Published in final edited form as:

Drug News Perspect. 2002 May ; 15(4): 226–232.

Diabetic Retinopathy and the NMDA receptor

Sylvia B. Smith

Department of Cellular Biology and Anatomy and Department of Ophthalmology Medical College of Georgia, Augusta, GA 30912-2000

Introduction

Diabetic retinopathy is a major sight-threatening disease and is a leading cause of blindness [1]. The purpose of this review is to describe that which is known and is conjectured about the involvement of the NMDA receptor and diabetic retinopathy. To this end, the discussion that follows has been organized to familiarize the reader with the characteristic features of the normal mammalian retina, followed by a discussion of the altered features of the diabetic retina. The discussion will then focus on the non-vascular changes associated with diabetic retinopathy with an emphasis on loss of certain neuronal cell types, particularly the retinal ganglion cells. As will be discussed below, there is a body of literature accumulating that suggests that glutamate accumulates in the diabetic retina and the implications are that the accumulation of this excitatory amino acid may activate the NMDA receptor with subsequent deleterious consequences for cells such as the retinal ganglion cell.

The normal retina

The retina is the neural stratum of the eyeball lying internal to the sclera/corneal layer and the uveal (or vascular) layer. A diagram of the eye is shown in figure 1 depicting the retina as the most internal of the three “tunics” of the eye. In the human, the retina is approximately 0.5 mm in thickness in the central region and only about 0.1 mm thick at the periphery. The posterior region of the eye, termed the macula contains the fovea, which is the thinnest portion of the retina and the area of best visual acuity. About 3 mm nasal to the fovea is the region known as the optic disk, which is the point at which the optic nerve leaves the retina to carry impulses to the brain. In the center of the disc are the central retinal artery and vein. In the normal ophthalmologic exam the optic disk is pink, though it can be grayish or white.

The retina develops from two layers of the invaginated optic vesicle, the outer lamina becomes a single layer of epithelial cells, which is pigmented and is referred to as the retinal pigment epithelial (RPE) cell layer. The remainder of the retina, which is referred to collectively as the “neural retina” or the “retina proper,” contains a variety of cell types. These include photoreceptor cells (rods and cones), their first order neuronal cells (bipolar cells) and the second order neurons in the visual pathway (the ganglion cells). A diagram of the retina is provided in figure 2. In panel A (Fig. 2), a photomicrograph of a histological section of a mouse retina is shown. In panel B (Fig. 2), a diagram of the major retinal cell types is provided so that the histological organization of the retina can be considered. The outermost layer of the retina is the RPE. Adjacent to these cells are the outer segments and inner segments of the rod (R) and cone (C) photoreceptor cells. The photoreceptor cells synapses with bipolar cells (B), the first order neurons of the visual pathway, in the outer

plexiform layer. Processes of other cells are present also in the OPL including those of horizontal cells. Horizontal cells (H) are inhibitory neurons and modulate activity of the bipolar cells. The nuclei of the bipolar and horizontal cells are found in the inner nuclear layer. Another cell type whose nuclei are present in the nuclear layer is the amacrine cell (A). Amacrine cells modulate the synaptic transmission from rod bipolar cells to ganglion cells. An important glial cell in the retina, termed the Müller cell (M), has its cell body within the inner nuclear layer and sends processes longitudinally toward the photoreceptor cells as well as toward the ganglion cells. Indeed the foot processes of these glial cells form the inner and outer limiting membranes of the retina. The Müller cell is the predominant glial element in the retina, comprising 90% of the glial cells. The Müller cell subserves many of the metabolic, ionic and extracellular buffering requirements of those retinal neurons with which it shares a common progenitor. It provides neurons with energy metabolites and therefore is active in glucose uptake. Müller cells participate also in lactate exchange and glycogen storage and metabolism. They play a key role in clearing toxic substances in the retina. The inner plexiform layer of the retina is a synaptic layer formed by processes from the amacrine, bipolar and ganglion cells. The innermost nuclear layer of the retina is the ganglion cell layer, the primary occupants of which are ganglion cells (G). Ganglion cells are the second order neurons of the visual pathway. Studies indicate that displaced amacrine cells may be found also in the ganglion cell layer. The axons of the ganglion cells join together to form the optic nerve, which leaves the eyeball at the optic disk.

Clinical and histopathologic features of the retina during diabetic retinopathy

Diabetic retinopathy is a complex degenerative disease involving all elements of the retina [2]. Vascular abnormalities have been studied most comprehensively, however new evidence is emerging that numerous cell types of the retina are involved. The vascular alterations are described briefly below, and have been reviewed extensively by others [3, 4, 5,].

Vascular changes in diabetic retinopathy

The clinical features most commonly recognized in patients with diabetes involve the capillaries of the retina [6–11]. The earliest clinical feature observed is the microaneurysm, followed by “dot” and “blot” hemorrhages, venous dilatations and beading, hard exudates, cotton wool patches and macular edema or swelling in the macular region of the retina. Advanced retinopathy is characterized by neovascularization within the retina, on the surface of the retina and on the optic disk. Fibrous tissue then proliferates, there can be hemorrhage into the vitreous and the retina can detach. The microangiopathy that characterizes diabetic retinopathy is sight threatening when it leads to macular edema or retinal ischemia. The histopathologic changes that correlate with these clinical observations include thickening of the basement membrane of capillaries, loss of pericytes (termed pericyte ghosts), and capillary sheathing. The small hemorrhages observed clinically occur in the middle layers of the retina, particularly in the inner nuclear layer. The neovascularization involves endothelial cell proliferation on the retinal side of the internal limiting membrane. The endothelial buds give rise to neovascular tufts that can grow onto the internal surface of the retina.

Non-vascular changes in diabetic retinopathy

In addition to the observations of vascular changes in diabetes, there have been many reports that vision and electrophysiological processing of visual stimuli by the retina are at risk within the first few years of the onset of diabetes and may precede the vascular changes.

Loss of color [12,13] and contrast [14,15] sensitivity are early signs of neural retinal dysfunction in humans that occur within 2 years of diabetes. Alterations of the b-wave of the electroretinogram occur after only a few years of the onset of diabetes in human patients [16] and within 2 weeks in rat models of diabetes [17]. It has been proposed by Bresnick [18,19] that diabetic retinopathy is a primary neurosensory disorder that precedes vasculopathy by many years.

As reviewed by Lorenzi and Gerhardinger [20], diabetes affects the neural and glial cells of the retina. These cells appear to be affected very early in the course of diabetes. It has been speculated by some that these early changes may contribute to the microangiopathy associated with diabetes. Amacrine cells, which are known to contain nNOS, are in close proximity to retinal vessels. The number of amacrine cells in the retina decreases in retinas of diabetic rats [21]. In addition, the transporter for glucose, GLUT1 is altered in retinas of these animals [21]. There is evidence also that Müller cells are altered during diabetes [22,23]. The expression of glutamine synthetase, the enzyme that converts glutamate to glutamine, is decreased [23]. In addition, in diabetic rats the expression of anti-apoptotic molecule Bcl-2 is increased along with the intermediate filament protein GFAP (glial fibrillary acid protein) [23]. GFAP expression is increased also in the retinas of human diabetic patients [23].

Ganglion cell death in diabetic retinopathy

A very dramatic alteration of the retina in diabetes is the increased frequency of apoptosis of retinal cells. Barber and co-workers [24] documented increased apoptosis of neural retinal cells in experimental diabetes in rats and diabetes mellitus in humans. At particular risk were retinal ganglion cells, which demonstrated a 10% decrease in cell number in streptozotocin-induced diabetic rats compared with controls. Work in our laboratory has demonstrated the same phenomenon in the retinas of streptozotocin-induced diabetic mice (Moore and Smith, unpublished observations). Pictured in figure 3 is photomicrograph of the retina of a mouse that has been diabetic for 12 weeks compared to the retina of an age-matched control mouse. Note that in the normal retina, the cells in the ganglion cell layer are tightly packed and uniformly organized, while in the diabetic retina, there are areas in which the cells are absent. (The arrow is pointing to an area where there are no ganglion cells.) We have analyzed the mechanism of this ganglion cell loss by TUNEL analysis, expression of caspase-3 and ultrastructural studies. We have found an increase in the number of TUNEL positive and caspase-3 positive cells in retinas of diabetic mice (manuscript in preparation). Indeed, in the retinas of mice that have been diabetic for 12–14 weeks, with no insulin therapy, there is an approximate 20–25% decrease in ganglion cells compared to controls.

Other studies are consistent with early degeneration of ganglion cells and their axons, which form the nerve fiber layer and eventually the optic nerve, in human diabetic patients. These include the findings of a progressive delay in visual evoked potentials of diabetic patients especially during the first 6 years after diabetes onset [25,26]. These studies showed that the visual evoked potentials of diabetic patients frequently have delayed latencies. The stimuli excite the smaller nerve fibers and thus it appears that these fibers are the first to be compromised in diabetes. Studies by Chihara and colleagues [27] have shown defects in the nerve fiber layer of patients with no evidence of microaneurysms and in more than half of patients with microaneurysms. In spontaneous diabetic BB/W rats the optic nerve fibers are significantly smaller, the nerve fiber layers show atrophic changes and the ganglion cells are dystrophic [28]. Interestingly in other studies of diabetic rats a decrease in the percentage volume of axons and an increase in the percentage volume of glial elements accompanied an increase in blood vessels. No difference was found in the spectrum of fiber diameters [29]. Thus there is an emerging body of data that suggests that neuronal changes occur in the diabetic retina.

Mechanism of ganglion cell death in diabetic retinopathy

There is evidence that the ganglion cell death in diabetic retinopathy occurs via glutamate-mediated toxicity [30]. Glutamate is the major excitatory neurotransmitter in the retina and is involved in neurotransmission from photoreceptors to bipolar cells and from bipolar cells to ganglion cells. Elevated levels of glutamate are implicated in neurodegeneration and glutamate is a well-known excitotoxin associated with a number of pathologic conditions. The toxic effects of glutamate on the retina, particularly the retinal ganglion cells are well-established [31–37]. In both human and experimental diabetes, glutamate levels are elevated. Within 3 months of the onset of diabetes in the streptozotocin-induced rat model, levels of glutamate increase significantly [30]. Recent studies by Kowluru et al [38] have confirmed an elevation of glutamate in the retina of diabetic rats. An elevation of glutamate is associated with human diabetic patients as well. When undiluted vitreous samples from diabetic and non-diabetic patients who had undergone pars plana vitrectomy were analyzed, levels of glutamate were 2.5 times greater in patients with diabetes [39].

As reviewed by Sucher et al [37] the neurotoxicity of ganglion cells is caused by excessive stimulation of receptors for excitatory amino acids predominantly through the N-methyl-D aspartate (NMDA) subtype of glutamate receptor.

NMDA receptor

The receptors for glutamate have been divided into two broad categories, ionotropic that directly gate channels and metabotropic that indirectly gate changes through second messengers. There are three types of ionotropic glutamate receptors, AMPA, kainate and NMDA. The NMDA receptor was named by the synthetic agonist that activates it, N-methyl-D-aspartate. The action of glutamate on the NMDA receptor is always excitatory. The NMDA receptor-channel contributes to the late component of the EPSP (excitatory post-synaptic potential). Figure 4 depicts the NMDA receptor and illustrates its three important properties. First, the receptor controls a cation channel of high conductance that is permeable to Ca^{2+} as well as to Na^{+} and K^{+} . Second, the opening of the channel depends upon membrane voltage as well as the chemical transmitter. Third, the opening of the channel requires extracellular glycine as a cofactor. It is noteworthy that recent studies from Snyder's lab have demonstrated that D-serine is an endogenous ligand for the glycine binding site of the NMDA receptor [40].

The NMDA-receptor-mediated toxicity in retinal ganglion cells is dependent on the influx of extracellular Ca^{2+} [37]. The increase in intracellular Ca^{2+} concentration acts as a second messenger that sets in motion the cascade leading to eventual cell death. Glutamate further stimulates its own release in a positive feedback loop by its interaction with the non-NMDA receptor subtypes. Ca^{2+} -induced Ca^{2+} release and further influx of Ca^{2+} through voltage-gated Ca^{2+} channels after glutamate-induced depolarization contribute to glutamate toxicity. Among the many consequences of increased Ca^{2+} are stimulation of protein kinase C, phospholipases, proteases, endonucleases, nitric oxide synthases and free radical production.

It is relevant that it is the increased *extracellular* accumulation of glutamate that has untoward consequences on the retinal ganglion cells. Typically the intracellular concentration of glutamate in brain tissue for example is 10 mmol per liter [41]. The extracellular glutamate levels is estimated at 0.6 μmol per liter [42,43]. It is not surprising therefore that extracellular glutamate levels in retina are tightly regulated by the action of potent uptake systems and degradative pathways present in neurons and Müller cells [44–51]. Neurons and Müller cells express high affinity glutamate transporter proteins that can rapidly clear glutamate from the extracellular environment. Within neurons glutamate can be recycled, but in Müller cells glutamate appears to be inactivated by conversion to

metabolites. Müller cells are equipped not only with high affinity glutamate uptake systems, but also glutamine synthetase and thus an enzymatic mechanism to metabolize glutamate to glutamine, a less toxic substance.

Nitric oxide (NO) and homocysteine are two other molecules that feed into the glutamate excitotoxic pathway and potentiate the ability of glutamate to activate the NMDA receptor. NO is a gaseous molecule known to possess a multitude of biological actions [52–54]. NO is a key factor leading to neuronal death triggered by glutamate-induced stimulation of the NMDA receptor. This has been demonstrated in many neuronal cell types [55–59] including retinal neurons [60]. It is noteworthy that in the studies of retinal neurons, a low concentration of NO played a protective role in glutamate neurotoxicity by closing the NMDA receptor gated ion channel, but elevated levels of NO, interacting with oxygen radicals, became toxic and mediated glutamate-induced neurotoxicity [60]. NO levels are increased in diabetes [61,62], including in patients with diabetic retinopathy [63]. An additional factor associated with diabetes and stimulation of the NMDA receptor is increased levels of homocysteine, termed hyperhomocysteinemia. Hyperhomocysteinemia is an independent risk factor in the development of diabetic retinopathy [64,65]. Excess levels of homocysteine induce neuronal cell death via stimulation of NMDA receptors [66]. Homocysteine is a homolog of cysteine produced by the demethylation of methionine. We have *in vivo* data demonstrating that homocysteine, like glutamate, is toxic to retinal ganglion cells [67]. Intravitreal injections of homocysteine led to apoptotic cell death as evidenced by increased numbers of TUNEL-positive cells, caspase-3 positive cells and ultrastructural evidence of apoptosis.

In summary, diabetic retinopathy is a complex disease characterized not only by the well-recognized vascular changes, but also by alterations of functions of other retinal cells. Evidence has emerged recently that several cell types are affected in diabetic retinopathy including the ganglion cells and the degeneration of these cells is thought to occur via overstimulation of the NMDA subtype of glutamate receptor. The diagram in figure 5 provides a working hypothesis for the retinal ganglion cell (RGC) death in diabetic retinopathy. Despite the evidence that neuronal alterations are an early phenomenon in the diabetic retina, it has not been investigated whether interfering with these neuronal changes would have any impact on the later vascular changes that are sight-threatening. Nor is it known whether altering the levels of glutamate, or other excitotoxic compounds, or interfering with their stimulation of the NMDA receptor might be beneficial in delaying the vasculopathy. Future work in our laboratory will explore those questions.

References

1. Wu, G. Retina: The Fundamentals. Philadelphia, Pa: WB Saunders; 1995. Diabetic retinopathy; p. 31
2. Bloodworth JMB. Diabetic retinopathy. Diabetes. 1962; 2:1–22. [PubMed: 13870120]
3. Frank RN. Diabetic Retinopathy. Progress in Retinal and Eye Research. 1995; 14:361–392.
4. Klein R, Klein BEK, Moss SE, Davis MD, DeMets DL. The Wisconsin epidemiologic study of diabetic retinopathy II. Prevalence and risk of diabetic retinopathy when age at diagnosis is less than 30 years. Arch Ophthalmol. 1984; 102:520–526. [PubMed: 6367724]
5. Ferris FL 3rd, Davis MD, Aiello LM. Treatment of diabetic retinopathy. N Engl J Med. 1999; 341:667–78. [PubMed: 10460819]
6. Larson HW. Diabetic retinopathy: an ophthalmoscopic study with a discussion of the morphologic changes and the pathogenetic factors in this disease. Acta Ophthalmol Scand. 1960; 60:1–89.
7. Dobree JH. Proliferative diabetic retinopathy: evolution of the retinal lesions. Br J Ophthalmol. 1964; 48:637–49. [PubMed: 14249185]

8. Davis MD. Vitreous contraction in proliferative diabetic retinopathy. *Arch Ophthalmol*. 1965; 74:741–51. [PubMed: 5846553]
9. Davis, MD. The natural course of diabetic retinopathy. In: Kimura, SJ.; Caygill, WM., editors. *Vascular complications of diabetes mellitus: with special emphasis on microangiopathy of the eye*. St. Louis: C.V. Mosby; 1967. p. 139-69.
10. Davis, MD.; Kern, TS.; Rand, LI. Diabetic retinopathy. In: Alberti, KGMM.; Zimmet, P.; DeFronzo, RA., editors. *International textbook of diabetes mellitus*. 2. Vol. 2. Chichester, England: John Wiley; 1997. p. 1413-46.
11. Donnelly R, Emslie-Smith AM, Gardner ID, Morris AD. ABC of arterial and venous disease: vascular complications of diabetes. *Brit Med J*. 2000; 320:1062–66. [PubMed: 10764371]
12. Daley M, Watzke R, Riddle M. Early loss of blue-sensitive color vision in patients with type 1 diabetes. *Diabetes Care*. 1987; 10:777–81. [PubMed: 3501362]
13. Roy M, Gunkel R, Podgor M. Color vision defects in early diabetic retinopathy. *Arch Ophthalmol*. 1986; 104:225–28. [PubMed: 3484948]
14. Sokol S, Moskowitz A, Skarf B. Contrast sensitivity in diabetics with and without background retinopathy. *Arch Ophthalmol*. 1985; 103:51–54. [PubMed: 3977675]
15. Hirsh, J.; Puklin, J. In: Henkind, P., editor. *Reduced contrast sensitivity may precede clinically observable retinopathy in type 1 diabetes*; Acta XXIV International Congress of Ophthalmology; San Francisco. New York: Lippincott; 1982. p. 719-724.
16. Coupland SG. Comparison of oscillatory potential and pattern electroretinogram measures in diabetic retinopathy. *Doc Ophthalmol*. 1987; 66:207–18. [PubMed: 3428075]
17. Sakai H, Tani Y, Shirasawa E, Shirao Y, Kawasaki K. Development of electroretinographic alterations in streptozotocin-induced diabetes. *Ophthalmic Res*. 1995; 27:57–63. [PubMed: 7596561]
18. Bresnick GH. Diabetic retinopathy viewed as a neurosensory disorder. *Arch Ophthalmol*. 1986; 104:989–90. [PubMed: 3729794]
19. Bresnick GH. Excitotoxins: a possible new mechanism for the pathogenesis of ischemic retinal damage. *Arch Ophthalmol*. 1989; 107:339–41. [PubMed: 2564272]
20. Lorenzi M, Gerhardinger C. Early cellular and molecular changes induced by diabetes in the retina. *Diabetologia*. 2001; 44:791–804. [PubMed: 11508263]
21. Roufail E, Soulis T, Boel E, Cooper ME, Rees S. Depletion of nitric oxide synthase-containing neurons in the diabetic retina: reversal by aminoguanidine. *Diabetologia*. 1998; 12:1419–25. [PubMed: 9867208]
22. Nork TM, Wallow IH, Sramek SJ, Anderson G. Muller's cell involvement in proliferative diabetic retinopathy. *Arch Ophthalmol*. 1987; 105:1424–9. [PubMed: 3117025]
23. Mizutani M, Gerhardinger C, Lorenzi M. Müller cell changes in human diabetic retinopathy. *Diabetes*. 1998; 47:445–49. [PubMed: 9519752]
24. Barber AJ, Lieth E, Khin SA, Antonetti DA, Buchanan AG, Gardner TW. Neural apoptosis in the retina during experimental and human diabetes. Early onset and effect of insulin. *J Clin Invest*. 1998; 102:783–91. [PubMed: 9710447]
25. Ponte F, Giuffrè G, Anastasi M, Lauricella M. Involvement of the visual evoked potentials in type I insulin-dependent diabetes. *Metab Pediatr Syst Ophthalmol*. 1986; 9:77–80. [PubMed: 3452012]
26. Anastasi M, Lauricella M, Giordano C, Galluzzo A. Visual evoked potentials in insulin-dependent diabetics. *Acta Diabetol Lat*. 1985; 22:343–49. [PubMed: 3832707]
27. Chihara E, Matsuoka T, Ogura Y, Matsumura M. Retinal nerve fiber layer defect as an early manifestation of diabetic retinopathy. *Ophthalmology*. 1993; 100:1147–51. [PubMed: 8341494]
28. Sima AA, Zhang WX, Cherian PV, Chakrabarti S. Impaired visual evoked potential and primary axonopathy of the optic nerve in the diabetic BB/W-rat. *Diabetologia*. 1992; 35:602–7. [PubMed: 1644237]
29. Scott TM, Foote J, Peat B, Galway G. Vascular and neural changes in the rat optic nerve following induction of diabetes with streptozotocin. *J Anat*. 1986; 144:145–52. [PubMed: 2961719]

30. Lieth E, Barber AJ, Xu B, Dice C, Ratz MJ, Tanase D, Strother JM. Glial reactivity and impaired glutamate metabolism in short-term experimental diabetic retinopathy. *Diabetes*. 1998; 47:815–20. [PubMed: 9588455]
31. Lucas DR, Newhouse JP. The toxic effect of sodium L-glutamate on the inner layers of the retina. *Arch Ophthalmol*. 1957; 58:193–201.
32. Olney JW. Glutamate-induced retinal degeneration in neonatal mice: Electron microscopy of the acutely evolving lesion. *J Neuropathol Exp Neurol*. 1969; 28:455–474. [PubMed: 5788942]
33. Hansson HA. Ultrastructural studies on the long-term effects of sodium glutamate on the rat retina. *Virchows Arch*. 1970; 6:1–11.
34. Lund-Karlsen RF, Fonnum F. The toxic effect of sodium glutamate on rat retina: Changes in putative transmitters and corresponding enzymes. *J Neurochem*. 1976; 27:1437–41. [PubMed: 12250]
35. Vorwerk CK, Lipton SA, Zurakowski D, Hyman BT, Sabel BA, Dreyer EB. Chronic low-dose glutamate is toxic to retinal ganglion cells: toxicity blocked by memantine. *Invest Ophthalmol Vis Sci*. 1996; 37:1618–24. [PubMed: 8675405]
36. Sisk DR, Kuwabara T. Histologic changes in the inner retina of albino rats following intravitreal injection of monosodium L-glutamate. *Graefes Arch Clin Exp Ophthalmol*. 1985; 223:250–258. [PubMed: 4065591]
37. Sucher NJ, Lipton SA, Dreyer EB. Molecular basis of glutamate toxicity in retinal ganglion cells. *Vis Res*. 1997; 37:3483–93. [PubMed: 9425525]
38. Kowluru RA, Engerman RL, Case GL, Kern TS. Retinal glutamate in diabetes and effect of antioxidants. *Neurochem Int*. 2001; 38:385–90. [PubMed: 11222918]
39. Ambati J, Chalam KV, Chawla DK, D'Angio CT, Guillet EG, Rose SJ, Vanderlinde RE, Ambati BK. Elevated gamma-aminobutyric acid, glutamate, and vascular endothelial growth factor levels in the vitreous of patients with proliferative diabetic retinopathy. *Arch Ophthalmol*. 1997; 115:1161–66. [PubMed: 9298058]
40. Schell MJ, Molliver ME, Snyder SH. D-serine, an endogenous synaptic modulator: localization to astrocytes and glutamate-stimulated release. *Proc Natl Acad Sci U S A*. 1995; 92:3948–52. [PubMed: 7732010]
41. Kvamme E, Schousboe A, Hertz L, Torgner IA, Svenneby G. Developmental change of endogenous glutamate and gamma-glutamyl transferase in cultured cerebral cortical interneurons and cerebellar granule cells, and in mouse cerebral cortex and cerebellum in vivo. *Neurochem Res*. 1985; 7:993–1008. [PubMed: 2864647]
42. Bouvier M, Szatkowski M, Amato A, Attwell D. The glial cell glutamate uptake carrier countertransports pH-changing anions. *Nature*. 1992; 360:471–4. [PubMed: 1448171]
43. Benveniste H, Drejer J, Schousboe A, Diemer NH. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. *J Neurochem*. 1984; 43:1369–74. [PubMed: 6149259]
44. Arriza JL, Eliasof S, Kavanaugh MP, Amara SG. Excitatory amino acid transporter 5, a retinal glutamate transporter coupled to a chloride conductance. *Proc Natl Acad Sci USA*. 1997; 94:4155–60. [PubMed: 9108121]
45. Vorwerk CK, Naskar R, Schuettauf F, Quinto K, Zurakowski D, Gochenauer G, Robinson MB, Mackler SA, Dreyer EB. Depression of retinal glutamate transporter function leads to elevated intravitreal glutamate levels and ganglion cell death. *Invest Ophthalmol Vis Sci*. 2000; 41:3615–21. [PubMed: 11006260]
46. Arriza JL, Fairman WA, Wadiche JI, Murdoch GH, Kavanaugh MP, Amara SG. Functional comparisons of three glutamate transporter subtypes cloned from human motor cortex. *J Neurosci*. 1994; 14:5559–69. [PubMed: 7521911]
47. Rauen T, Kanner BI. Localization of the glutamate transporter GLT-1 in rat and macaque monkey retinae. *Neurosci Lett*. 1994; 169:137–40. [PubMed: 8047270]
48. Rauen T, Rothstein JD, Wässle H. Differential expression of three glutamate transporter subtypes in the rat retina. *Cell Tissue Res*. 1996; 286:325–36. [PubMed: 8929335]

49. Pow DV, Barnett NL. Developmental expression of excitatory amino acid transporter 5: a photoreceptor and bipolar cell glutamate transporter in rat retina. *Neurosci Lett*. 2000; 280:21–24. [PubMed: 10696802]
50. Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW, Kanai Y, Hediger MA, Wang Y, Schielke JP, Welty DF. Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron*. 1996; 16:675–86. [PubMed: 8785064]
51. Trotti D, Rossi D, Gjesdal O, Levy LM, Racagni G, Danbolt NC, Volterra A. Peroxynitrite inhibits glutamate transporter subtypes. *J Biol Chem*. 1996; 271:5976–79. [PubMed: 8626378]
52. Dawson TM, Snyder SH. Gases as biological messengers: nitric oxide and carbon monoxide in the brain. *J Neurosci*. 1994; 14:5147–59. [PubMed: 8083727]
53. Schuman E, Madison D. Nitric oxide and synaptic function. *Annu Rev Neurosci*. 1994; 17:153–83. [PubMed: 7516125]
54. Moilanen E, Vapaatalo H. Nitric oxide in inflammation and immune responses. *Ann Med*. 1995; 27:359–67. [PubMed: 7546626]
55. Clementi E. Role of nitric oxide and its intracellular signalling pathways in the control of Ca^{2+} homeostasis. *Biochem Pharmacol*. 1998; 55:713–18. [PubMed: 9586942]
56. Leist M, Volbracht C, Kuhnle S, Fava E, Ferrando-May E, Nicotera P. Caspase-mediated apoptosis in neuronal excitotoxicity triggered by nitric oxide. *Molec Med*. 1997; 3:750–64. [PubMed: 9407551]
57. Suemaru K, Kawasaki H, Gomita Y, Tanizaki Y. Involvement of nitric oxide in development of tail-tremor induced by repeated nicotine administration in rats. *Eur J Pharmacol*. 1997; 335:139–43. [PubMed: 9369366]
58. Bonfoco E, Leist M, Zhivotovsky B, Orrenius S, Lipton SA, Nicotera P. Cytoskeletal breakdown and apoptosis elicited by NO donors in cerebellar granule cells require NMDA receptor activation. *J Neurochem*. 1996; 67:2484–93. [PubMed: 8931482]
59. Nei K, Matsuyama S, Shuntoh H, Tanaka CTI. NMDA receptor activation induces glutamate release through nitric oxide synthesis in guinea pig dentate gyrus. *Brain Res*. 1996; 28:105–10. [PubMed: 8864303]
60. Kashii S, Mandai M, Kikuchi M, Honda Y, Tamura Y, Kaneda K, Akaike A. Dual actions of nitric oxide in N-methyl-D-aspartate receptor-mediated neurotoxicity in cultured retinal neurons. *Brain Res*. 1996; 711:93–101. [PubMed: 8680879]
61. Bank N, Aynedjian HS. Role of EDRF (nitric oxide) in diabetic renal hyperfiltration. *Kidney Int*. 1993; 43:1306–12. [PubMed: 8315943]
62. Komers R, Allen TJ, Cooper ME. Role of endothelium-derived nitric oxide in the pathogenesis of renal hemodynamic changes of experimental diabetes. *Diabetes*. 1994; 43:1190–97. [PubMed: 7926287]
63. Schmetterer L, Findl O, Fasching P, Ferber W, Strenn K, Breiteneder H, Adam H, Eichler HG, Wolzt M. Nitric oxide and ocular blood flow in patients with IDDM. *Diabetes*. 1997; 46:653–58. [PubMed: 9075807]
64. Neugebauer SAU, Baba T, Kurokawa K, Watanabe T. Defective homocysteine metabolism as a risk factor for diabetic retinopathy. *Lancet*. 1997; 349:473–474. [PubMed: 9040583]
65. Vaccaro O, Ingrosso D, Rivellese A, Greco G, Riccardi G. Moderate hyperhomocysteinaemia and retinopathy in insulin-dependent diabetes. *Lancet*. 1997; 349:1102–03. [PubMed: 9107272]
66. Kim WK, Pae YS. Involvement of N-methyl-D-aspartate receptor and free radical in homocysteine-mediated toxicity on rat cerebellar granule cells in culture. *Neurosci Lett*. 1996; 216:117–20. [PubMed: 8904797]
67. Moore P, El-Sherbeny A, Roon P, Schoenlein PV, Ganapathy V, Smith SB. Apoptotic retinal ganglion cell death is induced in vivo by the excitatory amino acid homocysteine. *Exp Eye Res*. 2001; 73:45–57. [PubMed: 11428862]

Figure 1. Simplified schematic drawing of the eyeball

The eye is comprised of three “tunics” or layers. The outermost is the sclera which continues anteriorly as the transparent cornea. Internal to the sclera is the vascular tunic, which includes the choroid, a series of blood vessels. Internal to the choroid is the retina, which lines the posterior 5/6th of the eye. The innermost region of the retina has cells whose axons join to form the optic nerve. The optic nerve exits the eyeball at its posterior pole and carries neural impulses to the brain.

Figure 2. Histology of the retina

The left panel is a photomicrograph of a toluidine-blue stained section of a normal mouse retina. The eye was fixed in 2% glutaraldehyde/2% paraformaldehyde in 0.1 M cacodylate buffer, embedded in EPON and cut at 2 μ m thickness. The labeling on the left indicates the various layers of the retina. The innermost layer of the retina, labeled (1) is the inner limiting membrane and is the portion of the retina closest to the anterior portion of the eye. Deep to this layer is the nerve fiber layer (2) which represents the axons of the ganglion cells. Their fibers join to form the optic nerve. The cell bodies of the ganglion cells are in the Ganglion cell layer (3). These are the neurons that die by apoptosis in diabetic retinopathy. Ganglion cells synapse in the inner plexiform layer (4) with bipolar cells and amacrine cells, which have their cell bodies in the inner nuclear layer (5). Horizontal cells and Müller cells, the major glial cell of the retina, also have their cell bodies in the inner nuclear layer. The cells of the inner nuclear layer also synapse via the outer plexiform layer (6) with photoreceptor (rod and cone) cells of the outer nuclear layer (7). The photoreceptor cells have inner segments (9a) and outer segments (9b). The outer segments are enveloped by microvillous processes of the RPE (10) cells. The right panel is a schematic of the various cellular and synaptic processes of the retina. Several cell types are labeled: G = ganglion cell, M = Müller cell, B = bipolar cell, H = horizontal cell, A = amacrine cell, C = cone cell, R = rod cell.

Figure 3. Hematoxylin and eosin (H&E)-stained cryosection of retinas of a normal mouse and a diabetic mouse

C57Bl/6 mice were made diabetic by injecting them at 3 weeks of age with streptozotocin (75 mg/kg) for three consecutive days. For histological analysis, mice were euthanized, eyes were removed and immediately frozen in OCT embedding compound and sectioned using a cryomicrotome. Cryosections were either stained with H&E for analysis of numbers of retinal ganglion cells (RGCs) or were subjected to assays for apoptosis such as the TUNEL assay (not shown in this figure). In the left panel is an H&E-stained section of a normal mouse. In the right panel, the retina of a mouse that had been diabetic for 12 weeks is shown; arrows point to regions where the RGCs are no longer present. RGCs, which were apoptotic at earlier ages post-onset of diabetes, have died and been removed. Compare the uniform layer of ganglion cells in the RGC layer of the normal mouse (left) to non-uniform layer of the diabetic mouse (right).

Figure 4. Schematic diagram of the N-methyl-D aspartate (NMDA) subtype of glutamate receptor

The receptor, positioned in the plasma membrane of cells, has a number of subunits and controls a cation channel, which is permeable to Ca^{2+} as well as to Na^{+} and K^{+} . The receptor is stimulated by glutamate, as well as homocysteine and the pharmacological substrate, NMDA. The opening of the channel requires extracellular glycine as a cofactor; new data has emerged indicating that D-serine is also an endogenous ligand for the receptor and binds at the glycine binding site.

Figure 5. Hypothesized mechanism for retinal ganglion cell death in diabetes

In diabetes, levels of glutamate are elevated in the retina and vitreous. Increased extracellular levels of glutamate, and possibly homocysteine, activate the NMDA-subtype of glutamate receptor leading to an increase in levels of intracellular calcium (Ca^{2+}_i). The increased intracellular calcium initiates a cascade of events that lead to retinal ganglion cell (RGC) death including an increase in nitric oxide (NO) levels which can in turn cause additional activation of the NMDA receptor.