Intraretinal Oxygen Distribution in the Rat with Graded Systemic Hyperoxia and Hypercapnia

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Purpose. To describe the nature of oxygen level changes in specific layers in the rat retina under graded levels of systemic hyperoxia, with and without hypercapnia.

Methods. Oxygen-sensitive microelectrodes were used to measure oxygen tension as a function of depth through the retina of anesthetized, mechanically ventilated rats. Breathing mixtures were manipulated to produce stepwise increments in systemic oxygen levels, with or without 5% CO_2. Retinal arteriovenous oxygen differences were also measured as an indicator of oxygen delivery through the retinal circulation. Systemic blood gas levels were measured under each condition.

Results. Hyperoxia increases Po_2 throughout the retina to a varying extent in different retinal layers, with the increase more pronounced in the outer retina than in the inner retina. Simultaneous hypercapnia results in further increases in retinal oxygen levels. The lowest intraretinal oxygen level was consistently found in the inner plexiform layer (IPL), between the two capillary layers that support this region. There was a greater than fourfold increase in oxygen supply from the choroid with hyperoxia but, remarkably, the retinal circulation continued to provide a net delivery of oxygen to the retina.

Conclusions. Hyperoxia results in a significant but nonuniform increase in oxygen level in all layers of the rat retina, which is augmented by hypercapnia. The persistence of a minimum oxygen level in the IPL, despite the dramatic increase in oxygen flux from the choroid, suggests that oxygen consumption increases significantly in the IPL under hyperoxic conditions. (*Invest Ophthalmol Vis Sci.* 1999;40:2082–2087)

Oxygen is the primary biologic oxidant for energy production in mammalian cells.[1] Alterations in oxygen supply to the retina are thought to play a role in many retinal diseases, and there have been numerous attempts at manipulation of the intraretinal oxygen environment as part of a therapeutic strategy. Retinal vascular occlusion has been treated with systemic hyperoxia alone[2] (to increase retinal oxygen levels) or in combination with hypercapnia (to prevent the oxygen-induced contraction of the vessels), but the effect that this therapy has on intraretinal oxygen levels has yet to be quantified, even in a normal retina. Supplemental oxygen therapy in animal models of degenerative retinal diseases has recently been reported to slow down the progression of the disease if delivered at the appropriate stage.[3] Oxygen therapy has also been reported to be beneficial in human patients with RP.[4] In the developing retina in man and animals, the oversupply of oxygen can also lead to sight-threatening complications, and strategies for ameliorating the retinal consequences of systemic oxygen level changes have been put forward.[5,6]

Given the potential importance of strategies intended to manipulate the retinal oxygen environment by adjusting systemic parameters such as arterial oxygen and CO_2 levels, it would be valuable if we were able to predict reliably the effect that such manipulations create within the retina. However, there are several confounding factors that make this task more difficult than might be anticipated.

The retina in man and most mammals receives its oxygen from both the retinal and the choroidal vascular systems, which have markedly different regulatory properties. There is also evidence that the two distinct capillary layers of the retinal circulation may react differently to systemic perturbations.[7] The relationship between oxygen content of the blood and the Po_2 level is greatly influenced by the properties of the hemoglobin saturation curve. The heterogeneous nature of the oxygen uptake in different retinal layers has been confirmed for the outer retina[8] and may well be a feature of the inner retinal layers. The possibility that oxygen uptake may be influenced by oxygen level per se[9,10] is a further confounding factor that limits our ability to predict intraretinal oxygen distribution in the face of systemic manipulations.

The growing use of rat models of retinal disease[11,12] makes understanding the systemic parameters that influence the intraretinal oxygen environment in this species particularly important if subtle differences between healthy and diseased animals are to be exposed.[11–13] The rat, in common with humans and primates, has a central retinal artery that branches out to form the retinal vascular network. This is not the case in most other animals, such as the cat and pig, in which the majority of intraretinal measurements of this type have been made.[14–16]

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Because microelectrode-based technologies can be used to measure intraretinal oxygen tension as a function of retinal depth, direct measurements can be made under each of the systemic conditions of interest. We have demonstrated that such measurements can be closely correlated with key intraretinal landmarks such as the inner limiting membrane (ILM), the superficial and deep retinal capillary layers, the localized oxygen uptake of the inner segments of the photoreceptors, and the penetration of Bruch's membrane in the rat.[7] Comparison with histologic sections can then be used to determine the oxygen environment within the vascular structures and in each of the cell layers.[7] To date, there has been no reported measurement of intraretinal oxygen distribution during stepwise increases in systemic arterial oxygen levels, nor has there been an intraretinal investigation into the effects of systemic hypercapnia. This report is intended to fill this gap in our knowledge and to serve as baseline data for comparison with future studies in rat models of retinal disease. This information is also likely to improve our understanding of the basic physiology of the mammalian retina in terms of the oxygen environment and the oxygen requirements of different retinal cell classes.

METHODS

Animal Preparation

The rats (male, Sprague–Dawley) were housed two per cage on sawdust with a 12 hour–12 hour light–dark cycle. Ambient light levels were approximately 50 lux. They were fed standard laboratory rat chow with water ad libitum. On the day of the experiment the rat was anesthetized with an intraperitoneal injection of 100 mg/kg 5-ethyl-5-(1′-methyl-propyl)-2-thiobarbiturate (Inactin, Byk Gulden, Konstantz, Germany). Atropine sulfate (20 mg) was administered intramuscularly to minimize salivation. The trachea was cannulated for mechanical ventilation, the left internal jugular vein for venous infusion, and the femoral artery for continuous blood pressure monitoring and occasional aspiration of arterial blood (60 μl) for blood gas analysis (model 238; Ciba–Corning, Medfield, MA). The rat was then mounted prone in a modified Stellar stereotaxic system (model 51400; Stoelting, Chicago, IL) and the head fixed in position. The rat was paralyzed with a loading dose of 8 to 16 mg gallamine triethiodide, (40 mg/ml, Flaxedil; May and Baker, Dagenham, UK) into the jugular vein and artificially respired (rodent respirator, model 683; Harvard Apparatus, Holliston, MA) with a ventilation rate of 90 breaths/min and a tidal volume appropriate to ensure normal arterial P_{CO_2} levels with air ventilation. Rectal temperature was monitored and maintained at 37.5°C by a homeothermic blanket (Harvard Apparatus). All procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Ocular Surgery

The left eye was used for all experiments. The pupil was dilated with 1% tropicamide (Mydriacyl; Alcon, Sydney, Australia). The upper eyelid was partially removed, and an eye ring was sutured to the conjunctiva at the limbus and fixed to the stereotaxic framework. A small incision was made in the superior nasal quadrant with a diamond knife, just posterior to the limbus to allow entry of the microelectode. Damage to the larger choroidal vessels or posterior lens capsule was avoided. A planoconcave contact lens was placed on the cornea to allow the vitreous and the fundus to be visualized using an operating microscope during all intraocular manipulations.

Intraretinal Oxygen Profiles

The microelectrode techniques were similar to those reported in our earlier publications,[7,11,17] except that our electrode orientation and manipulation system was upgraded to a fully motorized system[18] under either handheld joystick control or under automatic computer control during a measuring sequence. Whalen-type recessed oxygen-sensitive microelectrodes[19] were manufactured and calibrated in our own laboratory. The microelectrode entered the left eye through the entry hole, which was also the locus of rotation of our microsurgical system, so that rotation of the positioning system pivots about the entry point into the eye. The small size of the electrode tip (1 μm) coupled with electrode beveling techniques and the high-acceleration piezoelectric translation of the electrode produced highly reproducible measurements of intraretinal and preretinal oxygen distribution. Intraretinal oxygen profiles were measured in the inferior retina, approximately 2 to 3 disc diameters from the disc margin. Experience has shown that highly reproducible oxygen measurements and identification of key intraretinal landmarks can be made in this region.[7] The electrode tip was placed at the surface of the chosen area of retina under microscope observation. The electrode was then stepped through the retina, under computer control, until a peak oxygen level was reached within the choroid. The measurement was repeated during stepwise withdrawal of the electrode. Although very close agreement between the insertion and withdrawal profiles was routinely achieved, the withdrawal profiles were used for data analysis, because they tended to be less influenced by artifacts associated with mechanical stress on the electrode tip during penetration. Preretinal vitreous measurements were performed by orienting the electrode to touch the surface of a retinal artery or vein. The oxygen tension measured by the microelectrode and systemic conditions such as arterial blood pressure and rectal temperature were recorded continuously on an eight-channel chart recorder (LR8100, Yokogawa, Tokyo, Japan). The readings of each channel were also accessed every two seconds through a computer interface (GPIB; IEEE) and the data logged directly to a spreadsheet along with the relative position of the microelectrode. All experiments were performed in photopic conditions.

Systemic Conditions

Ventilation mixtures were selected in increasing percentages of oxygen from 20% to 100% in increments of 20%, in either the presence or absence of 5% CO_2. No correction to the oxygen percentage was applied when 5% CO_2 was used; thus, the corresponding oxygen percentages in the hypercapnia trial ranged from 19% to 95% oxygen. Intraretinal and vitreal oxygen measurements and blood gas analysis were repeated under each ventilatory condition. Experiments usually lasted 8 hours, after which the rat was killed with an anesthetic overdose.

Statistics

All average values are stated as means ± SE. Significant differences were determined using Student's *t*-test, with $P < 0.05$ accepted as significant. Linear regression curve fits were per-

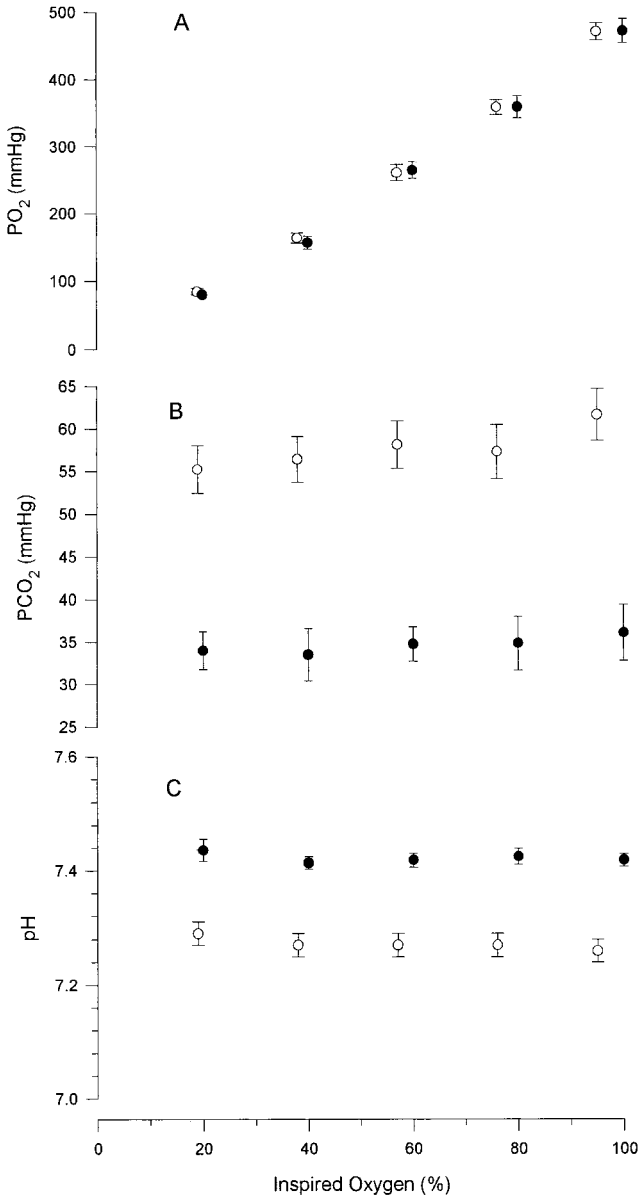

FIGURE 1. Mean systemic arterial blood gases at each ventilatory oxygen level under normocapnic (filled circles; $n = 8$, ±SEM) and hypercapnic (open circles; $n = 11$; ±SEM) conditions. **(A)** Po_2, **(B)** Pco_2, **(C)** pH.

formed using commercial software (SigmaPlot; Jandel Scientific, Corte Madera, CA).

RESULTS

Blood Gas Data

The systemic arterial blood gas data for increasing levels of ventilatory oxygen percentage without any additional CO_2 are shown as filled circles in Figure 1 ($n = 8$). The air-breathing values of Pao_2 80.5 ± 3.6 mm Hg, $Paco_2$ 34.0 ± 2.3 mm Hg, pH 7.44 ± 0.02, were in agreement with our earlier work on Sprague–Dawley rats.[12] As anticipated, increasing levels of inspired oxygen led to an approximately linear increase in systemic arterial oxygen levels, up to 473 ± 17.9 mm Hg, with $Paco_2$ and pH not significantly affected. The systemic arterial blood gas data for increasing levels of ventilatory oxygen percentage in the presence of 5% CO_2 are also shown in Figure 1 (empty circles; $n = 11$). The 19% O_2-5% CO_2 values were Pao_2 84.9 ± 4.9 mm Hg, $Paco_2$ 55.3 ± 2.8 mm Hg, pH 7.29 ± 0.02. Increasing levels of inspired oxygen led to an approximately linear increase in systemic arterial oxygen levels, up to 472 ± 12.9 mm Hg, with $Paco_2$ and pH not significantly affected. Systemic arterial Pao_2 was not significantly influenced by hypercapnia, but the Pco_2 was elevated and the pH reduced (both $P < 0.001$).

Intraretinal Oxygen Distribution

Combined intraretinal profile data for all animals in the hyperoxia–normocapnia trial ($n = 10$) are shown in Figure 2. The increase in inner retinal Po_2 was muted compared with that in the choroid, and the intraretinal minimum was preserved under all breathing conditions. The mean Po_2 at the retinal surface under air breathing conditions was 18.6 ± 1.3 mm Hg, which then decreased to a minimum (4.5 ± 0.9 mm Hg at 180 μm) within the inner plexiform layer (IPL) before increasing again through the plateau region to peak in the choroid at 42.3 ± 2.0 mm Hg. These three retinal landmarks were identifiable at all levels of oxygen ventilation. With 100% oxygen ventilation the corresponding values were, retinal surface 53.1 ± 2.3 mm Hg, minimum 25.1 ± 6.1 mm Hg, and peak choroidal Po_2 228 ± 21 mm Hg. The oxygen gradients in the outermost retina were significantly increased during hyperoxic ventilation.

Combined intraretinal profile data for all animals in the hyperoxia–hypercapnia trial ($n = 6$) are shown in Figure 3.

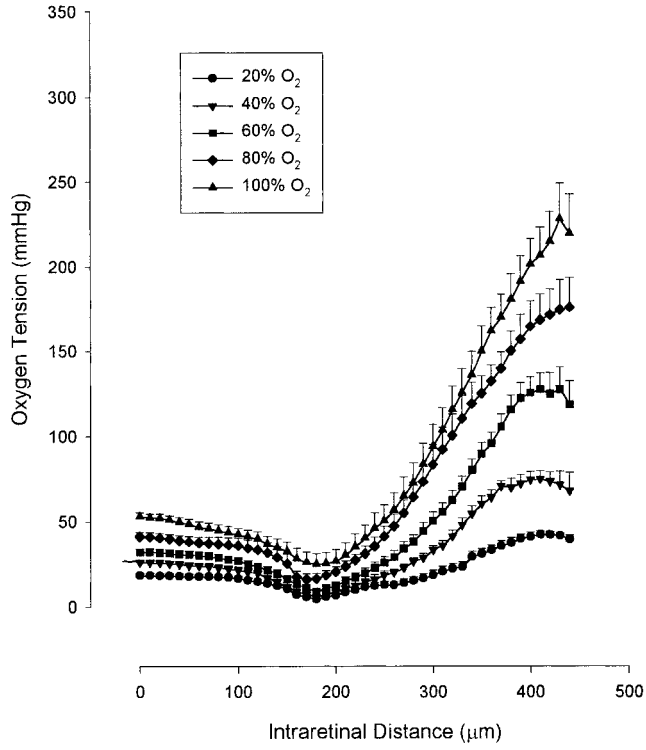

FIGURE 2. Mean intraretinal oxygen profiles as a function of track distance through the retina and choroid at each level of inspired oxygen across all animals under normocapnic conditions ($n = 10$; ±SEM).

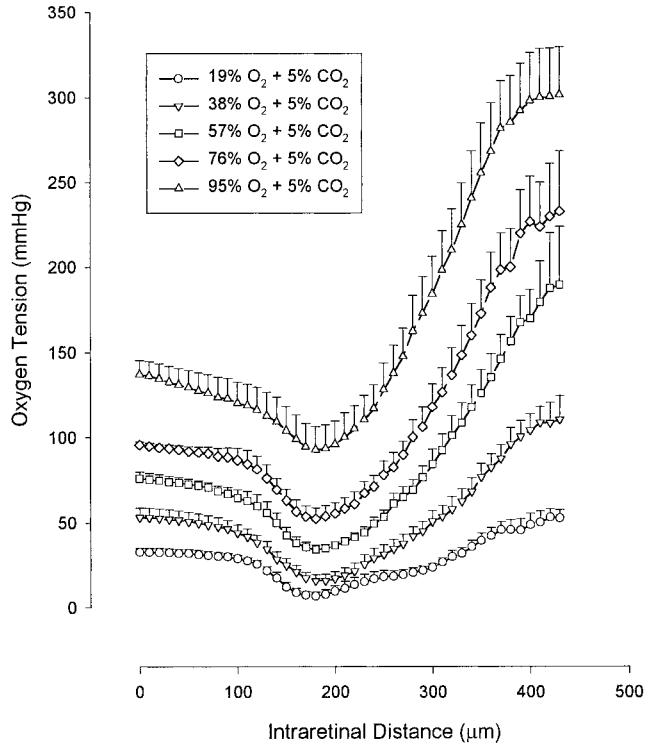

FIGURE 3. Mean intraretinal oxygen profiles as a function of track distance through the retina and choroid at each level of inspired oxygen across all animals under hypercapnic (5% CO_2) conditions (n = 6; ±SEM).

The mean Po_2 at the retinal surface under 19% O_2–5% CO_2 breathing conditions was 32.6 ± 2.2 mm Hg, which then decreased to a minimum (6.6 ± 2.4 mm Hg at 180 μm) within the IPL before increasing again through the plateau region to peak in the choroid at 52.8 ± 5.0 mm Hg. With 95% O_2–5% CO_2 ventilation the corresponding values were, retinal surface 137.4 ± 8.2 mm Hg, minimum 92.9 ± 13.7 mm Hg, and peak choroidal Po_2 of 302 ± 28 mm Hg. The intraretinal minimum was again evident at all levels of inspired oxygen under hypercapnic conditions. The increase in inner retinal oxygen level was still muted with respect to the large increases in choroidal Po_2 but less so than in the normocapnic case. The oxygen gradients in the outermost retina are similarly increased during hyperoxic ventilation in the presence of hypercapnia.

The oxygen level at the retinal surface (ILM), at the oxygen minimum (IPL), and in the choroid under each ventilatory condition are summarized in Figure 4, which highlights the muted nature of the absolute increase in intraretinal oxygen level compared with that in the choroid. Linear regression analysis of the individual data sets for hyperoxia alone (filled symbols) indicated slopes of 0.42 for the ILM, 0.26 for the minimum oxygen level in the IPL, and 2.4 in the choroid (all slopes are millimeters of mercury/percentage increase in oxygen ventilation). In the presence of hyperoxia with hypercapnia (open symbols), the slopes were 1.3 for the ILM, 1.1 for the minimum oxygen level in the IPL, and 3.3 in the choroid. A comparison of the oxygen levels at these locations under normocapnic and hypercapnic conditions reveals a highly significant difference at all oxygen levels at the ILM and in the choroid ($P < 0.001$). At the minimum oxygen tension in the IPL the normocapnia–hypercapnia effect was not significant at 20% oxygen ventilation, but it was significant at 40% and above.

Under all conditions the oxygen level within the retina and choroid was significantly lower than that in the femoral artery ($P < 0.001$).

Retinal Artery and Vein Po_2 Levels

Under air-breathing conditions, the average oxygen tension in the preretinal vitreous adjacent to a retinal artery was 31.8 ± 2.1 mm Hg, whereas next to a vein it was 18.7 ± 1.7 mm Hg. At increasing inspired oxygen levels, the oxygen level at the surface of the artery and vein increased. With 100% oxygen ventilation, the mean arterial value was 207 ± 9.4 mm Hg, and the vein was 53.3 ± 5.0 mm Hg.

For 19% O_2–5% CO_2 breathing the mean retinal artery contact value was 49.5 ± 4.4 mm Hg, whereas that for the vein was 28.6 ± 4.6. At increasing inspired oxygen levels the oxygen level at the surface of the artery and vein increased. With 95% O_2–5% CO_2 ventilation the arterial value was 296 ± 9.7 mm Hg, and the vein was 76.9 ± 1.9 mm Hg. Under all conditions the oxygen level adjacent to the retinal artery was significantly less than that in the femoral artery ($P < 0.001$). Under hypercapnic conditions the retinal artery oxygen level was higher than with hyperoxia alone. The oxygen level adjacent to the retinal vein was also significantly increased by hypercapnia, except at the 19%O_2–5%CO_2 level, at which the increase was not statistically significant. Under both normocapnic and hypercapnic conditions the retinal arteriovenous oxygen difference increased with each increment in oxygen ven-

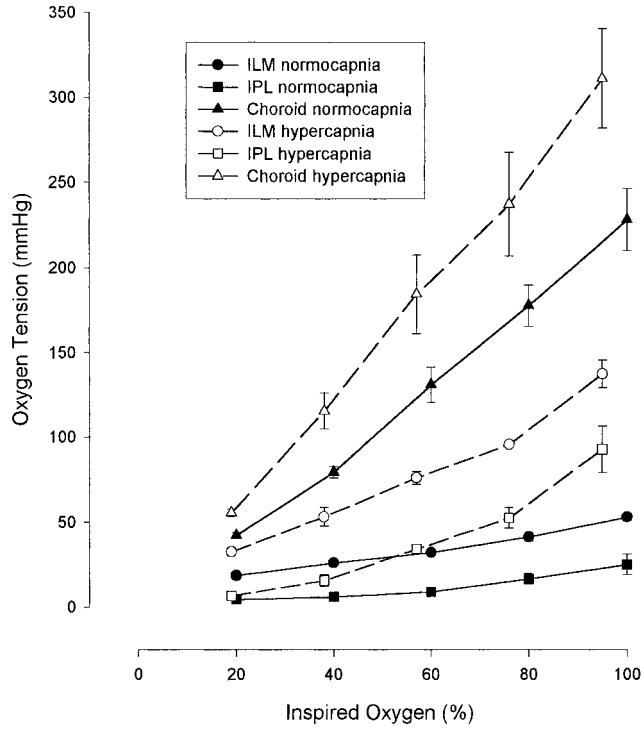

FIGURE 4. Mean values of oxygen level at the ILM, at the site of the minimum Po_2 in the IPL, and in the choroid, as a function of inspired oxygen percentage under normocapnic (filled symbols; n = 10; ±SEM) and hypercapnic (open symbols; n = 6; ±SEM) conditions.

tilation ($P < 0.05$). The arteriovenous oxygen differences were significantly increased under hypercapnic conditions.

Discussion

The feasibility of raising intraretinal oxygen levels by systemic manipulation of ventilatory parameters was well demonstrated in the present study. Although supplemental oxygen ventilation increased oxygen levels throughout the retina, the effect was further enhanced by hypercapnia. This was most likely because of the vasodilating influence of the pH changes associated with hypercapnia, particularly in the retinal circulation in which autoregulatory mechanisms are more highly developed.[20–22] Hypercapnia produced a clearly visible dilatation of the retinal vasculature in each animal in our study.

Oxygen Uptake in the IPL

A feature of every intraretinal oxygen measurement in air-breathing rats was the presence of a minimum oxygen tension lying between the superficial and the deep capillary layers of the retinal circulation. The oxygen contribution from both the superficial and the deep capillary layers is evident from the nature of the oxygen gradients flowing into this region. This region encompasses the IPL. The IPL contains an abundance of synaptic processes between the bipolar cells and the retinal ganglion cells, and synaptic connections from the amacrine cells are also present. The main function of the inner retina, similar to that of the central nervous system, is the generation, processing, and transmission of nerve impulses. This activity can only be accomplished if the required ionic gradients are maintained, which requires considerable energy.[23]

Increased Oxygen Uptake in Hyperoxia

The striking feature of the hyperoxia measurements was the maintenance of the intraretinal oxygen minimum in the IPL, even in the face of dramatically increased oxygen flux from the choroid. This increased oxygen flux did not reach the inner retinal boundary with the vitreous, because the oxygen gradients indicated a sustained flow of oxygen into the retina from the superficial capillary bed. The most likely explanation for this phenomenon is that the oxygen uptake of the retina increased under hyperoxic conditions. The possibility of the extra oxygen being washed out by the retinal circulation is excluded by the observation of a significant arteriovenous oxygen difference, with the arteries having a higher oxygen level than the veins under all conditions. This is consistent with other work in the rat in which arterial and venous oxygen levels were measured noninvasively.[24] Although the presence of the retinal circulation precludes a quantitative analysis of intraretinal oxygen consumption,[25] there are still several points that can be made in an attempt to understand our experimental results. There is general agreement that the oxygen uptake of the inner half of the rat retina is of the same order as that of the outer retina.[26,27] A similar finding has been reported for the pig retina.[28] If the oxygen requirements of the inner and outer halves of the retina are assumed to be equal, then a doubling of the oxygen flux from the choroid would be expected to support the entire retinal thickness in the event that the contribution from the retinal circulation were removed. This assumes that the retinal ischemia per se does not increase the oxygen demand because of the restricted supply of other metabolites such as glucose. Although we think that this assumption is unlikely to be correct, for the present discussion it still serves a useful purpose. It is clear from our observations that the oxygen flux from the choroid increased by a factor of more than four in hyperoxia (based on outer retinal gradients, Figs. 2, 3). Because the oxygen contribution from the retinal circulation is still present, at least to some degree, the total use of oxygen within the retina must have increased.

Oxygen Uptake in Different Retinal Layers

Enzymes associated with oxidative metabolism, most notably cytochrome oxidase, have been shown to be concentrated in specific retinal layers, the inner segments of the photoreceptors, the outer plexiform layer, the IPL, and the ganglion cells. The highest concentration is found in the inner segments of the photoreceptors, but a considerably thicker region of moderate staining encompassing the IPL is also evident.[6] The existence of an oxygen-consuming layer in the relatively avascular region between the two retinal capillary beds would account for the consistent appearance of the minimum oxygen tension in this area. Although cytochrome oxidase staining in the outer plexiform layer is significant, this layer is very thin in the rat, and any consumption is masked by the presence of the deep retinal capillary bed in this region in our chosen retinal location (2–3 disc diameters). This also applies to the relatively sparse ganglion cell layer with the adjacent superficial retinal capillaries.

Oxygen-Dependent Uptake

A further requirement to explain our observations under hyperoxic conditions would be a relationship between oxygen level and oxygen uptake.[10,27] This topic has been the focus of much interest, because such a mechanism also fulfills the role of an oxygen sensor, which is known to be an important element in blood flow control in many organs.[29,30] It has been suggested that the 50% metabolic rate for mitochondria inside an intact cell lies between an oxygen level of 10 to 15 mm Hg.[10] This suggests that the oxygen uptake of the IPL may not be saturated at the oxygen levels that were encountered in the IPL of the rat in our studies. Thus, the muted increase in oxygen level in the IPL when compared with that in the choroid and the retinal artery, may have been caused by an oxygen-level regulation of oxygen uptake in this region. Increased oxygen uptake by the photoreceptor inner segments may also be present during hyperoxic conditions, although Linsenmeier and Yancey[31] found no increase in outer retinal oxygen uptake in hyperoxia in the cat. However, others speculated that outer retinal oxygen consumption is increased in hyperoxia, having noted a similar increase in oxygen flux from the choroid in hyperoxic pigs.[16,21] Re-examining their oxygen profiles in the light of our own findings indicates an involvement of the inner retina in the increased oxygen consumption in their study.

Under hypercapnic conditions it may be anticipated that the relationship between oxygen level and oxygen consumption may be influenced by the consequent pH changes.[30] In our study, the increase in retinal arteriovenous oxygen difference in hypercapnia, despite the visible dilatation of the retinal vasculature, suggests that the oxygen delivery from the retinal circulation is increased when compared with hyperoxia alone.

Without quantitative blood flow information, this point cannot be proved, but there is a strong possibility that inner retinal oxygen uptake is increased under hypercapnic conditions.

Disease Models

It is interesting to note that the oxygen uptake of the IPL is no longer evident in the urethane model of photoreceptor degeneration.[11] This is unlikely to be because of a loss of signal input from the degenerated photoreceptors, as the Royal College of Surgeons (RCS) rat model of photoreceptor degeneration shows a significant oxygen uptake of the remaining inner retina in vitro.[27] We have also confirmed this result in measurements in vivo of oxygen uptake in the inner retina of the RCS rat (unpublished observations). The maintenance of oxygen metabolism in the inner retina in the absence of a functional outer retina is an area requiring further study.

The present study quantified the influence of systemic manipulation of oxygen levels in the rat on intraretinal oxygen levels. The enhancement of these effects by hypercapnia was also demonstrated. The apparently large oxygen uptake by the IPL and the increase in this oxygen uptake as more oxygen is made available are novel observations that require more detailed studies to elucidate the importance of such mechanisms in normal and ischemic retinas.

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