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Mini Review

Oxygen delivery, consumption, and conversion to reactive oxygen species in experimental models of diabetic retinopathy

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ABSTRACT

Retinal tissue receives its supply of oxygen from two sources – the retinal and choroidal circulations. Decreases in retinal blood flow occur in the early stages of diabetes, with the eventual development of hypoxia thought to contribute to pathological neovascularization. Oxygen consumption in the retina has been found to decrease in diabetes, possibly due to either a reduction in neuronal metabolism or to cell death. Diabetes also enhances the rate of conversion of oxygen to superoxide in the retina, with experimental evidence suggesting that mitochondrial superoxide not only drives the overall production of reactive oxygen species, but also initiates several pathways leading to retinopathy, including the increased activity of the polyol and hexosamine pathways, increased production of advanced glycation end products and expression of their receptors, and activation of protein kinase C.

Oxygen supply to the retina

The oxygen demand of the retina is high, with the retina among the most metabolically active tissues in the body. The retina is in a constant state of activity, converting light into neural signals, and with the activity continuing (and oxygen consumption even increasing) in the dark. The cells of the retina require a constant supply of oxygen; however, a dense vascular network anterior to the photoreceptors would interfere with light transmission to these light-sensing cells. To circumvent this potential interference, the outer one-third to one-half of the retina receives almost all of its oxygen via the choroidal microcirculation, which is just posterior to the retinal tissue (see Fig. 1). The choroidal circulation branches from the posterior ciliary arteries, which in turn derive from the ophthalmic artery. The inner one-half to two-thirds of the retina receives its requirement of oxygen from the retinal circulation, which is a separate branch (via the central retinal artery) of the ophthalmic circulation.

The retinal and choroidal circulations differ in their vascular density, autoregulatory control, and their arterio-venous oxygen differences, but both circulations are critical for supplying sufficient oxygen to the entire retina. Using microelectrodes, Yu et al. [1,2] have measured oxygen partial pressures in the retinas of mice and rats, and have found a very sharp decline from the retinal tissue near the choroid (~45 mmHg; see Fig. 1), with oxygen tension dropping to ~10 to 15 mmHg just anterior to the photoreceptors, consistent with the high metabolic rate of these neurons. Similar profiles have been obtained for pigs and cats [3]. The high consumption of oxygen in the outer retina prevents the choroidal circulation from providing sufficient oxygen to the inner retina in most mammals, excluding rabbits and guinea pigs. The contribution of the retinal microcirculation to the oxygenation of the inner retina was demonstrated by Yu et al. [2], with experiments in which the retinal circulation of rats was occluded by laser (retinal ischemia in Fig. 1), and measurements of oxygen tension were close to 0 mmHg throughout the inner retina. This experimental protocol additionally allowed the researchers to model the oxygen consumption of the inner retina, since the presence of flowing vessels in the tissue interferes with such analysis. Their analysis indicated that oxygen consumption in the inner retina is substantial, and of the same magnitude as that of the outer retina.

The choroidal circulation is much more dense than the retinal circulation (Fig. 1), with the sparse nature of the latter important for light transmission through the retina as mentioned previously. However, the limited retinal circulation has been speculated to contribute to the vulnerability of the retina to vascular disease [4].

Utilization of oxygen in the retina

As mentioned, oxygen profiles have helped to identify the sites of oxygen usage in the retina, with three dominant layers of consumption identified in the rat as the inner segment of the photoreceptors (where the mitochondria are localized), the outer plexiform layer (OPL in Fig. 1) and the deeper region of the inner plexiform layer (IPL)

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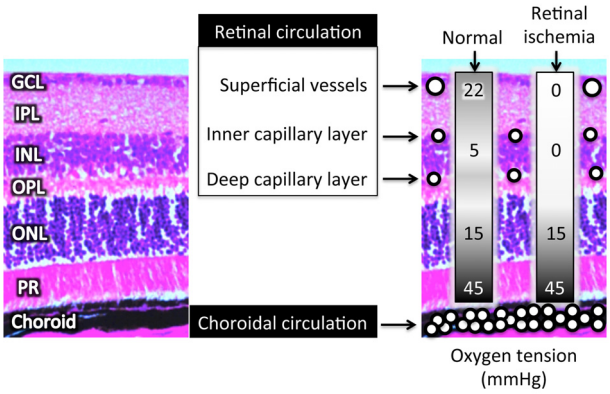

Fig. 1. Shown to the left in the figure are the distinct layers of the retina, as seen in a hematoxylin–eosin stained cross-section of a mouse. The layers are the ganglion cell layer (GCL), the inner plexiform layer (IPL), the inner nuclear layer (INL), the outer plexiform layer (OPL), the outer nuclear layer (ONL), and the photoreceptors (PR). The retina receives its oxygen from two different circulations, the retinal circulation that feeds the inner retina, and the choroidal circulation that feeds the outer retina, with cross-sectional depictions of these blood vessels superimposed on the picture at the right. Values of retinal oxygen tension are shown in the boxes to the right, with the values demonstrating the importance of the retinal circulation to the inner retina as described further in the text.

[4]. Accordingly, these three layers of the retina are also where retinal cross-sections stain most intensely for activity of cytochrome oxidase [5], the enzyme in the final step of the mitochondrial respiratory chain in which oxygen is consumed in the production of ATP. Much of the oxygen may be used for synaptic transmission [2], Na–K ATPase activity, phototransduction [6], and processes that require substantial amounts of energy such as the maintenance of the dark current and the generation of guanosine triphosphate [3].

Oxygen consumption increases in the dark, with this increase resulting in almost complete tissue anoxia in certain areas of the cat retina [7]. However, according to the data of Yu and Cringle [8], the development of anoxia in the dark is not a general property of mammalian retinas, in as much as oxygen tension in the outer retina does not decrease below 5 mmHg in dark-adapted rats, despite an approximate 50% increase in oxygen consumption. Yu and Cringle explained the lack of dark-induced anoxia in the rat retina as being due to a significant increase in oxygen delivery. In animals in which darkness induces hypoxia, it could be speculated that the hypoxia and/or the subsequent reoxygenation could induce an oxidative stress; however, it is not clear whether experimental evidence has supported this possibility.

Regulation of retinal blood flow by oxygen

Oxygen concentrations help regulate retinal blood flow. Not only does hypoxia stimulate increased perfusion and potentially angiogenesis, but the reverse also is true, whereby hyperoxia decreases retinal blood flow. The latter phenomenon has been demonstrated by Zhu et al. [9] in experiments in which newborn pigs breathed 100% oxygen, causing retinal blood flow to decrease by ~40% within 5–10 min. This response was attenuated by treatments aimed at several endogenous vasoconstrictors including thromboxane, endothelin, and 20-HETE (20-hydroxyeicosatetraeonic acid), but not by the antioxidant combination of superoxide dismutase and catalase. In comparison, diabetes-induced decreases in retinal blood flow rate in rodents (decreases of ~33%) have been found to be attenuated not only by targeting thromboxane [10–13], endothelin [14], 20-HETE [15], and angiotensin II [11], but also by scavenging superoxide with tempol [16], with the latter implicating reactive oxygen species (ROS) in retinal blood flow control during diabetes. Whether or not oxygen levels regulate these vasoconstrictor pathways in the diabetic retina has yet to be clarified.

Altered metabolism in the diabetic retina

Diabetes-induced decreases in retinal oxygen consumption have been reported for rabbits [17,18] and cats [19], but with conflicting reports of arterio-venous oxygen differences and consumption in the diabetic rat retina [20–22]. However, Obrosova et al. [23] have studied retinal metabolism in streptozotocin (STZ)-induced diabetes in both rats and also mice, at a time point of 6 weeks. They measured several metabolites in the retina including pyruvate, lactate, glutamate, α-ketoglutarate, ammonia, and free NAD^+/NADH. In rats, each of these were decreased significantly by ~30% to 50%, but only two of the metabolites were decreased in mice (pyruvate by 10%, and ammonia by 15%). In humans, arterio-venous oxygen differences have been reported to decrease throughout all stages of retinopathy [24]. In as much as early decreases in retinal blood flow have been found in diabetic patients [25], it is likely that oxygen consumption rates simultaneously decrease as is the case in several experimental models of the disease. Any decrease in retinal oxygen consumption, whether transient or sustained, could result in a decrease in retinal blood flow, with Small et al. [26] and Rimmer and Linsenmeier [27] speculating that this mechanism could be operative in the diabetic retina.

Neuronal cell death may contribute to the decrease in metabolism reported in the diabetic retina. STZ-induced diabetes in the rat has been found in one study to produce retinal cell death in the time frame of 1–12 months [28], and in another study, the number of apoptotic photoreceptors in the STZ rat continued to increase throughout the period from 4–24 weeks of hyperglycemia [29]. The increase in neuronal cell death in the retina also has been reported in mice injected with STZ [30,31], and in the $Ins2^{Akita}$ diabetic mouse [31]. Cell death contributes to the phenomenon of retinal thinning, which is found for both rodent [30,32] and human [33–36] diabetes.

Altered oxygen levels in the diabetic retina

The development of diabetic retinopathy is hypothesized, in part, to be a function of retinal hypoxia leading to the uncontrolled growth of new retinal blood vessels [37]. Interestingly, investigators using oxygen microelectrodes have found no signs of retinal hypoxia within the first year of experimentally induced diabetes in cats and dogs [38–40]. Our own lab has studied diabetic mice and rats, and despite finding 20–45% decreases in retinal blood flow rates [10–16], we have not seen significant signs of hypoxia (measured with the probe pimonidazole, and by immunostaining for the hypoxia-inducible factor HIF-1α) early in the progression of hyperglycemia (1–6 months) [41–43]. In fact, we have detected a possible phase of increased retinal oxygen in rats at a time point of 3 months diabetes [41], with this surprising finding also observed in the inner retina by Lau and Linsenmeier [44] at the same time point, using a more direct measurement with oxygen microelectrodes. However, other groups have found significant increases in HIF-1α and/or pimonidazole staining [45–48], and therefore, more studies may be required to resolve the time course of either hypoxia or hyperoxia early in the progression of experimental and human diabetes.

Even though the most direct measure of retinal oxygen, with microelectrodes, has not found hypoxia during the early weeks to months in experimental diabetes, Linsenmeier et al. [19] used this technique to reveal significant hypoxia at a much longer duration (6–7 years of diabetes) in cats, with inner retinal oxygen tension decreased by almost 50% compared to controls.