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REVIEW ARTICLE

Photoreceptors in diabetic retinopathy

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ABSTRACT

Although photoreceptors account for most of the mass and metabolic activity of the retina, their role in the pathogenesis of diabetic retinopathy has been largely overlooked. Recent studies suggest that photoreceptors might play a critical role in the diabetes-induced degeneration of retinal capillaries, and thus can no longer be ignored. The present review summarizes diabetes-induced alterations in photoreceptor structure and function, and provides a rationale for further study of a role of photoreceptors in the pathogenesis of the retinopathy.

INTRODUCTION

Diabetic retinopathy (DR), a leading cause of visual impairment and blindness, is clinically defined as a microvascular disease, but the unique susceptibility of the retina (compared to other tissues) to this disease has never been explained[1]. Here, we review the accumulating data that suggests a new hypothesis that photoreceptors in the outer retina might play an important role in the development of the retinopathy. Photoreceptors are light-sensing cells unique to the retina. The present review will summarize current data linking retinal photoreceptors in the pathogenesis of early stages of diabetic retinopathy. The discussion will focus primarily on rods, as most of the animal-based work on this topic has been carried out in rodents (which have a rod-rich retina)[2]. Less is known about how cone function is affected with diabetes, although new animal models (such as *nrl*$^{-/-}$ mice[3,4]) might be useful in addressing this in the future.

EVIDENCE SUGGESTING THAT PHOTORECEPTORS CONTRIBUTE TO VASCULAR DISEASE IN DIABETIC RETINOPATHY

Photoreceptors of the outer retina have not usually been regarded as important in the pathogenesis of early diabetic retinopathy, likely due in part to the substantial distance between the photoreceptors and the retinal microvasculature that is affected by diabetes (Figure 1). Nevertheless, available evidence raises a possibility that the unique susceptibility of the retina to injury in diabetes could in fact be as a result of the presence of photoreceptors. In support of the photoreceptor hypothesis, Arden *et al.*[5] sent a survey sent to a group of diabetic patients who also had retinitis pigmentosa. The results of those responses suggested that DR was less severe in patients who also had retinitis pigmentosa (and therefore, photoreceptor degeneration). Stitt *et al.*[6] subsequently reported that diabetes did not cause the expected decrease in density of the retinal microvasculature in mice lacking rhodopsin (Rho$^{-/-}$), and thus lacking most photoreceptors. These data suggest that loss of photoreceptors in the outer retina reduced the severity of vascular degeneration in that model of diabetic retinopathy.

There are at least two hypotheses to explain how photoreceptors might influence the development of DR, and they are not mutually exclusive:

Hypoxia

It is well known that photoreceptors account for much of the oxygen consumed by the retina, and that this metabolism is increased in the dark[7,8], when the rod dark current becomes maximal[9–11]. Studies in cat and macaque retinae, in which oxygen microelectrodes were inserted into the retina, found a 30–40% PO_2 difference between the inner retina and the vicinity of rods[12,13]. There was no detectable oxygen next to dark-adapted rods. In the dark, oxygen consumption is greater than any other cell in the retina[14].

Arden[8,15,16] incorporated available data showing the high metabolic activity of photoreceptors at night (dark current), and postulated that in the presence of a compromised retinal vasculature (such as in diabetes), photoreceptor activity in the dark would make the retina even more hypoxic than usual.

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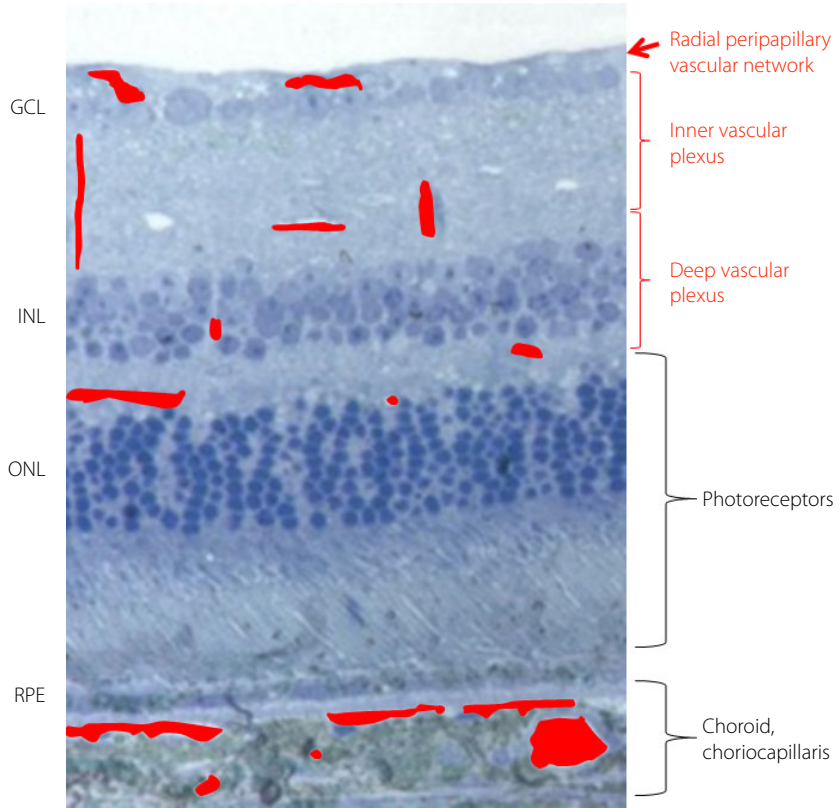

Figure 1 | Structure of the mouse retina, and localization of photoreceptors and microvasculature within the retina. The retina is highly organized, and cells in the ganglion cell layer (GCL), inner nuclear layer (INL) and outer nuclear layer (ONL) appear in discrete layers. Between these nuclear layers are plexiform layers where processes from various neural and glial cell types interdigitate. Retinal photoreceptors (that absorb light) interact with the retinal pigment epithelium (RPE) to maintain the visual cycle, and thus, vision. The vasculature supplying the retina comes from two different sides, with the photoreceptors supplied by choroidal vessels below the retina, and the inner retina supplied by interconnected vascular networks (radial peripapillary network, and inner and deep vascular networks). The retinal microvasculature is a major site of damage in diabetes. These vascular beds are shown in cartoon form (red) on the figure.

The extent to which this hypothesis applies also to the development of early diabetic retinopathy (before the vasculature is compromised) requires additional study.

Oxidative Stress

Diabetes results in increased generation of superoxide and other reactive oxygen species in the retina[17]. This oxidative stress is important in the pathogenesis of at least the vascular lesions of diabetic retinopathy, because inhibition of oxidative stress has been shown to inhibit development of inflammation and subsequent vascular lesions of early DR[18–21]. It has commonly been assumed that the diabetes-induced increases in oxidative stress arises in retinal cells known to be affected by diabetes (including endothelial cells and pericytes), but recent data shows that photoreceptors are the major site of superoxide generation in diabetes[17]. Consistent with a role of photoreceptors in oxidative stress, the presence or absence of light affects retinal oxidative stress (oxidative stress caused by diabetes is worsened in the dark), and oxidative stress contributes to the induction of pro-inflammatory proteins (which participate in the development of retinal microvascular pathology in diabetes)[17]. Photoreceptors express nicotinamide adenine dinucleotide phosphate oxidase[22], and contain most of the mitochondria found in the retina[23], and both of these sources of reactive oxygen species seem to contribute to the observed retinal superoxide generation in diabetes[17,24,25].

Based on these considerations, our working model of how photoreceptors play a critical role in the pathogenesis of diabetic retinopathy is summarized in Figure 2. Work is ongoing to elucidate how oxidative stress, inflammation and microvascular disease in diabetes are linked, and these are not the central topic of the present review. Here, we will largely focus on the impact of diabetes on rod morphology, cell biology and function as they related to oxidative stress generation in early stages of diabetic retinopathy.

MORPHOLOGICAL CHANGES TO PHOTORECEPTORS, RETINAL PIGMENT EPITHELIUM AND CHOROID IN DIABETES

A number of animal studies (summarized here) have reported that at least some photoreceptors degenerate in diabetes.

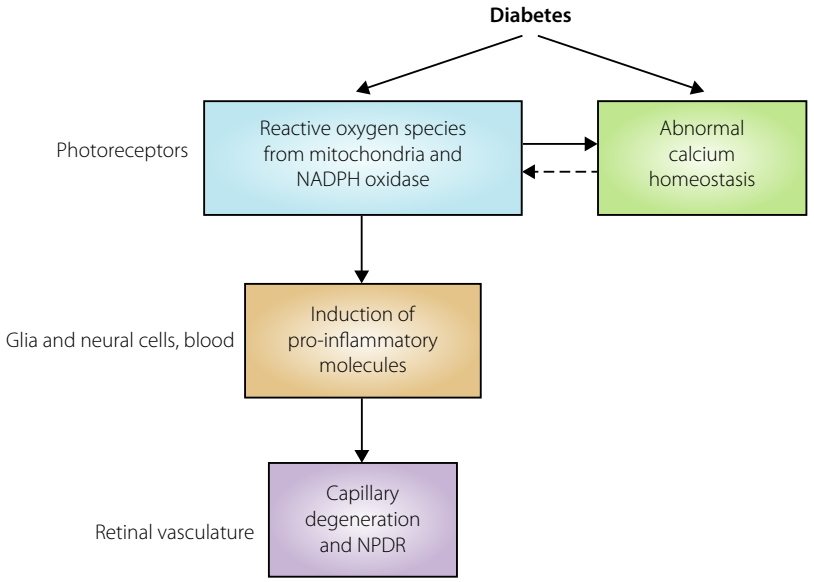

Figure 2 | Postulated mechanism by which retinal photoreceptors contribute to the development of the vascular lesions that are typical of the non-proliferative stage of diabetic retinopathy (NPDR). Diabetes causes oxidative stress and perhaps other adaptive changes in photoreceptors, in part through diabetes-induced alterations in ion flux. These abnormalities likely affect intermediate cells (such as Müller cells and leukocytes), which result in characteristic pathological alterations to the retinal vasculature, including increased permeability and non-perfusion. NADPH, nicotinamide adenine dinucleotide phosphate.

Nevertheless, it is important to recognize that diabetes has not been reported to cause widespread degeneration of photoreceptors, unlike in several other important retinal diseases.

Animal Studies

Some studies in diabetic rodents have reported photoreceptor degeneration early in the course of diabetes. Retinas from diabetic rats have been found to have increased caspase-3, as well as photoreceptor atrophy[26]. A reduction in the thickness of the outer nuclear layer was seen 24 weeks after the onset of diabetes, resulting in only half of the normal cellular layers in the outer nuclear layer remaining at 24 weeks of diabetes[27]. A few photoreceptors showed evidence of apoptosis at 4 weeks of diabetes, and the number of apoptotic photoreceptors increased thereafter[27]. Diabetes has also been reported to cause a reduction in the length of the rod outer segments[27] in male Sprague–Dawley rats over a study duration of 24 weeks. Morphological signs of degeneration in the outer segments of rods, most M-cones and some S-cones has been reported in Male Wistar and Sprague–Dawley rats killed 12 weeks after the induction of diabetes[28,29].

These photoreceptor abnormalities seem not to be secondary to chemical induction of diabetes, because they have also been detected in spontaneously diabetic animal models. A spontaneous model of type 1 diabetes in Ins2A-kita diabetic mice has been reported as showing cone, but not rod, photoreceptor loss after just 3 months of diabetes, and severe impairment of synaptic connectivity at the outer plexiform layer was detected in 9-month-old animals, suggesting cone photoreceptor degeneration[30]. A model of type 2 diabetes, the *db*/*db* mouse, showed thinning of the inner and outer nuclear (photoreceptor) layers, with defects in the integrity of the retinal pigment epithelium (RPE) over 8–24 weeks of diabetes[31,32].

Photoreceptors in less-studied animal models have also been reported to be affected by diabetes or experimental hyperglycemia. In Otsuka Long-Evans Tokushima Fatty (OLETF) rats (duration of diabetes not reported), the number of photoreceptor cell nuclei decreased, RPE decreased in height and basal infoldings were poorly developed[33]. Retinas from (mRen2)27 rats (a transgenic model showing greater than normal plasma prorenin levels) who were diabetic for just 3 weeks showed increased apoptotic cell death of both inner retinal neurons and photoreceptors[34]. Diabetes narrowed the layers of rods and cones after 6 weeks in rabbits, and these changes were exacerbated after 3–6 months of diabetes (including atrophy of the RPE and damage to photoreceptor discs)[35,36]. Adult zebrafish, in which the zebrafish were subjected to oscillating hyperglycemia for 30 days, showed degeneration of cone photoreceptor neurons and dysfunction of cone-mediated electroretinograms[37].

Diabetes-induced defects or degeneration of photoreceptors in animals have been reported to be inhibited therapeutically. These defects have been reported to be inhibited by administration of hesperetin[26], wolfberry[31], aliskiren[34] or exendin-4a, an agonist of glucagon-like factor-1[29].

Not all studies show photoreceptor death in diabetes. Studies of male Wistar and Sprague–Dawley rats diabetic for 12 weeks reported that retinal thickness, the number of apoptotic cells,

and the density of cones expressing middle (M)- and shortwave (S)-sensitive opsins were similar in diabetic and control retinas[28]. In male C57BL/6J mice diabetic for 2 months, no significant difference in the number of layers in the outer nuclear layer was detected[17]. Other morphological studies at substantially longer durations of diabetes and in multiple species have not found evidence of photoreceptor loss[38], or have not commented on (or noticed) it[39–42]. The lack of consistent conclusions among investigators about whether or not photoreceptor loss occurs in diabetes raises possibilities that some reports of photoreceptor loss might be due less to diabetes than to other differences (including strain differences), or that duration of diabetes plays an important role in the process.

Patient Studies

Evidence showing photoreceptor death is even less abundant in diabetic patients. Occasional case reports suggest photoreceptor loss in diabetes or diabetic macular edema (DME)[43], but there has been no systematic demonstration that photoreceptors are lost in diabetic patients, with the exception of autopsy evidence showing that the S-cones selectively are lost in DR[44].

Less severe changes to photoreceptor morphology have been associated with changes in visual acuity in diabetes[45–48]. Also, the photoreceptor inner and outer segment junction, and external limiting membrane have been identified as useful parameters for optical coherence tomography evaluation of foveal photoreceptor layer integrity in DME[46,47,49]. In DME, photoreceptor outer segment length of the central subfield was less[48] than the mean cone OS length in the fovea of healthy subjects[50], suggesting shortening of the photoreceptor outer segment length in diabetes or macular edema.

Summarizing

Anatomical changes in the photoreceptors elicited by diabetes appear modest, but this needs to be studied more, especially in patients.

MOLECULAR CHANGES IN PHOTORECEPTORS IN DIABETES

Animal Studies

Molecular techniques provide evidence that proteins important for photoreceptor function become altered before the appearance of microangiopathy in diabetes. For example, the content of rhodopsin[51], transducin[28,52,53], recoverin[53] and optical density of photopigment have been reported to become subnormal in diabetes. Reduced levels of genes involved in the phototransduction pathway [photoreceptor-specific opsin (Opn1mw), arrestin (Arr3) and increased transducin (Gnb3)] also suggests altered photoreceptor function[54], and whole transcriptome rinonucleic acid (RNA) sequencing (RNA-seq) has identified changes in transcripts including cyclic nucleotide gated channel (Cngb3), arrestin (Arr), guanine nucleotide binding protein (Gnb3) and phosphodiesterase (Pde6 h). Marginal decreases were also noticed in messenger RNA (mRNA) for RPE65, c transducin (Gnat2) and Crxos1[55]. A significant decrease in RPE65 protein immunoreactivity was apparent in Wistar rats diabetic for 12 weeks, but was less evident in diabetic Sprague–Dawley rats[28]. Rhodopsin kinase (Grk1) mRNA was subnormal in diabetic Brown Norway and Sprague–Dawley rats (but not in diabetic Long Evans rats), but expression of rhodopsin kinase protein was reported to be increased in the retinas of Sprague–Dawley rats diabetic for 6 weeks[53]. Despite the changes in rhodopsin kinase and arrestin identified above, diabetes of 12 weeks' duration in rats did not alter the rate of deactivation of the photoresponse[56].

Notably, insulin (independently of glucose uptake) has direct effects on photoreceptors. Insulin directly binds to photoreceptors, and initiates signaling within those cells[57–62]. Photoactivation of rhodopsin causes tyrosine phosphorylation of the insulin receptor and subsequent activation of phosphoinositide 3-kinase, a neuron survival factor[62,63]. This activation has been speculated to protect the photoreceptors from light damage. The retinal insulin receptor shows a high level of basal autophosphorylation, and this autophosphorylation is reduced in diabetic mouse retinas[64]. Thus, the absence or relative absence of insulin in diabetes might have effects on photoreceptors that have not been fully characterized yet.

Na^+/K^+-adenosine 5′-triphosphatase activity, which is concentrated in outer segments of rods, plays a major role in a-wave maintenance, and is responsible for sustaining the dark current[65]. Na^+/K^+-adenosine 5′-triphosphatase activity has been found to be impaired in diabetes[66–69]. It is possible that this diminished activity contributes to the diabetes-induced reduction in photoreceptor amplitude.

Not all defects affecting photoreceptor function in diabetes directly involve the photoreceptors. Some investigators have shown that the availability of vitamin A (retinol; the parent compound for retinoids) is subnormal in diabetes[70,71].

Summarizing

Diabetes causes a number of molecular alterations within photoreceptors, but there is not yet a clear understanding of how these changes occur, or their significance with regard to photoreceptor (and retinal) function. Whether these abnormalities are a cause or result of the oxidative stress that develops in photoreceptors in diabetes is not known.

CHANGES IN PHOTORECEPTOR/RPE UNIT FUNCTION IN DIABETES

Photoreceptors are the most metabolically active neuron in the central nervous system[72]. One common method for evaluating the function of photoreceptors non-invasively is by electroretinogram (ERG), and specifically by analysis of the ERG a-wave[73–75].

Animal Studies

Diabetes-induced defects in both amplitude and latency of the a-wave have been detected in some studies of diabetic rats. This

defect has been reported to develop as rapidly as 2 days after the onset of diabetes[76], but whether this rapid development of a functional defect was a result of diabetes or the rapidly changing metabolic mileau immediately after the initiation of hyperglycemia and insulin deficiency is not yet clear. Defects have also been reported at 4[77] and 12 weeks of diabetes[76], and the defects in photoreceptor function detected at 12 weeks of diabetes in rats encompassed several different parameters, including abnormal response amplitudes in the presence of normal sensitivity[76,77]. Diabetes did not affect deactivation of the photoreceptor response, and dark adaptation occurred faster than normal in those diabetic animals[56]. The authors interpreted these data as likely showing a decrease in the amount of rhodopsin present in the rod outer segments associated with a proportional decrease in outer segment lengths.

Likewise, some studies involving diabetic mice showed defects in the a-wave. Diabetic *db*/*db* mice showed significant a-wave amplitude and implicit time defect in the interval of 8–24 weeks of diabetes[32]. Spontaneously diabetic Ins2akita mice showed subnormal a-wave amplitude and implicit time at 9 months-of-age, but not at 3 or 6 months-of-age[30].

Diabetes has been reported to result in subnormal rhodopsin generation[51,78]. Rhodopsin regeneration was also reported to be impaired by decreased pH in rod photoreceptors based on studies in the excised mouse eye[51,78]. These data appear consistent with recent data from Linsenmeier *et al.*[79], who reported a significant acidosis in rod nuclei of rats diabetic for 1 month.

Not all investigators detected diabetes-induced alterations in a-wave. Responses of the a-wave were not significantly reduced by experimental diabetes of 3 months' duration in male Sprague–Dawley rats[80] or in male Long Evans rats[81]. Likewise, the a-wave at the brightest luminous energy was unaffected by 12 weeks of diabetes in male SD rats[82], and amplitudes in such rats were significantly reduced only at 10 and 15 weeks of diabetes, but not at 2, 6, 20 or 25 weeks[83]. No significant differences were observed in the sensitivity or amplitude of the a- or b-wave components of the ERG between female diabetic and control rats[84], but this might be due to the less severe diabetes that developed in the female rats (compared with male rats). A-wave amplitudes were not subnormal in C57Bl/6J mice[85] tested at 22 weeks of diabetes. Thus, there seems to be no consensus on a-wave involvement in diabetic rodents at present.

Patient Studies

Clinical data provide evidence for rod and cone receptor defects in patients with diabetic retinopathy. Studies of diabetic patients by Holopigian *et al.*[86] detected both rod-isolated and cone-isolated changes in a-wave that were primarily in the log S (sensitivity) parameter. Based on the mathematical model that they used to interpret the results, changes in the sensitivity parameter show that the receptors could have transduction abnormalities, although this was not confirmed experimentally. Losses of selective S-cone pathway sensitivity[87] have been identified in diabetic patients. Alterations in rod and cone signaling have been detected in newly onset type 2 diabetes patients with normal fundus appearance[88]. Patients with diabetes show retinal regions with early neuroretinal dysfunction that are predictive of the eventual locations that develop microvascular histopathology[89,90], but the contributions of photoreceptors to the multifocal ERG signal remains unclear. More light than usual is required to bleach an equivalent amount of photopigment in some diabetic patients, suggesting that the photopigment is not bleaching normally[91].

Elevations of glucose in diabetes seems itself to play an important role in the development of photoreceptor defects, as rod adaptation (but not cone adaptation) was enhanced by transiently increased blood glucose[92].

Photoreceptors and the RPE have multiple close interactions related to many important functions of the outer retina including recovery of photoreceptor sensitivity after a bleach[93].

Rod sensitivity was subnormal in patients with early diabetic retinopathy, and mean thresholds were abnormal at all eccentricities and in all four quadrants of retina[94]. Abnormalities in dark adaptation and absolute threshold have also been reported in human subjects with diabetes[7,94–96]. Electrooculogram amplitudes (thought to reflect ionic fluxes across the RPE) have been shown to fluctuate with elevation of blood glucose in healthy human subjects[97]. In addition, the RPE response was found to be abnormal in diabetic mice with prolonged diabetes[85].

Summarizing

The electrophysiology data suggest that photoreceptors and/or RPE show variable impairments in diabetes. Whether or not these changes can serve as biomarkers for impending development of aspects of diabetic retinopathy is still unclear.

DIABETES-INDUCED ALTERATIONS IN ION FLUX IN PHOTORECEPTORS

As discussed here, electrophysiological and biochemical (adenosine 5′-triphosphatase) evidence suggests that diabetes alters photoreceptor ion homeostasis. However, these data focus on the entire retina and movement of monovalent ions, such as sodium. L-type calcium channels (LTCCs) are the major entry route of calcium into photoreceptors, and play a major role in photoreceptor function. For example, sustained influx of calcium into photoreceptors through open LTCCs is essential for the regulated release of the neurotransmitter glutamate (among many other critical functions)[98]. Photoreceptors also have a relatively weak calcium buffering capacity[99–101], and contain at least 75% of total retinal mitochondria[102–106]. Together, these calcium handling features greatly facilitate rapid signaling in photoreceptors, but also substantially promote susceptibility to increased reactive oxygen species production relative to other cell types in the retina.

Manganese-enhanced magnetic resonance imaging (MEMRI) is a new method that measures aspects of photoreceptor function not evaluated using electrophysiology, such as the influx of divalent ions like calcium into central retinal photoreceptors of

awake and freely moving animals. Manganese (Mn^{2+}, a strong magnetic resonance imaging contrast agent) is a calcium ion surrogate that is taken into excitable cells through LTCCs[20,107–116]. After systemic injection of a non-toxic dose of $MnCl_2$, manganese uptake into photoreceptors and other retinal cells can be non-invasively and quantitatively measured using MEMRI. This technique is being used to investigate diabetes-induced changes in calcium channels in photoreceptors.

Early in the course of diabetes, MEMRI studies have shown that photoreceptor uptake of manganese is significantly reduced in dark-adapted mice and rats, suggesting that diabetes causes a paradoxical closure of LTCCs in the dark (as if the photoreceptors were light adapted)[20,111,117]. Because these ion channels are essential for regulated release of neurotransmitter at the photoreceptor synapse, paradoxically closed photoreceptor LTCCs in the dark (together with the normally closed LTCCs in the light) likely have significant consequences on function of photoreceptors and the whole retina.

Several possibilities exist as to how diabetes might inhibit opening of ion channels in dark:

1 The diabetes-induced defect in photoreceptor ion channel regulation is apparently secondary to oxidative stress. Preventing oxidative stress in diabetic mice or rats, using either genetic overexpression of Cu,Zn superoxide dismutase or systemic administration of α-lipoic acid, respectively, corrected the diabetes-induced reduction in ion flux into photoreceptors in the dark[20,111]. Interestingly, both of these treatments also have been shown to inhibit the diabetes-induced degeneration of retinal capillaries[20,118]. Ongoing experiments are testing the possibility that closed LTCCs might also contribute to the oxidative stress.
2 Diabetes alters electron chain efficiency, resulting in excessive generation of superoxide. Thus, the reduction in mitochondrial function might reduce the energy available for keeping the cyclic guanosine monophosphate channels open in the dark[111,119]. Available data does not provide support for this hypothesis, however, as retinal adenosine triphosphate levels (measured during daylight hours) have not been found to be abnormal in diabetes[69,120]. Furthermore, 11-*cis*-retinal supplementation partly restored manganese uptake, suggesting that enough energy was available to maintain open channels, at least to some degree[117].
3 Activated protein kinase C suppresses LTCC activity (at least in cardiac tissue)[121]. Protein kinase C activity is known to be increased in the retina in diabetes patients, and has been implicated in diabetes-induced reductions in visual function[122,123].

Summarizing

Accumulating evidence shows that diabetes alters ion flux in photoreceptors, and that these abnormalities are linked to oxidative stress. The contribution of photoreceptor calcium channels and ion flux to the oxidative stress and to the development of the lesions clinically accepted as diabetic retinopathy is vastly unexplored, and is actively being investigated.

CONCLUSION

Photoreceptors are unique to the retina, and thus might account for the unique susceptibility of the retina to damage in diabetes. Although photoreceptors account for most of the mass and metabolic activity of the retina, and they clearly influence the function of all other cell types in the retina, their role in DR has not been clearly delineated. The present review provides a rationale for further study of a role of photoreceptors in the pathogenesis of diabetic retinopathy. The contributions of surrounding cells, such as RPE and choriocapillaris, to the photoreceptor alterations in diabetes remain to be investigated.

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