiological differences between untreated ZDF rats and controls preceded an activation of Müller cells in the ZDF rats (upregulation of GFAP), which was attenuated by insulin treatment. There were otherwise no signs of cell death or morphological alterations in ZDF groups (Johnson et al., 2013). These data show that under chronic hyperglycemia, the ZDF rat retina became abnormally sensitive to variations in

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substrate supply. In diabetes, a similar inability to cope with intensive glucose lowering could render the retina susceptible to damage.

s0150 ### 3.2.3 Spontaneously diabetic Torii rats

p0835 A new spontaneously diabetic strain of the Sprague–Dawley rat was established in 1997 and named the spontaneously diabetic Torii (SDT) rats. The SDT rat strain should be a useful model to understand the pathology of the diabetic disease. An SDT rat spontaneously develops hyperglycemia resulting from a defect of insulin secretion due to tissue damage and dysfunction of the pancreatic islets. Glucose tolerance in SDT rats was impaired in correspondence with the decreased insulin secretion. Diabetes in SDT rats directly results from absolute decrease of insulin secreted from pancreatic β cell. The body mass of the SDT rats decreased after the onset of hyperglycemia, but the SDT rats did not show obesity throughout their lives. The SDT rats exhibited reduced pancreatic insulin content at 20 weeks and a decreased mass of β cell at 10 weeks. The β cells of the SDT rats are in the sense of "overworking" to fulfill the insulin demand of the peripheral tissues with a limited number of β cells and increased body mass before the onset of diabetes. Males spontaneously develop hyperglycemia predominantly due to an insulin secretory defect resulting from pathological damage to the pancreatic islets (Masuyama et al., 2004). Glucose tolerance in SDT rats was impaired in correspondence with the decreased insulin secretion. The age of onset of diabetes in SDT rats depends on the magnitude of glucose intolerance observed prior to the onset of diabetes (Masuyama et al., 2003). Inflammation could be observed in the SDT rats, such as lymphocyte infiltration consistently observed in autoimmune diabetes (Komeda et al., 1998; Like et al., 1982). Male SDT rats spontaneously develop hyperglycemia without obesity after 20 weeks of age with an incident rate reaching 100% at 40 weeks of age. At 38 weeks, almost all the β cells disappear from the pancreatic islets of SDT rats that show overt hyperglycemia and marked hypoinsulinemia accompanied by decreased body weight and body mass index (Masuyama et al., 2004). SDT rats develop severe diabetic ocular complications such as cataract and proliferative retinopathy (by 40 weeks of age) and tractional retinal detachment with fibrous proliferation (by 70 weeks of age) and massive hemorrhaging in the anterior chamber (by 77 weeks of age), which resemble human diabetic ocular complications (Shinohara et al., 2000). The SDT rat is a useful animal model to elucidate the underlying mechanism of nonobese type 2 diabetes mellitus where the pancreatic islet tissue is thought to play the main role in the development of diabetes.

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p0840 The SDT rat is considered to be a potentially useful model for studies of DR encountered in humans (Shinohara et al., 2000). This notion is supported by the fact that large retinal folds mimicking diabetic tractional retinal detachment were observed in SDT rat retinas like retinal hemorrhages, a neovascular fibrous membrane around the iris, a massive anterior chamber hemorrhage, an area of nonperfusion and/or extensive hyperfluorescence, acellular capillaries and pericyte loss, and retinal changes (Kakehashi et al., 2006). Apoptotic cells in the GCL and the INL were numerous in 40-week-old SDT rat retinas; GFAP immunoreactivity spanned the whole retina in SDT rats. The perivascular AQPs shifted from AQP4 to AQP1 in 40-week-old SDT rats that exhibited marked hyperglycemia. Thus, the development of diabetes increases neuroretinal apoptosis and coincides with an altered expression pattern of GFAP and water-selective channels AQP1 and AQP4 in SDT rats (Fukuda et al., 2010). In 44-week-old animals, a and b waves and the OPs were significantly reduced with prolonged implicit times in the SDT rats compared with Sprague–Dawley rats (Okuno et al., 2008). The depressed ERG may reflect vascular and neuronal damage throughout the retina as are seen in the advanced stages of human DR. Further, in SDT rat, hyperglycemia-induced abnormal retinal vascular bed was formed and the optic disc protruded (Sasase et al., 2009). Thus, the SDT rat can be used to study the physiology of DR.

s0155 ### 3.2.4 GK rats

p0845 The GK rat is a spontaneous model of noninsulin-dependent diabetes mellitus without obesity, which was developed by repeated selective breeding of normal Wistar rats using glucose intolerance as a selection index (Goto and Kakizaki, 1981). Glucose intolerance appears after 2 weeks of age (Goto and Kakizaki, 1981) and a significant hyperglycemia is found as early as 4 weeks of age, and the animals show hyperinsulinemia and decreased pancreatic insulin stores. Development of type 2 diabetes mellitus in the GK model results from the complex interaction of multiple events such as (i) the presence of several susceptibility loci containing genes responsible for some diabetic traits, (ii) gestational metabolic impairment inducing an epigenetic programming of the offspring pancreas and the major insulin target tissues, and (iii) environmentally induced loss of β cell differentiation due to chronic exposure to hyperglycemia/hyperlipidemia, inflammation, and oxidative stress (Portha et al., 2012). The adult GK body weight is 10–30% lower than that of age- and sex-matched control animals. Signs of early neuropathy (2 months) have been reported in GK adult rats, while

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nephropathy and retinopathy develop late (12 months; Portha, 2005). In the NFL, RPE, and choroid, strong VEGF immunoreactivity was noted only in the GK rat. Increased VEGF production in certain ocular tissue, similar to that in humans, is observed quite early, at least before the appearance of observable retinal changes in the diabetic GK rat (Sone et al., 1997).

p0850 GK rats do not exhibit cataracts for a long time because of their moderate diabetic state. However, they provide a useful model for the investigation of the retinal microcirculatory changes of diabetes mellitus over an extended time, since they show decreased retinal blood flow with reduced oxygen consumption without serious retinopathy; at the same time, decreased retinal metabolism, increased blood viscosity, and increased vascular resistance to flow can be detected in them (Miyamoto et al., 1996).

p0855 In the GK rats, immunoreactivity to L-glutamate and GABA was observed in the Müller and photoreceptor cells in addition to the immunoreactivity in normal rats. These immunoreactivity patterns in the GK rat retina might be induced by ischemia associated with diabetes mellitus (Takeo-Goto et al., 2002). Morphological changes could be demonstrated in retinal vessel preparations of GK rats; the endothelial/pericyte ratio was found to be higher in GK rats aged 8 months and after 24–30 months compared with their matched controls. Furthermore, in 24- to 30-month-old GK rats, the endothelial/pericyte ratio was higher than in 8-month-old GK rats (Agardh et al., 1997). *In vivo* studies indicated that in GK rats, the BRB permeability was increased; *ex vivo* studies showed that in retinas from GK rats, NOS activity was also higher; there is an increased production of NO, which may contribute to the BRB breakdown (Carmo et al., 2000).

p0860 The amplitudes of ERG a and b waves and also the OPs of the GK rats were reduced between 4 and 48 weeks of age. The a wave latencies in GK rats were significantly prolonged, but not the implicit times of OPs. At 14 days of age, the a wave amplitudes were significantly smaller in GK rats than in Wistar rats. Functional abnormalities of photoreceptors might be induced by inheritable degeneration at an early age in the GK rat. Although hyperglycemia would cause retinal hypoxia, it would not be severe enough to disturb the generation of OPs (Matsubara et al., 2006). Taken together, the GK rat appears to be a suitable model for experimental studies of chronic complications of diabetes, including DR. These results also suggest that the GK rat can be used as a model of initial-phase or latent-phase DR.

s0160 *3.2.5 ob/ob mice*

p0865 ob/ob mice are hyperphagic, obese, hyperinsulinemic, and hyperglycemic, so they are used as a model for diabetes and obesity. ob/ob mice are

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indistinguishable from their lean littermates at birth, but later, they become heavier and develop hyperinsulinemia. These differences are much more pronounced after weaning, and overt hyperglycemia is observed during the fourth week. The blood glucose rises to reach the peak after 3–5 months when the mice also have very high food intake and a rapid growth. After that, blood glucose values decrease and eventually become nearly normal at old age. The animals remain insulin-resistant, but impaired glucose tolerance and glycosuria after a glucose load are observed mostly in the postweaning period of rapid growth, and this usually becomes normalized when the mice get older (Lindstrom, 2007). In ob/ob mice, insulin receptor, insulin receptor substrate 1, and insulin receptor substrate 2 proteins and phosphorylation were maintained or increased, while protein levels and phosphorylation of pyruvate dehydrogenase kinase and Akt were decreased in the retina. Interestingly, phosphorylations of p38 MAPK and extracellular signal-regulated kinase 1 (ERK1) were responsive to insulin in the retina. At the same time, hypoxia-inducible factor (HIF)-1α and VEGF were increased and endothelial NOS was decreased there (Kondo and Kahn, 2004).

s0165 ### 3.2.6 db/db mice

p0870 The db/db mice are perfect animal models of type 2 diabetes. The phenotypes of severe obesity, hyperphagia, polydipsia, and polyuria are due to a spontaneous mutation of leptin receptor. The db/db mice are differentiated by the spontaneous mutations in different sites of leptin receptors. The db/db mice have abnormal phenotypes in their metabolic, reproductive, and immune systems (Wua et al., 2013). The genetically diabetic db/db mouse is a model of type 2 diabetes, where nephropathy and neuropathy, but not retinopathy, were observed in early examinations. Midena and coworkers (1989) showed a marked increase in the ratio of endothelial cells to intramural pericytes in diabetic mice compared with controls. This increase resulted from a selective and highly significant loss of pericytes in db/db mice. Some strand-like and relatively acellular capillaries were also observed. Basement membrane thickening and an accumulation of basement membrane material in the capillaries of the OPL of retinas from diabetic db/db mice were observed. When the thickness of the whole retina, in particular the INL and ONL, and the integrity of the RPE and the RGC numbers were examined, all were found decreased in db/db mice (Tang et al., 2011). At the same time, expression levels of Nox4 and VEGF were significantly increased in their retinas (Li et al., 2010).

p0875 In 15-month-old db/db mice, signs of DR, including BRB breakdown, loss of pericytes, neuroretinal apoptosis, glial reactivation, and proliferation

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of blood vessels, were evident. These changes in the diabetic retina were associated with increased expression of aldose reductase (Cheung et al., 2005). The db/db mice at the age of 19 weeks exhibited significantly increased retinal vascular leakage and decreased tight junction protein level in the retina. Moreover, the expression of proinflammatory factors, for example, ICAM-1 and TNFα, was drastically upregulated in diabetic retina (Li et al., 2009).

p0880 The altered visual functions of RGCs were characterized by the reduced receptive field center size, elevated luminance response, and attenuated contrast gain in 12- and 20-week db/db mice, respectively. These altered visual functions could, at least partly, be due to oxidative stress (Xiao et al., 2012a). Therefore, the db/db mouse may represent an adequate model for studies on the pathogenesis of DR.

s0170 3.2.7 $Ins2^{Akita}$ mice

p0885 An autosomal-dominant mutation that produces juvenile-onset hyperglycemia and insulinopenia in the absence of obesity was discovered in C57BL/6 mice. The $Ins2^{Akita}$ mutation disrupts normal insulin processing and causes a failure in secretion of mature insulins, which results in the early development of hyperglycemia (Mathews et al., 2002). The $Ins2^{Akita}$ mutation results in a single amino acid substitution in the insulin 2 gene and replaces a cysteine with tyrosine at the seventh amino acid of the A chain of the insulin 2 gene product, blocking the formation of an essential disulfide bond between the A and B chains of the mature protein, that causes misfolding of the insulin protein. The mutation arose and is maintained on the C57BL/6J background. Male mice heterozygous for this mutation have progressive loss of β cell function and decreased density with significant hyperglycemia as early as 4 weeks of age (Barber et al., 2005).

p0890 As we have mentioned earlier, dopaminergic and cholinergic amacrine cells are lost during the early stages of retinal neuropathy in diabetes. Loss of these neurons may play a critical role in the development of visual deficits (Gastinger et al., 2006). Within the first 3 months of diabetes, RGCs are lost from the peripheral retina of $Ins2^{Akita}$ mice, and the dendrites of surviving large ON-type cells undergo morphological changes. These abnormalities may explain some of the early anomalies in visual function induced by diabetes (Gastinger et al., 2008). $Ins2^{Akita}$ diabetic models are characterized by upregulation of α-, β-, and γ-crystallins in the retina. Despite being overexpressed, the molecular properties of α-crystallins are disrupted by diabetes and contribute to the loss of neuroprotective function. Identification and

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prevention of these alterations could lead to the emergence of new therapies for DR (Losiewicz and Fort, 2011). Although both choroidal and retinal blood flow and vision were altered after prolonged diabetes in the $Ins2^{Akita}$ mouse, choroidal blood flow was reduced early, suggesting that ocular blood flow deficit could be an early pathological change in DR (Muir et al., 2012).

p0895 Diabetes increases retinal vascular permeability in mice. The number of leukocytes adherent to the vascular wall was significantly elevated in the $Ins2^{Akita}$ mouse, confirming that the vascular inflammatory component of DR is present in this model. Astrocytes in some regions of the $Ins2^{Akita}$ mouse retinas contained reduced GFAP immunoreactivity with shorter processes, suggesting atrophy or loss of contact with blood vessels, but increased GFAP expression was not observed. Regions of the $Ins2^{Akita}$ mouse diabetic retinas contained microglia with swollen and contracted processes, suggesting a reactive state. Thinning of the inner layers of the retina suggests that chronic degeneration occurs after 22 weeks of hyperglycemia. The significantly reduced thickness of the peripheral INL suggests loss of horizontal, bipolar, and amacrine cell bodies. The thickness of the IPL was significantly reduced in all regions measured, indicating atrophy of the processes between neurons in all parts of the retina. There was no significant loss of the ONL and the OSs of the photoreceptors, suggesting that the degeneration predominantly occurs in the inner retina. Increased apoptosis was found in $Ins2^{Akita}$ mice after 4 weeks of hyperglycemia (Barber et al., 2005). No retinal thinning or disruption of retinal architecture was observed by optical coherence tomography or resin histology up to 6 months of age. In addition, no vascular changes were detected by fluorescein angiography or by scanning laser ophthalmoscopy. With the exception of microglial activation, reduced GFAP expression in astrocytes, and an increase in GFAP expression by Müller cells, no other changes were observed in the $Ins2^{Akita}$ mouse retina. These observations indicate that the classical clinical correlates of human DR are absent in $Ins2^{Akita}$ mice up to 6 months of age suggesting either that the histopathologic processes underlying the development of DR in this model require longer than 5 months of hyperglycemia to result in disruption of retinal architecture or that advanced DR is not a feature of the $Ins2^{Akita}$ diabetic mouse (McLenachan et al., 2013).

p0900 As can be seen from the aforementioned facts, the $Ins2^{Akita}$ mouse has several important advantages over other animal models. First, the autosomal-dominant mutation provides the opportunity to study heterozygotic animals. Second, the mice are fertile and breed well. Third, they have stable diabetes Au18 with insulin deficiency and can be maintained in a noncatabolic state without

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exogenous insulin. Fourth, the mechanism of diabetes onset does not involve systemic immunologic alterations, and it is therefore possible to evaluate the metabolic impact on the retina (Barber et al., 2005). Therefore, the use of the $Ins2^{Akita}$ mouse is an excellent choice to explore the molecular mechanisms involved in the initiation and progression of DR in older ages.

s0175 3.3. *Ex vivo* and *in vitro* models

p0905 There is increasing pressure from the society to find viable alternatives for animal research. Along with this requirement, scientists tried to set up experimental models for DR, which did not involve long-term keeping of sickened animals. In the succeeding text, we collected the results obtained in these models and evaluate how well they fare compared with *in vivo* models. Also, we try to pinpoint those aspects of DR, which can be studied using these approaches.

s0180 3.3.1 Retinal ganglion cell lines

p0910 The retinal ganglion cell line RGC-5 is a transformed line obtained from postnatal Sprague–Dawley rats. For studies of the effect of high glucose levels, cells were grown in a medium supplemented with 11 mM glucose (control) plus 34 mM mannitol or 28 mM glucose (medium glucose) plus 17 mM mannitol, 45 mM glucose (high glucose), and 10 μM succinate. Mannitol was added to control for osmolar effects (Hu et al., 2013; Ola et al., 2002). After incubation with succinate and various concentrations of glucose, the expression of VEGF in RGC-5 cells was elevated. The phosphorylation levels of ERK1/2, p38 MAPK, and JNK in RGC-5 cells after exposure to high glucose were increased (Hu et al., 2013). The type 1 sigma receptor (sigmaR1) has numerous pharmacological and physiological functions. This sigmaR1 is expressed under hyperglycemic conditions both *in vitro* and *in vivo* (Ola et al., 2002), and its expression has been demonstrated recently in RGCs, which undergo apoptosis early in DR via NMDA receptor-mediated overstimulation. It can be concluded that this model is suitable to study the molecular events of neuronal apoptosis in DR.

s0185 3.3.2 Müller glial cell lines

p0915 Isolated rat retinal Müller cells (rMC-1) (from 5-day-old Wistar rats) were cultured, and passaged cells were seeded for 2 weeks in a culture to mimic diabetic conditions. In other experiments, rMC-1s were plated on tissue culture plastic. When the cells reached 70% confluence, they were treated with different concentrations of glucose (normal group, 5 mM glucose, and

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high-glucose group, 25 mM glucose) in test medium for an indicated time (Du et al., 2003; Zhang et al., 2011). The cells in the glucose-treated group showed elevated VEGF immunoreactivity and significantly more VEGF mRNA than the control cells. The expression of VEGF in Müller cells increased after a short 3-day exposure to high glucose in culture (Ke et al., 2012). Hyperglycemia significantly increased the amount of GAPDH protein in the nucleus above normal within the first 48 h and induced apoptosis. The nuclear translocation of GAPDH is closely associated with the induction of apoptosis (Kusner et al., 2004). Glutaredoxin (Grx1) plays a key role in such regulation because it is a specific and efficient catalyst of deglutathionylation, and an increased level of Grx1 could be observed in retina of diabetic rats and in rMC-1 cultured in high glucose. Diabetes or incubation in elevated glucose concentration significantly increased superoxide production, which contributes to impaired viability and increased cell death under those circumstances (Du et al., 2003). The upregulation of Grx1 was concomitant with NF-κB activation and induction of ICAM-1, which play a central role in diabetic complications *in vivo* and also in these cell cultures (Shelton et al., 2009).

p0920 It is also possible to prepare freshly isolated human retinal Müller cell culture. The eyes were enucleated, packed on wet ice, and received in the laboratory within 12 h after death. Cells were plated on tissue culture plastic in the medium and allowed to attach and grow at 37 °C in a humidified chamber. Müller cells were isolated after the second passage of cells; cultures were 95% pure for Müller cells as assessed by immunohistochemistry using antibodies to GS, GFAP, and vimentin. Cells were switched to normal (7.8 mM) or high glucose (25 mM glucose). Cells were grown for up to 5 days (Kusner et al., 2004). These cell cultures are suitable to study Müller cell reactions to hyperglycemia and diabetes but obviously not fit for studying retinal changes as a whole, not to speak about the functional integrity of the retinal tissue.

s0190 *3.3.3 Retinal pigment epithelial cell lines*

p0925 Human retinal pigment epithelial (ARPE-19) cells were cultured at 37 °C, after the cells were grown to 90% confluence; they were cultured for another 48 h in the presence of D-glucose at a concentration of 5, 7.5, 12.5, or 17.5 mM, which we refer to as very low glucose, low glucose, medium glucose, or high-glucose culture medium, respectively. ARPE-19 cells were cultured for 72 h (Xie et al., 2012; Yokouchi et al., 2013). Other cultures were exposed to D-glucose at a final concentration of

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25 and 100 mM (corresponding to 2 h after meal plasma glucose level of diabetic patients and glucose level in uncontrolled diabetic patients, respectively) and compared with cultures exposed to 5.5 mM D-glucose as control (corresponding to fasting plasma glucose level of diabetes-free people). After exposure for at least 3 weeks, the monolayer cultures were used for further analysis (Chen et al., 2012b). A significant cellular damage was seen in ARPE-19 cells after a 48 h treatment with high glucose, accompanied by a decrease in SOD activity and glutathione concentration; high glucose also caused ARPE-19 cell apoptosis and activation of p38 MAPK and ERK (Xie et al., 2012). ANGPTL4, the major angiogenic factor released by ARPE-19, was induced by high glucose in RPE cells (Yokouchi et al., 2013). Fifty-six proteins showed significant changes in expression in cultured ARPE-19 cells. Significant changes in thiol reactivity were seen in response to high glucose concentration. Some of the identified proteins have been validated with clinical samples and provide potential targets for the prognosis and diagnosis of DR (Chen et al., 2012b). Thus, this methodology may provide molecular markers for improving diagnostic possibilities in DR.

s0195 ### *3.3.4 Retinal endothelial cell lines*

p0930 Bovine primary retinal endothelial cells were incubated in 5 or 25 mM glucose (Du et al., 2003). For high-glucose treatment, endothelial cells were cultured for 10 days in media supplemented with additional glucose to a final concentration of 25 mM. Control cells were simultaneously treated with either 25 mM L-glucose or 5 mM D-glucose. Primary retinal endothelial cells were used between passages 10 and 13 (Aranda et al., 2012). In another study, a transformed endothelial cell line was isolated from the BRB of transgenic mice. The cells were cultured in 5 or 25 mM glucose (Shelton et al., 2009). Rhesus retinal vascular endothelial cell line RF/6A is also thought to be a useful cell line for modeling DR (Hu et al., 2013). High-glucose treatment of the aforementioned retinal explants mimicked the diabetic phenotype. Similarly, primary retinal endothelial cells, which were subjected to high-glucose treatment, organized into tubes that were resistant to lysophosphatidic acid (LPA). Hyperglycemia caused LPA resistance within retinal endothelial cells by elevating ROS, which stimulated the ERK pathway, which antagonized LPA-mediated signaling events that were required for regression. This ROS/ERK pathway mechanism appeared to be the same route by which diabetes-induced LPA resistance of retinal neovessels. Au19 It is concluded that diabetes/hyperglycemia reprograms signaling pathways

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in retinal endothelial cells to induce a state of LPA resistance (Aranda et al., 2012). This and similar molecular pathways can be successfully studied to understand endothelial cell reactions in DR.

s0200 ### 3.3.5 Retinal explants

p0935 Retinal explants were obtained by gently peeling the choroid away from the RPE, leaving the RPE attached to the neurosensory retina. Control retinas were cut into 1 mm^2 pieces and placed in a collagen sandwich. Vessel formation was observed after 2–3 weeks. In some experiments to model diabetes, the medium was altered to achieve a final concentration of 25 mM D-glucose or L-glucose during the 2–3-week period of tube formation (Aranda et al., 2012; Lecleire-Collet et al., 2005). For other studies, rats were killed by CO_2 inhalation after 3 weeks of diabetes. Retinas were isolated 1.5 mm from the optic nerve head, excised into 36 small pieces, and placed in a liquid collagen solution. The retinal explants were cultured to examine the effect of diabetes on the number of regenerating neurites. The number of neurites per retinal explant was counted under a phase-contrast microscope on day 6 at which time point, their number was greatly increased (Oshitari and Roy, 2005).

p0940 In an 8-week-old female, C57BL/6 mouse diabetes was induced by STZ. Retinas were dissected from the eyeballs of both 10-week-old diabetic and control mice. Retinal pieces located at a distance of 1.0–1.5 mm from the optic disc were excised and cut into 16 pieces (approximately 500 μm^2) with a sharp razor blade. These retinal pieces were embedded in collagen gel and were cultured in a medium that contained 7 mM of glucose; for high-glucose medium, 50 mM of glucose was added (the final glucose concentration was 57 mM). The number of outgrowing neurites from the explants was counted under a phase-contrast light microscope after 3, 6, and 10 days in culture. TUNEL- and cleaved caspase 3-positive cells increased significantly in retinal explants incubated in glycated bovine serum albumin (BSA) (by 2.2- and 2.5-fold, respectively). The GCL was the most sensitive retinal layer to the glycated BSA. Neuronal damage was confirmed by the increased GFAP labeling in Müller glial cells from retinal explants (Lecleire-Collet et al., 2005). The number of regenerating neurites in the retinal explants of diabetic rats was significantly reduced (Oshitari and Roy, 2005). Treatment of retinal explants with high glucose concentration (57 mM) significantly diminished the number of regenerating neurites in the control mice, but not in the diabetic mice. These results suggest that retina in diabetic mice has impaired capability of neurite

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regeneration in a normal glucose environment but is adaptable to a high-glucose environment *in vitro*.

s0205 ### 3.3.6 Choroidal explants

p0945 Choroidal explants can be prepared after removing the cornea, lens, corpus vitreum, and retina from the inside of the globe. The posterior segment containing the sclera and the choroid is sectioned into either quadrants or thirds. After breaking any adhesions between the choroid and the sclera, the isolated segment of choroid was then sectioned into 1–2 mm^2 explants and placed in the collagen gel (Lameynardie et al., 2005). Angiogenesis was triggered by the injury of the dissection procedure and did not require stimulation by exogenous growth factors. Angiogenesis is enhanced in diabetic rats when compared with Wistar rats, in the presence of fetal bovine serum. Thereafter, explants prepared as described in the preceding text were cultured with different glucose concentration (Lameynardie et al., 2005). The assay model of choroidal angiogenesis was thus established by determining the number and length of microvessels in cultured choroidal explants. The STZ-induced diabetic states of Wistar and GK rats enhanced Au20 hyperglycemia-induced choroidal angiogenesis. This assay model is useful for determining angiogenic activity of growth factors and effective drugs in diabetic choroidopathy and retinopathy.

s0210 ## 4. EXPERIMENTAL APPROACHES TO THE TREATMENT OF DIABETIC RETINOPATHY

p0950 Several experimental therapies have been found to inhibit the development of DR in animal models. These included treatments with endogenous protective compounds, natural substances, and fully synthetic drugs (Table 1.1). If one tries to understand this enormous body of data, a more systematic approach is needed. Recently, Lu et al. (2013) had shown that suppression of GLUT1 is a new strategy to prevent diabetic complications. If one considers just the protection of RGCs, a plethora of approaches can be collected from the literature. For example, anti-inflammatory drugs such as nonsteroidal cyclooxygenase inhibitor nepafenac (Kern et al., 2007; Krady et al., 2005; Vincent and Mohr, 2007); several salicylates (Zhang et al., 2007); the glutamate NMDA receptor antagonist, memantine (Kusari et al., 2007); cannabidiol (CBD) (El-Remessy et al., 2006); NGF (Hammes et al., 1995b); IGF-1 (Seigel et al., 2006); aldose reductase inhibitors (Asnaghi et al., 2003); erythropoietin (EPO) (Zhang et al., 2008); and

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Au1 t0005 **Table 1.1** Effects of different compounds in experimental diabetic retinopathy.

Effects on		Compound/drug	Type 1 diabetes	Type 2 diabetes
Vascular	VEGF ↓	Insulin	Wang et al. (2007)	
		Telimsartan/valsartan	Nagai et al. (2007)	
		Candesartan		Fukumoto et al. (2008) Sugiyama et al. (2007)
		Perindopril	Zheng et al. (2009)	
		Prorenin receptor blocker	Satofuka et al. (2009)	
		Angiostatin	Sima et al. (2006)	
		Bevacizumab	Ma et al. (2010)	
		EPO	Mitsuhashi et al. (2013)	
		Melatonin		Salido et al. (2013)
		PEDF	Boehm et al. (2003) Liu et al. (2004) Yu et al. (2010)	
		Purearin	Chen et al. (2012a)	
		Aminoguanidine	Luo et al. (2012)	
		Baicalein	Yang et al. (2009)	

Continued

Table 1.1 Effects of different compounds in experimental diabetic retinopathy.—cont'd

Effects on	Compound/drug	Type 1 diabetes	Type 2 diabetes
	Eriodictyol	Bucolo et al. (2012)	
	Hesperidin	Shi et al. (2012)	
	GT	Kumar et al. (2012)	
	CGA	Shin et al. (2013)	
	Arctiin	Lu et al. (2012)	
	TC	Agrawal et al. (2012)	
	Oat diet	Al-Malki (2013)	
	GBE	Bucolo et al. (2013)	
	Zeaxanthin	Kowluru et al. (2008)	
	Cilostazol		Jung et al. (2013)
	Resveratrol	Kim et al. (2012)	
	Astragalin	Ke et al. (2012)	
	Fenugreek	Gupta et al. (2014)	
	Memantine	Kusari et al. (2007)	
	Vitamin D	Ren et al. (2012)	Ren et al. (2012)
	H_2S	Si et al. (2013)	

ICAM-1 ↓	Telimsartan/valsartan	Nagai et al. (2007)
	Prorenin receptor blocker	Satofuka et al. (2009)
	PEDF	Yamagishi et al. (2006b) Yu et al. (2010)
	Eriodictyol	Bucolo et al. (2012)
	Hesperidin	Shi et al. (2012)
	Zeaxanthin	Kowluru et al. (2008)
	Fasudil	Arita et al. (2009)
	Photobiomodulation	Tang et al. (2013)
Occludin ↑	PACAP	Scuderi et al. (2013)
	VIP	Scuderi et al. (2013)
	PEDF	Yu et al. (2010)
	GT	Silva et al. (2013)
	CGA	Shin et al. (2013)
	Sitagliptin	Gonçalves et al. (2012)

Continued

Table 1.1 Effects of different compounds in experimental diabetic retinopathy.—cont'd

Effects on	Compound/drug	Type 1 diabetes	Type 2 diabetes
Vascular permeability ↓	Angiotensin(1–7)	Verma et al. (2012)	
	Candesartan	Phipps et al. (2009)	
	Angiostatin	Sima et al. (2006)	
	PEDF	Boehm et al. (2003) Inagaki et al. (2003) Liu et al. (2004) Yamagishi et al. (2006a)	
	H(2) saline	Xiao et al. (2012b)	
Neovascularization ↓ Neoangiogenesis ↓	SST and SST analogs	Casini et al. (2005) Davis et al. (2001) Grant and Caballero (2002)	
	Angiostatin	Sima et al. (2004)	
	Decursin	Ahn et al. (1996) Yang et al. (2013b)	
	EPO	Watanabe (2007)	
	PEDF	Boehm et al. (2003)	
	Hesperidin	Shi et al. (2012)	
	TC	Agrawal et al. (2012) Rathi et al. (2002)	
	Vitamin D	Aksoy et al. (2000) Albert et al. (2007) Kaur et al. (2011)	Aksoy et al. (2000) Albert et al. (2007) Kaur et al. (2011)

BRB breakdown ↓ BRB dysfunction ↓	PACAP	Scuderi et al. (2013)	
	VIP	Scuderi et al. (2013)	
	Candesartan	Phipps et al. (2009)	
	EPO	Villa et al. (2003) Zhang et al. (2008)	
	PEDF	Liu et al. (2004)	
	Eriodictyol	Bucolo et al. (2012)	
	Hesperidin	Shi et al. (2012)	
	CGA	Shin et al. (2013)	
	Cannabidiol	El-Remessy et al. (2006)	
	RA	Miyajima et al. (2005) Nishikiori et al. (2007a)	
	Sitagliptin		Gonçalves et al. (2012)
	Memantine	Kusari et al. (2007)	
	H_2S	Si et al. (2013)	
	H(2) saline	Feng et al. (2013)	
Diabetes-related (retinal) edema ↓	EPO	Brines et al. (2008) McVicar et al. (2011)	
	Arctiin	Lu et al. (2012)	

Continued

Table 1.1 Effects of different compounds in experimental diabetic retinopathy.—cont'd

Effects on	Compound/drug	Type 1 diabetes	Type 2 diabetes
Vascular degenerative pathology ↓	Perindopril	Zheng et al. (2009)	
	Captopril	Zhang et al. (2007)	
	Bevacizumab	Ma et al. (2010)	
	EPO	Brines and Cerami (2008) Brines et al. (2008) McVicar et al. (2011) Wang et al. (2011) Watanabe (2007) Zhang et al. (2008)	
	NGF	Colafrancesco et al. (2011) Hammes et al. (1995b)	
	Aminoguanidine	Gardiner et al. (2003) Luo et al. (2012)	
	Baicalein	Yang et al. (2009)	
	TFF	Li et al. (2013b)	
	GT	Kumar et al. (2012)	
	CGA	Shin et al. (2013)	
	TC	Agrawal et al. (2012) Rathi et al. (2002)	
	Oat diet	Al-Malki (2013)	
	Cilostazol		Jung et al. (2013)

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		Resveratrol	Kim et al. (2012)
		Fenugreek	Gupta et al. (2014)
		Carnosine	Pfister et al. (2011)
		Nepafenac	Kern et al. (2007)
		Fasudil	Arita et al. (2009)
Cells and tissues	Neuroprotection	Insulin	Reiter et al. (2006)
		IGF analogs	Kummer et al. (2003)
		Rosiglitazone	Li et al. (2011)
		SST	Casini et al. (2005) Hernandez et al. (2013)
		PACAP	Szabadfi et al. (2012, 2014)
		EPO	Brines and Cerami (2008) Hernandez and Simo (2012) Wang et al. (2011) Zhang et al. (2008)
		rhEPO	Zhu et al. (2008)
		Melatonin	Kanter et al. (2006) Sudnikovich et al. (2007)
		E4	Zhang et al. (2011)

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Table 1.1 Effects of different compounds in experimental diabetic retinopathy.—cont'd

Effects on	Compound/drug	Type 1 diabetes	Type 2 diabetes
	NGF	Colafrancesco et al. (2011) Hammes et al. (1995b)	
	AG	Gardiner et al. (2003)	
	Baicalein	Yang et al. (2009)	
	TFF	Li et al. (2013b)	
	4-Methyl-2,6-diisobornylphenol	Logvinov et al. (2010)	
	GSPE	Li et al. (2008b)	
	Wolfberry		Tang et al. (2011)
	CBD	El-Remessy et al. (2006)	
	RA	Nishikiori et al. (2008)	
	H_2S	Si et al. (2013)	
Histological alterations ↓	Rosiglitazone	Li et al. (2011)	
	PACAP	Szabadfi et al. (2012)	
	Perindopril	Tikellis et al. (2004)	
	AG	Kern and Engerman (2001)	
	Hesperidin	Shi et al. (2012)	
	TFF	Li et al. (2013b)	
	GSPE	Li et al. (2008b)	
	Wolfberry		Tang et al. (2011)

	Zeaxanthin	Kowluru et al. (2008)	
	CBD	El-Remessy et al. (2010)	
	Nepafenac	Kern et al. (2007)	
	H(2) saline	Feng et al. (2013)	
Glial activation ↓ GFAP ↓	SST	Hernandez et al. (2013)	
	PACAP	Szabadfi et al. (2012)	
	EPO	Brines et al. (2008) McVicar et al. (2011)	
	rhEPO	Zhu et al. (2008)	
	Melatonin		Salido et al. (2013)
	E4	Zhang et al. (2011)	
	PEDF	Li et al. (2002)	
	Phlorizin		Zhang et al. (2013)
	GT	Silva et al. (2013)	
	Curcumin	Gupta et al. (2011) Zuo et al. (2013)	
	Cilostazol		Jung et al. (2013)
	CBD	El-Remessy et al. (2010)	

Continued

Table 1.1 Effects of different compounds in experimental diabetic retinopathy.—cont'd

Effects on	Compound/drug	Type 1 diabetes	Type 2 diabetes
Müller cell dysfunction ↓	Baicalein	Yang et al. (2009)	
Microglial and astroglial activation ↓	Baicalein	Yang et al. (2009)	
	N-acetylcysteine (NAC)	Tsai et al. (2009)	
Dopaminergic amacrine cell number ↑	PACAP	Szabadfi et al. (2012)	
	BDNF	Seki et al. (2004)	
Ganglion cell number ↑	Rosiglitazone	Li et al. (2011)	
	PACAP	Szabadfi et al. (2014)	
	EPO	Zhang et al. (2008)	
	Exenatide	Fu et al. (2012) Hao et al. (2012)	
	BDNF	Seki et al. (2004)	
	NGF	Colafrancesco et al. (2011)	
	4-Methyl-2,6-diisobornylphenol	Logvinov et al. (2010)	
	Astaxanthin		Dong et al. (2013)
	CBD	El-Remessy et al. (2006)	

	SNJ-1945 (calpain inhibitor)	Shanab et al. (2012)
	Memantine	Kusari et al. (2007)
	Nepafenac	Kern et al. (2007) Krady et al. (2005)
	Photobiomodulation	Tang et al. (2013)
Photoreceptor cells number ↑	PACAP	Szabadfi et al. (2012)
	4-Methyl-2,6-diisobornylphenol	Logvinov et al. (2010)
Loss of pericytes ↓	EPO	Garcia-Ramírez et al. (2011) Wang et al. (2010b)
	AG	Luo et al. (2012)
	Resveratrol	Kim et al. (2012)
	Nepafenac	Kern et al. (2007)
	N-acetylcysteine	Tsai et al. (2009)
Endothelial cell proliferation ↓	PACAP	Castorina et al. (2010)
	Bevacizumab	Ma et al. (2010)
	Aminoguanidine	Frank et al. (1997) Luo et al. (2012)

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Au2

Table 1.1 Effects of different compounds in experimental diabetic retinopathy.—cont'd

Effects on		Compound/drug	Type 1 diabetes	Type 2 diabetes
	Protect endothelial cells	PEDF	Inagaki et al. (2003) Yamagishi et al. (2006a)	
		Curcumin	Gupta et al. (2011)	
		Nepafenac	Kern et al. (2007)	
		N-acetylcysteine	Tsai et al. (2009)	
		Fasudil	Arita et al. (2009)	
	Protect RPE cells	PACAP	Szabadfi et al. (2014)	
		EPO	Garcia-Ramírez et al. (2011)	
		Wolfberry		Tang et al. (2011)
	TUNEL-positive cells ↓	IGF-1	Barber et al. (1998, 2001)	
		SST and SST analogs	Casini et al. (2005)	
		PACAP	Szabadfi et al. (2014)	
		Cilostazol		Jung et al. (2013)
	Retinal parenchyma thickening ↓	H(2) saline	Xiao et al. (2012b)	
Intracellular alterations	PI3K/Akt	Insulin/IGF-1	Barber et al. (2001) Reiter et al. (2003, 2006) Wang et al. (2004) Wu et al. (2004b)	
		PACAP	Szabadfi et al. (2014)	
		EPO	Garcia-Ramírez et al. (2011) Wang et al. (2010b)	

Au3

Bad ↓/Bax ↓	IGF-1	Barber et al. (2011) Galvan et al. (2003) Seigel et al. (2006)
	Exenatide	Fu et al. (2012) Hao et al. (2012)
	TFF	Li et al. (2013b)
Caspase 3 ↓	IGF-1	Barber et al. (2001)
	Rosiglitazone	Li et al. (2011)
	PACAP	Szabadfi et al. (2014)
	Melatonin	Li et al. (2013a)
	Exenatide	Fu et al. (2012) Hao et al. (2012)
	Nepafenac	Kern et al. (2007)
	H(2) saline	Xiao et al. (2012b)
Bcl 2 ↑	PACAP	Giunta et al. (2012) Szabadfi et al. (2014)
	Exenatide	Fu et al. (2012) Hao et al. (2012)
	TFF	Li et al. (2013b)
MAPK	PACAP	Szabadfi et al. (2014)
Misbalance between proapoptotic and survival signaling	SST	Hernandez et al. (2013)

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Table 1.1 Effects of different compounds in experimental diabetic retinopathy.—cont'd

Effects on	Compound/drug	Type 1 diabetes	Type 2 diabetes
Apoptotic process ↓	IGF-1	Barber et al. (2001) Kummer et al. (2003) Galvan et al. (2003)	
	SST	Hernandez et al. (2013)	
	Rosiglitazone	Li et al. (2011)	
	PACAP	Szabadfi et al. (2014)	
	Candesartan	Gao et al. (2009a)	
	EPO	Brines and Cerami (2005) Zhang et al. (2008)	
	Melatonin	Kanter et al. (2006) Li et al. (2013a) Sudnikovich et al. (2007)	
	E4	Zhang et al. (2009b, 2011)	
	NGF	Colafrancesco et al. (2011) Hammes et al. (1995b)	
	Phlorizin		Zhang et al. (2013)
	TFF	Li et al. (2013b)	
	Wolfberry		Tang et al. (2011)
	RA	Nishikiori et al. (2008)	
	Sitagliptin		Gonçalves et al. (2012)
	H(2) saline	Xiao et al. (2012b)	

	Cytokine level ↑	Tinospora cordifolia	Agrawal et al. (2012)	
	Cell survival pathways	PACAP	Szabadfi et al. (2014)	
		Melatonin	Li et al. (2013a)	
		Wolfberry		Tang et al. (2011)
Oxidative stress	Superoxide and other radicals ↓	EPO	Garcia-Ramírez et al. (2011) Wang et al. (2010a,b)	
		Zeaxanthin	Kowluru et al. (2008)	
		Lutein	Sasaki et al. (2010)	
		Astaxanthin		Dong et al. (2013)
		Nepafenac	Kern et al. (2007)	
		Photobiomodulation	Tang et al. (2013)	
	Effect on SOD and CAT enzymatic activity	Melatonin	Li et al. (2013a)	Salido et al. (2013)
		Hesperidin	Shi et al. (2012)	
		GT	Kumar et al. (2012)	
		Astaxanthin		Dong et al. (2013)
		Photobiomodulation	Tang et al. (2013)	

Continued

Table 1.1 Effects of different compounds in experimental diabetic retinopathy.—cont'd

Effects on	Compound/drug	Type 1 diabetes	Type 2 diabetes
Antioxidant mechanisms ↑ Oxidative stress ↓	Angiotensin(1–7)	Verma et al. (2012)	
	Candesartan	Gao et al. (2009a)	
	Melatonin	Li et al. (2013a)	
	PEDF	Yoshida et al. (2009)	
	Hesperidin	Shi et al. (2012)	
	GT	Kumar et al. (2012) Silva et al. (2013)	
	TC	Agrawal et al. (2012) Rathi et al. (2002)	
	Curcumin	Gupta et al. (2011) Zuo et al. (2013)	
	GBE	Bucolo et al. (2013)	
	Zeaxanthin	Kowluru et al. (2008)	
	Astaxanthin		Dong et al. (2013)
	CBD	El-Remessy et al. (2010)	
	Ginsenosides	Sen et al. (2013)	Sen et al. (2013)
	Fenugreek	Gupta et al. (2014)	
	H_2S	Si et al. (2013)	
	H(2) saline	Feng et al. (2013)	
	N-acetylcysteine	Tsai et al. (2009)	

	ROS ↓	Perindopril	Zheng et al. (2009)	
		GT	Silva et al. (2013)	
		Oat diet	Al-Malki (2013)	
		Wolfberry		Tang et al. (2011)
		H_2S	Si et al. (2013)	
	Glutathione peroxidase ↑ Balance pro- and antioxidative factors	EPO	Garcia-Ramírez et al. (2011) Wang et al. (2010a,b)	Au4
	NADPH oxidase ↓	Valsartan	Fukumoto et al. (2008)	
		Perindopril	Zheng et al. (2009)	
		PEDF	Inagaki et al. (2003) Yamagishi et al. (2006a) Yoshida et al. (2009)	
	Cytochrome *c* ↓	Melatonin	Li et al. (2013a)	
		Exenatide	Fu et al. (2012) Hao et al. (2012)	
Glutamate/ GLAST	Glutamate accumulation ↓, GLAST downregulation ↓	SST	Hernandez et al. (2013)	
		E4	Zhang et al. (2009b, 2011)	

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Table 1.1 Effects of different compounds in experimental diabetic retinopathy.—cont'd

Effects on		Compound/drug	Type 1 diabetes	Type 2 diabetes
AGE/RAGE	AGE ↓	Candesartan		Sugiyama et al. (2007)
		PEDF	Yoshida et al. (2009)	
		Aminoguanidine	Hammes et al. (1995a) Kern et al. (2000) Kern and Engerman (2001) Luo et al. (2012)	
		Phlorizin		Zhang et al. (2013)
		Hesperidin	Shi et al. (2012)	
		Oat diet	Al-Malki (2013)	
		GSPE	Li et al. (2008b)	
	RAGE ↓	Purearin, pyridoxamine	Chen et al. (2012a) Luo et al. (2012)	
Retinal lipid peroxidation	↓	Melatonin		Salido et al. (2013)
		Eriodictyol	Bucolo et al. (2012)	
		Astaxanthin		Dong et al. (2013)
		Ginsenosides	Kim and Park (2003)	

Other factors	BDNF ↑ CNTF ↑	EPO	Hu et al. (2011)
	BDNF ↑	H_2S	Si et al. (2013)
		H(2) saline	Feng et al. (2013)
	Synaptophysin ↑	Telimsartan/valsartan	Kurihara et al. (2008)
		H_2S	Si et al. (2013)
		H(2) saline	Feng et al. (2013)
Inflammation	Inflammatory process ↓	Angiotensin(1–7)	Verma et al. (2012)
		Prorenin receptor blocker	Satofuka et al. (2009)
		EPO	Villa et al. (2003) Zhang et al. (2008)
		PEDF	Shen et al. (2011)
		Baicalein	Yang et al. (2009)
		Hesperidin	Shi et al. (2012)
		GT	Kumar et al. (2012)
		TC	Agrawal et al. (2012) Rathi et al. (2002)
		Curcumin	Gupta et al. (2011) Zuo et al. (2013)

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Table 1.1 Effects of different compounds in experimental diabetic retinopathy.—cont'd

Effects on	Compound/drug	Type 1 diabetes	Type 2 diabetes
	GBE	Bucolo et al. (2013)	
	CBD	El-Remessy et al. (2006)	
	Fenugreek	Gupta et al. (2014)	
	Sitagliptin		Gonçalves et al. (2012)
	H_2S	Si et al. (2013)	
	N-acetylcysteine	Tsai et al. (2009)	
Leukostasis ↓ Leukocyte adhesion ↓	Valsartan/telmisartan	Nagai et al. (2007)	
	Captopril	Zhang et al. (2007)	
	Prorenin receptor blocker	Satofuka et al. (2009)	
	PEDF	Yamagishi et al. (2006b)	
	Fasudil	Arita et al. (2009)	
	Photobiomodulation	Tang et al. (2013)	
TNFα ↓ IL-1β ↓	Melatonin		Salido et al. (2013)
	Eriodictyol	Bucolo et al. (2012)	
	Hesperidin	Shi et al. (2012)	
	GT	Kumar et al. (2012)	
	TC	Agrawal et al. (2012)	
	Oat diet	Al-Malki (2013)	
	Fenugreek	Gupta et al. (2014)	

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Functional	ERG abnormalities ↓	SST	Hernandez et al. (2013)	
		Telimsartan/valsartan	Kurihara et al. (2008) Phipps et al. (2007)	
		Photobiomodulation	Tang et al. (2013)	
	Amplitudes of a wave ↑	Melatonin		Salido et al. (2013)
		PEDF	Li et al. (2002)	
		Memantine	Kusari et al. (2007)	
	Amplitudes of b wave ↑	rhEPO	Zhu et al. (2008)	
		Melatonin		Salido et al. (2013)
		E4	Zhang et al. (2011)	
		PEDF	Li et al. (2002)	
		Memantine	Kusari et al. (2007)	
		H(2) saline	Feng et al. (2013)	
		Photobiomodulation	Tang et al. (2013)	

Continued

Table 1.1 Effects of different compounds in experimental diabetic retinopathy.—cont'd

Effects on	Compound/drug	Type 1 diabetes	Type 2 diabetes
Amplitudes of OPs ↑	rhEPO	Zhu et al. (2008)	
	Melatonin		Salido et al. (2013)
	E4	Zhang et al. (2011)	
	Nepafenac	Kern et al. (2007)	
	H(2) saline	Feng et al. (2013)	

Abbreviations: AG, aminoguanidine; AGE, advanced glycosylated end product; BDNF, brain-derived neurotrophic factor; CBD, cannabidiol; CGA, chlorogenic acid; CNTF, ciliary neurotrophic factor; E4, exendin-4; EPO, erythropoietin; GBE, *Ginkgo biloba* leaf extract; GFAP, glial fibrillary acidic protein; GSPE, grape seed proanthocyanidin extracts; GT, green tea extract; ICAM-1, interstitial cell adhesion molecule 1; IGF-1, insulin-like growth factor 1; IL-1, interleukin-1; NGF, nerve growth factor; PACAP, pituitary adenylate cyclase-activating polypeptide; PEDF, pigment epithelium-derived factor; PI3K, phosphatidylinositide 3-kinase; RA, retinoic acid; RAGE, receptor of AGEs; ROS, reactive oxygen species; RPE, retinal pigment epithelium; SST, somatostatin; TC, *Tinospora cordifolia*; TNFα, tumor necrosis factor α; TUNEL, terminal dUTP nick-end labeling; VEGF, vascular endothelial growth factor; VEP, visually evoked potential; VIP, vasoactive intestinal peptide.

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peroxynitrite decomposition catalyst (El-Remessy et al., 2005) all have been found to inhibit RGC degeneration in diabetes. Aminoguanidine and aldose reductase inhibitors have been found to inhibit atrophy of optic nerve axons in diabetes (Ino-ue et al., 1998). Both the clinical trials and the laboratory investigations agree that besides the effectivity of the drug, the manner of administration may be the clue to treat the retinal complications, since many of the drugs do not cross the BRB, but intraocular injection may be effective. In the next paragraphs, we will therefore review both effectivity and administration of the different substances.

s0215 4.1. Insulin and IGFs

p0955 The retina is an insulin-sensitive tissue, especially regarding the anabolic effects of insulin (Reiter et al., 2003). All types of retinal cells express insulin receptors, with particularly high expression on Müller cells end feet and neuronal dendrites (Gosbell et al., 2002). However, insulin production within the retina has not been described to date. Exogenous insulin stimulation of whole retina activates the insulin receptor substrate/Akt branch of the insulin receptor signaling networks (Diaz et al., 2000; Reiter et al., 2003). Insulin provides trophic support for retinal neurons via phosphatidylinositide 3-kinase (PI3K)/Akt and P70S6 kinase pathways (Barber et al., 2001; Wu et al., 2004b), and this trophic function of insulin on retinal neurons is impaired by exposure to elevated glucose and glucosamine (Nakamura et al., 2001). Constitutive insulin receptor/Akt/p70S6K prosurvival signaling in retina is impaired by diabetes and may contribute to neuronal degeneration in DR. Vascular and neuronal survival could be compromised by reducing insulin receptor activity directly on these cells and/or indirectly by altering glial or microglial cell function, on which they depend. Systemic insulin therapy from the onset of diabetes prevents loss of retinal insulin receptor kinase activity, and importantly, for therapeutic implications, intraocular insulin injection restored that activity. Retinal insulin receptor signaling pathway provides neurotrophic support therefore DR may be considered as a neurotrophin-deficient and/or neurotrophin-resistant state (Reiter et al., 2006). Retinal apoptosis is increased 10-fold in rat retinas after at least 1 month of diabetes. Insulin implants significantly reduced the number of apoptotic cells (Barber et al., 1998). Indeed, insulin can rescue retinal neuronal cells from apoptosis through a PI3K/Akt-mediated mechanism, and systematically administered insulin activates the retinal insulin receptor, PI3K, and Akt in normal rats (Barber et al., 2001; Reiter et al., 2003). Many

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growth factors, including insulin, activate p70S6K in a PI3K-dependent manner resulting in retinal neuronal cell survival. This pathway works *in vivo* via the PI3K/Akt/mTOR signal transduction (Wu et al., 2004b).

p0960 Several authors argue that the best therapy for DR is the strict control of blood glucose, because insulin can decrease VEGF expression in the retina and protect retinal vessels from impairment in early STZ-induced diabetic rats (Wang et al., 2007); a similar conclusion has been reached recently regarding human DR (Hammes, 2013).

p0965 IGFs are neurotrophic factors that have been implicated in the pathogenesis of diabetic neurological disorders. The signaling pathway involves the type 1 IGF receptor, which binds both IGF-1 and IGF-2. Early systemic treatment with IGF or its structural analogs may prevent predegenerative changes that lead to the death of retinal cells in diabetes. IGF can prevent cell death in the nervous system in the context of diabetes despite the fact that IGF-1 treatment had no effect on severity of hyperglycemia or reduced body weight in diabetic rats (Seigel et al., 2006). IGF-1 mRNA content is reduced in the eye in the early stages of clinical and experimental diabetes (Gerhardinger et al., 2001), and IGF-1 levels are elevated in the vitreous due to variable disruption of the BRB in chronic disease. IGF replacement therapy can prevent or reverse diabetic neurological complications, such as early treatment with IGF-1 analogs could counteract certain proapoptotic abnormalities that precede retinal cell degeneration (Kummer et al., 2003). Further, systemic IGF-1 administration also prevents both the increase in p-Akt and TUNEL staining in retinal cells in diabetic rats. IGF-1 or IGF analog treatment in diabetic rats may rescue retinal cells from death by the inhibition of caspase 3 activation through the PI3K/Akt pathway (Barber et al., 2001). This treatment simultaneously reduced the elevation of Bad immunoreactivity in diabetes as well (Seigel et al., 2006). IGF-1 binding to its receptor blocks apoptosis by inducing the phosphorylation and inhibition of proapoptotic proteins such as Bad in all significantly affected retinal layers (INL, ONL, and GCL; Barber et al., 2001; Galvan et al., 2003).

p0970 Drugs acting along the insulin/IGF pathway may have similar mode of action. Rosiglitazone (with the trademark name Avandia) is an antidiabetic drug in the thiazolidinedione class of drugs. It works as an insulin sensitizer, by binding to peroxisome proliferator-activated receptors (PPARs) in fat cells and making the cells more responsive to insulin. After treatment with rosiglitazone in experimental STZ-induced diabetes, the thickness of the retina and the number of cells in the GCL were significantly greater. It also attenuated the diabetes-induced apoptosis of retinal neurons and

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mitochondrial metamorphosis in RGCs. Consistent with these effects, decreased cleaved caspase 3 and p-STAT3 levels and increased SOCS3 expression were observed (Li et al., 2011). Consequently, rosiglitazone might be used to prevent retinal neuronal damage in diabetes.

4.2. Neuropeptides

Apart from their well-known neuromodulatory actions in the retina, recent investigations revealed their role in retinal development (Bagnoli et al., 2003), and we have just started to appreciate their possible role in aging (Reglodi et al., 2012). When examining their role in DR, some substances of extraretinal origin must also be taken into account.

4.2.1 Somatostatin

Somatostatin (SST) is a neuropeptide widely distributed in the central and peripheral nervous system, where it plays a variety of biological roles (Blake et al., 2004; Olias et al., 2004). Two forms of SST have been identified—SST-14 and SST-28—the former is the one preferentially expressed in the mammalian retinal amacrine cells. It interacts with five main membrane receptors (sst1–sst5) that are coupled to different transduction pathways (Olias et al., 2004). Mostly, sst2 and sst4 receptors can be found in the retina. Expression of sst2 is the most abundant; it can be detected in virtually all layers (Casini et al., 2005; Thermos, 2003). sst1 and 5 receptors are also found (amacrine cells and RGCs). However, sst3 receptors are not expressed in the mammalian retina (Ke and Zhong, 2007; Thermos, 2003).

Potential therapeutic use of SST is based on the ability of SST to inhibit growth hormone secretion, which was implicated in the pathogenesis of DR (Kirkegaard et al., 1990). In some points of view, the neuronal death in diabetic retinas is caused by increased extracellular glutamate level. In the retina, SST inhibits glutamate accumulation and GLAST downregulation induced by diabetes (Hernandez et al., 2013). Indeed, potential neuroprotective roles of SST or its analogs may be mediated by inhibition of glutamate release through activation of K^+ channels. Further, SST has a dual action in DR, playing against both neoangiogenesis and excitotoxic neuronal death (Casini et al., 2005). SST is downregulated in the diabetic eye; its concentration is low in the vitreous body. SST-containing eye-drop treatment prevented ERG abnormalities, glial activation, apoptosis, and misbalance between proapoptotic and survival signaling in STZ-induced diabetic rats. Topical administration of SST or its analogs has a potent effect in preventing

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retinal neurodegeneration induced by diabetes (Hernandez et al., 2013). SST or SST analogs may counteract retinal damages in DR through a protective paracrine effect directly on retinal cells, which are known to express SST receptors. Several lines of clinical and experimental evidence suggest Au21 that SST analogs may be efficacious in inhibiting neovascularization associated with proliferative DR (Davis et al., 2001; Grant and Caballero, 2002).

p0990 Octreotide, a peptide analog of SST, can counteract vascular endothelial dysfunction. This drug proved to be effective in delaying or to some extent reverting symptoms of DR (Grant et al., 2000). Acting on sst2 receptors, it may possibly control inflammatory processes (Pinter et al., 2006), endothelial symptoms (Cervia et al., 2012), and also to some extent neural symptoms (Cervia et al., 2008; Vasilaki and Thermos, 2009; Vasilaki et al., 2002). Additional peptide and nonpeptide analogs should be involved in future experiments.

p0995 One of those may be cortistatin, which is produced mostly by a non-neural cell of the retina, the RPE. Its role may lie in the activation of glial cells under stress conditions (Carrasco et al., 2008). Its receptors are identical with those activated by SST. The influence of cortistatin on neural information processing is unknown at present. However, it is presumed that cortistatin is a part of a signaling system residing in the RPE of the retina, which shows some parallel properties with the hypothalamo–pituitary–adrenal axis (Zmijewski et al., 2007).

s0230 *4.2.2 Pituitary adenylate cyclase-activating polypeptide*

p1000 Pituitary adenylate cyclase-activating polypeptide (PACAP), a member of the vasoactive intestinal peptide (VIP)/secretin/glucagon peptide superfamily, is a neuropeptide with highly potent neuroprotective and general cytoprotective effects. PACAP and its receptors (PAC1, VPAC1, and VPAC2) occur in the retina (Atlasz et al., 2010), specifically in all retinal layers except the photoreceptors (Seki et al., 2000).

p1005 PACAP treatment has been proven to be protective in several retinal pathologies (Nakamachi et al., 2012). It attenuates retinal damage in excitotoxic, ischemic, traumatic, and UV light-induced retinal degeneration (Atlasz et al., 2010; Varga et al., 2011). Several lines of evidence suggest that PACAP might have therapeutic potential in the treatment of diabetes (Yamamoto et al., 2003) because of its effects on insulin secretion protection during β cell damage induced by various insults. Systemic PACAP treatment decreases the STZ-induced nephropathy (Li et al., 2008a) and experimental neuropathy (Dickinson et al., 1999), and PACAP inhibited the

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hyperglycemia-induced microvascular endothelial cell growth *in vitro* (Castorina et al., 2010).

p1010 This peptide effectively reduced the diabetes-induced symptoms by upregulating its own receptor, PAC1-R, and protecting several cell types (including the dopaminergic amacrine cells and RGCs; Szabadfi et al., 2012). An evidence was provided that RGCs, photoreceptors, and bipolar, horizontal, Müller glial, and amacrine cells underwent apoptosis and that PACAP treatment could attenuate this degeneration. Among amacrine cells, dopaminergic cells are certainly affected. PACAP has an ameliorating effect on dopaminergic cell degeneration. It has recently been suggested in an avian model of retinal development that PACAP may support the appearance of cells with newly acquired dopaminergic phenotype (Fleming et al., 2013). Although this possibility cannot be discounted in adult rat retinas with DR, it is less likely than protection mediated by PACAP in the case of the original dopaminergic cells.

p1015 Ultrastructural analysis revealed that retinal ribbon synapses and other synaptic profiles suffered alterations in diabetes and contained nontypical elements: empty profiles and blood vessels extending into the retina were also revealed. In PACAP-treated retinas, there were still some degenerative profiles in the OPL, but more ribbon synapses were found in the IPL. Noncharacteristic elements such as immune cells and blood vessels were present in both synaptic layers, similar to diabetic retinas (K. Szabadfi, unpublished results).

p1020 Breakdown of outer BRB is one of the major complications of DR, which is associated with the reduction of claudin-1 and ZO-1 expressions. This process was reversed by PACAP and VIP. Both peptides counteract hyperglycemia-induced damage in ARPE-19 cells, suggesting that they might be relevant to the maintenance of outer BRB function (Scuderi et al., 2013).

p1025 PACAP exerts its protective effects by increasing antiapoptotic factors and decreasing proapoptotic factors in different retinal and other injuries (Martin et al., 2004; Racz et al., 2006; Szabo et al., 2012; Vaudry et al., 2009). PACAP-induced pathways attenuate apoptosis in DR. Four pathways influenced by PACAP (MAPKs, PI3K/Akt, PKC, and inhibiting ER stress) converge to minimize apoptotic damage of retinal neurons in PACAP-treated diabetic retinas. PACAP (i) downregulated the precursor and active forms of caspase 8, caspase 3, and caspase 12 in diabetic retinas. PACAP treatment (ii) suppressed the expression and the phosphorylation of p38 MAPK and activated ERK1/2 in diabetes after 3 weeks of diabetes suggesting that the activity of MAPK pathways may account for at least part

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of the protection. It also potentiates (iii) PKC and Bcl-2 in early DR possibly accounting for some of the observed protection. PACAP reduces apoptosis via (iv) elevated level of p-Akt protein and its downstream target GSK3β phosphorylation. Akt signaling seems to play an important role in the neuroprotective effects of PACAP in DR (Szabadfi et al., 2014) and also other different retinal and other injuries (Lazarovici et al., 2012; Li et al., 2005; Racz et al., 2006; Szabo et al., 2012). Most of the cytoprotective effects of PACAP are mediated through activation of PAC1-R, which can induce a signaling cascade to stimulate protective factors and block caspase activation (Seaborn et al., 2011). PAC1-R-containing cells were not TUNEL-positive in any of the diabetic retinas, suggesting that PAC1-R-containing cells are more resistant (Szabadfi et al., 2014) than those without. In addition, mRNA and protein levels for PAC1-R are higher in diabetic retinas after PACAP treatment (Szabadfi et al., 2012). Tsutsumi et al. (2002) described that activation of VPAC1-R has been implicated in elevating glucose output, whereas activation of VPAC2-R may be involved in insulin secretion. PACAP exerts an inhibitory activity on hyperglycemia-induced endothelial cell proliferation, thus suggesting that the effect might be mediated by PAC1 and VPAC2 receptors (Castorina et al., 2010). We have also found unusual cells, like pericytes, granulocytes, and macrophages, in PACAP-treated diabetic retina (Szabadfi et al., 2012). According to our preliminary data, this can be correlated with the changing mRNA and protein levels of VPAC1-R and VPAC2-R, through which receptors of PACAP and VIP Au22
may have an action in inflammation. Thus, all three PACAP receptors may have positive contribution to fighting diabetes and its consequences (Giunta et al., 2012; Szabadfi et al., 2012, 2014).

p1030 The potency of PACAP in diabetes and related conditions in addition to its retinoprotective actions suggests that PACAP might have a therapeutic potential in the treatment of DR.

s0235 4.3. Therapies with vascular targets

p1035 Despite evidence that hyperactivity of the vasodeleterious axis (ACE/angiotensin II (Ang II)/angiotensin II type 1 receptor (AT1R)) of the renin–angiotensin system (RAS) is associated with the pathogenesis of DR, use of the inhibitors of this axis has had limited success in the control of this pathophysiology. Inhibition of the RAS has been shown to provide beneficial effects against DR, and AT1R activation may be a clue to retinal vascular dysfunction.

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s0240 ### 4.3.1 Angiotensin

p1040 DR is associated with impaired balance of RAS. Increased expression of ACE2/angiotensin(1–7) overcomes this imbalance and confers protection against DR. Intraocular administration of ACE2/angiotensin(1–7) by a viral vector resulted in significant reduction in diabetes-induced retinal vascular leakage, acellular capillaries, infiltrating inflammatory cells, and oxidative damage in both diabetic mice and rats. Thus, strategies enhancing the protective ACE2/angiotensin(1–7) axis of RAS in the eye could serve as a novel therapeutic target for DR (Verma et al., 2012).

s0245 ### 4.3.2 Angiotensin receptor blockers

p1045 Valsartan or telmisartan is an AT1R blocker. AT1R blockade significantly reversed the diabetes-induced electroretinographic changes and reduction of synaptic protein but not mRNA levels in the diabetic mouse and rat retina. Synaptophysin degradation and neuronal dysfunction ran parallel after AT1R activation in the diabetic retina, suggesting the possibility of the AT1R blockade as a novel neuroprotective treatment for DR (Kurihara et al., 2008; Phipps et al., 2007). Retinal leukocytes were significantly suppressed by AT1R blockade by valsartan. Administration of valsartan inhibited diabetes-induced retinal expression of ICAM-1 and VEGF (Nagai et al., 2007). Valsartan also decreased AQP1 expression in STZ-induced diabetic rats (Qin et al., 2012). Gao et al. (2009a) described a comprehensive proteomic analysis in diabetic mice in which the AT1R antagonist candesartan ameliorated the diabetes-induced changes of metabolism, oxidative phosphorylation, and apoptotic pathway-associated proteins. Further, candesartan treatment decreased diabetes-stimulated retinal vascular permeability suggesting that activation of AT1R contributes to BRB dysfunction (Phipps et al., 2009) and at the same time significantly reduced the levels of VEGF and NADPH oxidase subunits in type 2 DR (Fukumoto et al., 2008). Candesartan also inhibited the development of DR by reducing the accumulation of pentosidine (an AGE) and expression of VEGF (Sugiyama et al., 2007).

s0250 ### 4.3.3 Angiotensin-converting enzyme inhibitors

p1050 Perindopril is an angiotensin-converting enzyme (ACE) inhibitor (ACEI). It exerts a protective effect in DR by decreasing VEGF-to-PEDF ratio (downregulating VEGF and upregulating PEDF). The lowering of VEGF-to-PEDF ratio is significantly correlated with the relief of the vascular damage and was a result of reduced mitochondrial ROS production by

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increasing uncoupling protein 2 (UCP-2). ACEI can attenuate oxidative stress through both the NADPH oxidase pathway and the UCP-2/mitochondrial pathway. The upregulation of UCP-2 expression is mediated by PPARγ (Zheng et al., 2009). Connective tissue growth factor (CTGF) has been postulated to have prosclerotic and angiogenic properties. DR is associated with a greater than twofold increase in CTGF mRNA levels and immunoreactivity, which was attenuated by perindopril treatment. The protective effects of ACEIs on retinal pathology may be mediated via effects on retinal CTGF expression (Tikellis et al., 2004).

p1055 The ACE inhibitor captopril completely inhibited the diabetes-induced retinal capillary degeneration. Captopril inhibited hyperglycemia-induced leukostasis in the retinal vasculature (Zhang et al., 2007).

s0255 4.3.4 (Pro)Renin receptor blockers

p1060 The association of receptor-associated prorenin system was defined with diabetes-induced retinal inflammation. The administration of a prorenin receptor blocker inhibited the diabetes-induced retinal expression of VEGF and ICAM-1 in rats. Retinal adherent leukocytes were significantly suppressed with a (pro)renin receptor blocker. A significant contribution of the receptor-associated prorenin system to the pathogenesis of diabetes-induced retinal inflammation suggests the possibility of the prorenin receptor as a novel molecular target for the treatment of DR (Satofuka et al., 2009).

s0260 4.3.5 Antiangiogenic treatments

p1065 One of the major problems in DR is neovascularization. Any treatment preventing the formation of new vessels could prove to be antiangiogenic. The major factor driving the vascularization and neovascularization is VEGF. Reducing VEGF levels is therefore a major target in preventing DR. Anti-VEGF therapies in the treatment of proliferative DR and the use of intravitreal anti-VEGF therapy and anti-VEGF traps in clinical practice are now encouraged as adjuncts to corrective surgery (Hayden et al., 2011). It is safe and efficacious for macular condition in humans, although vitreal administration may be associated with an increased risk of systemic thromboembolism (Zhang et al., 2009a).

s0265 4.3.5.1 Angiostatin

p1070 Angiostatin is a naturally occurring protein found in several animal species, including humans. It is an endogenous angiogenesis inhibitor, which blocks

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the growth of new blood vessels, effecting vascular leakage in any tissue (Sima et al., 2004). Diabetic rats showed significant increases in vascular permeability in the retina and iris. Angiostatin could reverse this effect. Angiostatin downregulates VEGF expression and thus blocks the major cause of vascular leakage in the diabetic retina (Sima et al., 2006). Angiostatin may have a therapeutic potential in the treatment of diabetic macular edema and other diseases with vascular leakage.

s0270 #### 4.3.5.2 Decursin

p1075 Decursin is a compound isolated from the root of *Angelica gigas Nakai* that can be a novel compound for the antiangiogenesis approach of DR (Ahn et al., 1996). Targeting VEGFR-2 with decursin inhibits second messenger signaling in retinal cells (Kim et al., 2009a). The antiangiogenic effects of decursin have been largely attributed to reduced VEGFR-2 expression in endothelial cells. Even high doses of decursin did not induce apoptosis suggesting that it may be a safe therapeutic agent (Yang et al., 2013b).

s0275 #### 4.3.5.3 Anti-VEGF antibodies

p1080 Bevacizumab is a humanized anti-VEGF antibody. The efficacy of intravitreal injection of bevacizumab was evaluated as a preventive intervention of vascular endothelial cell proliferation in the rat retina after 2 months of diabetes. A single intraocular injection of bevacizumab may be beneficial as a therapy for preventing retinal vascular endothelial cell growth (Ma et al., 2010). Ranibizumab is a recombinant humanized monoclonal anti-VEGF antibody fragment developed for intravitreal use. It has the ability to bind to all biologically active isoforms of VEGF. These recent developments led to their widespread use in clinical trials and even in clinical practice (Gabriel, 2013; Hammes, 2013).

s0280 ## 4.4. Hormones

p1085 There are a few compounds that have been tried in DR that had not been localized to neurons in any parts of the nervous system to date and therefore by no means can be considered neuropeptides or neurohormones. Delivered via circulation to the target organs, by classical terminology, they can be considered hormones.

s0285 ### *4.4.1 Erythropoietin*

p1090 EPO is a glycoprotein hormone and a neurotrophic and endothelial survival factor that has both neuroprotective and vascular protective functions

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(Ghezzi and Brines, 2004; Zhong et al., 2007). EPO is upregulated in the eye in DR. It is considered to act in a dual way as a neuroprotective factor by inducing angiogenesis (Marti et al., 2000) and has been found in both RPE and neuroretina in diabetic eyes. Its overexpression is unrelated to mRNA expression of HIFs (Garcia-Ramirez et al., 2008; Hernandez et al., 2006). It is possible that higher concentrations of EPO can be neuroprotective under certain circumstances (Hernandez and Simo, 2012). In early diabetic retinas, upregulation of EPO-R mRNA and protein and increased expression of EPO-R in neurons in different layers were seen. In the experimental STZ-induced diabetic rat, intravitreal injection of EPO caused a dose-dependent inhibition of the breakdown of the BRB after a characteristic cytokine response (Zhang et al., 2008). The protective effects of EPO against the breakdown of BRB are related to the known anti-inflammatory effect of EPO (Villa et al., 2003). Inhibition of EPO synthesis or action reduces retinal neovascularization in proliferative DR and inhibits endothelial cell proliferation *in vitro* (Watanabe, 2007). EPO can prevent or delay neuronal apoptosis. Apoptotic neurons in the ONL were essentially undetectable in EPO-treated diabetic eyes up to 4 weeks. At 6 weeks, some cell death was detectable suggesting a therapeutic window for EPO in DR treatment. Treatment with EPO at the dose inducing erythropoiesis is beneficial not only for retinal vessels but also for retinal neuron survival (Zhang et al., 2008). Apoptosis in EPO-R-expressing cells could be aborted by rapid EPO binding (Brines and Cerami, 2005). Intravitreal injection of EPO resulted in downregulation of EPO-R, VEGF, and VEGF receptor at 4 weeks of DR (Mitsuhashi et al., 2013).

p1095 Exogenous EPO administration by intravitreal or intraperitoneal injection in early diabetes may prevent structural vascular and neuronal damage in STZ-induced diabetic rats (Wang et al., 2011; Zhang et al., 2008). Intravitreal EPO administration is able to upregulate brain-derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF) expressions (Hu et al., 2011). The EPO molecule has been successfully altered to selectively eliminate erythropoietic and prothrombotic potencies, while preserving tissue-protective activities (Brines and Cerami, 2008). Administration of the EPO helix-B domain inhibits diabetes-related edema and significantly protects against neuroglial and vascular degenerative pathology (Brines et al., 2008; McVicar et al., 2011). Administration of suberythropoietic amounts of EPO delta peptide reduces oxidative stress in target tissues and prevents pericyte loss in the diabetic retina (Wang et al., 2010b). It ameliorates prosurvival signals involving Akt and reduces the loss of pericytes in

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the diabetic retina. EPO can reduce oxidative stress via different mechanisms, acting as a direct and indirect antioxidant by reducing superoxide and other radicals; inducing glutathione peroxidase, which is downregulated in the diabetic retina, thus improving the balance between pro- and antioxidative factors; and stimulating p-Akt predominantly in cells of the INL in the diabetic retina. EPO treatment reduces pericyte dropout via reduction of angiotensin-2 upregulation. It also protects RPE cells against the increase of permeability induced by diabetic conditions, and this effect is mainly mediated by the downstream signaling of Janus kinase 2 and PI3K/Akt pathways (Garcia-Ramírez et al., 2011).

p1100 Recombinant human erythropoietin (rhEPO) has roles in preventing and reversing dysfunction of retinal neurons and glial cells in early STZ-induced diabetic rats. The amplitudes of b wave and OPs showed no decrease in diabetic rats with rhEPO injection. The administration of rhEPO may be useful in the neural treatment of DR at early stage (Zhu et al., 2008).

s0290 4.4.2 Melatonin

p1105 Indoleamine hormone melatonin, secreted by pinealocytes (Stehle et al., 2011), is a powerful free radical scavenger and lipophilic antioxidant agent (Galano et al., 2011; Reiter et al., 2002). Apart from its main biological function (regulating circadian cycle—e.g., Lanfumey et al., 2013), melatonin shows activity against oxidative stress and inflammatory and apoptotic processes in diabetic complications (Chang et al., 2008; Klepac et al., 2006; Zwirska-Korczala et al., 2005). In addition, melatonin has strong mitochondria-protective effects (Kim and Lee, 2008). Melatonin treatment increases the mRNA expression and the activity of both Cu–Zn SOD and Mn SOD; thus, their activity enables a prolonged antioxidant capability under high-glucose conditions. It also reduces the apoptosis of the retinal neurons and caspase 3 mRNA expression. Melatonin first reduces the oxidative damage to mitochondria, therefore reducing the release of cytochrome *c* (Li et al., 2013a). Melatonin has double solubility, a high lipophilic and partial hydrophilic property, which means that it has a high degree of dispersion; it can easily pass through the cell and mitochondrial membrane to exert antioxidant activity. Melatonin's effect on retinal cell apoptosis is not due to the hypoglycemic effect itself. Additionally, its antioxidative and neuroprotective effects also depend on the intervention time (Kanter et al., 2006; Sudnikovich et al., 2007). Melatonin prevents the decrease in the ERG a and b waves and OP amplitude and the increase in retinal lipid peroxidation, retinal catalase activity, NOS activity, and

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TNFα, GFAP, and VEGF levels in DR associated with type 2 diabetes (Salido et al., 2013). No toxicity has been reported after long-term use. Its safe administration makes this molecule an attractive agent for the treatment of early DR.

s0295 ### 4.4.2 Exendin-4 and its analogs

p1110 Exendin-4 (E4), a 39-amino acid peptide, is a cognate receptor (GLP-1R) agonist. It was found in the saliva of the *Gila monster*. E4 has been shown to bind to GLP-1R in pancreatic cells and to promote the proliferation of β cells in the pancreas (Lupi et al., 2008). It was reported that both GLP-1 and E4 have neuroprotective properties. Since GLP-1R is also expressed in the rat retina (Zhang et al., 2009b), primarily in the Müller cells, a study was carried out to test the effects of E4a in retinal protection when delivered intravitreally. It has been shown that intravitreal injection of E4a could protect the rat retinas from diabetic insults, but the effect was transient (Zhang et al., 2011). The amplitudes of both b waves and OPs were reduced in diabetic rats, which were greatly improved at 1 month after E4a treatment. However, such protective effects disappeared at 3 months after the treatment. Such action might have occurred through a local effect of E4a by its receptor GLP-1R because intravitreal injection of E4a had no effect on body weight or blood glucose level. Morphological examination also confirmed the protective effects of E4a. The maintenance of retinal neuronal cells by E4a might be achieved by its antiapoptotic function, but the pathways involved merit further exploration. E4a might exert its protective function by upregulating its GLP-1R and GLAST expression in the Müller cells (Zhang et al., 2009b, 2011).

p1115 Exenatide is a synthetic version of E4. The appropriate concentration of exenatide protects RGC-5 cells from high- or low-glucose-induced RGC impairment and mitochondrial changes. Exenatide improved the survival rate of the cells and suppressed changes in the mitochondrial morphology. Treatment with exenatide significantly inhibited cytochrome *c* release and decreased the intracellular expression levels of Bax and caspase 3, whereas Bcl-2 was increased. It is concluded that a mitochondrial mechanism might play a key role in the protective effect of exenatide on the RGC-5 cells, and exenatide might be beneficial for patients with DR (Fu et al., 2012; Hao et al., 2012).

s0300 ## 4.5. Neurotrophic factors

p1120 Neurotrophic factors are thought to play a major role in the development and maintenance of the neural connections. The possibility of their induction in

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the tissues and extrinsic application in degenerative disorders was coined long time ago (Semkova and Krieglstein, 1999). Here, we describe some of the research regarding the use of neurotrophic factors in DR.

s0305 ### 4.5.1 Brain-derived neurotrophic factor

p1125 In low quantities, BDNF is expressed in RGCs and Müller glia (Seki et al., 2003), and it is important for the survival of RGCs and amacrine cells (Cusato et al., 2002). Under normal conditions, it acts as a synaptic modulator in the retinal dopaminergic system (Cellerino et al., 1998). Seki et al. (2004) reported that the degeneration of dopaminergic amacrine and RGCs is accompanied by a reduction in BDNF levels in the retina of rats with STZ-induced diabetes and demonstrated the therapeutic potential of BDNF for treating neurodegeneration of dopaminergic amacrine cells in the diabetic retina by intraocular administration. BDNF protects the neurons through (i) TrkB receptors, (ii) insulin-responsive pathways, and (iii) reduction of systemic glucose level locally in the retina.

s0310 ### 4.5.2 Nerve growth factor

p1130 NGF is the first discovered and best-characterized member of a neurotrophin family and is produced by a number of different cells, including the cells of the visual system (Levi-Montalcini, 1987). However, there is no report on retinal production. Intraocularly injected NGF can reach the posterior portion of the eye and also the brain through the optic nerve. The level of NGF in the retina of rats with diabetes decreased significantly compared with the level of NGF of normal animals. The expression of NGF receptor, TrkA, is markedly reduced in RGCs. NGF treatment prevents both diabetes-induced programmed cell death in the neuroretina and diabetes-specific pathology in the vascular retina in experimental diabetic models (Colafrancesco et al., 2011; Hammes et al., 1995b).

p1135 Topical NGF administration (delivered as eye drops) can prevent the deleterious events that can lead the progressive RGC death in diabetes. The protective action of NGF on RGCs suggests a potential benefit of NGF-based therapy for ocular disorders such as DR (Colafrancesco et al., 2011). This observation also suggests that NGF in cooperation with other factors can be part of a permanently active endogenous retinoprotective mechanism.

s0315 ### 4.5.3 Pigment epithelium-derived factor

p1140 PEDF was first purified from the conditioned media of human RPE cells with neuronal differentiating activity (Barnstable and Tombran-Tink,

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2004). PEDF is a potent angiogenesis inhibitor in the mammalian eye. Its amount decreases in the retina and vitreous in DR, suggesting that loss of PEDF activity in the eye may contribute to development of proliferative DR (Boehm et al., 2003). Intravitreal injection of PEDF can suppress BRB breakdown and vascular permeability induced by VEGF (Liu et al., 2004), which implies that PEDF takes part in regulating vascular permeability. Single intravitreal injection of PEDF mRNA delivered with the help of a viral vector relieved BRB breakdown in STZ-induced diabetic rats for 6 months. The effect was associated with the downregulation of retinal VEGF mRNA and ICAM-1 expression and concomitant upregulation of occludin expression (Yu et al., 2010). PEDF inhibits retinal leukostasis in diabetic rats by reducing ICAM-1 expression via suppressing oxidative stress generation (Yamagishi et al., 2006b).

p1145 Diabetes decreases PEDF and GS levels in the retina. PEDF increases expression of GS against the effect of IL-1β in early DR acting as an anti-inflammatory factor in retinal Müller cells (Shen et al., 2011). PEDF not only inhibits AGE-induced endothelial cell damage but also prevents AGE-elicited retinal vascular hyperpermeability in rats by suppressing NADPH oxidase activity (Inagaki et al., 2003; Yamagishi et al., 2006a). The inhibition of NADPH oxidase is a molecular target for the antioxidative and protective properties of PEDF in early DR (Yoshida et al., 2009). PEDF application may offer a promising strategy for halting the development of DR through its antioxidative properties and by blocking the harmful action of AGE on diabetic retinas (Yoshida et al., 2009). Besides vascular responses, PEDF administration restored the decrease in amplitudes of a and b waves of ERG, which was associated with suppression of GFAP expression (Li et al., 2002). Thus, PEDF application may be useful for long-term preventive or adjunctive therapy for DR (Yu et al., 2010).

s0320 4.6. Others

p1150 A plethora of studies offer miscellaneous approaches to treating DR. These range from chemical inhibition of certain metabolic processes through using natural compounds to photomodulation. Here, we provide a short summary of these less systematic approaches.

s0325 *4.6.1 Inhibition of metabolic changes*

p1155 RAGE is a signal-transducing receptor for AGEs. The engagement of RAGE or AGEs elicits the diabetic complications; thus, the inhibition of RAGE expression might represent a potential target for DR treatment.

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Various inhibitors of AGE–RAGE system have therapeutic utility for DR (Chen et al., 2013).

p1160 One pharmacological strategy for the treatment of DR is to utilize the small nucleophilic hydrazine compound, aminoguanidine (AG). This drug is a selective inhibitor of AGEs and has been shown to prevent a range of diabetic vascular complications, including DR (Hammes et al., 1995a; Kern and Engerman, 2001; Kern et al., 2000). AG effectively prevents capillary closure, microaneurysm formation, and the depletion of NOS-containing neurons in the diabetic retina (Gardiner et al., 2003). The beneficial effect of AG has been underlined by the fact that AG is a non-AGE-specific inhibitor with antioxidant or inducible NOS properties (Sakata et al., 1999). AG inhibited the development of retinal lesions in diabetic rats (Kern and Engerman, 2001). AG delays the development of experimental DR in early stages by reducing AGE deposition in retinal blood vessels and ameliorating the ultrastructural pathological changes in retinal capillaries in diabetic rats. AG treatment prevented pericyte loss, endothelial cell proliferation, capillary occlusion, and acellular capillary formation. AG exerts protective effects through specific inhibition of AGEs (Luo et al., 2012). AG inhibits VEGF through inhibition of AGEs and VEGF-induced endothelial cell proliferation and migration (Luo et al., 2012). However, as we mentioned earlier, although AG is a useful inhibitor of protein glycation, it may be toxic from the viewpoints of vitamin B6 metabolism and also by inhibiting pyridoxal phosphate-dependent enzymes (Okada and Ayabe, 1995). AG is known to inhibit diamine oxidase that catalyzes determination of diamines such as histamine. This effect on histamine could be a problem if high-histamine-generating food is consumed, but again only if one is taking insufficient vitamin B6 (Taylor, 1986). For the aforementioned reason, AG is not used in human therapy because of it has serious toxicity issues, which are undetectable except by specific tests that are not part of standard clinical blood tests.

p1165 Similar to AG, pyridoxamine and purearin are also AGE inhibitors. Au23 These compounds have been evaluated for treatment of diabetic complications (Chen et al., 2012a; Luo et al., 2012). Both substances downregulate the expression levels of RAGE and VEGF in diabetic rat retina providing the opportunity for management of DR.

s0330 *4.6.2 Compounds and extracts from plants*

p1170 Using nonsynthetic compounds of natural origin is a popular approach these days in treating any diseases. In the PubMed database alone, there are more

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than 500,000 entries found for the isolation and use of such substances. Applications include everything from antimicrobial use to cancer research. Some have been tried in DR too; we found more than 200 entries for that alone. Here is a selection of those entries, which met at least some success.

s0335 #### 4.6.2.1 Flavonoids

p1175 Flavonoids acting as antioxidants may function as terminators of free radical chains and as chelators of redox-active metal ions that are capable of catalyzing lipid peroxidation. Flavonoids have the ability to act as a scavenger of reactive radical species and prevent the Fenton reaction.

p1180 Baicalein (5,6,7-trihydroxy-2-phenyl-4H-1-benzopyran-4-one) is a type of flavonoid, originally isolated from the roots of *Scutellaria baicalensis*. It has been used for centuries as a folk medicine in China and Japan, among others, for the treatment of inflammatory disease. Given that inflammatory processes play an important role in the pathogenesis of DR, baicalein with its anti-inflammatory properties blocks high-glucose-induced microglial and astroglial activation, thereby preventing the secretion of inflammatory and/or cytotoxic factors. Baicalein treatment inhibited high-glucose-induced Müller cell dysfunction and VEGF overexpression and consequently protected neurons and vasculature from damage in DR (Yang et al., 2009).

p1185 Phlorizin is a 2′-glucoside of phloretin. It belongs to the group of dihydrochalcones, a group of flavonoids. Phlorizin is effective in treating diabetic complications. It significantly reduces fasting blood glucose concentrations and levels of AGE and remarkably inhibits apoptosis and the expression of GFAP in the retinas of db/db mice. From the 1636 proteins that were indentified from the retina tissue, in total, 348 proteins were differentially expressed in db/db mice compared with the controls. Only 60 proteins in the retinas of db/db mice were found to be differentially changed following phlorizin treatment, including 33 proteins that were downregulated and 27 proteins that were upregulated (Zhang et al., 2013).

p1190 Eriodictyol is a strong antioxidative flavonoid extracted from *Eriodictyon californicum*. Eriodictyol has effects on retinal TNFα, VEGF, ICAM-1, and eNOS formation as well as the plasma lipid peroxidation and BRB integrity in STZ-induced diabetic rats. Treatment with eriodictyol reduces TNFα, VEGF, ICAM-1, and eNOS in the diabetic rat retina and suppresses diabetes-related BRB breakdown. It does not have hypoglycemic effects but is rather protective due to an unknown molecular mechanism (Bucolo et al., 2012).

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p1195 Hesperidin is a flavanone glycoside found abundantly in citrus fruits. It significantly suppresses BRB breakdown and increases the retina thickness, reduces blood glucose and aldose reductase activity, and downregulates retinal TNFα, ICAM-1, VEGF, IL-1β, and AGEs levels. Furthermore, treatment with hesperidin significantly reduces plasma malondialdehyde content and increases SOD activity in diabetic rats. Hesperidin therefore attenuates retinal abnormalities via antiangiogenic, anti-inflammatory, and antioxidative effects and through an inhibitory effect on polyol pathway and AGE accumulation (Shi et al., 2012).

p1200 The total flavonoids extracted from Flos Puerariae (TFF; *Pueraria* sp.) have potential protective effects in DR of diabetic mice. The thickness of the retina was significantly increased and the retinal capillary basement membrane thickness was reduced in the TFF-treated diabetic mice. It also attenuated the diabetes-induced apoptosis of retinal neurons with decreased Bax and increased ratio of Bcl-2 to Bax (Li et al., 2013b).

s0340 #### 4.6.2.2 Polyphenols

p1205 Green tea (GT), widely studied for its beneficial properties protecting against brain ischemia, is a rich source of polyphenols. Diabetic rats received GT orally for 12 weeks, which fully restored GFAP, oxidative markers (ROS), glutamine synthetase, occludin, glutamate transporter, and receptor levels in the retina. The protective effects of GT were also evaluated *in vitro* in Müller and ARPE-19 cell lines. GT treatment decreased the level of ROS and restored the glutamate transporter levels in both cell lines. GT protects the retina against diabetes-induced glutamate toxicity via an antioxidant mechanism (Silva et al., 2013). GT also acts against retinal oxidative stress and proinflammatory parameters in diabetic rats. Both SOD and CAT enzymatic activities were restored close to normal in GT-treated STZ-induced diabetic animals. Expression of proinflammatory parameters (TNFα and VEGF) was significantly inhibited in GT-treated diabetic retinas, and GT prevented retinal capillary basement membrane thickening as well (Kumar et al., 2012).

p1210 Chlorogenic acid (CGA) is a polyphenol found in various agricultural products such as coffee, beans, potatoes, and apples and is formed by esterification of caffeic and quinic acid. It has antibacterial, anti-inflammatory, antioxidant, and anticarcinogenic activities and beneficial effects on glucose metabolism (Dos Santos et al., 2006; Kim et al., 2010). Treatment with CGA has a dose-dependent protective effect on the vascular system in DR; it reduces VEGF expression, restores occludin, and decreases BRB

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breakdown. Partial restoration of claudin-5 level was also observed, but this effect was not significant. At the same time, ZO-1 level was not affected by CGA treatment (Shin et al., 2013).

p1215 Additionally, 4-methyl-2,6-diisobornylphenol facilitated reductions in the diabetes-induced degradation of photoreceptors and RGCs (Logvinov et al., 2010), which is probably associated mainly with the antioxidant properties of this agent.

s0345 #### 4.6.2.3 Other plant extracts

p1220 Arctiin, a bioactive compound isolated from dry seeds of *Arctium lappa* L. (Fructus Arctii), has been reported to have antidiabetic activity. At week 16 in STZ-induced diabetes, the glycosylated hemoglobin level was significantly decreased in all of arctiin-treated groups, and the serum glucose level was also decreased in the rats treated with a high dose. Treatment with arctiin ameliorated retinal edema, detachment of the retina, and VEGF expression in the retina. Arctiin decreases the severity of diabetic complications, demonstrating the importance of this compound as an inhibitor of DR (Lu et al., 2012). Au24

p1225 The total triterpenic acid mixture, isolated from *Cornus mas* (Fructus Corni), could reverse the abnormalities of the diabetic retina, such as the upregulation of mRNA levels of endothelin receptor and iNOS. It markedly reduced vasodilatation mediated by acetylcholine and NO (Su et al., 2007).

p1230 *Tinospora cordifolia* (TC), commonly known as Guduchi from the Menispermaceae family, has a long history of use in Ayurvedic medicine. TC contains many pharmacologically active ingredients, such as alkaloids, glycosides, and steroids. TC plays a role in the prevention and management of DR due to its antihyperglycemic, antiangiogenic, anti-inflammatory, and antioxidant properties and prevents cataract and vascular changes (Agrawal et al., 2012; Rathi et al., 2002). The possible mechanism of its hypoglycemic action is that TC may potentiate insulin effect (Stanely et al., 2000). Apart from its antihyperglycemic actions, TC has also shown some promising effects in preventing diabetic complications. TC reduced VEGF levels in rats (Agrawal et al., 2012). This reduction might be due to the presence of an active ingredient, octacosanol, which is reported to downregulate VEGF gene expression by inhibiting matrix metalloproteinases and nuclear translocation of NF-κB and reduce its DNA binding activity (Agrawal et al., 2012). The expression of VEGF in diabetic retina can be regulated by PKC, whose activation is related to many vascular abnormalities. TC significantly reduced PKC activation and level.

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There was also an effect on the retinal cytokine elevation (Agrawal et al., 2012). The inhibition of TNFα- and IL-1β-induced inflammation by TC in DR may be attributed to the action of one or more of these compounds blocking the binding of cytokines to their specific surface receptors. Both glutathione and catalase levels increased in TC-treated group as compared with diabetics (Agrawal et al., 2012).

p1235 Treatment with curcumin showed significant hypoglycemic activity compared with the diabetic group. Curcumin positively modulated the antioxidant system; the elevation of proinflammatory cytokines and oxidative stress were prevented by curcumin. Curcumin prevented the structural degeneration of endothelial cell organelles and the increase in capillary basement membrane thickness and retinal Müller glial cells (prevents upregulation of GFAP and downregulation of GS) in the diabetic rat retina. Curcumin may have potential benefits in the prevention of DR (Gupta et al., 2011; Zuo et al., 2013).

p1240 Several studies demonstrated that dietary fiber can significantly reduce the risk of cardiovascular diseases and type 2 diabetes (Wursch and Pi-Sunyer, 1997). Oat is a paradigm natural food supplement with a broad spectrum of beneficial biochemical and cell biological effects, based on its ability to reduce hyperglycemia-induced ROS overproduction. The interference in the overproduction of ROS by oat in diabetic rats normalizes parameters of oxidative stress in the retina and prevents the activation of major pathways involved in hyperglycemia-induced vascular damage. Oat could have potential benefits in the prevention of the onset and progression of DR. Oat reduced and even normalized downstream effectors of vascular response to injury. Besides reduction of ROS overproduction, it also has an indirect AGE-inhibiting effect; it disrupts the detrimental AGE–RAGE–NF-κB pathways. Oat treatment also attenuated the increased VEGF and TNFα levels (Al-Malki, 2013).

p1245 Grape seed proanthocyanidin extracts (GSPEs) have been reported to possess a variety of potent properties including antioxidant, anti-inflammation, radical scavenging, and antitumor activities (Houde et al., 2006; Shao et al., 2003; Vayalil et al., 2004). GSPE-treated diabetic rats showed significant reductions in AGE levels and an antinonenzymatic glycation effect compared with untreated diabetic rats. GSPE had protective effects on the retinal tissue of diabetic rats. Some affected proteins returned to normal levels accompanying AGE recovery after GSPE therapy in DR. These proteins are most likely to participate in the regulation of small heat-shock proteins (Li et al., 2008b).

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p1250 Wolfberry (*Lycium barbarum*) is a fruit, which contains large amounts of polysaccharides, phenolics, lutein, and zeaxanthin in ester forms, which are neuroprotective in the progression of macular degeneration (Faitova et al., 2006; Inbaraj et al., 2008). Dietary wolfberry and its bioactive constituents, zeaxanthin and lutein, functioned as modulators of cell survival/death signaling pathways through targeting pathways in cAMP-activated protein kinase and forkhead O transcription factor 3α signaling, resulting in normalization of cellular ROS and subsequent attenuation of endoplasmatic reticulum stress. This could lead to prevention of apoptosis and restoration of retinal structure in type 2 diabetic db/db mouse. RPE integrity and retinal structure were altered at early stages of diabetes in the db/db mouse model, and this abnormality could be attenuated by dietary wolfberry, which prevented or delayed the onset of the disease of DR (Tang et al., 2011).

p1255 *Ginkgo biloba* leaf extract (GBE) contains many different flavone glycosides and terpenoids, which have antioxidant action and anti-inflammatory effects, suppressing the production of reactive oxygen and nitrogen species (Ilieva et al., 2004). GBE inhibits the increase of low-density lipoprotein (LDL) and prevents oxidative damage to mitochondria, suggesting that its beneficial effects on neurodegenerative diseases are related to prevention of chronic oxidative damage (Yoshikawa et al., 1999). GBE blunts some of negative effects due to hyperglycemia, such as oxidation, inflammation, and VEGF expression, which are the main causes of DR (Bucolo et al., 2013).

p1260 Zeaxanthin is one of the dietary carotenoids that are specifically concentrated in the retina, especially in the macular region. Zeaxanthin significantly inhibits diabetes-induced retinal oxidative damage and elevation of VEGF and ICAM-1, abnormalities that are associated with the pathogenesis of DR. These results suggest that zeaxanthin supplementation has the potential to inhibit the development of retinopathy in diabetes (Kowluru et al., 2008). Lutein also prevents diabetes-induced visual dysfunction. It inhibits oxidative stress, thereby preserving the integrity of the neuroprotective pathways in early diabetic retina (Sasaki et al., 2010).

p1265 Astaxanthin is a carotenoid with powerful antioxidant properties that exists naturally in various plants, algae, and seafood (salmon, trout, krill, crayfish, shrimp, and other crustaceans), yeast, and the feathers of some birds. Astaxanthin was shown to reduce the apoptosis of RGCs and improve the levels of oxidative stress markers, including superoxide anion, malondialdehyde (a marker of lipid peroxidation), 8-hydroxy-2-deoxyguanosine (indicator of oxidative DNA damage), and MnSOD activity in the retinal tissue of db/db

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mouse. Therefore, astaxanthin may be further developed as an antioxidant drug to treat DR (Dong et al., 2013).

p1270 Cilostazol is a quinolinone-derivative medication used in the alleviation of the symptoms of intermittent claudication in individuals with peripheral vascular disease. In the retinas of OLETF rats, cilostazol treatment reduced GFAP and VEGF expression and the number of TUNEL-positive cells (Jung et al., 2013).

p1275 Berberine and rosiglitazone significantly decreased PPARγ expression in diabetic retina, while berberine and fenofibrate obviously increased PPARα and PPARδ expressions in diabetic retina. Berberine modulates PPAR$\alpha/\gamma/\delta$ protein levels in diabetic retina, which may help to ameliorate retinopathy complication induced by STZ-induced diabetes and a high-carbohydrate/high-fat diet. Berberine might be a more beneficial drug to treat diabetic retinal complication compared with fenofibrate and rosiglitazone (Zhou and Zhou, 2007).

p1280 Cannabinoids are known to possess therapeutic properties including antioxidant, anti-inflammatory, and NMDA receptor activation-blocking activity (Hampson et al., 1998; Marsicano et al., 2002). Nonpsychotropic CBD demonstrated its neuroprotective effects via anti-inflammatory and BRB-preserving effects in diabetic rats along with a prominent Müller glial cell activation (El-Remessy et al., 2010). Exposure of retinal Müller glial cells to high glucose levels stimulates oxidative stress and peroxynitrite formation (Shelton et al., 2007). Diabetes-induced oxidative and nitrative stresses alter the function of Müller cells by impairing GS activity, leading to glutamate neurotoxicity and sustaining retinal neuronal cell death. Treating diabetic animals with CBD blocked the increases in oxidative and nitrative stresses and significantly reduced the number of apoptotic cells. CBD restores GS activity by reducing its tyrosine nitration in diabetic animals. This effect was associated with a significant reduction in Müller glial cell activation, which confirms the preservation of its morphology and function in diabetic animals (El-Remessy et al., 2010). CBD represents a novel therapeutic agent in the treatment of diabetes and stress-mediated retinal damage.

p1285 Resveratrol, a natural plant-derived phytoalexin, treatment effectively blocked the diabetes-induced increase of vessel leakage, pericyte loss, and VEGF protein levels in the mouse retinas. Resveratrol is effective in decreasing vascular lesions and VEGF induction in mouse retinas of early diabetes (Kim et al., 2012).

p1290 Astragalin, a 3-*O*-glucoside extracted from *Astragalus membranaceus* and *Astragalus propinquus*, has a history of use as a herbal medicine in systems

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of traditional Chinese medicine and has many pharmacological properties. It has been shown that astragalin decreases the overexpression of VEGF in cultured Müller glial cells after 3 days of treatment and alleviates the effects of high glucose. Astragalin has promising applications in preventing and treating DR (Ke et al., 2012).

p1295 Ginseng plants (Araliaceae), including North American ginseng (*Panax quinquefolius*) root extracts has multiple pharmacological actions because of their diverse phytochemical constituents. Ginsenosides are its major bioactive factors. Ginsenosides or panaxosides are the derivatives of protopanaxatriol, a class of steroid glycosides, and triterpene saponins (Wang et al., 2008). Bioactives of ginseng possess antioxidant properties, quenching free radicals, protecting LDLs from oxidation, and inhibiting lipid peroxidation (Kim and Park, 2003). Alcoholic ginseng root extract (per os daily) was administered for models of both type 1 (C57BL/6 mice with STZ-induced diabetes) and type 2 diabetes (db/db mice) for 2 or 4 months. In both the heart and retina of diabetic animals, ginseng treatment significantly prevented oxidative stress and diabetes-induced upregulation of extracellular matrix proteins and vasoactive factors. These data indicate that North American ginseng prevents the diabetes-induced retinal and cardiac biochemical and functional changes probably through inhibition of oxidative stress (Sen et al., 2013).

p1300 The extract, named fenugreek, of *Trigonella foenum-graecum* L. contains simple alkaloids (trigonelline, choline, gentianine, and carpaine) and other pyridines and pyrroles. Treatment with fenugreek resulted in marked inhibition in the expression of inflammatory (TNFα and IL-1β) and angiogenic molecular biomarkers (VEGF and PKCβ) and positive modulatory effects on retinal oxidative stress. The fenugreek-treated retinas did not show vascular leakage with and had relatively reduced thickening of capillary basement membrane. Fenugreek has great potential in preventing diabetes-induced retinal degeneration in humans with regular consumption in the specified dosage (Gupta et al., 2014).

s0350 ### *4.6.3 Miscellaneous compounds*

p1305 Several compounds of miscellaneous origin have been tested to counteract experimental DR symptoms. An incomplete list of these follows.

s0355 #### 4.6.3.1 Retinoid acids

p1310 Retinoic acid (RA) and its derivatives are essential signaling molecules throughout life and may play an important role in the induction of glial cell Au25

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line-derived neurotrophic factor (GDNF; Nishikiori et al., 2007a; Thang et al., 2000), which is a differentiation factor in the retina (Thang et al., 2000; Wu et al., 2004a). RA inhibits the apoptosis; a marked decrease in apoptotic cell number could be observed during the development of DR after treatment with RA (Nishikiori et al., 2008). A possible protective mechanism might be that RA protects retinal cells by inducing GDNF production in glial cells in the retina (Nishikiori et al., 2007a,b). RAs are promising drugs for diabetic ophthalmic disease because they have anticataractogenic effects for diabetic cataracts (Nishikiori et al., 2007b), BRB-protective effects for vascular integrity (Miyajima et al., 2005; Nishikiori et al., 2007a), and neuroprotective effects against apoptosis in DR (Nishikiori et al., 2008).

s0360 #### 4.6.3.2 Calpain inhibitors

p1315 Calpains are a family of 14 calcium-regulated, intracellular cysteine proteases, which modulate cellular functions by limited specific proteolysis (Huang and Wang, 2001). Calpains are activated by locally increased Ca^{2+} levels through calcium channels and intracellular stores (Camins et al., 2006). Inhibition of calpain signaling is a therapeutic target for several pathological conditions, including DR. Calpain plays a crucial role in metabolically induced RGC degeneration caused by DR and oxidative stress. The combination of antioxidants and calpain inhibition offers important opportunities for future neuroprotective treatment against RGC death. Levels of calpain are regulated by an endogenous specific inhibitor, calpastatin. An exogenous calpain inhibitor, SNJ-1945, has shown strong ability to penetrate BRB after oral administration (Shirasaki et al., 2005). Four weeks after the induction of diabetes, degeneration of RGCs and their axons was successfully prevented by SNJ-1945. This compound was able to suppress mRNA overexpression of calpain-1, and it has a significant preventive effect against diabetic changes to RGC synapses in DR. Proapoptotic end products of cleaved α-fodrin were significantly reduced in DR by oral administration of SNJ-1945 (Shanab et al., 2012).

s0365 #### 4.6.3.3 Sitagliptin

p1320 Sitagliptin is an oral antihyperglycemic drug of the dipeptidyl peptidase-4 inhibitor class. Treatment with sitagliptin prevented the changes in the endothelial subcellular distribution of the tight junction proteins (occludin and claudin-5) induced by diabetes. Sitagliptin decreased the nitrosative stress, the inflammatory state, and cell death in diabetic retinas. It allowed

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the recovery of a number of CD34+ cells present in the bloodstream with levels similar to their number in controls and increased the adhesion ability of endothelial progenitor cells to the retinal vessels and exerted beneficial effects on the BRB integrity in ZDF rat retinas (Gonçalves et al., 2012).

s0370 #### 4.6.3.4 Carnosine

p1325 Carnosine (beta-alanyl-L-histidine) is an antiglycating dipeptide of the amino acids beta-alanine and histidine. It is highly concentrated in muscle and brain tissues. Carnosine has a number of antioxidant properties and has been proven to scavenge ROS and alpha–beta-unsaturated aldehydes formed from peroxidation of cell membrane fatty acids during oxidative stress. Oral carnosine treatment prevented retinal vascular damage after 6 months of experimental hyperglycemia. The protection was associated with a significant induction of Hsp27 in activated glial cells and normalization of increased angiotensin-2 levels in diabetic retinas. Oral carnosine treatment protects retinal capillary cells in experimental DR, independently of its other biochemical functions (Pfister et al., 2011).

s0375 #### 4.6.3.5 Memantine

p1330 Vitreoretinal glutamate levels are elevated in experimental diabetes (Lieth et al., 1998). The expression of the *N*-methyl-D-aspartate (NMDA)-type glutamate receptors is upregulated in the diabetic retina suggesting a role of glutamate excitotoxicity. Memantine is an NMDA receptor blocker and acts as an uncompetitive antagonist (Smith, 2002). Long-term treatment with memantine significantly improves retinal function and prevents RGC loss in STZ-induced diabetic rats. Memantine also significantly reduces elevated VEGF protein levels in the retina and vitreous fluid and BRB breakdown in the retinas of diabetic animals. Memantine significantly improved amplitudes of ERG a and b waves. Another possible mechanism of the neuroprotective effect of memantine in the retinas of STZ-induced diabetic rats could be the inhibition of retinal type 3 serotonin and nicotinic Ach receptors, although elevated serotonin and acetylcholine levels have not been demonstrated in diabetic retina (Kusari et al., 2007).

s0380 #### 4.6.3.6 Nepafenac

p1335 Nepafenac is a prodrug of amfenac, a nonsteroidal anti-inflammatory drug that inhibits COX-1 and COX-2 and the synthesis of proinflammatory prostaglandins (Kapin et al., 2003). The administration of nepafenac inhibits functional and morphological lesions characteristic to the early stages of DR.

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It also inhibited the diabetes-induced apoptosis of endothelial cells and pericytes and the degeneration of retinal capillaries. In the retina, nepafenac partially inhibits the diabetes-induced activation of executioner caspases, such as caspase 3 and caspase 6. It may also be a direct scavenger of superoxide. Topically applied nepafenac decreased the OP latency despite persistent hyperglycemia. Nepafenac administered via eye drops reaches the retina of rats in sufficient concentration to inhibit multiple biochemical and morphological abnormalities in diabetes (Kern et al., 2007).

s0385 #### 4.6.3.7 Vitamin D

p1340 Many studies provide evidence that lack of vitamin D has a role in pathogenesis of both type 1 and type 2 diabetes (Takiishi et al., 2010), including their accompanied syndromes such as DR (Aksoy et al., 2000; Albert et al., 2007; Kaur et al., 2011). Vitamin D is a potent inhibitor of retinal neovascularization in DR (Albert et al., 2007) through decreasing the level of VEGF (Ren et al., 2012).

s0390 #### 4.6.3.8 Hydrogen sulfide

p1345 Hydrogen sulfide (H_2S) is the most recent addition to endogenous gasotransmitter family. It played beneficial roles in several diseases, and a lower level of H_2S was observed in the blood of diabetic patients and STZ-treated rats (Jain et al., 2010). Treatment with exogenous H_2S prevented diabetic neurodegeneration and enhanced expressions of synaptophysin and BDNF in retinas. Reduction was seen in BRB permeability and the number of acellular capillaries following treatment with exogenous H_2S in retinas of STZ-induced diabetic rats. This could be explained by the concomitant reduction in vitreous VEGF content and gene expression of HIF-1α and VEGF-R2 and increased expression of occludin. Treatment with H_2S not only functioned as a direct scavenger of ROS but also influenced some important enzymes associated with oxidative stress. Treatment with H_2S or NaHS, a donor of H_2S, attenuated STZ-induced retinopathy, possibly through abating oxidative stress and suppressing inflammation (Si et al., 2013).

s0395 #### 4.6.3.9 Hydrogen saline

p1350 If hydrogen is dissolved in physiological saline under high pressure to a supersaturated level, it becomes hydrogen saline (H(2)saline or hydrogen-rich saline; Sun et al., 2011). It has considerable antioxidant and anti-inflammatory properties, and it also suppresses oxidative stress-induced injury. In the retina, it has protective effects against glutamate-induced

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toxicity (Wei et al., 2012). H(2)saline treatment could depress caspase 3 activity, reduce retinal apoptosis and vascular permeability, and prominently attenuate the retinal parenchyma thickening that resulted from DR (Xiao et al., 2012b). In H(2)saline-treated STZ-induced diabetic rats, the diabetes-induced reduction of b wave amplitudes and OPs was restored, and the BRB breakdown and histological changes in the inner retina were reversed. Furthermore, H(2)saline reduced oxidative stress, increased antioxidant enzyme activities, and preserved synaptophysin and BDNF levels in diabetic rat retina (Feng et al., 2013).

s0400 #### 4.6.3.10 *N*-acetylcysteine

p1355 Macrophage/microglia activation, pericyte loss, and endothelial/perivascular cell changes occur early in the pathogenesis of DR. These changes are associated with an increase in plasma markers of oxidative stress and inflammation and are minimized by treatment with a well-known free radical scavenger, *N*-acetylcysteine (Tsai et al., 2009).

s0405 #### 4.6.3.11 Fasudil

p1360 Fasudil protects the vascular endothelium by inhibiting neutrophil adhesion and reducing neutrophil-induced endothelial injury. Intravitreal administration significantly increased eNOS phosphorylation, whereas it reduced ICAM-1 expression, leukocyte adhesion, and the number of damaged endothelial cells. Neutrophils from DR patients showed significantly higher adhesion to cultured endothelium and caused endothelial apoptosis, which was significantly reduced by fasudil. Fasudil may alter the Rho/Rho kinase pathway, which plays a critical role in diabetic retinal microvasculopathy; therefore, Rho kinase inhibition may become a new strategy in the management of DR, especially in its early stages (Arita et al., 2009).

s0410 #### 4.6.3.12 Photobiomodulation

p1365 Daily 670 nm photobiomodulation treatment resulted in a significant inhibition in diabetes-induced RGC death and a 50% improvement of the ERG amplitude, especially photopic b wave responses. Photobiomodulation did not alter cytochrome oxidase activity in the retina or in the cultured retinal cells, but inhibited diabetes-induced superoxide production and preserved MnSOD expression *in vivo*. It essentially prevented the diabetes-induced increase in leukostasis and expression of ICAM-1. It is a noninvasive, inexpensive, and easily administered simple adjunct therapy to attenuate the development of DR (Tang et al., 2013).

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5. CONCLUDING REMARKS

As it has been shown in the preceding text, there are numerous open options to research DR and its treatment possibilities. Perhaps, it is conspicuous for the reader that symptoms of DR are identical regardless of which type of diabetes causes this syndrome. Therefore, both type 1 and type 2 diabetes models can be used to study treatment options. However, not all the aforementioned diabetes models are equally efficient in producing all the symptoms observed in human patients. In our opinion, there can be two main causes of this mismatch: (i) In humans, diabetes in most cases is controlled (i.e., patients use medications to lower their blood glucose levels to acceptable concentrations). Early good glycemic control and prevention of the abnormalities of apoptosis are important for diabetic patients to prevent the development and progression of sight-threatening DR (Gao et al., 2009b; Hammes, 2013). Also, (ii) the relatively short life span of the most often used animal models may be a problem. Rats and mice (especially those inflicted with diabetes) do not live long enough to produce all the alterations in circulation and develop compensatory mechanisms for the diabetic damage, and experimenters very often use young adult specimens for their studies. Therefore, efforts should be made to develop chronic models with controlled blood sugar levels and to achieve long-term survival (more than a year after diabetes induction) of experimental animals to produce all the symptoms (or as many of them as possible). These constrains may also explain why mice have been used less frequently as models in studying DR. Genetically modified transgenic or knockout mice are widely used in other areas of biomedical research. Several inbred strains are also used for studying diabetes and DR, but they could be more extensively studied if models were chosen carefully. Thus, considering all the requirements, long-term STZ-induced diabetes in rats with carefully controlled blood glucose level could be one of the best options to study diabetic complications such as DR. Among the genetically diabetic strains, spontaneously type 2 diabetic rats could be the best for this purpose; our bias is clearly toward the OLETF and SDT rats, because they produce the symptoms that are closest to the human disease. *Ex vivo* and *in vitro* models may be useful in studying certain aspects of pathogenesis of DR and test drug candidates but may not be sufficiently sophisticated to study all aspects, especially not those related to real functional and electrophysiological properties.

As for the protective compounds, besides the strict blood glucose control, two major lines of research should be pursued in the future: (i) to find

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extremely selective blockers of specific antiapoptotic pathways or generate a mixture of neurotrophic factors to keep nerve cells alive under damaging metabolic conditions and (ii) to provide general protection against ischemia, which facilitates the formation of ROS, AGE, and advanced lipoxidation end product (ALE). For the first approach, compounds acting at different levels of the signal transduction pathway have been tried. Among inhibitors of neuron loss, octreotide, a peptide analog of SST acting at sst2A receptors, has been found the most effective. If a nonpeptide analog could be developed for this receptor, chances for routine medicinal use would definitely increase. The similar notion is true for PACAP. Currently, only shortened or modified versions of the peptide are available for experimentation (Bourgault et al., 2008); nonpeptide PACAP agonists do not exist. Experiments are in progress to show if PACAP is protective in the form of artificial tears (D. Reglodi, unpublished results). Likewise, if an optimal mixture of trophic factors (IGF, NGF, BDNF, and PEDF) could be created, it could be applied topically. PEDF is a particularly promising candidate, and since its production is restricted to the retina, unwanted side effects can be minimal. Hormones and hormone-like materials (angiotensin and EPO) present in several tissues of the body carry less hope because of their potentially strong side effects. A more promising way is to fight angiogenesis with anti-VEGF compounds or antibodies. In fact, the latter form of treatment gains momentum in clinical trials either alone or in combination with surgical treatments in the clinical practice. A similarly promising approach is to fight ROS, AGE, and ALE formation. GT, ginseng and *Ginkgo* extracts, curcumin, and astragalin are particularly potent. Their administration is simple, but it should be noted that these compounds have to be administered over a long period of time. This can be done in the form of nutritional supplements. Adverse action or side effects are improbable.

p1380 As the final point, a desirable experimental design will be described here, which may lead to results that can be successfully translated to humans. Aged OLETF and/or SDT rats or, alternatively, animals with STZ-induced controlled long-term diabetes (at least 1-year duration) should be used. Metabolic parameters should be monitored every day, blood sugar level should be kept around 10–15 mmol/l. Control ERG measurements should be performed before DR develops. When early symptoms of DR (e.g., vascular leakage, retinal edema, and deteriorating ERG) appear, protective treatment regimes should be initiated. Besides keeping an untreated diabetic group alive as long as possible, one experimental group could receive specific neuroprotective/antiapoptotic treatment and the other nonselective anti-ROS

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compound(s). The possible protective agents should be administered either topically (e.g., in case of neuropeptide analogs or growth factors) in a predetermined manner (e.g., monthly intravitreal injection) or per os daily (anti-ROS compounds). This way, the efficacy of specific and generic treatment strategies and their long-term manageability could be determined. The animals that die during the experimental period should be taken to autopsy to assess the severity of diabetes and DR. There are obviously several difficulties in executing such a wide-scope and long-term experiment with enough animals to draw valid conclusions. However, at this stage of DR research, one or more research groups have to take the risk to produce translatable results.

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REFERENCES

Abu El-Asrar, A.M., Dralands, L., Missotten, L., Geboes, K., 2007. Expression of anti-apoptotic and proapoptotic molecules in diabetic retinas. Eye (Lond). 21 (2), 238–245.

Agardh, C.D., Agardh, E., Zhang, H., Ostenson, C.G., 1997. Altered endothelial/pericyte ratio in Goto-Kakizaki rat retina. J. Diabetes Complications 11 (3), 158–162.

Agrawal, S.S., Naqvi, S., Gupta, S.K., Srivastava, S., 2012. Prevention and management of diabetic retinopathy in STZ diabetic rats by Tinospora cordifolia and its molecular mechanisms. Food Chem. Toxicol. 50 (9), 3126–3132.

Ahn, K.S., Sim, W.S., Kim, I.H., 1996. Decursin: a cytotoxic agent and protein kinase C activator from the root of Angelica gigas. Planta Med. 62 (1), 7–9.

Aizu, Y., Oyanagi, K., Hu, J., Nakagawa, H., 2002. Degeneration of retinal neuronal processes and pigment epithelium in the early stage of the streptozotocin-diabetic rats. Neuropathology 22 (3), 161–170.

Aksoy, H., Akçay, F., Kurtul, N., Baykal, O., Avci, B., 2000. Serum 1,25 dihydroxy vitamin D (1,25(OH)2D3), 25 hydroxy vitamin D (25(OH)D) and parathormone levels in diabetic retinopathy. Clin. Biochem. 33 (1), 47–51.

Albert, D.M., Scheef, E.A., Wang, S., Mehraein, F., Darjatmoko, S.R., Sorenson, C.M., Sheibani, N., 2007. Calcitriol is a potent inhibitor of retinal neovascularization. Invest. Ophthalmol. Vis. Sci. 48 (5), 2327–2334.

Al-Gayyar, M.M., Mysona, B.A., Matragoon, S., Abdelsaid, M.A., El-Azab, M.F., Shanab, A.Y., Ha, Y., Smith, S.B., Bollinger, K.E., El-Remessy, A.B., 2013. Diabetes and overexpression of proNGF cause retinal neurodegeneration via activation of RhoA pathway. PLoS One 8 (1), e54692.

Ali, T.K., Matragoon, S., Pillai, B.A., Liou, G.I., El-Remessy, A.B., 2008. Peroxynitrite mediates retinal neurodegeneration by inhibiting nerve growth factor survival signaling in experimental and human diabetes. Diabetes 57 (4), 889–898.

Al-Malki, A.L., 2013. Oat attenuation of hyperglycemia-induced retinal oxidative stress and NF-κB activation in streptozotocin-induced diabetic rats. Evid. Based Complement. Alternat. Med. 2013, 983923.

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Amandio, M., Bucolo, C., Leggio, G.M., Drago, F., Govoni, B., Pascale, A., 2010. The PKCB/HuR/VEGF pathway in diabetic retinopathy. Biochem. Pharmacol. 80, 1230–1237.

Ambati, J., Chalam, K.V., Chawla, D.K., D'Angio, C.T., Guillet, E.G., Rose, S.J., Vanderlinde, R.E., Ambati, B.K., 1997. Elevated gamma-aminobutyric acid, glutamate, and vascular endothelial growth factor levels in the vitreous of patients with proliferative diabetic retinopathy. Arch. Ophthalmol. 115 (9), 1161–1166.

Amthor, F.R., Keyser, K.T., Dmitrieva, N.A., 2002. Effects of the destruction of starburst-cholinergic amacrine cells by the toxin AF64A on rabbit retinal directional selectivity. Vis. Neurosci. 19 (4), 495–509.

Aranda, J., Motiejunaite, R., Im, E., Kazlauskas, A., 2012. Diabetes disrupts the response of retinal endothelial cells to the angiomodulator lysophosphatidic acid. Diabetes 61 (5), 1225–1233.

Arita, R., Hata, Y., Nakao, S., Kita, T., Miura, M., Kawahara, S., Zandi, S., Almulki, L., Tayyari, F., Shimokawa, H., Hafezi-Moghadam, A., Ishibashi, T., 2009. Rho kinase inhibition by fasudil ameliorates diabetes-induced microvascular damage. Diabetes 58 (1), 215–226.

Arjamaa, O., Nikinmaa, M., 2006. Oxygen-dependent diseases in the retina: role of hypoxia-inducible factors. Exp. Eye Res. 83 (3), 473–483.

Asnaghi, V., Gerhardinger, C., Hoehn, T., Adeboje, A., Lorenzi, M., 2003. A role for the polyol pathway in the early neuroretinal apoptosis and glial changes induced by diabetes in the rat. Diabetes 52, 506–511.

Atlasz, T., Szabadfi, K., Kiss, P., Babai, N., Koszegi, Z., Tamas, A., Reglodi, D., Gabriel, R., 2008. PACAP-mediated neuroprotection of neurochemically identified cell types in MSG-induced retinal degeneration. J. Mol. Neurosci. 36, 97–104.

Atlasz, T., Szabadfi, K., Kiss, P., Racz, B., Gallyas, F., Tamas, A., Gaal, V., Marton, Zs, Gabriel, R., Reglodi, D., 2010. Review of pituitary adenylate cyclase activating polypeptide in the retina: focus on the retinoprotective effects. Ann. N.Y. Acad. Sci. 1200, 128–139.

Aveleira, C.A., Lin, C.M., Abcouwer, S.F., Ambrósio, A.F., Antonetti, D.A., 2010. TNFα signals through PKCζ/NF-κB to alter the tight junction complex and increase retinal endothelial cell permeability. Diabetes 59 (11), 2872–2882.

Badr, G.A., Tang, J., Ismail-Beigi, F., Kern, T.S., 2000. Diabetes downregulates GLUT1 expression in the retina and its microvessels but not in the cerebral cortex or its microvessels. Diabetes 49 (6), 1016–1021.

Bagnoli, P., Dal Monte, M., Casini, G., 2003. Expression of neuropeptides and their receptors in the developing retina of mammals. Histol. Histopathol. 18, 1219–1242.

Barba, I., Garcia-Ramírez, M., Hernandez, C., Alonso, M.A., Masmiquel, L., García-Dorado, D., Simo, R., 2010. Metabolic fingerprints of proliferative diabetic retinopathy: an 1H-NMR-based metabonomic approach using vitreous humor. Invest. Ophthalmol. Vis. Sci. 51 (9), 4416–4421.

Barber, A.J., Lieth, E., Khin, S.A., Antonetti, D.A., Buchanan, A.G., Gardner, T.W., 1998. Neural apoptosis in the retina during experimental and human diabetes. Early onset and effect of insulin. J. Clin. Invest. 102, 783–791.

Barber, A.J., Nakamura, M., Wolpert, E.B., Reiter, C.E., Seigel, G.M., Antonetti, D.A., Gardner, T.W., 2001. Insulin rescues retinal neurons from apoptosis by a phosphatidylinositol 3-kinase/Akt-mediated mechanism that reduces the activation of caspase 3. J. Biol. Chem. 276 (35), 32814–32821.

Barber, A.J., Antonetti, D.A., Kern, T.S., Reiter, C.E., Soans, R.S., Krady, J.K., Levison, S.-W., Gardner, T.W., Bronson, S.K., 2005. The Ins2Akita mouse as a model of early retinal complications in diabetes. Invest. Ophthalmol. Vis. Sci. 46, 2210–2218.

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:37 Page Number: 98

B978-0-12-800179-0.00001-5, 00001

Barber, A.J., Gardner, T.W., Abcouwer, S.F., 2011. The significance of vascular and neural apoptosis to the pathology of diabetic retinopathy. Invest. Ophthalmol. Vis. Sci. 52 (2), 1156–1163.

Barnstable, C.J., Tombran-Tink, J., 2004. Neuroprotective and antiangiogenic actions of PEDF in the eye: molecular targets and therapeutic potential. Prog. Retin. Eye Res. 23 (5), 561–577.

Behl, Y., Krothapalli, P., Desta, I., DiPiazza, A., Roy, S., Graves, D.I., 2008. Diabetes-enhanced tumor necrosis factor alpha production promotes apoptosis and the loss of retinal microvascular cells in type 1 and type 2 models of diabetic retinopathy. Am. J. Pathol. 172, 1411–1418.

Bhutto, I.A., Lu, Z.Y., Takami, Y., Amemiya, T., 2002. Retinal and choroidal vasculature in rats with spontaneous diabetes type 2 treated with the angiotensin-converting enzyme inhibitor cilazapril: corrosion cast and electron-microscopic study. Ophthalmic Res. 34 (4), 220–231.

Biro, K., Palhalmi, J., Toth, A.J., Kukorelli, T., Juhasz, G., 1998. Bimoclomol improves early electrophysiological signs of retinopathy in diabetic rats. Neuroreport 9 (9), 2029–2033.

Blake, A.D., Badway, A.C., Strowski, M.Z., 2004. Delineating somatostatin's neuronal actions. Curr. Drug Targets CNS Neurol. Disord. 3 (2), 153–160.

Boehm, B.O., Lang, G., Volpert, O., Jehle, P.M., Kurkhaus, A., Rosinger, S., Lang, G.K., Bouck, N., 2003. Low content of the natural ocular anti-angiogenic agent pigment epithelium-derived factor (PEDF) in aqueous humor predicts progression of diabetic retinopathy. Diabetologia 46 (3), 394–400.

Bourgault, S., Vaudry, D., Guilhaudis, L., Raoult, E., Couvineau, A., Laburthe, M., Ségalas-Milazzo, I., Vaudry, H., Fournier, A., 2008. Biological and structural analysis of truncated analogs of PACAP27. J. Mol. Neurosci. 36, 260–269.

Brines, M., Cerami, A., 2005. Emerging biological roles for erythropoietin in the nervous system. Nat. Rev. Neurosci. 6 (6), 484–494.

Brines, M., Cerami, A., 2008. Erythropoietin-mediated tissue protection: reducing collateral damage from the primary injury response. J. Intern. Med. 264 (5), 405–432.

Brines, M., Patel, N.S., Villa, P., Brines, C., Mennini, T., De Paola, M., Erbayraktar, Z., Erbayraktar, S., Sepodes, B., Thiemermann, C., Ghezzi, P., Yamin, M., Hand, C.C., Xie, Q.W., Coleman, T., Cerami, A., 2008. Nonerythropoietic, tissue-protective peptides derived from the tertiary structure of erythropoietin. Proc. Natl. Acad. Sci. U.S.A. 105 (31), 10925–10930.

Bringmann, A., Wiedemann, P., 2012. Müller glial cells in retinal disease. Ophthalmologica 227, 1–12.

Bringmann, A., Pannicke, T., Grosche, J., Francke, M., Wiedemann, P., Skatchkov, S.N., Osborne, N.N., Reichenbach, A., 2006. Müller cells in the healthy and diseased retina. Prog. Retin. Eye Res. 25 (4), 397–424.

Brownlee, M., 2001. Biochemistry and molecular cell biology of diabetic complications. Nature 414 (6865), 813–820.

Brownlee, M., 2005. The pathobiology of diabetic complications: a unifying mechanism. Diabetes 54 (6), 1615–1625.

Bucolo, C., Leggio, G.M., Drago, F., Salomone, S., 2012. Eriodictyol prevents early retinal and plasma abnormalities in streptozotocin-induced diabetic rats. Biochem. Pharmacol. 84 (1), 88–92.

Bucolo, C., Marrazzo, G., Platania, C.B., Drago, F., Leggio, G.M., Salomone, S., 2013. Fortified extract of red berry, Ginkgo biloba, and white willow bark in experimental early diabetic retinopathy. J. Diabetes Res. 2013, 432695.

Camins, A., Verdaguer, E., Folch, J., Pallàs, M., 2006. Involvement of calpain activation in neurodegenerative processes. CNS Drug Rev. 12 (2), 135–148.

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:38 Page Number: 99

B978-0-12-800179-0.00001-5, 00001

Carmo, A., Cunha-Vaz, J.G., Carvalho, A.P., Lopes, M.C., 2000. Nitric oxide synthase activity in retinas from non-insulin-dependent diabetic Goto-Kakizaki rats: correlation with blood-retinal barrier permeability. Nitric Oxide 4 (6), 590–596.

Carrasco, E., Hernandez, C., de Torres, I., Farrés, J., Simo, R., 2008. Lowered cortistatin expression is an early event in the human diabetic retina and is associated with apoptosis and glial activation. Mol. Vis. 14, 1496–1502.

Casini, G., Catalani, E., Dal Monte, M., Bagnoli, P., 2005. Functional aspects of the somatostatinergic system in the retina and the potential therapeutic role of somatostatin in retinal disease. Histol. Histopathol. 20, 615–632.

Castorina, A., Giunta, S., Mazzone, V., Cardile, V., D'Agata, V., 2010. Effects of PACAP and VIP on hyperglycemia-induced proliferation in murine microvascular endothelial cells. Peptides 31, 2276–2283.

Cellerino, A., Pinzón-Duarte, G., Carroll, P., Kohler, K., 1998. Brain-derived neurotrophic factor modulates the development of the dopaminergic network in the rodent retina. J. Neurosci. 18 (9), 3351–3362.

Cervia, D., Martini, D., Ristori, C., Catalani, E., Timperio, A.M., Bagnoli, P., Casini, G., 2008. Modulation of the neuronal response to ischemia by somatostatin analogues in wild-type and knock-out mouse retinas. J. Neurochem. 106, 2224–2235.

Cervia, D., Catalani, E., Dal Monte, M., Casini, G., 2012. Vascular endothelial growth factor in the ischemic retina and its regulation by somatostatin. J. Neurochem. 120, 818–829.

Chang, H.M., Huang, Y.L., Lan, C.T., Wu, U.I., Hu, M.E., Youn, S.C., 2008. Melatonin preserves superoxide dismutase activity in hypoglossal motoneurons of adult rats following peripheral nerve injury. J. Pineal Res. 44 (2), 172–180.

Chen, H., Jia, W., Xu, X., Fan, Y., Zhu, D., Wu, H., Xie, Z., Zheng, Z., 2008. Upregulation of PEDF expression by PARP inhibition contributes to the decrease in hyperglycemia-induced apoptosis in HUVECs. Biochem. Biophys. Res. Commun. 369 (2), 718–724.

Chen, F., Zhang, H.Q., Zhu, J., Liu, K.Y., Cheng, H., Li, G.L., Xu, S., Lv, W.H., Xie, Z.G., 2012a. Puerarin enhances superoxide dismutase activity and inhibits RAGE and VEGF expression in retinas of STZ-induced early diabetic rats. Asian Pac. J. Trop. Med. 5 (11), 891–896.

Chen, Y.H., Chen, J.Y., Chen, Y.W., Lin, S.T., Chan, H.L., 2012b. High glucose-induced proteome alterations in retinal pigmented epithelium cells and its possible relevance to diabetic retinopathy. Mol. Biosyst. 8 (12), 3107–3124.

Chen, M., Curtis, T.M., Stitt, A.W., 2013. Advanced glycation end products and diabetic retinopathy. Curr. Med. Chem. 20 (26), 3234–3240.

Cheung, A.K., Fung, M.K., Lo, A.C., Lam, T.T., So, K.F., Chung, S.S., Chung, S.K., 2005. Aldose reductase deficiency prevents diabetes-induced blood-retinal barrier breakdown, apoptosis, and glial reactivation in the retina of db/db mice. Diabetes 54 (11), 3119–3125.

Colafrancesco, V., Coassin, M., Rossi, S., Aloe, L., 2011. Effect of eye NGF administration on two animal models of retinal ganglion cells degeneration. Ann. Ist. Super. Sanita 47 (3), 284–289.

Cringle, S.J., Yu, D.Y., Alder, V.A., Su, E.N., 1993. Retinal blood flow by hydrogen clearance polarography in the streptozotocin-induced diabetic rat. Invest. Ophthalmol. Vis. Sci. 34 (5), 1716–1721.

Curtis, T.M., Scholfield, C.N., 2004. The role of lipids and protein kinase Cs in the pathogenesis of diabetic retinopathy. Diabetes Metab. Res. Rev. 20 (1), 28–43, Review.

Cusato, K., Bosco, A., Linden, R., Reese, B.E., 2002. Cell death in the inner nuclear layer of the retina is modulated by BDNF. Brain Res. Dev. Brain Res. 139 (2), 325–330.

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:38 Page Number: 100

B978-0-12-800179-0.00001-5, 00001

Dagher, Z., Park, Y.S., Asnaghi, V., Hoehn, T., Gerhardinger, C., Lorenzi, M., 2004. Studies of rat and human retinas predict a role for the polyol pathway in human diabetic retinopathy. Diabetes 53 (9), 2404–2411.

Das Evcimen, N., King, G.L., 2007. The role of protein kinase C activation and the vascular complications of diabetes. Pharmacol. Res. 55 (6), 498–510.

Davis, M.I., Wilson, S.H., Grant, M.B., 2001. The therapeutic problem of proliferative diabetic retinopathy: targeting somatostatin receptors. Horm. Metab. Res. 33 (5), 295–299.

D'Cruz, T.S., Weibley, B.N., Kimball, S.R., Barber, A.J., 2012. Post-translational processing of synaptophysin in the rat retina is disrupted by diabetes. PLoS One 7 (9), e44711.

DeBosch, B.J., Deo, B.K., Kumagai, A.K., 2002. Insulin-like growth factor-1 effects on bovine retinal endothelial cell glucose transport: role of MAP kinase. J. Neurochem. 81 (4), 728–734.

Denis, U., Lecomte, M., Paget, C., Ruggiero, D., Wiernsperger, N., Lagarde, M., 2002. Advanced glycation end-products induce apoptosis of bovine retinal pericytes in culture: involvement of diacylglycerol/ceramide production and oxidative stress induction. Free Radic. Biol. Med. 33 (2), 236–247.

Diaz, B., Serna, J., De Pablo, F., de la Rosa, E.J., 2000. *In vivo* regulation of cell death by embryonic (pro)insulin and the insulin receptor during early retinal neurogenesis. Development 127 (8), 1641–1649.

Dickinson, T., Mitchell, R., Robberecht, P., Fleetwood-Walker, S.M., 1999. The role of VIP/PACAP receptor subtypes in spinal somatosensory processing in rats with an experimental peripheral mononeuropathy. Neuropharmacology 38, 167–180.

Do Carmo, A., Ramos, P., Reis, A., Proença, R., Cunha-vaz, J.G., 1998. Breakdown of the inner and outer blood retinal barrier in streptozotocin-induced diabetes. Exp. Eye Res. 67 (5), 569–575.

Dong, C.J., Agey, P., Hare, W.A., 2004. Origins of the electroretinogram oscillatory potentials in the rabbit retina. Vis. Neurosci. 21 (4), 533–543.

Dong, L.Y., Jin, J., Lu, G., Kang, X.L., 2013. Astaxanthin attenuates the apoptosis of retinal ganglion cells in db/db mice by inhibition of oxidative stress. Mar. Drugs 11 (3), 960–974.

Dos Santos, M.D., Almeida, M.C., Lopes, N.P., de Souza, G.E., 2006. Evaluation of the anti-inflammatory, analgesic and antipyretic activities of the natural polyphenol chlorogenic acid. Biol. Pharm. Bull. 29 (11), 2236–2240.

Drel, V.R., Xu, W., Zhang, J., Kador, P.F., Ali, T.K., Shin, J., Julius, U., Slusher, B., El-Remessy, A.B., Obrosova, I.G., 2009. Poly(ADP-ribose)polymerase inhibition counteracts cataract formation and early retinal changes in streptozotocin-diabetic rats. Invest. Ophthalmol. Vis. Sci. 50 (4), 1778–1790.

Du, Y., Smith, M.A., Miller, C.M., Kern, T.S., 2002. Diabetes-induced nitrative stress in the retina, and correction by aminoguanidine. J. Neurochem. 80 (5), 771–779.

Du, Y., Miller, C.M., Kern, T.S., 2003. Hyperglycemia increases mitochondrial superoxide in retina and retinal cells. Free Radic. Biol. Med. 35 (11), 1491–1499.

Eichler, W., Kuhrt, H., Hoffmann, S., Wiedemann, P., Reichenbach, A., 2000. VEGF release by retinal glia depends on both oxygen and glucose supply. Neuroreport 11, 3533–3537.

El-Remessy, A.B., Behzadian, M.A., Abou-Mohamed, G., Franklin, T., Caldwell, R.W., Caldwell, R.B., 2003a. Experimental diabetes causes breakdown of the blood-retina barrier by a mechanism involving tyrosine nitration and increases in expression of vascular endothelial growth factor and urokinase plasminogen activator receptor. Am. J. Pathol. 162 (6), 1995–2004.

El-Remessy, A.B., Khalil, I.E., Matragoon, S., Abou-Mohamed, G., Tsai, N.J., Roon, P., Caldwell, R.B., Caldwell, R.W., Green, K., Liou, G.I., 2003b. Neuroprotective effect

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:38 Page Number: 101

B978-0-12-800179-0.00001-5, 00001

of (-)Delta9-tetrahydrocannabinol and cannabidiol in N-methyl-D-aspartate-induced retinal neurotoxicity: involvement of peroxynitrite. Am. J. Pathol. 163 (5), 1997–2008.

El-Remessy, A.B., Bartoli, M., Platt, D.H., Fulton, D., Caldwell, R.B., 2005. Oxidative stress inactivates VEGF survival signaling in retinal endothelial cells via PI 3-kinase tyrosine nitration. J. Cell Sci. 118 (Pt. 1), 243–252.

El-Remessy, A.B., Al-Shabrawey, M., Khalifa, Y., Tsai, N.T., Caldwell, R.B., Liou, G.I., 2006. Neuroprotective and blood-retinal barrier-preserving effects of cannabidiol in experimental diabetes. Am. J. Pathol. 168 (1), 235–244.

El-Remessy, A.B., Khalifa, Y., Ola, S., Ibrahim, A.S., Liou, G.I., 2010. Cannabidiol protects retinal neurons by preserving glutamine synthetase activity in diabetes. Mol. Vis. 16, 1487–1495.

Elsner, M., Guldbakke, B., Tiedge, M., Munday, R., Lenzen, S., 2000. Relative importance of transport and alkylation for pancreatic beta-cell toxicity of streptozotocin. Diabetologia 43 (12), 1528–1533.

Engerman, R.L., Kern, T.S., 1995. Retinopathy in animal models of diabetes. Diabetes Metab. Rev. 11 (2), 109–120, Review.

Faitova, J., Krekac, D., Hrstka, R., Vojtesek, B., 2006. Endoplasmic reticulum stress and apoptosis. Cell. Mol. Biol. Lett. 11 (4), 488–505.

Feng, Y., Wang, R., Xu, J., Sun, J., Xu, T., Gu, Q., Wu, X., 2013. Hydrogen-rich saline prevents early neurovascular dysfunction resulting from inhibition of oxidative stress in STZ-diabetic rats. Curr. Eye Res. 38 (3), 396–404.

Fernandes, R., Suzuki, K., Kumagai, A.K., 2003. Inner blood-retinal barrier GLUT1 in long-term diabetic rats: an immunogold electron microscopic study. Invest. Ophthalmol. Vis. Sci. 44 (7), 3150–3154.

Fernandes, R., Hosoya, K., Pereira, P., 2011. Reactive oxygen species downregulate glucose transport system in retinal endothelial cells. Am. J. Physiol. Cell Physiol. 300 (4), C927–C936.

Fleming, R.L., Silveira, M.S., Santos, L.E., Henze, I.P., Gardino, P.F., de Mello, M.C., de Mello, F.G., 2013. Pituitary adenylyl cyclase-activating polypeptide receptor re-sensitization induces plastic changes in the dopaminergic phenotype in the mature avian retina. J. Neurochem. 124 (5), 621–631.

Fong, D.S., Aiello, L.P., Ferris 3rd., F.L., Klein, R., 2004. Diabetic retinopathy. Diabetes Care 27, 2540–2553.

Fox, A., Eastwood, C., Gentry, C., Manning, D., Urban, L., 1999. Critical evaluation of the streptozotocin model of painful diabetic neuropathy in the rat. Pain 81 (3), 307–316.

Fu, Z., Kuang, H.Y., Hao, M., Gao, X.Y., Liu, Y., Shao, N., 2012. Protection of exenatide for retinal ganglion cells with different glucose concentrations. Peptides 37 (1), 25–31.

Fukuda, M., Nakanishi, Y., Fuse, M., Yokoi, N., Hamada, Y., Fukagawa, M., Negi, A., Nakamura, M., 2010. Altered expression of aquaporins 1 and 4 coincides with neurodegenerative events in retinas of spontaneously diabetic Torii rats. Exp. Eye Res. 90 (1), 17–25.

Fukumoto, M., Takai, S., Ishizaki, E., Sugiyama, T., Oku, H., Jin, D., Sakaguchi, M., Sakonjo, H., Ikeda, T., Miyazaki, M., 2008. Involvement of angiotensin II-dependent vascular endothelial growth factor gene expression via NADPH oxidase in the retina in a type 2 diabetic rat model. Curr. Eye Res. 33, 885–891.

Fulton, A.B., Akula, J.D., Mocko, J.A., Hansen, R.M., Benador, I.Y., Beck, S.C., Fahl, E., Seelinger, M.W., Moskowitz, A., Harris, M.E., 2009. Retinal degenerative and hypoxic ischemic disease. Doc. Ophthalmol. 118, 55–61.

Gabriel, R., 2013. Neuropeptides and diabetic retinopathy. Br. J. Clin. Pharmacol. 75 (5), 1189–1201.

Galano, A., Tan, D.X., Reiter, R.J., 2011. Melatonin as a natural ally against oxidative stress: a physicochemical examination. J. Pineal Res. 51, 1–16.

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:39 Page Number: 102

B978-0-12-800179-0.00001-5, 00001

Galloway, N.R., Wells, M., Barber, C., 1971. Changes in the oscillatory potential in relation to different types diabetic retinography. Vision Res. 11, 1218.

Galvan, V., Logvinova, A., Sperandio, S., Ichijo, H., Bredesen, D.E., 2003. Type 1 insulin-like growth factor receptor (IGF-1R) signaling inhibits apoptosis signal-regulating kinase 1 (ASK1). J. Biol. Chem. 278 (15), 13325–13332.

Gao, G., Li, Y., Zhang, D., Gee, S., Crosson, C., Ma, J., 2001. Unbalanced expression of VEGF and PEDF in ischemia-induced retinal neovascularization. FEBS Lett. 489 (2–3), 270–276.

Gao, G., Li, Y., Fant, J., Crosson, C.E., Becerra, S.P., Ma, J.X., 2002. Difference in ischemic regulation of vascular endothelial growth factor and pigment epithelium-derived factor in brown norway and sprague dawley rats contributing to different susceptibilities to retinal neovascularization. Diabetes 51 (4), 1218–1225.

Gao, B.B., Phipps, J.A., Bursell, D., Clermont, A.C., Feener, E.P., 2009a. Angiotensin AT1 receptor antagonism ameliorates murine retinal proteome changes induced by diabetes. J. Proteome Res. 8 (12), 5541–5549.

Gao, X.Y., Kuang, H.Y., Zou, W., Liu, X.M., Lin, H.B., Yang, Y., 2009b. The timing of re-institution of good blood glucose control affects apoptosis and expression of Bax and Bcl-2 in the retina of diabetic rats. Mol. Biol. Rep. 36 (7), 1977–1982.

Garcia-Ramirez, M., Hernandez, C., Simo, R., 2008. Expression of erythropoietin and its receptor in the human retina: a comparative study of diabetic and nondiabetic subjects. Diabetes Care 31 (6), 1189–1194.

Garcia-Ramírez, M., Hernandez, C., Ruiz-Meana, M., Villarroel, M., Corraliza, L., Garcia-Dorado, D., Simo, R., 2011. Erythropoietin protects retinal pigment epithelial cells against the increase of permeability induced by diabetic conditions: essential role of JAK2/PI3K signaling. Cell. Signal. 23 (10), 1596–1602.

Gardiner, T.A., Anderson, H.R., Stitt, A.W., 2003. Inhibition of advanced glycation end-products protects against retinal capillary basement membrane expansion during long-term diabetes. J. Pathol. 201 (2), 328–333.

Gastinger, M.J., Singh, R.S., Barber, A.J., 2006. Loss of cholinergic and dopaminergic amacrine cells in streptozotocin-diabetic rat and Ins2Akita-diabetic mouse retinas. Invest. Ophthalmol. Vis. Sci. 47 (7), 3143–3150.

Gastinger, M.J., Kunselman, A.R., Conboy, E.E., Bronson, S.K., Barber, A.J., 2008. Dendrite remodeling and other abnormalities in the retinal ganglion cells of Ins2 Akita diabetic mice. Invest. Ophthalmol. Vis. Sci. 49 (6), 2635–2642.

Gaucher, D., Chiappore, J.A., Paques, M., Simonutti, M., Boitard, C., Sahel, J.A., Massin, P., Picaud, S., 2007. Microglial changes occur without neural cell death in diabetic retinopathy. Vision Res. 47, 612–623.

Geraldes, P., King, G.L., 2010. Activation of protein kinase C isoforms and its impact on diabetic complications. Circ. Res. 106 (8), 1319–1331.

Gerhardinger, C., McClure, K.D., Romeo, G., Podestà, F., Lorenzi, M., 2001. IGF-1 mRNA and signaling in the diabetic retina. Diabetes 50 (1), 175–183.

Gerhardinger, C., Costa, M.B., Coulombe, M.C., Toth, I., Hoehn, T., Grosu, P., 2005. Expression of acute-phase response proteins in retinal Müller cells in diabetes. Invest. Ophthalmol. Vis. Sci. 46 (1), 349–357.

Ghezzi, P., Brines, M., 2004. Erythropoietin as an antiapoptotic, tissue-protective cytokine. Cell Death Differ. 11 (Suppl. 1), S37–S44.

Gibson, C.J., 1988. Diurnal alterations in retinal tyrosine level and dopamine turnover in diabetic rats. Brain Res. 454 (1–2), 60–66.

Giunta, S., Castorina, A., Bucolo, C., Magro, G., Drago, F., D'Agata, V., 2012. Early changes in pituitary adenylate cyclase-activating peptide, vasoactive intestinal peptide and related receptors expression in retina of streptozotocin-induced diabetic rats. Peptides 37 (1), 32–39.

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:39 Page Number: 103

B978-0-12-800179-0.00001-5, 00001

Goh, S.Y., Cooper, M.E., 2008. Clinical review: the role of advanced glycation end products in progression and complications of diabetes. J. Clin. Endocrinol. Metab. 93 (4), 1143–1152, Review.

Gonçalves, A., Leal, E., Paiva, A., Teixeira Lemos, E., Teixeira, F., Ribeiro, C.F., Reis, F., Ambrósio, A.F., Fernandes, R., 2012. Protective effects of the dipeptidyl peptidase IV inhibitor sitagliptin in the blood-retinal barrier in a type 2 diabetes animal model. Diabetes Obes. Metab. 14 (5), 454–463.

Gorg, B., Wettstein, M., Metzger, S., Schliess, F., Haussinger, D., 2005. Lipopolysaccharide-induced tyrosine nitration and inactivation of hepatic glutamine synthetase in the rat. Hepatology 41 (5), 1065–1073.

Gorg, B., Qvartskhava, N., Voss, P., Grune, T., Haussinger, D., Schliess, F., 2007. Reversible inhibition of mammalian glutamine synthetase by tyrosine nitration. FEBS Lett. 581 (1), 84–90.

Gosbell, A.D., Favilla, I., Jablonski, P., 2002. The location of insulin receptors in bovine retina and isolated retinal cells. Clin. Experiment. Ophthalmol. 30 (2), 124–130.

Goto, Y., Kakizaki, M., 1981. The spontaneous-diabetes rat: a model of noninsulin dependent diabetes mellitus. Proc. Jpn Acad. 57, 381–384.

Goto, R., Doi, M., Ma, N., Semba, R., Uji, Y., 2005. Contribution of nitric oxide-producing cells in normal and diabetic rat retina. Jpn. J. Ophthalmol. 49 (5), 363–370.

Grant, M.B., Caballero, S., 2002. Somatostatin analogues as drug therapies for retinopathies. Drugs Today (Barc). 38 (11), 783–791.

Grant, M.B., Mames, R.N., Fitzgerald, C., Hazariwala, K.M., Cooper-DeHohh, R., Caballero, S., Estes, K.S., 2000. The efficacy of octreotide in the therapy of severe proliferative and early non-proliferative diabetic retinopathy. Diabetes Care 23, 504–509.

Grunwald, J.E., Brucker, A.J., Braunstein, S.N., Schwartz, S.S., Baker, L., Petrig, B.L., Riva, C.E., 1994. Strict metabolic control and retinal blood flow in diabetes mellitus. Br. J. Ophthalmol. 78 (8), 598–604.

Gupta, S.K., Kumar, B., Nag, T.C., Agrawal, S.S., Agrawal, R., Agrawal, P., Saxena, R., Srivastava, S., 2011. Curcumin prevents experimental diabetic retinopathy in rats through its hypoglycemic, antioxidant, and anti-inflammatory mechanisms. J. Ocul. Pharmacol. Ther. 27, 123–130.

Gupta, S.K., Kumar, B., Nag, T.C., Srinivasan, B.P., Srivastava, S., Gaur, S., Saxena, R., Au27
2014. Effects of Trigonella foenum-graecum (L.) on retinal oxidative stress, and proinflammatory and angiogenic molecular biomarkers in streptozotocin-induced diabetic rats. Mol. Cell. Biochem. 388, 1–9.

Hajna, Z., Szabadfi, K., Gabriel, R., Balla, Z., Biro, Z., Degrell, P., Koszegi, T., Tekus, V., Helyes, Z., Dobos, A., Farkas, S., Szucs, G., Pinter, E., 2013. Establishment of a chronic animal model for comprehensive preclinical investigation of drugs against diabetes-related complications. In preparation.

Hammes, H.P., 2013. Optimal treatment of diabetic retinopathy. Ther. Adv. Endocrinol. Metab. 4 (2), 61–71.

Hammes, H.P., Ali, S.S., Uhlmann, M., Weiss, A., Federlin, K., Geisen, K., Brownlee, M., 1995a. Aminoguanidine does not inhibit the initial phase of experimental diabetic retinopathy in rats. Diabetologia 38, 269–273.

Hammes, H.P., Federoff, H.J., Brownlee, M., 1995b. Nerve growth factor prevents both neuroretinal programmed cell death and capillary pathology in experimental diabetes. Mol. Med. 1, 527–534.

Hampson, A.J., Grimaldi, M., Axelrod, J., Wink, D., 1998. Cannabidiol and (-)Delta9-tetrahydrocannabinol are neuroprotective antioxidants. Proc. Natl. Acad. Sci. U.S.A. 95 (14), 8268–8273.

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:39 Page Number: 104

B978-0-12-800179-0.00001-5, 00001

Hancock, H.A., Kraft, T.W., 2004. Oscillatory potential analysis and ERGs of normal and diabetic rats. Invest. Ophthalmol. Vis. Sci. 45 (3), 1002–1008.

Hao, M., Kuang, H.Y., Fu, Z., Gao, X.Y., Liu, Y., Deng, W., 2012. Exenatide prevents high-glucose-induced damage of retinal ganglion cells through a mitochondrial mechanism. Neurochem. Int. 61 (1), 1–6.

Hartnett, M.E., Lappas, A., Darland, D., McColm, J.R., Lovejoy, S., D'Amore, P.A., 2003. Retinal pigment epithelium and endothelial cell interaction causes retinal pigment epithelial barrier dysfunction via a soluble VEGF-dependent mechanism. Exp. Eye Res. 77 (5), 593–599.

Hayden, M.R., Sowers, K.M., Pulakat, L., Joginpally, T., Krueger, B., Whaley-Connell, A., Sowers, J.R., 2011. Possible mechanisms of local tissue renin-angiotensin system activation in the cardiorenal metabolic syndrome and type 2 diabetes mellitus. Cardiorenal Med. 1 (3), 193–210.

Hazin, R., Colyer, M., Lum, F., Barazi, M.K., 2011. Revisiting Diabetes 2000: challenges in establishing nationwide diabetic retinopathy prevention programs. Am J. Ophthalmol. 152 (5), 723–729.

Hernandez, C., Simo, R., 2012. Neuroprotection in diabetic retinopathy. Curr. Diab. Rep. 12 (4), 329–337.

Hernandez, C., Fonollosa, A., Garcia-Ramirez, M., Higuera, M., Catalan, R., Miralles, A., Garcia-Arumi, J., Simo, R., 2006. Erythropoietin is expressed in the human retina and it is highly elevated in the vitreous fluid of patients with diabetic macular edema. Diabetes Care 29 (9), 2028–2033.

Hernandez, C., Garcia-Ramirez, M., Corraliza, L., Fernandez-Carneado, J., Farrera-Sinfreu, J., Ponsati, B., Gonzalez-Rodriguez, A., Martinez-Valverde, A., Simo, R., 2013. Topical administration of somatostatin prevents retinal neurodegeneration in experimental diabetes. Diabetes 62, 2569–2578. Au28

Hollborn, M., Dukic-Stefanovic, S., Pannicke, T., Ulbricht, E., Reichenbach, A., Wiedemann, P., Bringmann, A., Kohen, L., 2011. Expression of aquaporins in the retina of diabetic rats. Curr. Eye Res. 36 (9), 850–856.

Houde, V., Grenier, D., Chandad, F., 2006. Protective effects of grape seed proanthocyanidins against oxidative stress induced by lipopolysaccharides of periodontopathogens. J. Periodontol. 77 (8), 1371–1379.

Hu, L.M., Luo, Y., Zhang, J., Lei, X., Shen, J., Wu, Y., Qin, M., Unver, Y.B., Zhong, Y., Xu, G.T., Li, W., 2011. EPO reduces reactive gliosis and stimulates neurotrophin expression in Müller cells. Front. Biosci. 3, 1541–1555.

Hu, J., Wu, Q., Li, T., Chen, Y., Wang, S., 2013. Inhibition of high glucose-induced VEGF release in retinal ganglion cells by RNA interference targeting G protein-coupled receptor 91. Exp. Eye Res. 109, 31–39.

Huang, Q., Sheibani, N., 2008. High glucose promotes retinal endothelial cell migration through activation of Src, PI3K/Akt1/eNOS, and ERKs. Am. J. Physiol. Cell Physiol. 295 (6), C1647–C1657.

Huang, Y., Wang, K.K., 2001. The calpain family and human disease. Trends Mol. Med. 7 (8), 355–362.

Huang, L., Yu, W., Li, X., Xu, Y., Niu, L., He, X., Dong, J., Yan, Z., 2009a. Expression of Robo4 in the fibrovascular membranes from patients with proliferative diabetic retinopathy and its role in RF/6A and RPE cells. Mol. Vis. 15, 1057–1069.

Huang, L., Xu, Y., Yu, W., Li, X., Liqun, C., He, X., Peiying, H., 2009b. Robo1: a potential role in ocular angiogenesis. Curr. Eye Res. 34 (12), 1019–1029.

Huang, L., Xu, Y., Yu, W., Li, Y., Chu, L., Dong, J., Li, X., 2010. Effect of Robo1 on retinal pigment epithelial cells and experimental proliferative vitreoretinopathy. Invest. Ophthalmol. Vis. Sci. 51 (6), 3193–3204.

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:40 Page Number: 105

B978-0-12-800179-0.00001-5, 00001

Huebschmann, A.G., Regensteiner, J.G., Vlassara, H., Reusch, J.E., 2006. Diabetes and advanced glycoxidation end products. Diabetes Care 29 (6), 1420–1432.

Ibrahim, A.S., El-Remessy, A.B., Matragoon, S., Zhang, W., Patel, Y., Khan, S., Al-Gayyar, M.M., El-Shishtawy, M.M., Liou, G.I., 2011. Retinal microglial activation and inflammation induced by amadori-glycated albumin in a rat model of diabetes. Diabetes 60 (4), 1122–1133.

Ilieva, I., Ohgami, K., Shiratori, K., Koyama, Y., Yoshida, K., Kase, S., Kitamei, H., Takemoto, Y., Yazawa, K., Ohno, S., 2004. The effects of Ginkgo biloba extract on lipopolysaccharide-induced inflammation *in vitro* and *in vivo*. Exp. Eye Res. 79 (2), 181–187.

Inagaki, Y., Yamagishi, S., Okamoto, T., Takeuchi, M., Amano, S., 2003. Pigment epithelium-derived factor prevents advanced glycation end products-induced monocyte chemoattractant protein-1 production in microvascular endothelial cells by suppressing intracellular reactive oxygen species generation. Diabetologia 46 (2), 284–287.

Inbaraj, B.S., Lu, H., Hung, C.F., Wu, W.B., Lin, C.L., Chen, B.H., 2008. Determination of carotenoids and their esters in fruits of Lycium barbarum Linnaeus by HPLC-DAD-APCI-MS. J. Pharm. Biomed. Anal. 47 (4–5), 812–818.

Inokuchi, N., Ikeda, T., Imamura, Y., Sotozono, C., Kinoshita, S., Uchihori, Y., Nakamura, K., 2001. Vitreous levels of insulin-like growth factor-I in patients with proliferative diabetic retinopathy. Curr. Eye Res. 23 (5), 368–371.

Ino-ue, M., Yokogawa, H., Yamamoto, M., Naka, H., Kuriyama, H., 1998. Structural impairments in optic nerve of diabetic rats ameliorated with the aldose reductase inhibitor. Exp. Eye Res. 66 (4), 397–401.

Ishikawa, A., Ishiguro, S., Tamai, M., 1996. Accumulation of gamma-aminobutyric acid in diabetic rat retinal Müller cells evidenced by electron microscopic immunocytochemistry. Curr. Eye Res. 15 (9), 958–964.

Islam, M.S., 2013. Animal models of diabetic neuropathy: progress since 1960s. J. Diabetes Res. 2013, 149452.

Izuta, H., Matsunaga, N., Shimazawa, M., Sugiyama, T., Ikeda, T., Hara, H., 2010. Proliferative diabetic retinopathy and relations among antioxidant activity, oxidative stress, and VEGF in the vitreous body. Mol. Vis. 16, 130–136.

Jain, S.K., Bull, R., Rains, J.L., Bass, P.F., Levine, S.N., Reddy, S., McVie, R., Bocchini, J.-A., 2010. Low levels of hydrogen sulfide in the blood of diabetes patients and streptozotocin-treated rats causes vascular inflammation? Antioxid. Redox Signal. 12, 1333–1337.

Jansson, L., Naeser, P., 1987. An increased number of mast cells in the sclerae of alloxan-diabetic mice. Acta Ophthalmol. (Copenh) 65, 203–205.

Jo, D.H., Cho, C.S., Kim, J.H., Jun, H.O., Kim, J.H., 2013. Animal models of diabetic retinopathy: doors to investigate pathogenesis and potential therapeutics. J. Biomed. Sci. 20, 38.

Johnson, L.E., Larsen, M., Perez, M.T., 2013. Retinal adaptation to changing glycemic levels in a rat model of type 2 diabetes. PLoS One 8, e55456.

Joussen, A.M., Poulaki, V., Le, M.I., Koizumi, K., Esser, C., Janicki, H., Schraer Meyer, U., Kociok, N., Fauser, S., Kichhof, B., Kern, T.S., Adamis, A.P., 2004. A central role for inflammation in the pathogenesis of diabetic retinopathy. FASEB J. 18, 1450–1452.

Joussen, A.M., Doehmen, S., Le, M.L., Koizumi, K., Radetzky, S., Krohne, T.U., Poulaki, V., Semkova, I., Kociok, N., 2009. TNF-alpha mediated apoptosis plays an important role in the development of early diabetic retinopathy and long-term histopathological alterations. Mol. Vis. 15, 1418–1428.

Jung, K.I., Kim, J.H., Park, H.Y., Park, C.K., 2013. Neuroprotective effects of cilostazol on retinal ganglion cell damage in diabetic rats. J. Pharmacol. Exp. Ther. 345 (3), 457–463.

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:40 Page Number: 106

B978-0-12-800179-0.00001-5, 00001

Kakehashi, A., Saito, Y., Mori, K., Sugi, N., Ono, R., Yamagami, H., Shinohara, M., Tamemoto, H., Ishikawa, S.E., Kawakami, M., Kanazawa, Y., 2006. Characteristics of diabetic retinopathy in SDT rats. Diabetes Metab. Res. Rev. 22 (6), 455–461.

Kamijo, M., Cherian, P.V., Sima, A.A., 1993. The preventive effect of aldose reductase inhibition on diabetic optic neuropathy in the BB/W-rat. Diabetologia 36 (10), 893–898.

Kanter, M., Uysal, H., Karaca, T., Sagmanligil, H.O., 2006. Depression of glucose levels and partial restoration of pancreatic beta-cell damage by melatonin in streptozotocin-induced diabetic rats. Arch. Toxicol. 80 (6), 362–369.

Kapin, M.A., Yanni, J.M., Brady, M.T., McDonough, T.J., Flanagan, J.G., Rawji, M.H., Dahlin, D.C., Sanders, M.E., Gamache, D.A., 2003. Inflammation-mediated retinal edema in the rabbit is inhibited by topical nepafenac. Inflammation 27 (5), 281–291.

Kaur, H., Donaghue, K.C., Chan, A.K., Benitez-Aguirre, P., Hing, S., Lloyd, M., Cusumano, J., Pryke, A., Craig, M.E., 2011. Vitamin D deficiency is associated with retinopathy in children and adolescents with type 1 diabetes. Diabetes Care 34 (6), 1400–1402.

Ke, J.B., Zhong, Y.M., 2007. Expression of somatostatin receptor subtype 5 in rat retinal amacrine cells. Neuroscience 144, 1025–1032.

Ke, M., Hu, X.Q., Ouyang, J., Dai, B., Xu, Y., 2012. The effect of astragalin on the VEGF production of cultured Müller cells under high glucose conditions. Biomed. Mater. Eng. 22 (1–3), 113–119.

Kern, T.S., 2007. Contributions of inflammatory processes to the development of the early stages of diabetic retinopathy. Exp. Diabetes Res. 2007, 95–103.

Kern, T.S., Barber, A.J., 2008. Retinal ganglion cells in diabetes. J. Physiol. 586 (Pt. 18), 4401–4408.

Kern, T.S., Engerman, R.L., 1994. Comparison of retinal lesions in alloxan-diabetic rats and galactose-fed rats. Curr. Eye Res. 13 (12), 863–867.

Kern, T.S., Engerman, R.L., 2001. Pharmacological inhibition of diabetic retinopathy: aminoguanidine and aspirin. Diabetes 50 (7), 1636–1642.

Kern, T.S., Tang, J., Mizutani, M., Kowluru, R.A., Nagaraj, R.H., Romeo, G., Podesta, F., Lorenzi, M., 2000. Response of capillary cell death to aminoguanidine predicts the development of retinopathy: comparison of diabetes and galactosemia. Invest. Ophthalmol. Vis. Sci. 41 (12), 3972–3978.

Kern, T.S., Miller, C.M., Du, Y., Zheng, L., Mohr, S., Ball, S.L., Kim, M., Jamison, J.A., Bingaman, D.P., 2007. Topical administration of nepafenac inhibits diabetes-induced retinal microvascular disease and underlying abnormalities of retinal metabolism and physiology. Diabetes 56 (2), 373–379.

Kim, S.H., Lee, S.M., 2008. Cytoprotective effects of melatonin against necrosis and apoptosis induced by ischemia/reperfusion injury in rat liver. J. Pineal Res. 44 (2), 165–171.

Kim, S.H., Park, S.H., 2003. Effects of Panax ginseng extract on lipid metabolism in humans. Pharmacol. Res. 48, 511–513.

Kim, Y.H., Kim, Y.S., Noh, H.S., Kang, S.S., Cheon, E.W., Park, S.K., Lee, B.J., Choi, W.-S., Cho, G.J., 2005. Changes in rhodopsin kinase and transducin in the rat retina in early-stage diabetes. Exp. Eye Res. 80 (6), 753–760.

Kim, J.H., Kim, J.H., Lee, Y.M., Ahn, E.M., Kim, K.W., Yu, Y.S., 2009a. Decursin inhibits retinal neovascularization via suppression of VEGFR-2 activation. Mol. Vis. 15, 1868–1875.

Kim, J.H., Kim, J.H., Yu, Y.S., Cho, C.S., Kim, K.W., 2009b. Blockade of angiotensin II attenuates VEGF-mediated blood-retinal barrier breakdown in diabetic retinopathy. J. Cereb. Blood Flow Metab. 29 (3), 621–628.

Kim, C., Yu, H.G., Sohn, J., 2010. The anti-angiogenic effect of chlorogenic acid on choroidal neovascularization. Korean J. Ophthalmol. 24 (3), 163–168.

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:40 Page Number: 107

B978-0-12-800179-0.00001-5, 00001

Kim, Y.H., Kim, Y.S., Roh, G.S., Choi, W.S., Cho, G.J., 2012. Resveratrol blocks diabetes-induced early vascular lesions and vascular endothelial growth factor induction in mouse retinas. Acta Ophthalmol. 90 (1), e31–e37.

Kirkegaard, C., Nørgaard, K., Snorgaard, O., Bek, T., Larsen, M., Lund-Andersen, H., 1990. Effect of one year continuous subcutaneous infusion of a somatostatin analogue, octreotide, on early retinopathy, metabolic control and thyroid function in Type I (insulin-dependent) diabetes mellitus. Acta Endocrinol. (Copenh) 122 (6), 766–772.

Kishi, S., Numaga, T., Yamazaki, S., 1982. Structure of the inner retinal surface in simple diabetic retinopathy. Jpn. J. Ophthalmol. 26 (2), 141–149.

Klepac, N., Rudes, Z., Klepac, R., 2006. Effects of melatonin on plasma oxidative stress in rats with streptozotocin induced diabetes. Biomed. Pharmacother. 60 (1), 32–35.

Kohzaki, K., Vingrys, A.J., Armitage, J.A., Bui, B.V., 2012. Electroretinography in streptozotocin diabetic rats following acute intraocular pressure elevation. Graefes Arch. Clin. Exp. Ophthalmol. 251 (2), 529–535.

Komeda, K., Noda, M., Terao, K., Kuzuya, N., Kanazawa, M., Kanazawa, Y., 1998. Establishment of two substrains, diabetes-prone and non-diabetic, from Long-Evans Tokushima Lean (LETL) rats. Endocr. J. 45 (6), 737–744.

Kondo, T., Kahn, C.R., 2004. Altered insulin signaling in retinal tissue in diabetic states. J. Biol. Chem. 279 (36), 37997–38006.

Kong, L.L., Wu, H., Cui, W.P., Zhou, W.H., Luo, P., Sun, J., Yuan, H., Miao, L.N., 2013. Advances in murine models of diabetic nephropathy. J. Diabetes Res. 2013, 797548.

Kowluru, R.A., 2013. Mitochondria damage in the pathogenesis of diabetic retinopathy and in the metabolic memory associated with its continued progression. Curr. Med. Chem. 20 (26), 3226–3233.

Kowluru, R.A., Abbas, S.N., 2003. Diabetes-induced mitochondrial dysfunction in the retina. Invest. Ophthalmol. Vis. Sci. 44 (12), 5327–5334.

Kowluru, R.A., Engerman, R.L., Case, G.L., Kern, T.S., 2001. Retinal glutamate in diabetes and effect of antioxidants. Neurochem. Int. 38 (5), 385–390.

Kowluru, R.A., Menon, B., Gierhart, D.L., 2008. Beneficial effect of zeaxanthin on retinal metabolic abnormalities in diabetic rats. Invest. Ophthalmol. Vis. Sci. 49 (4), 1645–1651.

Krady, J.K., Basu, A., Allen, C.M., Xu, Y., LaNoue, K.F., Gardner, T.W., Levison, S.W., 2005. Minocycline reduces proinflammatory cytokine expression, microglial activation, and caspase 3 activation in a rodent model of diabetic retinopathy. Diabetes 54 (5), 1559–1565.

Kumagai, A.K., 1999. Glucose transport in brain and retina: implications in the management and complications of diabetes. Diabetes Metab. Res. Rev. 15 (4), 261–273.

Kumar, B., Gupta, S.K., Nag, T.C., Srivastava, S., Saxena, R., 2012. Green tea prevents hyperglycemia-induced retinal oxidative stress and inflammation in streptozotocin-induced diabetic rats. Ophthalmic Res. 47 (2), 103–108.

Kummer, A., Pulford, B.E., Ishii, D.N., Seigel, G.M., 2003. Des(1-3)IGF-1 treatment normalizes type 1 IGF receptor and phospho-Akt (Thr 308) immunoreactivity in predegenerative retina of diabetic rats. Int. J. Exp. Diabesity Res. 4 (1), 45–57.

Kurihara, T., Ozawa, Y., Nagai, N., Shinoda, K., Noda, K., Imamura, Y., Tsubota, K., Okano, H., Oike, Y., Ishida, S., 2008. Angiotensin II type 1 receptor signaling contributes to synaptophysin degradation and neuronal dysfunction in the diabetic retina. Diabetes 57 (8), 2191–2198.

Kusari, J., Zhou, S., Padillo, E., Clarke, K.G., Gil, D.W., 2007. Effect of memantine on neuroretinal function and retinal vascular changes of streptozotocin-induced diabetic rats. Invest. Ophthalmol. Vis. Sci. 48 (11), 5152–5159.

Kusner, L.L., Sarthy, V.P., Mohr, S., 2004. Nuclear translocation of glyceraldehyde-3-phosphate dehydrogenase: a role in high glucose-induced apoptosis in retinal Müller cells. Invest. Ophthalmol. Vis. Sci. 45 (5), 1553–1561.

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:41 Page Number: 108

B978-0-12-800179-0.00001-5, 00001

Laabich, A., Cooper, N.G., 2000. Neuroprotective effect of AIP on N-methyl-D-aspartate-induced cell death in retinal neurons. Brain Res. Mol. Brain Res. 85 (1–2), 32–40.

Lameynardie, S., Chiavaroli, C., Travo, P., Garay, R.P., Parés-Herbuté, N., 2005. Inhibition of choroidal angiogenesis by calcium dobesilate in normal Wistar and diabetic GK rats. Eur. J. Pharmacol. 510 (1–2), 149–156.

Lanfumey, L., Mongeau, R., Hamon, M., 2013. Biological rhythms and melatonin in mood disorder treatments. Pharmacol. Ther. 138, 176–184.

Lazarovici, P., Cohen, G., Arien-Zakay, H., Chen, J., Zhang, C., Chopp, M., Jiang, H., 2012. Multimodal neuroprotection induced by PACAP38 in oxygen-glucose deprivation and middle cerebral artery occlusion stroke models. J. Mol. Neurosci. 48, 526–540.

Lecleire-Collet, A., Tessier, L.H., Massin, P., Forster, V., Brasseur, G., Sahel, J.A., Picaud, S., 2005. Advanced glycation end products can induce glial reaction and neuronal degeneration in retinal explants. Br. J. Ophthalmol. 89 (12), 1631–1633.

Levi-Montalcini, R., 1987. The nerve growth factor 35 years later. Science 237 (4819), 1154–1162, Review.

Li, Q., Puro, D.G., 2002. Diabetes-induced dysfunction of the glutamate transporter in retinal Müller cells. Invest. Ophthalmol. Vis. Sci. 43 (9), 3109–3116.

Li, Q., Zemel, E., Miller, B., Perlman, I., 2002. Early retinal damage in experimental diabetes: electroretinographical and morphological observations. Exp. Eye Res. 74 (5), 615–625.

Li, M., David, C., Kikuta, T., Somogyvari-Vigh, A., Arimura, A., 2005. Signaling cascades involved in neuroprotection by subpicomolar pituitary adenylate cyclase-activating polypeptide 38. J. Mol. Neurosci. 27, 91–105.

Li, M., Maderdrut, J.L., Lertora, J.J., Arimura, A., Batuman, V., 2008a. Renoprotection by pituitary adenylate cyclase-activating polypeptide in multiple myeloma and other kidney diseases. Regul. Pept. 145, 24–32.

Li, M., Ma, Y.B., Gao, H.Q., Li, B.Y., Cheng, M., Xu, L., Li, X.L., Li, X.H., 2008b. A novel approach of proteomics to study the mechanism of action of grape seed proanthocyanidin extracts on diabetic retinopathy in rats. Chin. Med. J. (Engl.) 121 (24), 2544–2552.

Li, J., Wang, J.J., Chen, D., Mott, R., Yu, Q., Ma, J.X., Zhang, S.X., 2009. Systemic administration of HMG-CoA reductase inhibitor protects the blood-retinal barrier and ameliorates retinal inflammation in type 2 diabetes. Exp. Eye Res. 89 (1), 71–78.

Li, J., Wang, J.J., Yu, Q., Chen, K., Mahadev, K., Zhang, S.X., 2010. Inhibition of reactive oxygen species by Lovastatin downregulates vascular endothelial growth factor expression and ameliorates blood-retinal barrier breakdown in db/db mice: role of NADPH oxidase 4. Diabetes 59 (6), 1528–1538.

Li, P., Xu, X., Zheng, Z., Zhu, B., Shi, Y., Liu, K., 2011. Protective effects of rosiglitazone on retinal neuronal damage in diabetic rats. Curr. Eye Res. 36 (7), 673–679.

Li, X., Zhang, M., Tang, W., 2013a. Effects of melatonin on streptozotocin-induced retina neuronal apoptosis in high blood glucose rat. Neurochem. Res. 38 (3), 669–676.

Li, D., Yang, F., Cheng, H., Liu, C., Sun, M., Wu, K., Ai, M., 2013b. Protective effects of total flavonoids from Flos Puerariae on retinal neuronal damage in diabetic mice. Mol. Vis. 19, 1999–2010.

Lieth, E., Barber, A.J., Xu, B., Dice, C., Ratz, M.J., Tanase, D., Strother, J.M., 1998. Glial reactivity and impaired glutamate metabolism in short-term experimental diabetic retinopathy. Diabetes 47 (5), 815–820.

Lieth, E., LaNoue, K.F., Antonetti, D.A., Ratz, M., 2000. Diabetes reduces glutamate oxidation and glutamine synthesis in the retina. The Penn State Retina Research Group. Exp. Eye Res. 70 (6), 723–730.

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:41 Page Number: 109

B978-0-12-800179-0.00001-5, 00001

Like, A.A., Butler, L., Williams, R.M., Appel, M.C., Weringer, E.J., Rossini, A.A., 1982. Spontaneous autoimmune diabetes mellitus in the BB rat. Diabetes 31 (Suppl. 1 Pt. 2), 7–13.

Lindstrom, P., 2007. The physiology of obese-hyperglycemic mice [ob/ob mice]. Sci. World J. 7, 666–685.

Liu, H., Ren, J.G., Cooper, W.L., Hawkins, C.E., Cowan, M.R., Tong, P.Y., 2004. Identification of the antivasopermeability effect of pigment epithelium-derived factor and its active site. Proc. Natl. Acad. Sci. U.S.A. 101 (17), 6605–6610.

Liu, X., Mameza, M.G., Lee, Y.S., Eseonu, C.I., Yu, C.R., Kang Derwent, J.J., Egwuagu, C.E., 2008. Suppressors of cytokine-signaling proteins induce insulin resistance in the retina and promote survival of retinal cells. Diabetes 57, 1651–1658.

Logvinov, S.V., Plotnikov, M.B., Zhdankina, A.A., Smolyakova, V.I., Ivanov, I.S., Kuchin, A.V., Chukicheva, I.V., Varakuta, E.Y., 2010. Morphological changes in retinal neurons in streptozotocin-induced diabetes mellitus and their correction with an isobornylphenol derivative. Neurosci. Behav. Physiol. 40 (7), 779–782.

Lorenzi, M., Gerhardinger, C., 2001. Early cellular and molecular changes induced by diabetes in the retina. Diabetologia 44 (7), 791–804, Review.

Losiewicz, M.K., Fort, P.E., 2011. Diabetes impairs the neuroprotective properties of retinal alpha-crystallins. Invest. Ophthalmol. Vis. Sci. 52 (9), 5034–5042.

Lu, Z.Y., Bhutto, I.A., Amemiya, T., 2003. Retinal changes in Otsuka long-evans Tokushima Fatty rats (spontaneously diabetic rat)—possibility of a new experimental model for diabetic retinopathy. Jpn. J. Ophthalmol. 47 (1), 28–35.

Lu, L.C., Zhou, W., Li, Z.H., Yu, C.P., Li, C.W., Luo, M.H., Xie, H., 2012. Effects of arctiin on streptozotocin-induced diabetic retinopathy in Sprague-Dawley rats. Planta Med. 78 (12), 1317–1323.

Lu, L., Seidel, C.P., Iwase, T., Stevens, R.K., Gong, Y.Y., Wang, X., Hackett, S.F., Campochiaro, P.A., 2013. Suppression of GLUT1; a new strategy to prevent diabetic complications. J. Cell. Physiol. 228 (2), 251–257.

Lundkvist, A., Reichenbach, A., Betsholtz, C., Carmeliet, P., Wolburg, H., Pekny, M., 2004. Under stress, the absence of intermediate filaments from Müller cells in the retina has structural and functional consequences. J. Cell Sci. 117, 3481–3488.

Luo, D., Fan, Y., Xu, X., 2012. The effects of aminoguanidine on retinopathy in STZ-induced diabetic rats. Bioorg. Med. Chem. Lett. 22 (13), 4386–4390.

Lupi, R., Mancarella, R., Del Guerra, S., Bugliani, M., Del Prato, S., Boggi, U., Mosca, F., Filipponi, F., Marchetti, P., 2008. Effects of exendin-4 on islets from type 2 diabetes patients. Diabetes Obes. Metab. 10, 515–519.

Ly, A., Yee, P., Vessey, K.A., Phipps, J.A., Jobling, A.I., Fletcher, E.L., 2011. Early inner retinal astrocyte dysfunction during diabetes and development of hypoxia, retinal stress, and neuronal functional loss. Invest. Ophthalmol. Vis. Sci. 52 (13), 9316–9326.

Ma, J., Zhu, T., Tang, X., Ye, P., Zhang, Z., 2010. Effect of an intravitreal injection of bevacizumab on the expression of VEGF and CD34 in the retina of diabetic rats. Clin. Exp. Ophthalmol. 38 (9), 875–884.

Maahs, D.M., West, N.A., Lawrence, J.M., Mayer-Davis, E.J., 2010. Epidemiology of type 1 diabetes. Endocrinol. Metab. Clin. North Am. 39 (3), 481–497.

Mantovani, A., Sica, A., Sozzani, S., Allavena, P., Vecchi, A., Locati, M., 2004. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol. 25, 677–686.

Marsicano, G., Moosmann, B., Hermann, H., Lutz, B., Behl, C., 2002. Neuroprotective properties of cannabinoids against oxidative stress: role of the cannabinoid receptor CB1. J. Neurochem. 80 (3), 448–456.

Marti, H.H., Bernaudin, M., Petit, E., Bauer, C., 2000. Neuroprotection and angiogenesis: dual role of erythropoietin in brain ischemia. News Physiol. Sci. 15, 225–229.

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:41 Page Number: 110

B978-0-12-800179-0.00001-5, 00001

Martin, P.M., Roon, P., Van Ells, T.K., Ganapathy, V., Smith, S.B., 2004. Death of retinal neurons in streptozotocin-induced diabetic mice. Invest. Ophthalmol. Vis. Sci. 45 (9), 3330–3336.

Masuyama, T., Fuse, M., Yokoi, N., Shinohara, M., Tsujii, H., Kanazawa, M., Kanazawa, Y., Komeda, K., Taniguchi, K., 2003. Genetic analysis for diabetes in a new rat model of nonobese type 2 diabetes. Spontaneously Diabetic Torii rat. Biochem. Biophys. Res. Commun. 304 (1), 196–206.

Masuyama, T., Komeda, K., Hara, A., Noda, M., Shinohara, M., Oikawa, T., Kanazawa, Y., Taniguchi, K., 2004. Chronological characterization of diabetes development in male Spontaneously Diabetic Torii rats. Biochem. Biophys. Res. Commun. 314 (3), 870–877.

Mathews, C.E., Langley, S.H., Leiter, E.H., 2002. New mouse model to study islet transplantation in insulin-dependent diabetes mellitus. Transplantation 73 (8), 1333–1336.

Matsubara, H., Kuze, M., Sasoh, M., Ma, N., Furuta, M., Uji, Y., 2006. Time-dependent course of electroretinograms in the spontaneous diabetic Goto-Kakizaki rat. Jpn. J. Ophthalmol. 50 (3), 211–216.

Matsuura, T., Yamagishi, S., Kodama, Y., Shibata, R., Ueda, S., Narama, I., 2005. Otsuka Long-Evans Tokushima fatty (OLETF) rat is not a suitable animal model for the study of angiopathic diabetic retinopathy. Int. J. Tissue React. 27 (2), 59–62.

McLenachan, S., Chen, X., McMenamin, P.G., Rakoczy, E.P., 2013. Absence of clinical correlates of diabetic retinopathy in the Ins2Akita retina. Clin. Experiment. Ophthalmol. 41 (6), 582–592.

McVicar, C.M., Hamilton, R., Colhoun, L.M., Gardiner, T.A., Brines, M., Cerami, A., Stitt, A.W., 2011. Intervention with an erythropoietin-derived peptide protects against neuroglial and vascular degeneration during diabetic retinopathy. Diabetes 60 (11), 2995–3005.

Meyer-Rusenberg, B., Pavlidis, M., Stupp, T., Thanos, S., 2007. Pathological changes in human retinal ganglion cells associated with diabetic and hypertensive retinopathy. Graefes Arch. Clin. Exp. Ophthalmol. 245 (7), 1009–1018.

Midena, E., Segato, T., Radin, S., di Giorgio, G., Meneghini, F., Piermarocchi, S., Belloni, A.S., 1989. Studies on the retina of the diabetic db/db mouse. I. Endothelial cell-pericyte ratio. Ophthalmic Res. 21 (2), 106–111.

Mima, A., Qi, W., Hiraoka-Yamomoto, J., Park, K., Matsumoto, M., Kitada, M., Li, Q., Mizutani, K., Yu, E., Shimada, T., Lee, J., Shoelson, S.E., Jobin, C., Rask-Madsen, C., King, G.L., 2012. Retinal not systemic oxidative and inflammatory stress correlated with VEGF expression in rodent models of insulin resistance and diabetes. Invest. Ophthalmol. Vis. Sci. 53 (13), 8424–8432.

Mitsuhashi, J., Morikawa, S., Shimizu, K., Ezaki, T., Yasuda, Y., Hori, S., 2013. Intravitreal injection of erythropoietin protects against retinal vascular regression at the early stage of diabetic retinopathy in streptozotocin-induced diabetic rats. Exp. Eye Res. 106, 64–73.

Miyajima, H., Osanai, M., Chiba, H., Nishikiori, N., Kojima, T., Ohtsuka, K., Sawada, N., 2005. Glyceraldehyde-derived advanced glycation end-products preferentially induce VEGF expression and reduce GDNF expression in human astrocytes. Biochem. Biophys. Res. Commun. 330 (2), 361–366.

Miyamoto, K., Ogura, Y., Nishiwaki, H., Matsuda, N., Honda, Y., Kato, S., Ishida, H., Seino, Y., 1996. Evaluation of retinal microcirculatory alterations in the Goto-Kakizaki rat. A spontaneous model of non-insulin-dependent diabetes. Invest. Ophthalmol. Vis. Sci. 37 (5), 898–905.

Mizuno, A., Noma, Y., Kuwajima, M., Murakami, T., Zhu, M., Shima, K., 1999. Changes in islet capillary angioarchitecture coincide with impaired B-cell function but not with insulin resistance in male Otsuka-Long-Evans-Tokushima fatty rats: dimorphism of the diabetic phenotype at an advanced age. Metabolism 48 (4), 477–483.

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:42 Page Number: 111

B978-0-12-800179-0.00001-5, 00001

Mizutani, M., Kern, T.S., Lorenzi, M., 1996. Accelerated death of retinal microvascular cells in human and experimental diabetic retinopathy. J. Clin. Invest. 97 (12), 2883–2890.

Moreo, G., Mariani, E., Pizzamiglio, G., Colucci, G.B., 1995. Visual evoked potentials in NIDDM: a longitudinal study. Diabetologia 38 (5), 573–576.

Muir, E.R., Rentería, R.C., Duong, T.Q., 2012. Reduced ocular blood flow as an early indicator of diabetic retinopathy in a mouse model of diabetes. Invest. Ophthalmol. Vis. Sci. 53 (10), 6488–6494.

Mysona, B., Dun, Y., Duplantier, J., Ganapathy, V., Smith, S.B., 2009. Effects of hyperglycemia and oxidative stress on the glutamate transporters GLAST and system xc- in mouse retinal Müller glial cells. Cell Tissue Res. 335 (3), 477–488.

Nagai, N., Izumi-Nagai, K., Oike, Y., Koto, T., Satofuka, S., Ozawa, Y., Yamashiro, K., Inoue, M., Tsubota, K., Umezawa, K., Ishida, S., 2007. Suppression of diabetes-induced retinal inflammation by blocking the angiotensin II type 1 receptor or its downstream nuclear factor-kappaB pathway. Invest. Ophthalmol. Vis. Sci. 48 (9), 4342–4350.

Nakamachi, T., Matkovits, A., Seki, T., Shioda, S., 2012. Distribution and protective function of pituitary adenylate cyclase-activating polypeptide in the retina. Front. Endocrinol. (Lausanne) 3, 145.

Nakamura, M., Barber, A.J., Antonetti, D.A., LaNoue, K.F., Robinson, K.A., Buse, M.G., Gardner, T.W., 2001. Excessive hexosamines block the neuroprotective effect of insulin and induce apoptosis in retinal neurons. J. Biol. Chem. 276 (47), 43748–43755.

Nishikiori, N., Osanai, M., Chiba, H., Kojima, T., Mitamura, Y., Ohguro, H., Sawada, N., 2007a. Glial cell-derived cytokines attenuate the breakdown of vascular integrity in diabetic retinopathy. Diabetes 56 (5), 1333–1340.

Nishikiori, N., Osanai, M., Chiba, H., Kojima, T., Ohguro, H., Sawada, N., 2007b. Inhibitory effects of retinoic acid receptor alpha stimulants on murine cataractogenesis through suppression of deregulated calpains. Invest. Ophthalmol. Vis. Sci. 48 (5), 2224–2229.

Nishikiori, N., Osanai, M., Chiba, H., Kojima, T., Inatomi, S., Ohguro, H., Sawada, N., 2008. Experimental effect of retinoic acids on apoptosis during the development of diabetic retinopathy. Clin. Ophthalmol. 2 (1), 233–235.

Obrosova, I.G., Kador, P.F., 2011. Aldose reductase/polyol inhibitors for diabetic retinopathy. Curr. Pharm. Biotechnol. 12 (3), 373–385, Review.

Okada, M., Ayabe, Y., 1995. Effects of aminoguanidine and pyridoxal phosphate on glycation reaction of aspartate aminotransferase and serum albumin. J. Nutr. Sci. Vitaminol. (Tokyo) 41 (1), 43–50.

Okuno, T., Oku, H., Sugiyama, T., Ikeda, T., 2008. Electroretinographic study of spontaneously diabetic Torii rats. Doc. Ophthalmol. 117 (3), 191–196.

Ola, M.S., Moore, P., Maddox, D., El-Sherbeny, A., Huang, W., Roon, P., Agarwal, N., Ganapathy, V., Smith, S.B., 2002. Analysis of sigma receptor (sigmaR1) expression in retinal ganglion cells cultured under hyperglycemic conditions and in diabetic mice. Brain Res. Mol. Brain Res. 107 (2), 97–107.

Ola, M.S., Nawaz, M.I., Siddiquei, M.M., Al-Amro, S., Abu El-Asrar, A.M., 2012. Recent advances in understanding the biochemical and molecular mechanism of diabetic retinopathy. J. Diabetes Complications 26 (1), 56–64, Review.

Olias, G., Viollet, C., Kusserow, H., Epelbaum, J., Meyerhof, W., 2004. Regulation and function of somatostatin receptors. J. Neurochem. 89 (5), 1057–1091.

Omri, S., Omri, B., Savoldelli, M., Jonet, L., Thillaye-Goldenberg, B., Thuret, G., Gain, P., Jeanny, J.C., Crisanti, P., Behar-Cohen, F., 2010. The outer limiting membrane (OLM) revisited: clinical implications. Clin. Ophthalmol. 4, 183–195.

Omri, S., Behar-Cohen, F., de Kozak, Y., Sennlaub, F., Verissimo, L.M., Jonet, L., Savoldelli, M., Omri, B., Crisanti, P., 2011. Microglia/macrophages migrate through retinal epithelium barrier by a transcellular route in diabetic retinopathy: role of PKCζ in the Goto Kakizaki rat model. Am. J. Pathol. 179 (2), 942–953.

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:49 Page Number: 112

B978-0-12-800179-0.00001-5, 00001

Osborne, N.N., Casson, R.J., Wood, J.P., Chidlow, G., Graham, M., Melena, J., 2004. Retinal ischemia: mechanisms of damage and potential therapeutic strategies. Prog. Retin. Eye Res. 23, 91–147.

Oshitari, T., Roy, S., 2005. Diabetes: a potential enhancer of retinal injury in rat retinas. Neurosci. Lett. 390 (1), 25–30.

Pannicke, T., Iandiev, I., Wurm, A., Uckermann, O., vom Hagen, F., Reichenbach, A., Wiedemann, P., Hammes, H.P., Bringmann, A., 2006. Diabetes alters osmotic swelling characteristics and membrane conductance of glial cells in rat retina. Diabetes 55 (3), 633–639.

Park, B.H., Rho, H.W., Park, J.W., Cho, C.G., Kim, J.S., Chung, H.T., Kim, H.R., 1995. Protective mechanism of glucose against alloxan-induced pancreatic beta-cell damage. Biochem. Biophys. Res. Commun. 210 (1), 1–6.

Patel, J.I., Tombran-Tink, J., Hykin, P.G., Gregor, Z.J., Cree, I.A., 2006. Vitreous and aqueous concentrations of proangiogenic, antiangiogenic factors and other cytokines in diabetic retinopathy patients with macular edema: implications for structural differences in macular profiles. Exp. Eye Res. 82 (5), 798–806.

Pfister, F., Riedl, E., Wang, Q., vom Hagen, F., Deinzer, M., Harmsen, M.C., Molema, G., Yard, B., Feng, Y., Hammes, H.P., 2011. Oral carnosine supplementation prevents vascular damage in experimental diabetic retinopathy. Cell. Physiol. Biochem. 28 (1), 125–136.

Phipps, J.A., Wilkinson-Berka, J.L., Fletcher, E.L., 2007. Retinal dysfunction in diabetic ren-2 rats is ameliorated by treatment with valsartan but not atenolol. Invest. Ophthalmol. Vis. Sci. 48 (2), 927–934.

Phipps, J.A., Clermont, A.C., Sinha, S., Chilcote, T.J., Bursell, S.E., Feener, E.P., 2009. Plasma kallikrein mediates angiotensin II type 1 receptor-stimulated retinal vascular permeability. Hypertension 53 (2), 175–181.

Pinter, E., Helyes, Z., Szolcsanyi, J., 2006. Inhibitory effect of somatostatin on inflammation and nociception. Pharmacol. Ther. 112, 440–456.

Portha, B., 2005. Programmed disorders of beta-cell development and function as one cause for type 2 diabetes? The GK rat paradigm. Diabetes Metab. Res. Rev. 21 (6), 495–504.

Portha, B., Giroix, M.H., Tourrel-Cuzin, C., Le-Stunff, H., Movassat, J., 2012. The GK rat: a prototype for the study of non-overweight type 2 diabetes. Methods Mol. Biol. 933, 125–159.

Puro, D.G., 2002. Diabetes-induced dysfunction of retinal Müller cells. Trans. Am. Ophthalmol. Soc. 100, 339–352.

Qazi, Y., Maddula, S., Ambati, B.K., 2009. Mediators of ocular angiogenesis. J. Genet. 88, 495–515, Review.

Qin, Y., Ren, H., Hoffman, M.R., Fan, J., Zhang, M., Xu, G., 2012. Aquaporin changes during diabetic retinopathy in rats are accelerated by systemic hypertension and are linked to the renin-angiotensin system. Invest. Ophthalmol. Vis. Sci. 53, 3047–3053.

Racz, B., Gallyas Jr., F., Kiss, P., Toth, G., Hegyi, O., Gasz, B., Borsiczky, B., Ferencz, A., Roth, E., Tamas, A., Lengvari, I., Lubics, A., Reglodi, D., 2006. The neuroprotective effects of PACAP in monosodium glutamate-induced retinal lesion involve inhibition of proapoptotic signaling pathways. Regul. Pept. 137, 20–26.

Rathi, S.S., Grover, J.K., Vikrant, V., Biswas, N.R., 2002. Prevention of experimental diabetic cataract by Indian Ayurvedic plant extracts. Phytother. Res. 16, 774–777.

Reglodi, D., Kiss, P., Szabadfi, K., Atlasz, T., Gabriel, R., Horvath, G., Szakaly, P., Sandor, B., Lubics, A., Laszlo, E., Farkas, J., Matkovits, A., Brubel, R., Hashimoto, H., Ferencz, A., Vincze, A., Helyes, Z., Welke, L., Lakatos, A., Tamas, A., 2012. PACAP is an endogenous neuroprotective factor—insights from PACAP deficient mice. J. Mol. Neurosci. 48, 482–492.

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:49 Page Number: 113

B978-0-12-800179-0.00001-5, 00001

Reiter, R.J., Tan, D.X., Burkhardt, S., 2002. Reactive oxygen and nitrogen species and cellular and organismal decline: amelioration with melatonin. Mech. Ageing Dev. 123 (8), 1007–1019.

Reiter, C.E., Sandirasegarane, L., Wolpert, E.B., Klinger, M., Simpson, I.A., Barber, A.J., Antonetti, D.A., Kester, M., Gardner, T.W., 2003. Characterization of insulin signaling in rat retina *in vivo* and *ex vivo*. Am. J. Physiol. Endocrinol. Metab. 285, E763–E774.

Reiter, C.E., Wu, X., Sandirasegarane, L., Nakamura, M., Gilbert, K.A., Singh, R.S., Fort, P.E., Antonetti, D.A., Gardner, T.W., 2006. Diabetes reduces basal retinal insulin receptor signaling: reversal with systemic and local insulin. Diabetes 55 (4), 1148–1156.

Ren, Z., Li, W., Zhao, Q., Ma, L., Zhu, J., 2012. The impact of 1,25-dihydroxy vitamin D3 on the expressions of vascular endothelial growth factor and transforming growth factor-β(1) in the retinas of rats with diabetes. Diabetes Res. Clin. Pract. 98 (3), 474–480.

Robinson, R., Barathi, V.A., Chaurasia, S.S., Wong, T.Y., Kern, T.S., 2012. Update on animal models of diabetic retinopathy: from molecular approaches to mice and higher mammals. Dis. Model Mech. 5 (4), 444–456.

Rossini, A.A., Appel, M.C., Williams, R.M., Like, A.A., 1977. Genetic influence of the streptozotocin-induced insulitis and hyperglycemia. Diabetes 26, 916–920.

Sakata, N., Imanaga, Y., Meng, J., Tachikawa, Y., Takebayashi, S., Nagai, R., Horiuchi, S., 1999. Increased advanced glycation end products in atherosclerotic lesions of patients with end-stage renal disease. Atherosclerosis 142 (1), 67–77.

Sakurai, K., Ogiso, T., 1995. Effect of ferritin on lambda DNA strand breaks in the reaction system of alloxan plus NADPH-cytochrome P450 reductase: ferritin's role in diabetogenic action of alloxan. Biol. Pharm. Bull. 18 (2), 262–266.

Salido, E.M., Bordone, M., De Laurentiis, A., Chianelli, M., Keller Sarmiento, M.I., Dorfman, D., Rosenstein, R.E., 2013. Therapeutic efficacy of melatonin in reducing retinal damage in an experimental model of early type 2 diabetes in rats. J. Pineal Res. 54 (2), 179–189.

Samuels, I.S., Lee, C.A., Petrash, J.M., Peachey, N.S., Kern, T.S., 2012. Exclusion of aldose reductase as a mediator of ERG deficits in a mouse model of diabetic eye disease. Vis. Neurosci. 29 (6), 267–274.

Sandbach, J.M., Coscun, P.E., Grossniklaus, H.E., Kokoszka, J.E., Newman, N.J., Wallace, D.C., 2001. Ocular pathology in mitochondrial superoxide dismutase (Sod2)-deficient mice. Invest. Ophthalmol. Vis. Sci. 42 (10), 2173–2178.

Sandler, S., Swenne, I., 1983. Streptozotocin, but not alloxan, induces DNA repair synthesis in mouse pancreatic islets *in vitro*. Diabetologia 25 (5), 444–447.

Sarthy, V.P., Harris, R., 2001. The Retinal Müller Cell: Structure and Function (Perspectives in Vision Research). Kluwer Academic/Plenum Publishers, New York.

Sasaki, M., Ozawa, Y., Kurihara, T., Kubota, S., Yuki, K., Noda, K., Kobayashi, S., Ishida, S., Tsubota, K., 2010. Neurodegenerative influence of oxidative stress in the retina of a murine model of diabetes. Diabetologia 53 (5), 971–979.

Sasase, T., Morinaga, H., Abe, T., Miyajima, K., Ohta, T., Shinohara, M., Matsushita, M., Kakehashi, A., 2009. Protein kinase C beta inhibitor prevents diabetic peripheral neuropathy, but not histopathological abnormalities of retina in Spontaneously Diabetic Torii rat. Diabetes Obes. Metab. 11 (11), 1084–1087.

Satofuka, S., Ichihara, A., Nagai, N., Noda, K., Ozawa, Y., Fukamizu, A., Tsubota, K., Itoh, H., Oike, Y., Ishida, S., 2009. (Pro)renin receptor-mediated signal transduction and tissue renin-angiotensin system contribute to diabetes-induced retinal inflammation. Diabetes 58 (7), 1625–1633.

Scuderi, S., D'Amico, A.G., Castorina, A., Imbesi, R., Carnazza, M.L., D'Agata, V., 2013. Ameliorative effect of PACAP and VIP against increased permeability in a model of outer blood retinal barrier dysfunction. Peptides 39, 119–124.

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:50 Page Number: 114

B978-0-12-800179-0.00001-5, 00001

Seaborn, T., Masmoudi-Kouli, O., Fournier, A., Vaudry, H., Vaudry, D., 2011. Protective effects of pituitary adenylate cyclase-activating polypeptide (PACAP) against apoptosis. Curr. Pharm. Des. 17, 204–214.

Segawa, Y., Shirao, Y., Yamagishi, S., Higashide, T., Kobayashi, M., Katsuno, K., Iyobe, A., Harada, H., Sato, F., Miyata, H., Asai, H., Nishimura, A., Takahira, M., Souno, T., Segawa, Y., Maeda, K., Shima, K., Mizuno, A., Yamamoto, H., Kawasaki, K., 1998. Upregulation of retinal vascular endothelial growth factor mRNAs in spontaneously diabetic rats without ophthalmoscopic retinopathy. A possible participation of advanced glycation end products in the development of the early phase of diabetic retinopathy. Ophthalmic Res. 30 (6), 333–339.

Seigel, G.M., Lupien, S.B., Campbell, L.M., Ishii, D.N., 2006. Systemic IGF-1 treatment inhibits cell death in diabetic rat retina. J. Diabetes Complications 20 (3), 196–204.

Seki, T., Izumi, S., Shioda, S., Zhou, C.J., Arimura, A., Koide, R., 2000. Gene expression for PACAP receptor mRNA in the rat retina by in situ hybridization and in situ RT-PCR. Ann. N.Y. Acad. Sci. 921, 366–369.

Seki, M., Nawa, H., Fukuchi, T., Abe, H., Takei, N., 2003. BDNF is upregulated by postnatal development and visual experience: quantitative and immunohistochemical analyses of BDNF in the rat retina. Invest. Ophthalmol. Vis. Sci. 44 (7), 3211–3218.

Seki, M., Tanaka, T., Nawa, H., Usui, T., Fukuchi, T., Ikeda, K., Abe, H., Takei, N., 2004. Involvement of brain-derived neurotrophic factor in early retinal neuropathy of streptozotocin-induced diabetes in rats: therapeutic potential of brain-derived neurotrophic factor for dopaminergic amacrine cells. Diabetes 53 (9), 2412–2419.

Semkova, I., Krieglstein, J., 1999. Neuroprotection mediated via neurotrophic factors and induction of neurotrophic factors. Brain Res. Rev. 30, 176–188.

Sen, S., Chen, S., Wu, Y., Feng, B., Lui, E.K., Chakrabarti, S., 2013. Preventive effects of North American ginseng (*Panax quinquefolius*) on diabetic retinopathy and cardiomyopathy. Phytother. Res. 27 (2), 290–298.

Shanab, A.Y., Nakazawa, T., Ryu, M., Tanaka, Y., Himori, N., Taguchi, K., Yasuda, M., Watanabe, R., Takano, J., Saido, T., Minegishi, N., Miyata, T., Abe, T., Yamamoto, M., 2012. Metabolic stress response implicated in diabetic retinopathy: the role of calpain, and the therapeutic impact of calpain inhibitor. Neurobiol. Dis. 48 (3), 556–567.

Shanmugam, N., Figarola, J.L., Li, Y., Swiderski, P.M., Rahbar, S., Natarajan, R., 2008. Proinflammatory effects of advanced lipoxidation end products in monocytes. Diabetes 57 (4), 879–888.

Shao, Z.H., Becker, L.B., Vanden Hoek, T.L., Schumacker, P.T., Li, C.Q., Zhao, D., Wojcik, K., Anderson, T., Qin, Y., Dey, L., Yuan, C.S., 2003. Grape seed proanthocyanidin extract attenuates oxidant injury in cardiomyocytes. Pharmacol. Res. 47 (6), 463–469.

Shelton, M.D., Kern, T.S., Mieyal, J.J., 2007. Glutaredoxin regulates nuclear factor kappa-B and intercellular adhesion molecule in Müller cells: model of diabetic retinopathy. J. Biol. Chem. 282 (17), 12467–12474.

Shelton, M.D., Distler, A.M., Kern, T.S., Mieyal, J.J., 2009. Glutaredoxin regulates autocrine and paracrine proinflammatory responses in retinal glial (Müller) cells. J. Biol. Chem. 284 (8), 4760–4766.

Shen, X., Xie, B., Cheng, Y., Jiao, Q., Zhong, Y., 2011. Effect of pigment epithelium derived factor on the expression of glutamine synthetase in early phase of experimental diabetic retinopathy. Ocul. Immunol. Inflamm. 19 (4), 246–254.

Shi, X., Liao, S., Mi, H., Guo, C., Qi, D., Li, F., Zhang, C., Yang, Z., 2012. Hesperidin prevents retinal and plasma abnormalities in streptozotocin-induced diabetic rats. Molecules 17 (11), 12868–12881.

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:50 Page Number: 115

B978-0-12-800179-0.00001-5, 00001

Shin, J.Y., Sohn, J., Park, K.H., 2013. Chlorogenic acid decreases retinal vascular hyperpermeability in diabetic rat model. J. Korean Med. Sci. 28 (4), 608–613.

Shinohara, M., Masuyama, T., Shoda, T., Takahashi, T., Katsuda, Y., Komeda, K., Kuroki, M., Kakehashi, A., Kanazawa, Y., 2000. A new spontaneously diabetic non-obese Torii rat strain with severe ocular complications. Int. J. Exp. Diabetes Res. 1 (2), 89–100.

Shirao, Y., Kawasaki, K., 1998. Electrical responses from diabetic retina. Prog. Retin. Eye Res. 17 (1), 59–76, Review.

Shirasaki, Y., Miyashita, H., Yamaguchi, M., Inoue, J., Nakamura, M., 2005. Exploration of orally available calpain inhibitors: peptidyl alpha-ketoamides containing an amphiphile at P3 site. Bioorg. Med. Chem. 13 (14), 4473–4484.

Si, Y.F., Wang, J., Guan, J., Zhou, L., Sheng, Y., Zhao, J., 2013. Treatment with hydrogen sulfide alleviates streptozotocin-induced diabetic retinopathy in rats. Br. J. Pharmacol. 169 (3), 619–631.

Silva, K.C., Rosales, M.A., Hamassaki, D.E., Saito, K.C., Faria, A.M., Ribeiro, P.A., Faria, J.B., Faria, J.M., 2013. Green tea is neuroprotective in diabetic retinopathy. Invest. Ophthalmol. Vis. Sci. 54 (2), 1325–1336.

Sima, J., Zhang, S.X., Shao, C., Fant, J., Ma, J.X., 2004. The effect of angiostatin on vascular leakage and VEGF expression in rat retina. FEBS Lett. 564 (1–2), 19–23.

Sima, J., Ma, J., Zhang, S.X., Guo, J., 2006. Study of the influence of angiostatin intravitreal injection on vascular leakage in retina and iris of the experimental diabetic rats. Yan Ke Xue Bao 22 (4), 252–258.

Simo, R., Villarroel, M., Corraliza, L., Hernandez, C., Garcia-Ramirez, M., 2010. The retinal pigment epithelium: something more than a constituent of the blood-retinal barrier-implications for the pathogenesis of diabetic retinopathy. J. Biomed. Biotechnol. 2010, 190724.

Smith, S.B., 2002. Diabetic retinopathy and the NMDA receptor. Drug News Perspect. 15 (4), 226–232.

Sone, H., Kawakami, Y., Okuda, Y., Sekine, Y., Honmura, S., Matsuo, K., Segawa, T., Suzuki, H., Yamashita, K., 1997. Ocular vascular endothelial growth factor levels in diabetic rats are elevated before observable retinal proliferative changes. Diabetologia 40 (6), 726–730.

Stanely, P., Prince, M., Menon, V.P., 2000. Hypoglycaemic and other related actions of Tinospora cordifolia roots in alloxan-induced diabetic rats. J. Ethnopharmacol. 70 (1), 9–15.

Stehle, J.H., Saade, A., Rawashdeh, O., Ackermann, K., Jilg, A., Sebesteny, T., Maronde, E., 2011. A survey of molecular details in the human pineal gland in the light of phylogeny, structure, function and chronobiological diseases. J. Pineal Res. 51 (1), 17–43.

Stitt, A.W., Bhaduri, T., McMullen, C.B., Gardiner, T.A., Archer, D.B., 2000. Advanced glycation end products induce blood-retinal barrier dysfunction in normoglycemic rats. Mol. Cell Biol. Res. Commun. 3 (6), 380–388.

Stitt, A.W., Frizzell, N., Thorpe, S.R., 2004. Advanced glycation and advanced lipoxidation: possible role in initiation and progression of diabetic retinopathy. Curr. Pharm. Des. 10 (27), 3349–3360, Review.

Su, W., Dai, D.Z., Liu, H.R., Na, T., Dai, Y., 2007. Upregulated endothelin system in diabetic vascular dysfunction and early retinopathy is reversed by CPU0213 and total triterpene acids from Fructus Corni. Clin. Exp. Pharmacol. Physiol. 34 (12), 1228–1233.

Sudnikovich, E.J., Maksimchik, Y.Z., Zabrodskaya, S.V., Kubyshin, V.L., Lapshina, E.A., Bryszewska, M., Reiter, R.J., Zavodnik, I.B., 2007. Melatonin attenuates metabolic disorders due to streptozotocin-induced diabetes in rats. Eur. J. Pharmacol. 569 (3), 180–187.

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:50 Page Number: 116

B978-0-12-800179-0.00001-5, 00001

Sugiyama, T., Okuno, T., Fukuhara, M., Oku, H., Ikeda, T., Obayashi, H., Ohta, M., Fukui, M., Hasegawa, G., Nakamura, N., 2007. Angiotensin II receptor blocker inhibits abnormal accumulation of advanced glycation end products and retinal damage in a rat model of type 2 diabetes. Exp. Eye Res. 85 (3), 406–412.

Sun, Q., Cai, J., Zhou, J., Tao, H., Zhang, J.H., Zhang, W., Sun, X.J., 2011. Hydrogen-rich saline reduces delayed neurologic sequelae in experimental carbon monoxide toxicity. Crit. Care Med. 39 (4), 765–769.

Szabadfi, K., Atlasz, T., Kiss, P., Reglodi, D., Szabo, A., Kovacs, K., Szalontai, B., Setalo Jr., G., Banki, E., Csanaky, K., Tamas, A., Gabriel, R., 2012. Protective effects of the neuropeptide PACAP in diabetic retinopathy. Cell Tissue Res. 348 (1), 37–46.

Szabadfi, K., Szabo, A., Kiss, P., Reglodi, D., Setalo Jr., G., Kovacs, K., Tamas, A., Toth, G., Gabriel, R., 2014. PACAP promotes neuron survival in early experimental diabetic retinopathy. Neurochem. Int. 64, 84–91. Au27

Szabo, A., Danyadi, B., Bognar, E., Szabadfi, K., Fabian, E., Kiss, P., Mester, L., Sridharan, M., Atlasz, T., Gabriel, R., Toth, G., Tamas, A., Reglodi, D., Kovacs, K., 2012. Effect of PACAP on MAP kinases, Akt and cytokine expressions in rat retinal hypoperfusion. Neurosci. Lett. 523, 93–98.

Szkudelski, T., 2001. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol. Res. 50 (6), 537–546.

Takeo-Goto, S., Doi, M., Ma, N., Goto, R., Semba, R., Uji, Y., 2002. Immunohistochemical localization of amino acids in the diabetic retina of Goto-Kakizaki rats. Ophthalmic Res. 34 (3), 139–145.

Takiishi, T., Gysemans, C., Bouillon, R., Mathieu, C., 2010. Vitamin D and diabetes. Endocrinol. Metab. Clin. North Am. 39 (2), 419–446.

Tang, J., Kern, T.S., 2011. Inflammation in diabetic neuropathy. Prog. Retinal Eye Res. 30, 343–358.

Tang, L., Zhang, Y., Jiang, Y., Willard, L., Ortiz, E., Wark, L., Medeiros, D., Lin, D., 2011. Dietary wolfberry ameliorates retinal structure abnormalities in db/db mice at the early stage of diabetes. Exp. Biol. Med. (Maywood) 236 (9), 1051–1063.

Tang, J., Du, Y., Lee, C.A., Talahalli, R., Eells, J.T., Kern, T.S., 2013. Low-intensity far-red light inhibits early lesions that contribute to diabetic retinopathy: *in vivo* and *in vitro*. Invest. Ophthalmol. Vis. Sci. 54 (5), 3681–3690.

Taylor, S.L., 1986. Histamine food poisoning: toxicology and clinical aspects. Crit. Rev. Toxicol. 17 (2), 91–128.

Thang, S.H., Kobayashi, M., Matsuoka, I., 2000. Regulation of glial cell line-derived neurotrophic factor responsiveness in developing rat sympathetic neurons by retinoic acid and bone morphogenetic protein-2. J. Neurosci. 20 (8), 2917–2925.

Thermos, K., 2003. Functional mapping of somatostatin receptors in the retina: a review. Vision Res. 43 (17), 1805–1815, Review.

Thoreson, W.B., Witkovsky, P., 1999. Glutamate receptors and circuits in the vertebrate retina. Prog. Retin. Eye Res. 18 (6), 765–810.

Tikellis, C., Cooper, M.E., Twigg, S.M., Burns, W.C., Tolcos, M., 2004. Connective tissue growth factor is upregulated in the diabetic retina: amelioration by angiotensin-converting enzyme inhibition. Endocrinology 145 (2), 860–866.

Tsai, G.Y., Cui, J.Z., Syed, H., Xia, Z., Ozerdem, U., McNeill, J.H., Matsubara, J.A., 2009. Effect of N-acetylcysteine on the early expression of inflammatory markers in the retina and plasma of diabetic rats. Clin. Exp. Ophthalmol. 37 (2), 223–231.

Tsutsumi, M., Claus, T.H., Liang, Y., Li, Y., Yang, L., Zhu, J., Dela Cruz, F., Peng, X., Chen, H., Yung, S.L., Hamren, S., Livingston, J.N., Pan, C.Q., 2002. A potent and highly selective VPAC2 agonist enhances glucose-induced insulin release and glucose disposal: a potential therapy for type 2 diabetes. Diabetes 51 (5), 1453–1460.

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:51 Page Number: 117

B978-0-12-800179-0.00001-5, 00001

van Gurp, M., Festjens, N., van Loo, G., Saelens, X., Vandenabeele, P., 2003. Mitochondrial intermembrane proteins in cell death. Biochem. Biophys. Res. Commun. 304 (3), 487–497.

VanGuilder, H.D., Brucklacher, R.M., Patel, K., Ellis, R.W., Freeman, W.M., Barber, A.J., 2008. Diabetes downregulates presynaptic proteins and reduces basal synapsyn I phosphorylation in rat retina. Eur. J. Neurosci. 28, 1–11.

Varga, B., Szabadfi, K., Kiss, P., Fabian, E., Tamas, A., Griecs, M., Gabriel, R., Reglodi, D., Kemeny-Beke, A., Pamer, Z., Biro, Z., Tosaki, A., Atlasz, T., Juhasz, B., 2011. PACAP improves functional outcome in excitotoxic retinal lesion: an electroretinographic study. J. Mol. Neurosci. 43, 44–50.

Vasilaki, A., Thermos, K., 2009. Somatostatin analogues as therapeutics in retinal disease. Pharmacol. Ther. 122, 324–333.

Vasilaki, A., Mouratidou, M., Schulz, S., Thermos, K., 2002. Somatostatin mediates nitric oxide production by activating sst2 receptors in the rat retina. Neuropharmacology 43, 899–909.

Vaudry, D., Falluel-Morel, A., Bourgault, S., Basille, M., Burel, D., Wurtz, O., Fournier, A., Chow, B.K., Hashimoto, H., Galas, L., Vaudry, H., 2009. Pituitary adenylate cyclase activating polypeptide and its receptors: 20 years after the discovery. Pharmacol. Rev. 61, 283–357.

Vayalil, P.K., Mittal, A., Katiyar, S.K., 2004. Proanthocyanidins from grape seeds inhibit expression of matrix metalloproteinases in human prostate carcinoma cells, which is associated with the inhibition of activation of MAPK and NF kappa B. Carcinogenesis 25 (6), 987–995.

Verma, A., Shan, Z., Lei, B., Yuan, L., Liu, X., Nakagawa, T., Grant, M.B., Lewin, A.S., Hauswirth, W.W., Raizada, M.K., Li, Q., 2012. ACE2 and Ang-(1-7) confer protection against development of diabetic retinopathy. Mol. Ther. 20 (1), 28–36.

Villa, P., Bigini, P., Mennini, T., Agnello, D., Laragione, T., Cagnotto, A., Viviani, B., Marinovich, M., Cerami, A., Coleman, T.R., Brines, M., Ghezzi, P., 2003. Erythropoietin selectively attenuates cytokine production and inflammation in cerebral ischemia by targeting neuronal apoptosis. J. Exp. Med. 198 (6), 971–975.

Villaroel, M., Ciudin, A., Hernandez, C., Simo, R., 2010. Neurodegeneration: an early event of diabetic retinopathy. World J. Diabetes 1, 57–64.

Vincent, J.A., Mohr, S., 2007. Inhibition of caspase-1/interleukin-1beta signaling prevents degeneration of retinal capillaries in diabetes and galactosemia. Diabetes 56 (1), 224–230.

Wang, Y., Ma, P., Li, B., Lin, S., Wu, Y., Feng, J., Tang, S., 2007. The role of glucose control with intensive insulin therapy on retinal vessels of early diabetic rats. Yan Ke Xue Bao 23 (4), 205–211.

Wang, C., Aungi, H.H., Zhang, B., Sun, S., Li, X.L., He, H., Xie, J.T., He, T.C., Du, W., Yuan, C.S., 2008. Chemopreventive effects of heat-processed Panax quinquefolius root on human breast cancer cells. Anticancer Res. 28, 2545–2552.

Wang, J., Xu, X., Elliott, M.H., Zhu, M., Le, Y.Z., 2010a. Müller cell-derived VEGF is essential for diabetes-induced retinal inflammation and vascular leakage. Diabetes 59 (9), 2297–2305.

Wang, Q., Pfister, F., Dorn-Beineke, A., vom Hagen, F., Lin, J., Feng, Y., Hammes, H.P., 2010b. Low-dose erythropoietin inhibits oxidative stress and early vascular changes in the experimental diabetic retina. Diabetologia 53 (6), 1227–1238.

Wang, Q., Gorbey, S., Pfister, F., Höger, S., Dorn-Beineke, A., Krügel, K., Berrone, E., Wu, L., Korff, T., Lin, J., Busch, S., Reichenbach, A., Feng, Y., Hammes, H.P., 2011. Long-term treatment with suberythropoietic Epo is vaso- and neuroprotective in experimental diabetic retinopathy. Cell. Physiol. Biochem. 27 (6), 769–782.

Ward, M.M., Jobling, A.I., Kalloniatis, M., Fletcher, E.L., 2005. Glutamate uptake in retinal glial cells during diabetes. Diabetologia 48 (2), 351–360.

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:51 Page Number: 118

B978-0-12-800179-0.00001-5, 00001

Wassle, H., 2004. The parallel processing in the mammalian retina. Nat. Rev. 5, 1–11.
Watanabe, D., 2007. Erythropoietin as a retinal angiogenic factor in proliferative diabetic retinopathy. Nippon Ganka Gakkai Zasshi 111, 892–898.
Weaver, D.C., McDaniel, M.L., Lacy, P.E., 1978. Alloxan uptake by isolated rat islets of Langerhans. Endocrinology 102 (6), 1847–1855.
Wei, L., Ge, L., Qin, S., Shi, Y., Du, C., Du, H., Liu, L., Yu, Y., Sun, X., 2012. Hydrogen-rich saline protects retina against glutamate-induced excitotoxic injury in guinea pig. Exp. Eye Res. 94 (1), 117–127.
Wolff, B.E., Bearse, M.A., Schneck, M.E., Barez, S., Adams, A.J., 2010. Multifocal VEP (mfVEP) reveals abnormal neuronal delays in diabetes. Doc. Ophthalmol. 121, 189–196.
Wu, W.C., Lai, C.C., Chen, S.L., Sun, M.H., Xiao, X., Chen, T.L., Tsai, R.J., Kuo, S.W., Tsao, Y.P., 2004a. GDNF gene therapy attenuates retinal ischemic injuries in rats. Mol. Vis. 10, 93–102.
Wu, X., Reiter, C.E., Antonetti, D.A., Kimball, S.R., Jefferson, L.S., Gardner, T.W., 2004b. Insulin promotes rat retinal neuronal cell survival in a p70S6K-dependent manner. J. Biol. Chem. 279 (10), 9167–9175.
Wua, J., Wang, H.M., Li, J., Men, X.L., 2013. The research applications of db/db mouse. Sheng Li Ke Xue Jin Zhan 44 (1), 12–18.
Wursch, P., Pi-Sunyer, F.X., 1997. The role of viscous soluble fiber in the metabolic control of diabetes. A review with special emphasis on cereals rich in beta-glucan. Diabetes Care 20 (11), 1774–1780.
Xiao, C., He, M., Nan, Y., Zhang, D., Chen, B., Guan, Y., Pu, M., 2012a. Physiological effects of superoxide dismutase on altered visual function of retinal ganglion cells in db/db mice. PLoS One 7 (1), e30343.
Xiao, X., Cai, J., Xu, J., Wang, R., Cai, J., Liu, Y., Xu, W., Sun, X., Li, R., 2012b. Protective effects of hydrogen saline on diabetic retinopathy in a streptozotocin-induced diabetic rat model. J. Ocul. Pharmacol. Ther. 28 (1), 76–82.
Xie, P., Fujii, I., Zhao, J., Shinohara, M., Matsukura, M., 2012. A novel polysaccharide compound derived from algae extracts protects retinal pigment epithelial cells from high glucose-induced oxidative damage in vitro. Biol. Pharm. Bull. 35 (9), 1447–1453.
Xu, H., Chen, M., Forrester, J.V., 2009. Para-inflammation in the aging retina. Prog. Retin. Eye Res. 28, 348–368.
Yale, J.F., Marliss, E.B., 1984. Altered immunity and diabetes in the BB rat. Clin. Exp. Immunol. 57 (1), 1–11.
Yamagishi, S., Nakamura, K., Matsui, T., Inagaki, Y., Takenaka, K., Jinnouchi, Y., Yoshida, Y., Matsuura, T., Narama, I., Motomiya, Y., Takeuchi, M., Inoue, H., Yoshimura, A., Bucala, R., Imaizumi, T., 2006a. Pigment epithelium-derived factor inhibits advanced glycation end product-induced retinal vascular hyperpermeability by blocking reactive oxygen species-mediated vascular endothelial growth factor expression. J. Biol. Chem. 281 (29), 20213–20220.
Yamagishi, S., Matsui, T., Nakamura, K., Takeuchi, M., Imaizumi, T., 2006b. Pigment epithelium-derived factor (PEDF) prevents diabetes- or advanced glycation end products (AGE)-elicited retinal leukostasis. Microvasc. Res. 72 (1–2), 86–90.
Yamagishi, S., Ueda, S., Matsui, T., Nakamura, K., Okuda, S., 2008a. Role of advanced glycation end products (AGEs) and oxidative stress in diabetic retinopathy. Curr. Pharm. Des. 14 (10), 962–968, Review.
Yamagishi, S., Nakamura, K., Matsui, T., Ueda, S., Fukami, K., Okuda, S., 2008b. Agents that block advanced glycation end product (AGE)-RAGE (receptor for AGEs)-oxidative stress system: a novel therapeutic strategy for diabetic vascular complications. Expert. Opin. Investig. Drugs 17 (7), 983–996.
Yamamoto, K., Hashimoto, H., Tomimoto, S., Shintani, N., Miyazaki, J., Tashiro, F., Aihara, H., Nammo, T., Li, M., Yamagata, K., Miyagawa, J., Matsuzawa, Y.,

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:51 Page Number: 119

B978-0-12-800179-0.00001-5, 00001

Kawabata, Y., Fukuyama, Y., Koga, K., Mori, W., Tanaka, K., Matsuda, T., Baba, A., 2003. Overexpression of PACAP in transgenic mouse pancreatic beta-cells enhances insulin secretion and ameliorates streptozotocin-induced diabetes. Diabetes 52, 1155–1162.

Yang, Y.S., Danis, R.P., Peterson, R.G., Dolan, P.L., Wu, Y.Q., 2000. Acarbose partially inhibits microvascular retinopathy in the Zucker Diabetic Fatty rat (ZDF/Gmi-fa). J. Ocul. Pharmacol. Ther. 16 (5), 471–479.

Yang, L.P., Sun, H.L., Wu, L.M., Guo, X.J., Dou, H.L., Tso, M.O., Zhao, L., Li, S.M., 2009. Baicalein reduces inflammatory process in a rodent model of diabetic retinopathy. Invest. Ophthalmol. Vis. Sci. 50 (5), 2319–2327.

Yang, Y., Mao, D., Chen, X., Zhao, L., Tian, Q., Liu, C., Zhou, B.L.S., 2012. Decrease in retinal neuronal cells in streptozotocin-induced diabetic mice. Mol. Vis. 18, 1411–1420.

Yang, J.H., Kwak, H.W., Kim, T.G., Han, J., Moon, S.W., Yu, S.Y., 2013a. Retinal neurodegeneration in type II diabetic Otsuka Long-Evans Tokushima fatty rats. Invest. Ophthalmol. Vis. Sci. 54 (6), 3844–3851.

Yang, Y., Yang, K., Li, Y., Li, X., Sun, Q., Meng, H., Zeng, Y., Hu, Y., Zhang, Y., 2013b. Decursin inhibited proliferation and angiogenesis of endothelial cells to suppress diabetic retinopathy via VEGFR2. Mol. Cell. Endocrinol. 378 (1–2), 46–52.

Yokoi, N., Hoshino, M., Hidaka, S., Yoshida, E., Beppu, M., Hoshikawa, R., Sudo, K., Kawada, A., Takagi, S., Seino, S., 2013. A novel rat model of type 2 diabetes: the Zucker fatty diabetes mellitus ZFDM rat. J. Diabetes Res. 2013, 103731.

Yokouchi, H., Eto, K., Nishimura, W., Takeda, N., Kaburagi, Y., Yamamoto, S., Yasuda, K., 2013. Angiopoietin-like protein 4 (ANGPTL4) is induced by high glucose in retinal pigment epithelial cells and exhibits potent angiogenic activity on retinal endothelial cells. Acta Ophthalmol. 91 (4), e289–e297.

Yong, P.H., Zong, H., Medina, R.J., Limb, G.A., Uchida, K., Stitt, A.W., Curtis, T.M., 2010. Evidence supporting a role for N-(3-formyl-3,4-dehydropiperidino)lysine accumulation in Müller glia dysfunction and death in diabetic retinopathy. Mol. Vis. 16, 2524–2538.

Yoshida, Y., Yamagishi, S., Matsui, T., Jinnouchi, Y., Fukami, K., Imaizumi, T., Yamakawa, R., 2009. Protective role of pigment epithelium-derived factor (PEDF) in early phase of experimental diabetic retinopathy. Diabetes Metab. Res. Rev. 25 (7), 678–686.

Yoshikawa, T., Naito, Y., Kondo, M., 1999. Ginkgo biloba leaf extract: review of biological actions and clinical applications. Antioxid. Redox Signal. 1 (4), 469–480.

Yu, H., Chen, L., Jiang, J., 2010. Administration of pigment epithelium-derived factor delivered by adeno-associated virus inhibits blood-retinal barrier breakdown in diabetic rats. Mol. Vis. 16, 2384–2394.

Zeng, X.X., Ng, Y.K., Ling, E.A., 2000. Neuronal and microglial response in the retina of streptozotocin-induced diabetic rats. Vis. Neurosci. 17 (3), 463–471.

Zeng, H.Y., Green, W.R., Tso, M.O., 2008. Microglial activation in human diabetic retinopathy. Arch. Ophthalmol. 126, 227–232.

Zeng, K., Xu, H., Mi, M., Zhang, Q., Zhang, Y., Chen, K., Chen, F., Zhu, J., Yu, X., 2009. Dietary taurine supplementation prevents glial alterations in retina of diabetic rats. Neurochem. Res. 34 (2), 244–254.

Zhang, J.Z., Xi, X., Gao, L., Kern, T.S., 2007. Captopril inhibits capillary degeneration in the early stages of diabetic retinopathy. Curr. Eye Res. 32 (10), 883–889.

Zhang, J., Wu, Y., Jin, Y., Ji, F., Sinclair, S.H., Luo, Y., Xu, G., Lu, L., Dai, W., Yanoff, M., Li, W., Xu, G.T., 2008. Intravitreal injection of erythropoietin protects both retinal vascular and neuronal cells in early diabetes. Invest. Ophthalmol. Vis. Sci. 49 (2), 732–742.

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:52 Page Number: 120

B978-0-12-800179-0.00001-5, 00001

Zhang, X., Bao, S., Hambly, B.D., Gillies, M.C., 2009a. Vascular endothelial growth factor-A: a multifunctional molecular player in diabetic retinopathy. Int. J. Biochem. Cell Biol. 41 (12), 2368–2371.

Zhang, Y., Wang, Q., Zhang, J., Lei, X., Xu, G.T., Ye, W., 2009b. Protection of exendin-4 analogue in early experimental diabetic retinopathy. Graefes Arch. Clin. Exp. Ophthalmol. 247 (5), 699–706.

Zhang, Y., Zhang, J., Wang, Q., Lei, X., Chu, Q., Xu, G.T., Ye, W., 2011. Intravitreal injection of exendin-4 analogue protects retinal cells in early diabetic rats. Invest. Ophthalmol. Vis. Sci. 52 (1), 278–285.

Zhang, S.Y., Li, B.Y., Li, X.L., Cheng, M., Cai, Q., Yu, F., Wang, W.D., Tan, M., Yan, G., Hu, S.L., Gao, H.Q., 2013. Effects of phlorizin on diabetic retinopathy according to isobaric tags for relative and absolute quantification-based proteomics in db/db mice. Mol. Vis. 19, 812–821.

Zheng, Z., Chen, H., Ke, G., Fan, Y., Zou, H., Sun, X., Gu, Q., Xu, X., Ho, P.C., 2009. Protective effect of perindopril on diabetic retinopathy is associated with decreased vascular endothelial growth factor-to-pigment epithelium-derived factor ratio: involvement of a mitochondria-reactive oxygen species pathway. Diabetes 58 (4), 954–964.

Zhong, L., Bradley, J., Schubert, W., Ahmed, E., Adamis, A.P., Shima, D.T., Robinson, G.-S., Ng, Y.S., 2007. Erythropoietin promotes survival of retinal ganglion cells in DBA/2 J glaucoma mice. Invest. Ophthalmol. Vis. Sci. 48 (3), 1212–1218.

Zhou, J.Y., Zhou, S.W., 2007. Effect of berberine on PPARalpha/delta/gamma expression in type 2 diabetic rat retinae. Yao Xue Xue Bao 42 (12), 1243–1249.

Zhou, W., Yu, W., Xie, W., Huang, L., Xu, Y., Li, X., 2011. The role of SLIT-ROBO signaling in proliferative diabetic retinopathy and retinal pigment epithelial cells. Mol. Vis. 17, 1526–1536.

Zhu, B., Wang, W., Gu, Q., Xu, X., 2008. Erythropoietin protects retinal neurons and glial cells in early-stage streptozotocin-induced diabetic rats. Exp. Eye Res. 86 (2), 375–382.

Zmijewski, M.A., Sharma, R.K., Slominski, A.T., 2007. Expression of molecular equivalent of hypothalamic-pituitary-adrenal axis in adult retinal pigment epithelium. J. Endocrinol. 193, 157–169.

Zong, H., Ward, M., Stitt, A.W., 2011. AGEs, RAGE, and diabetic retinopathy. Curr. Diab. Rep. 11 (4), 244–252, Review.

Zuo, Z.F., Zhang, Q., Liu, X.Z., 2013. Protective effects of curcumin on retinal Müller cell in early diabetic rats. Int. J. Ophthalmol. 6 (4), 422–424.

Zwirska-Korczala, K., Jochem, J., Adamczyk-Sowa, M., Sowa, P., Polaniak, R., Birkner, E., Latocha, M., Pilc, K., Suchanek, R., 2005. Influence of melatonin on cell proliferation, antioxidative enzyme activities and lipid peroxidation in 3T3-L1 preadipocytes—an in vitro study. J. Physiol. Pharmacol. 56 (Suppl. 6), 91–99.

Comp. by: DElayaraja Stage: Proof Chapter No.: 1 Title Name: IRCMB
Date:21/4/14 Time:22:50:52 Page Number: 121

B978-0-12-800179-0.00001-5, 00001

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