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Review

Retinal metabolism: A comparative look at energetics in the retina

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ABSTRACT

The retina is part of the central nervous system, and shares the characteristically high metabolism of the brain. The high energy demand of the retina is normally matched with a large supply of metabolites. When supply does not equal demand (e.g. if retinal blood flow is impaired), retinal neurons are at risk of excitotoxic cell death and vision is impaired or lost. Understanding the energetic budget of the retina is therefore crucial for understanding the pathology and treatment of retinal disease. In this minireview I give an overview of the energetics of the retina, with a focus on lessons learnt from comparative physiology. Retinas of all species studied thus far receive blood flow from choroidal capillaries. Additionally, fish, reptiles, and birds each have unique structures to increase metabolite supply. Primates and some mammals also have intra- and supraretinal vasculature to supply the retina, while other mammals rely solely on the choroid at the cost of retinal thickness. Neuroglobin, an oxygen-binding protein, may assist in oxygen delivery to counteract large diffusion distances from capillaries to mitochondria. Energy demand differs among models, as does mitochondrial location. More ATP is consumed in the dark due to Na^+/K^+ ATPase activity to counteract the dark current, whereas phototransduction dominates ATP demand in the light. Photoreceptor metabolism is therefore especially high, and may be sustained with phosphocreatine and lactate shuttles. This comparative physiology approach raises new research questions, and suggests caution in comparing animal models of retinal disease, as they differ greatly in vasculature and energetics.

Contents

1. Introduction

The brain is energetically costly, and is often cited as accounting for 20% of total energy consumption in resting humans, despite weighing 2% of total mass (Erecińska and Silver, 1989; Herculano-Houzel, 2011; Kety, 1950). The retina is an embryological projection of the forebrain (Chuang and Raymond, 2001), and therefore shares the high metabolic demand of central nervous tissue (Ames, 1992; Wong-Riley, 2010). This is clinically important, because most retinal diseases (such as glaucoma and diabetic retinopathy, among others) include retinal ischemia (Kaur et al., 2008; Osborne et al., 2004; Schmidt et al., 2008), in which metabolites (e.g. glucose, O_2) are limited and energy supply is decreased. Understanding the energetic budget of the retina is therefore crucial for understanding the pathology and treatment of retinal disease.

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In this minireview I will give an overview of the energetics of the retina, with a focus on lessons learnt from comparative physiology. This discussion will begin with energy supply, including blood flow to the eye, and adaptations to facilitate O_2 diffusion and glucose transport. This review of supply will be followed by a discussion of demand, with a special focus on photoreceptor (PR) metabolism. The paper will conclude by exploring the metabolic disorders which arise when supply does not meet demand, such as when blood flow to the retina is limited.

2. Supply: how do the structures of the retina receive blood flow and O_2?

Blood reaches the retina differently in fishes, birds, reptiles, and mammals. In the back of the eye in all vertebrates, blood perfuses through a layer of capillaries called the choroidal layer (or the choroid) (Nickla and Wallman, 2010). The choroid is separated from other layers by Bruch's membrane, which lies closer to the centre of the eye (Booij et al., 2010), and by a retinal pigment epithelium (RPE) (Dowling, 1987; Kolb, 2003). Because the choroidal capillaries are fenestrated, cells of the RPE are bound by tight junctions to maintain a blood-retinal barrier (Vilchis and Salceda, 1996). The RPE is crucial for discarding damaged disks of membranes from PR outer segments, and for restoring light-absorbing chromophores to PRs (Klimanskaya, 2006).

Before further reviewing blood flow to the eye, it will be important to discuss the structure of the retina (shown in Fig. 1). The retina is located on the innermost layer of the eye, over Bruch's membrane and distal to the vitreous humor which bathes the retina. It has a laminate structure, with PRs layered closest to the RPE (Dowling, 1987). PRs have a unique, polarized cytostructure which can be divided into four sequential parts: the outer segments (which contain membranous disks with photosensitive chromophores) are closest to the RPE, followed by the mitochondrion-rich inner segments, then their somata, and then synaptic pedicles (Dowling, 1987). This structure is important in discussing the energetics of the retina because it affects the diffusion distance from capillaries: oxygen must pass across several layers (i.e. Bruch's membrane, the RPE, and PR outer segments) to reach mitochondria in the inner segments (Buttery et al., 1991; Wittenberg and Wittenberg, 1974).

Information about light stimuli is relayed from the outer retina to the inner retina, from PRs to bipolar cells, and ultimately to ganglion cells (Dowling, 1987; Kolb, 2003). Ganglion cells are closest to the vitreous-filled lumen of the eye. Ganglion cell axons coalesce to form a nerve fiber layer. They leave the eye at the lamina cribrosa (located at the optic disk), where they form the optic nerve (Schmidt et al., 2008).

Although the choroid is ubiquitous among vertebrates (Nickla and Wallman, 2010; Yu and Cringle, 2001), further vascularization in the eye poses several physical problems. First, blood vessels are opaque. Light must pass through the entire retina (e.g. through the nerve fiber layer and all cell layers, to reach PR outer segments), and so blood vessels anywhere proximal to the PR layer will interfere with the light path (Yu and Cringle, 2001). Secondly, the diffusion distance in the retina is extremely large in most species. Wittenberg and Wittenberg (1974) emphasize that in humans, this distance is 60 μm from the choroidal capillaries to the nearest mitochondria, in PR inner segments; they contrast this to 20 μm in muscle. Furthermore, O_2 and other metabolites would need to diffuse throughout the thickness of the retina, which is generally between 100 and 300 μm in mammals (Buttery et al., 1991). In a thorough experiment of 86 species of teleost fish, the average reti-

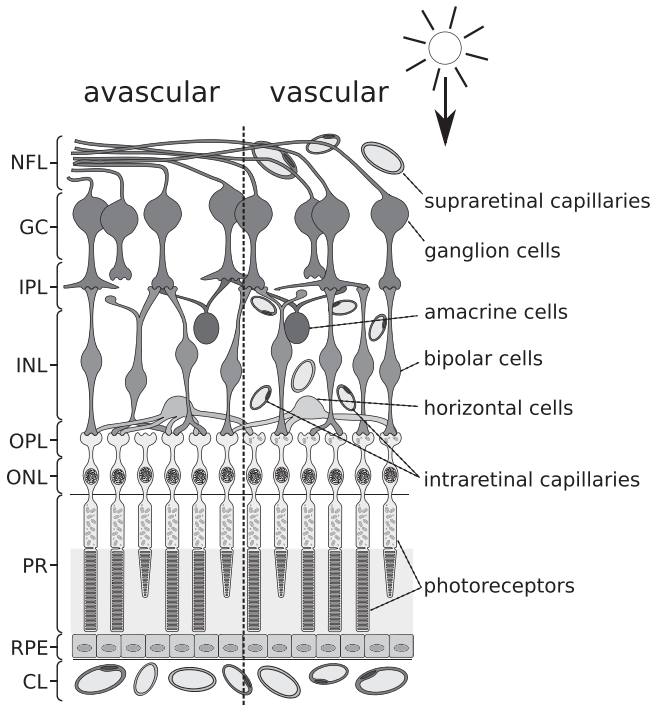

Fig. 1. Schematic of the vertebrate retina. *Left.* The choroidal capillaries are the sole sources of metabolites in avascular retinas. *Right.* Some mammals have vascularized retinas, in which additional supra- and intra-retinal blood vessels perfuse the inner retina. CL, choroidal layer; RPE, retinal pigment epithelium; PR, photoreceptors; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GC, ganglion cells; NFL, nerve fiber layer.

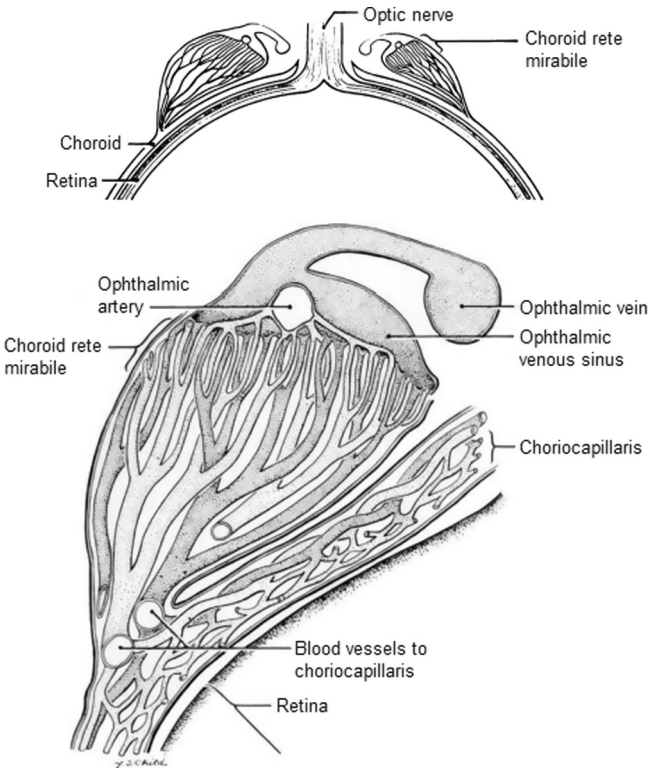

Fig. 2. Representative drawing of the position and structure of the choroid rete mirabile of teleost fish. Top: cross-section of the choroid rete and eye at the optic nerve. The choroid rete supplies the choroid, with highly oxygenated blood by means of the Root effect. Bottom: cross-section of the choroid rete and related structures. The fine arterial and venous capillaries are close to each other in the rete, allowing for transfer of CO_2 and protons from venous to arterial blood, and ultimately increasing arterial PO_2. Reprinted with permission from Wittenberg and Wittenberg (1974).

nal thickness (without Bruch's membrane or the RPE) was 360 μm (Wittenberg and Wittenberg, 1974). This calls into question whether the choroid is too distant to adequately supply such a metabolically active tissue. Therefore, the vertebrate retina must strike a balance between these two physical constraints: highly vascularized retinas would obscure light into the retina, while poor vascularization would impose limits on supply of metabolites, especially in the innermost retinal layers.

Vertebrates have adapted various solutions to this problem. In most teleost fish, blood from the ophthalmic artery passes through a rete system (the choroid rete mirabile, or the choroid rete) before reaching the choroid (Fig. 2) (Eastman and Lannoo, 2007; Wittenberg and Wittenberg, 1974). The choroid rete allows for increased partial pressure of O_2 (PO_2) in the eye by means of the Root effect, in which protons from venous capillaries can reach hemoglobin in arterial blood, and lower its binding affinity for O_2 (Waser and Heisler, 2005). It is unclear whether the rete developed primarily to increase PO_2 in the eye, or for some other function (e.g. making heat uniform throughout the choroidal layer) (Shih et al., 1993; Wittenberg and Wittenberg, 1974).

Fish with choroid retia attain massive PO_2 values in the retina – most often between 250 and 800 mmHg (Wittenberg and Wittenberg, 1974). For comparison, atmospheric PO_2 is ~155 mmHg, and the PO_2 of trout arterial blood is ~85 mmHg (Wittenberg and Wittenberg, 1974). Vitreal PO_2 also correlates with the size of the rete system supplying the choroid. Fish without retia, such as skates, rays, eels, and congers, have an average vitreal PO_2 of 8–18 mmHg (Wittenberg and Wittenberg, 1974). Certain predatory species, which rely heavily on sight, have eyes that bulge due to their large retia, and have a vitreal PO_2 of 1000–1300 mmHg (Wittenberg and Wittenberg, 1974). This might suggest that added O_2 improves retinal function and vision (Wittenberg and Wittenberg, 1974). Furthermore, choroid retia have evolved independently, in holosteans (in gar of the genus *Amia*) and in teleosts (Berenbrink et al., 2005; Wittenberg and Wittenberg, 1974).

The choroid rete is unique to fish, and so other vertebrates have different adaptations to perfuse the retina. Most birds and some reptiles have a structure called the pecten oculi, an outgrowth from the choroid whose role has been a mystery to scientists for over three centuries (Fig. 3) (Brach, 1977). Other reptiles have a related structure, the conus capillaris, whose role is at least as uncertain (Brach, 1976). The pecten is highly vascular. Its size is related to visual acuity (Kiama et al., 2001) and its capillaries have a unique morphology, with microfolds that drastically increase their surface area (Braekevelt, 1991). These features suggest that the pecten supplies metabolites to the retina, *en lieu* of blood vessels on the vitreal side (Braekevelt, 1991; Kiama et al., 2001). By removing the need for intraretinal vascularization, the pecten might partially explain visual acuity in certain birds like hawks (Ainsworth and Le Page, 2007), but it leaves a large blind spot in the visual field of birds (O'Rourke et al., 2010).

Interestingly, the pecten may agitate the vitreous humor to deliver metabolites to the retina. Using fluorescein angiography, Pettigrew et al. (1990) imaged the pecten. Fluorescein dye leaked slowly from the pecten into the nearby vitreous – within 0.5 mm of the pecten while the eye was still. However, saccadic eye movements caused the pecten to oscillate, fanning dye throughout the vitreous. This suggests that metabolites such as oxygen and glucose would diffuse from the pecten, and be propelled towards the retina by saccadic oscillations. Pecten oculi vary widely in size, and have been grouped into vaned (as in Fig. 3), cone-like, or pleated morphologies (Meyer, 1977). Pettigrew et al. (1990) propose that saccadic oscillations co-evolved with different pecten morphologies, to fan metabolites throughout the retina according to the anatomical and visual needs of the species.

Mammals do not have these specialized structures. Some mammals, such as guinea pigs and rabbits, have avascular or very poorly vascularized retinas, so that all metabolites arrive from the choroid (Fig. 1, left). This likely limits the thickness of the retina, to minimize diffusion distances (Yu and Cringle, 2001).

Other mammals (including mice, rats, and primates) have additional blood vessels on the inner, or vitreal, side of the retina (Huberman and Niell, 2011; Purnyn, 2013; Yu and Cringle, 2001) (Fig. 1, right). Among other factors, these vessels presumably affect visual acuity. For example, the vascularized periphery of the human retina has poor vision compared to the avascular fovea (Dowling, 1987; Kolb, 2003). Furthermore, mouse retinas have no fovea and are vascularized throughout, like the periphery of the human retina. This is thought to contribute in part to their extremely poor visual acuity (Huberman and Niell, 2011).

At least some retinas also have a high concentration of neuroglobin – a respiratory protein which is distantly related to myoglobin and hemoglobin (Schmidt et al., 2003). Its role in the retina is unclear, but by facilitating O_2 transport, neuroglobin may help to

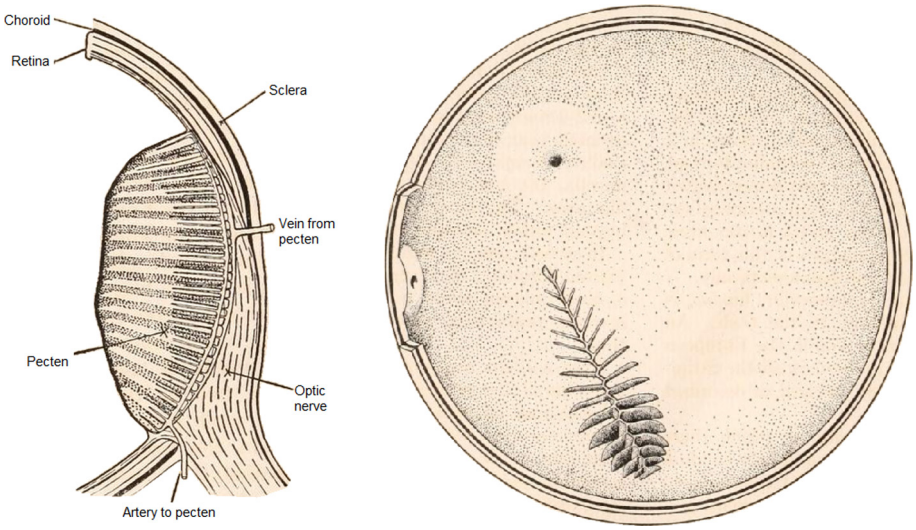

Fig. 3. The pecten oculi of birds. Left: cross-section of the pecten oculi of the sparrow (*Passer domesticus*). The artery to the pecten sends a branch along each fold; similarly, the vein from the pecten receives a branch from each fold. Right: the vaned pecten of the British Kingfisher (*Alcedo ispida*). Reprinted from C. A. Wood (1917).

overcome the large diffusion distance in the retina. This may explain the high expression of neuroglobin in the mouse eye (~100 μM) compared to the mouse brain (~1 μM). Neuroglobin may also be involved in hypoxia tolerance in some animals. For example, the hypoxia-tolerant goldfish has more than 3 times more neuroglobin in the eye compared to the less tolerant zebrafish (Roesner et al., 2008), but the mechanism of neuroprotection (if any) is unclear. It has also been suggested that respiratory proteins may allow for a temporary storage of oxygen, possibly for peaks of retinal activity (Roesner et al., 2008; Schmidt et al., 2003). Much more work is needed to clarify the role of neuroglobin, both in the eye and in neural tissue more generally.

At least in mammals, glucose supply to the retina is facilitated by ATP- and Na^+-dependent glucose transporters (GLUT) at the blood-retinal barriers (Kumagai, 1999; Wong-Riley, 2010). From the choroid, glucose passively diffuses across Bruch's membrane and is transported by GLUTs at the RPE (Booij et al., 2010; Vilchis and Salceda, 1996). In vascularized retinas, GLUTs also transport glucose across the non-fenestrated endothelial cells of vitreal blood vessels (Vilchis and Salceda, 1996). Once in the retina, most cells take up glucose with GLUT3, the common neuronal glucose transporter; Muller cells (the major retina glial cell) additionally have GLUT2 suggesting higher reliance on glucose (Kumagai, 1999). Glucose consumption is extremely high in the retina – threefold that of the cortex (Puchowicz et al., 2004). Compared to brain tissue, free glucose concentrations are higher in the retina (Tang et al., 2000). These high concentrations likely fuel glycolysis in times of high-demand, such as during flickering light (Ames, 1992).

3. Demand: what and where are the energy demands in the retina?

The brain is often touted as one of the most metabolically active human organ, and the retina may be the most demanding of the brain's tissues (Ames, 1992; Wong-Riley, 2010). The high cost of vision is exemplified by cases of regressive evolution: animals who evolve to live in darkness devolve their visual systems (Krishnan and Rohner, 2017; Rétaux and Casane, 2013). Most underground- or cave-dwellers regress their eyes, such as *Proteus* salamanders or the naked mole rat (*Spalax ehrenbergi*). This devolution is thought to spare the large energy cost of developing and maintaining retinas and visual processing centers in the brain (Rétaux and Casane, 2013). At least three variants of Mexican tetra (*Astyanax mexicanus*) have independently evolved to live in dark caves, away from a surface variant. Each cave variant has smaller optic tectums, small or absent eyes, and 5–15% less neural energy expenditure (Moran et al., 2015). This agrees with the notion that the retina is one of the most metabolically active tissues (Ames, 1992; Wong-Riley, 2010).

The biggest ATP demand comes from PRs and the RPE, which are both involved in phototransduction (Dowling, 1987). PRs are unique among sensory neurons, in that they are effectively turned off by their stimulus: they are depolarized in darkness by a high permeability to Na^+ (the "dark current"), and are repolarized by light (Hagins et al., 1970; Yau, 1994). PR outer segments have disks of membranes, packed with G-protein coupled receptors (GPCRs) containing light-sensitive photopigments (Ingram et al., 2016). When the photopigments absorb light, the GPCRs trigger a G-protein-dependent cascade that reduces the cytosolic concentration of cGMP. The reduced [cGMP] deprives Na^+-permeable cyclic nucleotide gated (CNG) channels of their ligand, which closes them and stops the dark current (Ingram et al., 2016).

This method of phototransduction has several implications for the energetics in the retina. First, energy expenditure in the retina is higher in darkness than in the light, because the dark current requires constant activity from the Na^+/K^+ ATPase (Ames, 1992). Indeed, in darkness, Na^+/K^+ ATPase activity accounts for over 50% of the total energy expenditure (Ames et al., 1992). Secondly, cGMP turnover consumes 13% of energy in the light (Ames, 1992). Thirdly, in light, NADPH-dependent restoration of chromophores and phosphorylation of the GPCRs like rhodopsin require further energy (Hemmer et al., 1993). The RPE also expends a substantial portion of energy supporting PR function: the RPE absorbs and degrades damaged disks from the outer segments; it is necessary for restoring the photopigment in cones; and it expends energy in transport to supply the retina with metabolites (Dowling, 1987; Kolb, 2003).

PR synaptic pedicles release neurotransmitter vesicles to bipolar and horizontal cells in darkness, at an enormous rate – enough that specialized organelles, called synaptic ribbons, have developed to facilitate increased release (Baden et al., 2013). In electron micrographs, these appear as electron-dense strips surrounded by arrays of vesicles (Haverkamp et al., 2000). Loading so many vesicles likely requires large amounts of ATP to power V-type ATPases (Warren et al., 2016). Key energy demands of PRs are illustrated in Fig.4A.

One might wonder, how do PRs supply the large energy requirement of their outer segments and pedicles, if PR mitochondria are located in their inner segments? It was thought that ATP and GTP had short diffusion lengths, so they could not travel from mitochondria directly to sites of energy use (Hemmer et al., 1993). Furthermore, ATP would be rapidly consumed by ion pumps in the synaptic pedicle, before reaching sites where vesicles would be loaded with neurotransmitter (Linton et al., 2010).

Instead, energy is transported to at least some of these areas by means of a phosphocreatine (PC) shuttle (Hemmer et al., 1993; Linton et al., 2010) (Fig. 4B). In support of this notion, Hemmer et al. (1993) found immunohistochemical evidence for two types of creatine kinase (CK) in bovine PRs: mitochondrial CK in inner segments, and brain-type CK in outer segments. Further evidence for CK in PRs came from creatine kinase activity in isolated bovine outer segments (Hemmer et al., 1993). This led these authors to propose a model in which mitochondrial CK phosphorylates creatine into PC, at the cost of ATP. In their model, brain-type CK catalyzes the opposite reaction, restoring ATP at sites of energy demand in the outer segments.

However, other studies in chick, salamander, zebrafish, and mice found no immunological or biochemical evidence for brain CK in outer segments (Linton et al., 2010; Sistermans et al., 1995; Wallimann et al., 1986). Instead, brain-type CK activity was shown to be necessary for vesicle release at the PR synapse: PR neurotransmitter release was inhibited by a specific CK inhibitor (fluorodinitrobenzene), and was abolished in brain-type CK knockout mice (Linton et al., 2010). Despite the disparity in outer segments among species, reports in all species suggest that PC is crucial for PR function.

Interestingly, Linton et al. (2010) found vascular retinas to have mitochondria in PR pedicles. This would reduce the need for the PC shuttle, but would instead derive ATP from the extra oxygen supplied to the retina.

In addition to the high demand of phototransduction, other regions of high energy demand include the two plexiform layers of the retina where most neurotransmission occurs (Yu and Cringle, 2001). The first of these layers (the outer plexiform layer) includes synapses between PRs, and bipolar and horizontal cells. The second (the inner plexiform layer) includes connections between ganglion cells, and bipolar and amacrine cells (Dowling, 1987; Kolb, 2003). These synapses require constant energy for neurotransmission and for maintaining membrane potential. Consequently, neuroglobin and mitochondria are prevalent in these

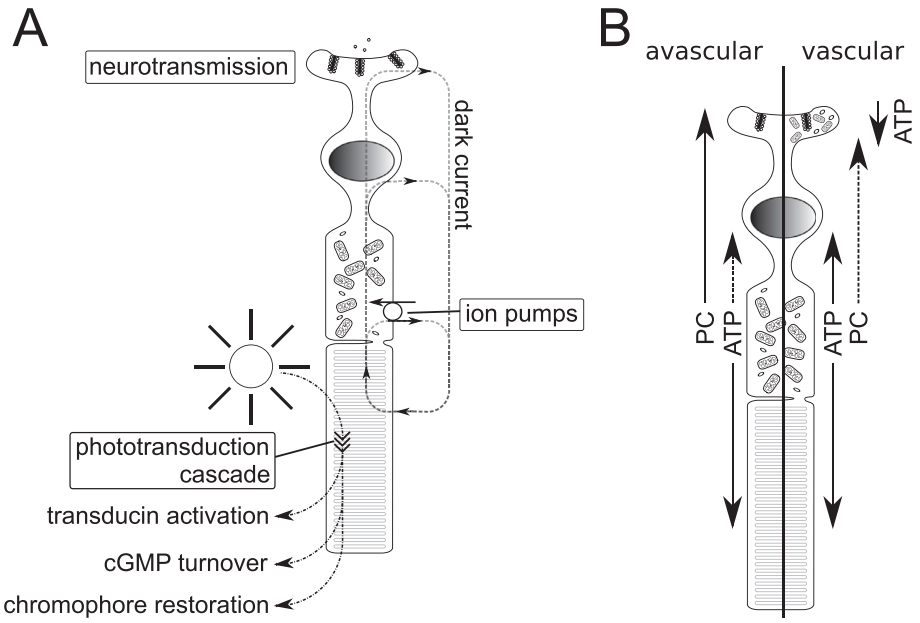

Fig. 4. *A.* Energy demands of vertebrate photoreceptors. In the light, the phototransduction cascade requires energy to activate transducin, support cGMP turnover, and restore chromophores (e.g. 11-cis-retinal). In the dark, cations enter through outer segment membranes, depolarizing the cell. Especially in the inner segment, soma, and pedicles, Na^+ and Ca^{2+} are actively transported (by ion pumps) out of the cell at the cost of ATP. At the synaptic pedicles, ATP is required in the dark for neurotransmission (e.g. loading neurotransmitters into vesicles). *B. Left:* Energy supply within a vertebrate photoreceptor. Mitochondria in the inner segment produces ATP to supply outer segments. Especially in avascular retinas, phosphocreatine (PC) is created by mitochondria in the inner segment and diffuses to the synaptic pedicle to restore ATP for neurotransmission. Some species may additionally shuttle PC to outer segments (not shown). *Right:* Vascular retinas have additional mitochondria in synaptic pedicles. There they produce ATP, so that PC shuttling is less important. Based off experiments in Hemmer et al., 1993 and Linton et al., 2010.

areas in vascular retinas, presumably to support their large energy demand (Bentmann et al., 2005; Kageyama and Wong-Riley, 1984; Schmidt et al., 2003). One caveat here is that avascular species have far fewer mitochondria, and they are located almost entirely in the PR inner segments (Bentmann et al., 2005). This difference in mitochondrial expression correlates with oxygen availability (Bentmann et al., 2005; Schmidt et al., 2003; Yu and Cringle, 2001). Avascular retinas rely on choroidal capillaries for oxygen, and thus have mitochondria only in the nearest retinal cells: the PRs. Vascularized retinas additionally receive oxygen from blood vessels in the plexiform and ganglion cell layers of the retina; accordingly, mitochondria have been localized to these layers (Bentmann et al., 2005).

One unique characteristic of retinal metabolism is a high usage of glucose (Kumagai, 1999; Tang et al., 2000) – a feature which has never been adequately explained. Glycolysis seems to be especially important in the retina, both during rest and during activity. For example, in the cat, glycolysis is responsible for 80% of glucose consumption in the retina, even in the presence of oxygen (Wang et al., 1997). Neurotransmission in the rabbit retina is thought to be largely dependent on glycolysis, and that bursts of flickering activity increased glycolysis by 48%, and increased lactate production 2.3-fold (Ames et al., 1992; Ames, 1992).

There are many mysteries regarding glycolysis and lactate production in the retina, especially regarding the main glial cell in the retina (Müller cells). Early studies tracked glucose consumption with ^{14}C-labelled glucose and ^{3}H-2-deoxyglucose in guinea pig retinas. They included evidence that lactate is produced by glycolysis in Müller cells, and is shuttled to PRs for conversion to pyruvate to fuel oxidative metabolism (Poitry-Yamate et al., 1995; Poitry-Yamate and Tsacopoulos (1992)). But a later study in mouse and rat retinas found that Müller cells were deficient in pyruvate kinase (and so could not produce pyruvate, a precursor for lactate). They also produced little lactate in culture, but were found to metabolize isotopically labelled lactate and aspartate produced in PRs (Lindsay et al., 2014). These authors proposed a detailed model in which PRs produce lactate, which Müller cells oxidize to pyruvate to sustain oxidative metabolism. Therefore, in both the guinea pig and the rat models, Müller cells and PRs have a lactate shuttle which supports oxidative metabolism – yet the producing and consuming cells are different. I propose that these models are compatible, if this is another difference due to vascularity: perhaps vascular retinas (like those of the rat) can afford to release lactate from PRs (Lindsay et al., 2014), as they have mitochondria to generate energy in their synaptic pedicles (Linton et al., 2010). The only mitochondria in avascular retinas (such as that of guinea pigs) are in inner segments (Bentmann et al., 2005). In these retinas, lactate is more likely to be produced in Müller cells (and possibly cells of the poorly perfused outer retina), to supply the mitochondria in PR inner segments. Such a 'lactate shuttle' has been proposed before in the retina, where O_2-poor cells would produce lactate through anaerobic glycolysis, which could then be shuttled for oxidation in O_2-rich cells closer to afferent blood vessels (Ames, 1992).

Lactate is readily and preferentially oxidized in mitochondria (Brooks, 2009), and evidence for lactate shuttles is growing in biology. For example, lactate produced during aerobic exercise was found to be a primary fuel source for the heart in dual-carbon labelled experiments (Gertz et al., 1988), and immunohistochemical and pharmacological evidence supports the presence of an astrocyte-neuron lactate shuttle in rats (Erlichman et al., 2008; Hashimoto et al., 2008). Therefore, the presence of lactate shuttles in the retina merits further study, and may prove to explain the high glucose consumption in the retina even in the presence of oxygen.

4. Supply and demand must be in equilibrium to prevent disease

ATP supply fails to match demand when oxygen or blood supply are restricted (ischemia). This is part of the pathology of numerous

retinal diseases, including diabetic retinopathy, glaucoma, retinopathy of prematurity, and retinal artery occlusions, among others (Almasieh et al., 2012; Osborne et al., 2004; Szabadfi et al., 2010). This section will briefly describe how ischemia or hypoxia arises in these major retinal diseases. It will conclude with how ischemia leads to neuronal death along a common pathway in each disease.

Although the biological basis for glaucoma is not completely understood, intraocular pressure (IOP) is a major and consistent risk factor (Weinreb et al., 2014). IOP is associated with poor drainage of aqueous humor from the anterior chamber of the eye (Almasieh et al., 2012; Brusini and Johnson, 2007; Weinreb et al., 2014). High IOP distends the lamina cribrosa – a flexible, mesh-like structure at the optic disk, where the optic nerve and the central retinal artery first enter the eye (Schmidt et al., 2008). As the lamina cribrosa is misshapen by IOP, it pinches axons of the optic nerve and constricts the central retinal artery (Schmidt et al., 2008). As a result, nerve growth factors and other cargo fail to travel along axons to maintain ganglion cells (Almasieh et al., 2012; Schmidt et al., 2008). Furthermore, the central retinal artery can no longer adequately supply blood vessels in the inner retina, decreasing metabolite supply, reducing ATP production, and triggering ischemic cell death (Almasieh et al., 2012).

Artery or vein occlusions can also deprive parts of the retina of blood. These occur when a thrombus or other embolus occludes a retinal artery, leading to ischemic damage and partial blindness (Hayreh and Zimmerman, 2005; Hayreh et al., 2009; Osborne et al., 2004). The most common type is a central retinal artery occlusion (CRAO), which starves the entire inner retina of blood flow. This can lead to complete blindness in that eye (Hayreh and Zimmerman, 2005). Branch retinal artery occlusion (BRAO) involves a blockade in a branch of the central retinal artery, and can lead to partial blindness in the ischemic areas (Hayreh et al., 2009).

Diabetic retinopathy starts with an oversupply of glucose. Hyperglycemia is thought to lead to biochemical and cellular changes in the retinal microvasculature, although the exact mechanisms are unknown (Caldwell et al., 2003; Puchowicz et al., 2004; Tang et al., 2000). Notably, pericytes which help contract retinal blood vessels are damaged or lost, so that the blood vessels cannot autoregulate to control blood flow (Caldwell et al., 2003; Osborne et al., 2004). This can lead to localized areas of ischemia, as well as leakage, edema, and angiogenesis – all of which are thought to impair vision (Caldwell et al., 2003).

Just as diabetic retinopathy starts with excess glucose, age-related macular degeneration (AMD) is thought to be caused by excess metabolic by-products (Jager et al., 2008; Schmidt et al., 2008). These by-products accumulate between the RPE and Bruch membrane, increasing the diffusion distance from the choroid to the PRs and thus making the outer retina hypoxic (Jager et al., 2008; Schmidt et al., 2008; Stefansson et al., 2011). Hypoxia also leads to angiogenesis, as in diabetic retinopathy (Stefansson et al., 2011).

Hypoxia is also part of the pathology of retinopathy of prematurity. Preterm neonates are regularly given supplemental oxygen after birth. Although the extra oxygen increases survival rates, it reduces endothelial growth factors such as erythropoietin and VEGF, and therefore arrests vascular development in the retina (Hellström et al., 2013). After removal from hyperoxia, the retina becomes hypoxic, which increases erythropoietin and VEGF and leads to a proliferation of blood vessels (Hellström et al., 2013). These new blood vessels are often leaky and can lead to retinal detachment (Repka et al., 2006; Hellström et al., 2013). It is noteworthy that hypoxia can induce angiogenesis and lead to vision loss in diabetic retinopathy, AMD, retinopathy of prematurity, and several other vascular eye diseases (Stahl et al., 2010).

In all the above conditions, ischemia contributes to a common outcome. Ischemic neurons are thought to die by a process called excitotoxicity, which occurs as follows (Choi, 1992; Lipton, 1999; Szabadfi et al., 2010): without O_2 and glucose from blood flow, ATP production declines. This reduction in cytosolic ATP starves Na^+/K^+ ATPases, leading to ion dysregulation across neuronal membranes. The resulting depolarization opens voltage-gated Ca^{2+} channels, which lead to a toxic level of cytosolic Ca^{2+}. Depolarization also releases more excitatory neurotransmitters, which further increase cytosolic Ca^{2+} levels (for example, through Ca^{2+}-permeable glutamate receptors). Without ATP to restore ion gradients, neurons can swell excessively due to osmosis, and Ca^{2+} can lead to neuronal death through Ca^{2+}-dependent lipases and proteases.

Not all cells are equally sensitive to ischemia or hypoxia, however. The inner retina seems especially susceptible to ischemia in a variety of preparations (Hughes, 1991; Peachey et al., 1993; Rosenbaum et al., 1998; Osborne et al., 2004). For example, in histological experiments in rats examining retinal histology after IOP-induced ischemia, inner retinal layers begin to lose thickness after 60 min, compared with 90 min in the outer layers (Hughes, 1991). In ERGs of isolated and arterially perfused cat eyes, ischemia decreased b-wave responses to 17% of their original value, compared to an a-wave decrease of 60%, suggesting that photoreceptor responses were less susceptible than downstream neurons (Peachey et al., 1993). There is no consensus as to how photoreceptors are more resistant to ischemia. It has been proposed that neuroglobin may play a role in oxygen consumption, as it localizes preferentially to areas of high oxygen consumption in the retina, including photoreceptor inner segments and the outer plexiform layer (Schmidt et al., 2003; Osborne et al., 2004).

The difference in susceptibility to ischemia or hypoxia may also relate to energy demands. Glutamate receptors such as NMDARs and certain AMPARs are Ca^{2+}-permeable (Szydlowska and Tymianski, 2010), so that after they are opened, ATP is required to extrude Ca^{2+} and restore cytosolic Ca^{2+} concentrations. It has been noted that ischemia-sensitive cells in the inner retina, such as ganglion cells and amacrine cells, often have high expressions of these excitatory neurotransmitter receptors; in contrast, more hypoxia-tolerant cells such as photoreceptors do not (Brandstätter et al., 1994; Osborne et al., 2004; Schmidt et al., 2008).

5. Conclusion

Retinal research is marked by a strong emphasis on comparative physiology. Blood flow to the eye is remarkably different among vertebrates, even within mammals. However, the effect of how vascularization affects metabolism in the retina is only starting to be understood (Bentmann et al., 2005; Yu and Cringle, 2001), and critical players such as neuroglobin have only recently been discovered (Schmidt et al., 2003). Metabolic demand in the eye is even more uncertain, especially in regard to the extraordinarily high dependence on glucose (Puchowicz et al., 2004; Tang et al., 2000; Vilchis and Salceda, 1996) and the enigmatic role of lactate (Lindsay et al., 2014; Poitry-Yamate et al., 1995; Poitry-Yamate and Tsacopoulos (1992)). It is quite possible that vascular and avascular retinas will have different energetic demands and adaptations (Bentmann et al., 2005; Yu and Cringle, 2001), marking a distinction that could change our use and comparison of animal models of retinal diseases. This is especially true for models of retinal diseases which involve ischemia and excitotoxic cell death: glaucoma, retinal artery occlusions, diabetic retinopathy, and AMD (Osborne et al., 2004; Schmidt et al., 2008; Stefansson et al., 2011). This could help us better understand, and prevent, several leading causes of blindness throughout the world.

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