Light and Choroidal Po₂ Modulation of Intraretinal Oxygen Levels in an Avascular Retina

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PURPOSE. To determine the influence that choroidal oxygen level and outer retinal oxygen demand have on oxygen availability to the inner retina when the choroid is the only source of retinal oxygenation. This condition prevails in avascular retinas and in vascularized retinas suffering vascular occlusion.

METHODS. Oxygen-sensitive microelectrodes were used to measure the oxygen tension as a function of depth in the naturally avascular retina of anesthetized and mechanically ventilated guinea pigs (n = 6). Choroidal Po₂ was manipulated by varying the ventilation gas mixture, and outer retinal oxygen consumption was modulated by light-dark adaptation. Individual Po₂ profiles were fitted to a multilayer mathematical model of Po₂ distribution, and pairs of profiles at different choroidal Po₂ levels, or under light and dark conditions, were fitted to an intraretinal Po₂ difference model. Both models reflect the purely choroidal supply of retinal oxygenation.

RESULTS. An increase in choroidal Po₂ produced an equivalent increase in all retinal layers. Light induced a decreased oxygen consumption in the region of the inner segments of the photoreceptors, which resulted in a significant increase in Po₂ in this layer, flowing on unattenuated to all inner retinal layers. The intraretinal Po₂ distribution and the light- and ventilatory-induced changes in Po₂ were consistent with theoretical predictions of the mathematical models.

CONCLUSIONS. The present experimental studies confirm that when the choroid is the only source of retinal oxygenation, the full effect of increased choroidal oxygen level or reduced uptake in the outer retina passes through to the inner retinal layers if the oxygen utilization by the inner retina remains constant. (Invest Ophthalmol Vis Sci. 1999;40:2307–2313)

Ischemia and hypoxia of the inner retinal layers are thought to be involved in many clinically important conditions, including diabetic retinopathy and retinal vascular occlusion. Many strategies have been devised for improving the oxygen supply to ischemic retina, and some are in clinical use. For example, panretinal photocoagulation is thought to create a reduction in outer retinal oxygen consumption and an increase in oxygen flux from the choriocapillaris to the ischemic inner retina. Light adaptation has also been put forward as a potential means of improving oxygen delivery to ischemic retina, similarly based on the reduced uptake of oxygen by the outer retina. Raised choroidal oxygen levels by oxygen or carbogen breathing has also been proposed as a therapy for retinal ischemia, and several attempts to increase this effect by hyperbaric oxygen therapy have been made.

There has also been much interest in limiting the effect that raised systemic arterial oxygen levels have on the developing retina, in relation to the development of retinopathy of prematurity. In the developing retina, the maturation and increased oxygen uptake by the photoreceptors are thought to lower oxygen levels in the inner retina and to create a necessary hypoxic stimulus for retinal vascular growth. Finding the right combination of arterial oxygen level that does not adversely affect the development of the retinal vasculature remains a major goal in neonatal care.

Thus, understanding how choroidal oxygen levels, or outer retinal oxygen demand, influence the oxygen levels in the ischemic or avascular inner retina is of relevance to retinal disease and is also central to our understanding of retinal vascular development.

Preretinal measurements of oxygen tension in animal models of retinal ischemia have produced conflicting results with regard to the influence of choroidal oxygen changes. Descriptions of the preretinal oxygen response to systemic hyperoxia have ranged from zero to extreme hyperoxic levels in front of the ischemic area, even in studies by the same group in the same species. A diminishing preretinal oxygen response to successive applications of systemic hyperoxia was reported in a kitten model of retinopathy of prematurity. The duration of the induced ischemia and hypoxia, and the effect that it has on inner retinal viability, may well be important parameters that have contributed to the diversity of results in apparently similar studies. However, the weight of evidence from these preretinal studies in the cat, pig, and primate supports the notion that raised choroidal oxygen levels can relieve the hypoxic component of retinal ischemia. A close relationship between systemic arterial oxygen level and preretinal oxygen tension in the ischemic region above a threshold arterial value suggests that increases in choroidal Po₂ flow on to all retinal layers. A similar finding in the avascular region of the rabbit retina suggests that this may be the case in
general when the choroid is the only source of retinal oxygenation. However, because choroidal oxygen levels may be significantly lower than in the systemic arteries, only direct measurements of oxygen distribution in the retina and choroid can confirm this result.

Intraretinal PO\textsubscript{2} studies in ischemic retinas are much rarer\textsuperscript{10,21,22} and have also produced contradictory results. The first studies in the pig\textsuperscript{10} and the cat\textsuperscript{21} suggest that in hyperoxic ventilation the choroid could easily supply enough oxygen to the ischemic inner retina. However, a recent more extensive intraretinal study in the cat\textsuperscript{22,23} found that 100% oxygen ventilation could prevent intraretinal hypoxia after retinal artery occlusion in only 50% of cases.\textsuperscript{22} Furthermore, a light-induced decrease in outer retinal oxygen consumption, and a consequent increase in choroidal oxygen level, was found not to result in an equivalent improvement in inner retinal oxygen levels, even though inner retinal oxygen consumption was said to remain constant. Rather, the effect was shown to taper off with distance from the choroid and to provide little if any benefit to the innermost retinal layers. If generally applicable to ischemic retinas, this finding has important implications for any strategies designed to improve oxygen supply to ischemic inner retinas that are dependent on raised choroidal PO\textsubscript{2}, or reduced outer retinal oxygen consumption. Although the findings of the study\textsuperscript{22} were subsequently questioned on intuitive and theoretical grounds,\textsuperscript{24} these issues are best resolved by direct experimentation in combination with theoretical analysis.

In an attempt to help resolve these important issues, we performed intraretinal measurements of oxygen tension in the naturally avascular retina of the guinea pig at different choroidal oxygen levels or under light and dark conditions. The avascular nature of the guinea pig retina offers some important advantages in such an investigation. First, the guinea pig retina is free of any influence of retinal vasculature without the need to artificially induce retinal ischemia, thereby eliminating any time-related effects. Second, by using a combination of hyperoxic and hypercapnic ventilation to raise choroidal oxygen levels it is possible to ensure that no region of inner retina is influenced by limited oxygen availability, even in the dark. Finally, the oxygen consumption of the inner retina in the guinea pig is very low\textsuperscript{25} when compared with that of the photoreceptors, so any potential light-induced, or oxygen level-induced, changes in inner retinal oxygen consumption would have little influence on a photoreceptor-mediated oxygen change. Thus, the guinea pig retina offers a useful model in which to assess the effect of induced changes in retinal oxygen supply and consumption without complications arising from inner retinal hypoxia or ischemia, or any oxygen level-related effects. Although the guinea pig retina represents a model of purely choroidal oxygen supply, the behavior of the inner retina may be different between a naturally avascular or an ischemic retina, in that the ischemic inner retina may well increase its oxygen uptake as more oxygen is made available. Nevertheless, the avascular retina provides a simplified “pure” model in which the influence of outer retinal oxygen supply and consumption on inner retinal oxygen levels can be tested experimentally and compared with predicted results from the available mathematical models.

**METHODS**

The experimental techniques were similar to those reported in our earlier publications.\textsuperscript{20,27} Adult male albino guinea pigs were anesthetized with an intraperitoneal injection of 100 mg/kg 5-ethyl-5-(1'-methyl-propyl)-2-thiobarbiturate (Inactin; Byk Gulden, Konstantz, West Germany) and atropine sulfate (20 μg). The trachea was cannulated, and the animals were artificially ventilated at 90 breaths/min (Harvard rodent respirator, model 683; Harvard Apparatus, Holliston, MA). The left jugular vein was cannulated for continuous monitoring of central venous pressure and for drug infusion. The right carotid artery was cannulated for blood pressure monitoring and occasional blood sampling for blood gas analysis. A paralyzing dose of 8 to 16 mg of gallamine triethiodide (40 mg/ml; Flaxedil; May & Baker, Dagenham, UK) was given to eliminate eye movements. The systemic blood pressure and heart rate were continuously monitored, and supplementary doses of Inactin (10 mg) given as necessary to maintain deep anesthesia. Rectal temperature was maintained between 36.5°C and 37.5°C with a homeothermic blanket (Harvard Apparatus). The animal was placed prone in a modified Stellar stereotaxic instrument (model 51400; Stoolting, Chicago, IL), and the head was clamped to the stereotaxic frame. An eye ring, rigidly attached to the stereotaxic frame, was sutured to the left eye through the conjunctiva at the limbus. The pupil was dilated with 1% Mydriacil (tropicamide; Alcon, Sydney, Australia). All procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Intraretinal Oxygen Profiles**

Oxygen-sensitive microelectrodes were used to measure oxygen tension as a function of depth through the retina. The electrode entered the eye through a small hole just behind the limbus. The small size of the electrode tip (1 μm) coupled with high acceleration piezoelectric translation of the electrode through the retina produced highly reproducible measurements of intraretinal oxygen distribution. Intraretinal oxygen profiles were measured in the inferior retina, approximately 2 to 3 disc diameters from the disc margin. The electrode tip was placed at the surface of the chosen area of the retina under microscope observation. The electrode was then stepped through the retina, under computer control, until a peak oxygen level within the choroid was reached. 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 influenced less by artifacts associated with mechanical stress on the electrode tip during penetration.

**Systemic Conditions and Retinal Illumination**

A combination of hyperoxic and hypercapnic ventilation was used to raise choroidal PO\textsubscript{2} levels to ensure that there was no possibility of inner retinal hypoxia influencing the results.\textsuperscript{27} The hypercapnia is necessary to override the ability of the guinea pig choroid to sustain close to normal oxygen levels with systemic hyperoxia alone.\textsuperscript{27} In six animals the intraretinal oxygen tension distribution was measured under systemic conditions, which raised inner retinal oxygen levels above zero, and the measurement repeated at a higher choroidal oxygen level produced by 95%O\textsubscript{2} /5%CO\textsubscript{2} ventilation.
In three animals the effect of retinal illumination was assessed by repeating the intraretinal profile measurement in the light and in the dark at a sufficiently high choroidal PO$_2$ level to ensure that inner retinal PO$_2$ did not fall to zero in the dark. The level of retinal illumination was altered by darkening the room and then gating filtered blue light (to avoid any heating effect) delivered via fiberoptic cable to provide 28 W/cm$^2$ (550 nm) at the cornea.

After ventilation or illumination changes the preretinal oxygen tension was monitored continuously, and the intraretinal PO$_2$ profiles were measured only when equilibrium had been reached.

**Mathematical Models**

**Intraretinal PO$_2$ Distribution Model.** A full derivation of a four-layer mathematical model specific for a purely choroidal source of retinal oxygenation is presented elsewhere.$^{28}$ The key difference between this model and those applied to vascularized retinas$^8,22$ is that our model allows oxygen to be provided from the choriocapillaris only, and the relatively large distances across the vitreous to any other significant oxygen sources or sinks.$^7$ It should be emphasized that there is no suggestion of an oxygen barrier at the retinal/vitreous interface, simply that oxygen tension will equilibrate to the same level on either side. Under these conditions the oxygen distribution across the retina can be described in terms of the supply PO$_2$, in the choriocapillaris ($P_c$) and the oxygen consumption ($Q$), diffusion ($D$), and solubility ($k$) properties in each retinal layer.

The distribution of oxygen across the retina is governed by Fick’s law.

$$\frac{Q}{Dk} \frac{dP}{dx} = a$$  \hspace{1cm} (1)

where $P$ is oxygen tension, $x$ is distance, and $Dk$ is the product of the oxygen diffusion and solubility coefficients, which are assumed to be uniform across the retina. Integrating this equation twice gives an equation for oxygen tension as a function of distance.

$$P(x) = \frac{Q}{2Dk} x^2 + \alpha x + \beta$$  \hspace{1cm} (2)

The constants $\alpha$ and $\beta$ for each layer are found by applying the appropriate boundary conditions and can be simplified considerably by setting $Q_1$ and $Q_4$ equal to zero, an assumption that has been shown to correspond well to the distribution of outer retinal oxygen uptake in all species studied.$^8,22,29$ The resultant constants are as follows:

$$\begin{align*}
\alpha_1 &= \frac{Q_2}{Dk} L_1 + \alpha_2, \\
\alpha_2 &= -\frac{Q_2}{Dk} L_2 + \alpha_4, \\
\beta_1 &= P_c, \\
\beta_2 &= \left(\frac{Q_2}{2Dk}\right) L_1^2 + \beta_1, \\
\beta_3 &= \left(\frac{Q_2}{2Dk}\right) L_2^2 + \beta_2, \\
\beta_4 &= \left(\frac{Q_1}{2Dk}\right) L_3^2 + \beta_3
\end{align*}$$

**Light-Induced Oxygen Consumption Change Model.**

Equations for the PO$_2$ change in each of the four layers due to a light-induced change in oxygen consumption ($\Delta Q_2$) in layer 2 can be derived from subtracting the equations for the distribution of PO$_2$ across the retina under the two conditions. The resultant equations are shown below and include the term $\Delta P_c$, which reflects a possible change in choriocapillaris PO$_2$ due to altered oxygen extraction from the choriocapillaris.

$$\Delta P_1(x) = \frac{\Delta Q_2}{Dk} (L_1 - L_2) x + \Delta P_c$$

$$\Delta P_2(x) = \frac{\Delta Q_2}{2Dk} x^2 - \frac{\Delta Q_1}{Dk} (L_2) x + \frac{\Delta Q_2}{2Dk} L_1^2 + \Delta P_c$$

$$\Delta P_3(x) = \frac{\Delta Q_2}{2Dk} (L_2 - L_3) + \Delta P_c$$

$$\Delta P_4(x) = \frac{\Delta Q_2}{2Dk} (L_3^2 - L_2^2) + \Delta P_c$$  \hspace{1cm} (3)
Similarly, the $P_{O2}$ change in each layer due to a change in choroidal $P_{O2}$ alone with all other parameters remaining constant is as follows:

$$\Delta P_1(x) = \Delta P_2(x) = \Delta P_3(x) = \Delta P_4(x) = \Delta P_c \quad (4)$$

**RESULTS**

**Effect of Increased Choroidal $P_{O2}$ on Intraretinal Oxygen Distribution**

Figure 2 shows the oxygen tension as a function of penetration depth through the retina and choroid at two different choroidal oxygen levels (57% and 95% $O_2$ ventilation). The fitted line is that from our four-layer avascular retina model (Eq. 2). Figure 3 shows the difference between profiles at two choroidal $P_{O2}$ levels from each of the six animals. The fitted lines in this case are a first-order linear fit to the $P_{O2}$ difference data. It is clear that in all cases the higher choroidal oxygen level flows on to all retinal layers equally. The wide range of choroidal $P_{O2}$ changes between animals reflects the differing response to carbogen ventilation.\(^2^7\)

**Effect of Illumination on Intraretinal Oxygen Distribution**

Figure 4 shows the intraretinal oxygen distribution at the same location in the dark and in the light in an animal breathing 95% $O_2$/5% $CO_2$. The best least mean squared fits of Eq. 2 to the data are also shown. Similar results were found in the two other animals tested in this manner. The change in intraretinal $P_{O2}$ for all three animals is shown in Figure 5, together with the best fit to the mathematical model for a change in outer retinal oxygen consumption (Eq. 3). The light exposure causes an increase in oxygen tension in the proximal region of the outer retina and a similar increase across the remaining inner retina.

**Agreement with Proposed Models**

In assessing the agreement between the experimental data and our proposed model of oxygen distribution specific to a purely choroidal source of retinal oxygen, an important observation is the lack of a significant oxygen gradient at the inner margin of the retina. This was true in all measurements for which sufficient time was allowed for equilibration, and localized gradients near the optic disc were avoided. Taking all the profiles in this study ($n = 18$) into consideration, the mean oxygen gradient in the innermost 50 $\mu$m of retina was only $0.016 \pm 0.018$ mm Hg/$\mu$m, which is only 1.3% of the oxygen gradient in the outermost retina. There is clearly an insignificant flux of oxygen across the retina/vitreous interface. The experimental $P_{O2}$ profiles were well fitted to the mathematical models of oxygen distribution across the retina (Eq. 2) and light-induced $P_{O2}$ change (Eq. 3). The average coefficient of determination ($R^2$) was $0.97 \pm 0.04$ ($n = 21$). The mean gradient of the linear
The central assumption of these models is that the source of retinal oxygenation have been presented previously.

The naturally avascular retina of the guinea pig presents a useful and simple model in which to study the intraretinal PO₂ changes due to increases in choroidal PO₂ or changes in outer retinal oxygen consumption. The absence of a retinal circulation avoids any requirement to render the inner retina ischemic to extend the oxygen consumption analysis to all retinal layers. Mathematical models specific to a purely choroidal source of retinal oxygenation have been presented previously. The central assumption of these models is that the oxygen gradient at the retina-vitreous boundary is negligible.

Thus, the disparate results in the two studies could be explained by the presence of a small region of anoxic retina in the study by Braun et al. in those three pairs of profiles on which their light-induced PO₂ change model is based. The PO₂ profiles in Figure 6 are mathematically generated using parameters appropriate for the cat retina.

These findings are not just relevant to avascular retinas. In terms of retinal oxygen supply and consumption, an analogy can be drawn between a naturally avascular retina and a retina with an occluded retinal circulation. However, the behavior of the inner retina in an ischemic condition may well be influenced by other factors related to ischemia and hypoxia, or by an interaction between oxygen level and oxygen consumption.

The only previous study of light-induced changes in intraretinal oxygenation in an ischemic retina concluded that raised choroidal PO₂ and reduced oxygen consumption of the photoreceptors had little or no influence on oxygen levels in the innermost retinal layers, even though the oxygen consumption of the inner retina was said to remain constant. This result is clearly at odds with the present investigation, which shows a significant and uniform increase in inner retinal oxygen level under these conditions. If results of Braun et al. were a general property of ischemic retinas then the usefulness of strategies to relieve inner retinal hypoxia by raising choroidal PO₂ or reducing outer retinal oxygen consumption would be in question.

What could the explanation be for such disparate findings in what appears to be analogous conditions in the two studies? Based on an argument restricted to intuitive and theoretical grounds we had proposed an alternative explanation of the results from the ischemic cat retina study. In the light of the present experimental results, which support the applicability of our mathematical models, it seemed worthwhile to determine whether these models could provide an alternative explanation that was consistent with the results of the ischemic retina study. The key factor here is the existence of very low oxygen levels in the inner retina in that study. Braun et al. reported that inner retinal oxygen tension was zero at some point in more than half of the profiles measured. More detailed examination of the three pairs of profiles used in their development of the light-dark model reveals that two were from the same animal, which had inner retinal oxygen levels very close to zero in the light-adapted state. We believe that this influenced their results more than they anticipated.

The PO₂ profiles in Figure 6 are mathematically generated profiles from three different animals under light- and dark-adapted conditions. The fitted lines are the best fits to the PO₂ difference model (Eq. 3) assuming that Q₂ and Pₑ may have changed.

Thus, the disparate results in the two studies could be explained by the presence of a small region of anoxic retina in the study by Braun et al. in those three pairs of profiles on which their light-induced PO₂ change model is based. The potential for a relatively small anoxic layer to influence the light-induced PO₂ change so markedly can be better understood by reference to Eq. (5) in which it is apparent that the
PO2 change due to a change in consumption in a given layer increases with the distance of that layer from the choroidal source. Thus, a relatively small increase in oxygen consumption of the innermost retina is able to mask a much larger decrease in oxygen consumption of the outer retina. In the example given, the additional consumption of oxygen by the innermost 25 \(\mu\)m of retina when oxygen is available represents a 34% increase in total inner retinal oxygen consumption. However, this is sufficient to totally mask the 70 mm Hg preretinal PO2 increase that would have occurred given a 64% reduction of outer retinal oxygen consumption and a 20 mm Hg rise in choriocapillaris PO2. These parameters are similar to those reported in the study by Braun et al. Thus, we have a model that both fits their experimental data and offers an explanation for the discrepancy between their findings, and what we assert to be the general case for a retina with a purely choroidal source of retinal oxygenation, and an inner retinal oxygen consumption that remains constant.

Alterations in inner retinal oxygen consumption due to oxygen availability may not be confined to the existence of an anoxic region. We have recently demonstrated in the rat that the oxygen uptake of the inner plexiform layer increases markedly as more oxygen is made available, even though there is no anoxia present.

Our findings of significant inner retinal PO2 changes in light and dark are consistent with studies in which preretinal PO2 was monitored during changes in retinal illumination in avascular retinas. A similar effect was seen in vascularized retinas of the monkey and cat when hyperoxic ventilation was used to increase the availability of choroidal oxygenation and to reduce the autoregulatory masking effect of the retinal circulation.

The nature of the intraretinal PO2 change that we have found as a result of reduced outer retinal oxygen consumption may have been predicted from an examination of the earliest intraretinal oxygen measurements, those in isolated fish retinas oxygenated from the receptor side only. Although the reasons for the reduced oxygen consumption were different, the effect on inner retinal oxygen distribution was remarkably similar to that seen in the present study.

Information from avascular retinas may also be relevant to developing retinas where the choroidal supply of oxygen pre-
cedes the maturing of the photoreceptors and the subsequent development of the retinal circulation. In retinopathy of prematurity, for example, the effect of raised chorioidal oxygen levels and the influence of outer retinal oxygen consumption, may be better understood. These are important issues that are currently under clinical investigation. The present results may also be applicable to the human macula, which is devoid of retinal capillaries. It is interesting that the only intraretinal oxygen measurement in a primate fovea showed an oxygen distribution not unlike that in the avascular guinea pig retina. It was noted that “diffusion of oxygen from the vascularized region around the fovea is minimal.” Thus, oxygen level changes in the choroid, or modulation of oxygen consumption in the tightly packed photoreceptors, may well influence the oxygen status of the inner retina in this region in a manner comparable to our observations in the avascular guinea pig retina.

In conclusion, we have shown that under conditions in which the choroid is the only source of retinal oxygenation, and the oxygen uptake of the inner retina remains constant, the full effect of a rise in choriocapillaris oxygen level passes to all retinal layers and that a reduction in outer retinal oxygen consumption produces a uniform increase in oxygenation of the inner retina. Understanding these effects may be important in aspects of retinal development and retinal pathology and in the clinical management of retinal vascular disease.

References