Intraretinal Oxygen Consumption in the Rat In Vivo

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Purpose. To make the first quantitative assessment of the rate of oxygen consumption in high oxygen-consuming layers of both the outer and inner retina of the rat in vivo.

Methods. Oxygen-sensitive microelectrodes were used to measure the oxygen tension as a function of depth through the retina in anesthetized rats. Individual PO_2 profiles were fitted to a multilayer mathematical model of PO_2 distribution that is able to determine the oxygen uptake in those retinal layers in which the oxygen supply is through diffusion from vascular layers of the retina. This includes the entire outer retina and the region of the inner retina containing the inner plexiform layer. Measurements were performed in the light (13 mW/cm^2 at the cornea) and in the dark and the amplitude and time constant for light-induced changes in outer retinal oxygenation determined.

Results. Under light-adapted conditions, the oxygen consumption of the outer retina was 148 ± 11 nL O_2/min/cm^2 ($n = 20$) and that of the included portion of the inner retina was 184 ± 17 nL O_2/min/cm^2. In the dark, outer retinal oxygen consumption increased by 47.8% ($P < 0.001$), and the time constant for the resultant PO_2 decrease in the outer retina was 14.9 ± 1.8 seconds ($n = 16$). There was no significant change in inner retinal oxygen consumption between light and dark conditions ($P = 0.89$).

Conclusions. Under light-adapted conditions the oxygen uptake by the selected region of the inner retina (primarily the inner plexiform layer) is greater than that of the outer retina ($P < 0.01$). Dark adaptation rapidly and significantly increases outer retinal oxygen consumption, but the inner retina remains unaffected. (*Invest Ophthalmol Vis Sci.* 2002;43:1922–1927)

It has been well demonstrated that oxygen is the most critical metabolite for preserving retinal function in humans,[1] and retinal hypoxia is thought to be a factor in many retinal diseases that have an ischemic component. Understanding the oxygen requirements of different retinal layers may identify those layers most susceptible to disrupted oxygen supply and provide better clues to the most appropriate therapeutic intervention strategy. The inner retinal layers may be particularly at risk to hypoxic insult, because they are supplied with oxygen from the retinal vasculature, which is relatively sparse compared with the choroidal circulation supplying the majority of the outer retina. Unfortunately, our understanding of the oxygen requirements of the inner retina is much less detailed than that for the outer retina. The avascular nature of the outer retina has allowed localized oxygen consumption rates to be calculated from microelectrode-based measurements of intraretinal oxygen distribution.[2,3] This has highlighted the marked heterogeneity of oxygen consumption in the outer retinal layers, with the oxygen consumption being dominated by the inner segments of the photoreceptors. Using such techniques, similar patterns of outer retinal oxygen consumption in vivo have been determined in the cat,[2] monkey,[4] and guinea pig,[5] but equivalent data on the distribution of outer retinal oxygen consumption are not currently available for the rat.

Partitioning the oxygen consumption of specific layers of the inner retina is more problematic. In those layers containing retinal vasculature, the intermingling of oxygen sources and sinks precludes such a simple analysis of the spatial distribution of oxygen consumption. Removing the influence of the retinal vasculature by occluding the retinal circulation is one way around the problem, but this comes at the expense of rendering the retina ischemic.[6–8] Even if the ensuing hypoxia is overcome by artificially increased oxygen delivery from the choroid, there is still the possibility that the ischemic component of the insult may influence inner retinal function and metabolism. Studying animals that have a naturally avascular retina allows the oxygen consumption analysis to be extended to include the inner retina,[5,9] but the oxygen requirements of the inner retina in such species seem unlikely to reflect those of vascularized retinas, such as those in humans.[9]

There is also increasing evidence that retinal oxygen uptake is not a static parameter but is greatly affected by the prevailing oxygenation conditions.[10] It may therefore be important that the oxygen uptake of the retina be assessed under normal physiological conditions. Although there have been many useful studies of oxygen uptake in preparations of the rat retina in vitro,[11,12] their relationship to normal physiological conditions must inevitably be questioned. In vitro studies have also confirmed a direct relationship between the oxygen uptake of the rat retina and the oxygen level of the perfusate medium.[13]

The present in vivo study takes advantage of the predominantly avascular nature of the rat retina in the layers between the superficial and deep capillary beds of the retinal circulation.[9] Our microelectrode-based measurements of intraretinal oxygen distribution in the rat have consistently shown that when the major retinal vessels are avoided, the intraretinal oxygen distribution reflects oxygen delivery from two layers of retinal capillaries.[9,14] This conclusion is also supported by the great similarity of profiles obtained from randomly selected areas. Such a distribution is amenable to a one-dimensional analysis of the intraretinal oxygen distribution to extract local oxygen consumption information.[15] In those layers containing retinal capillaries, only the "net" result of oxygen delivery and consumption can be extracted. However, in the effectively avascular layers, the oxygen consumption can be quantified. This allows us to examine the oxygen metabolism of the region of retina containing the inner plexiform layer. This may be particularly relevant, given that this region has been shown to consistently have the lowest oxygen level within the retina,[14] a property that is maintained even under hyperoxic ventilation conditions[10] and suggests that the inner plexiform layer may have a high rate of oxygen metabolism. The potential for alterations in inner retinal oxygen metabolism to play a role in

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retinal disease is evident both in ischemic retinal diseases and in models of retinal degeneration.[16–18] Because the rat is an animal model that is used increasingly for the investigation of many types of retinal disease, an understanding of the normal oxygen requirements of the rat retina is essential in assessing the potential impact of altered oxygen availability and/or oxygen consumption in retinal disease.

METHODS

The experimental techniques were similar to those reported in our earlier publications.[9,14] Adult male Sprague-Dawley rats were anesthetized (Inactin 100 mg/kg, intraperitoneally; Sigma, St. Louis, MO) and the femoral artery cannulated for systemic blood pressure monitoring and intermittent blood gas analysis. The animal was then placed in a robotic stereotaxic apparatus. The eye was stabilized by suturing to a fixed eye ring at the limbus. A small hole at the pars plana allowed entry of an oxygen-sensitive microelectrode. The electrodes were manufactured in our laboratory by using techniques based on those described by Whalen et al.[19] and calibrated in equilibrated saline solutions before use.[14] The electrode could be visualized inside the eye through a planoconcave contact lens and operating microscope. The electrode was positioned so that its tip was placed on the surface of the inferior retina in a region free of major retinal vessels. Piezoelectrically driven electrode movements and small tip size (1 μm) allow clean retinal penetration with minimal tissue damage.[14] All electrode movements during intraretinal penetrations were under computer control, with the oxygen level recorded at 10-μm intervals throughout the retina. The nonperpendicular nature of the penetration means that distances are expressed as track length through the retina, rather than as absolute retinal depth. For quantitative analysis of oxygen consumption, a penetration angle of 30° to the perpendicular was assumed, and the appropriate correction applied. Intraretinal measurements of oxygen distribution as a function of retinal depth were performed under light-adapted (13 mW/cm^2 white light at the cornea) and dark-adapted conditions. Light and dark profiles were measured in the same retinal location, and the sequence of light or dark measurements was randomized. In a subgroup of animals (n = 16), the electrode was then positioned close to the location of the minimum oxygen level in the outer retina (in the dark), and the oxygen level, as a function of time, was recorded during repeated light and dark exposure. These transient responses were fitted to a single exponential model (Sigmaplot, 2000; SPSS Science, Chicago, IL) to extract both the amplitude and the time constant for light-induced changes in oxygen level in the outer retina.

Blood gas analysis and blood pressure monitoring confirmed that the animals were under normal systemic conditions during all measurements. All procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Mathematical Model of Intraretinal Oxygen Consumption

Full details of the model are published elsewhere,[9] but the key elements of the model are outlined herein. The model has eight layers, based on the current knowledge of the anatomic and functional properties of the retina (Fig. 1).[9] Layer 1 contains the outer segments of the photoreceptors, layer 2 the inner segments, and layer 3 the outer nuclear layer, layer 4 the deep retinal capillaries and the outer plexiform layer, layer 5 the inner nuclear layer, layer 6 the outer region of the inner plexiform layer, layer 7 the inner region of the inner plexiform layer, and layer 8 the ganglion cell/nerve fiber layer and the superficial retinal capillaries. Advantage is taken of the observation that oxygen flux into or out of the vitreous is negligible under equilibrium, air-breathing conditions in regions away from major retinal vessels.[20]

Oxygen consumption (Q) is proportional to the rate of change of oxygen flux with distance:

$$Q = Dk \frac{d^2P}{dx^2}$$

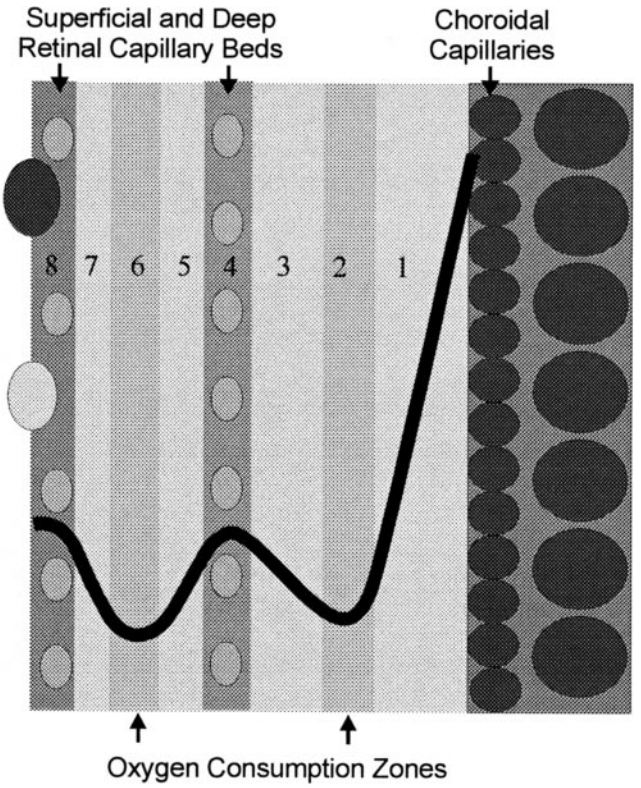

FIGURE 1. Schematic model of oxygen distribution across the eight layers of the retina. Layer 1: outer segments of the photoreceptors; layer 2: inner segments; layer 3: outer nuclear layer; layer 4: deep retinal capillaries and the outer plexiform layer; layer 5: inner nuclear layer; layer 6: outer region of the inner plexiform layer; layer 7: inner region of the inner plexiform layer; and layer 8: ganglion cell/nerve fiber layer and the superficial retinal capillaries. The oxygen consumption of the avascular region containing the inner segments of the photoreceptors (layer 2), and the predominantly avascular layer containing the inner plexiform layer (layer 6) can be determined.

where P is PO_2, x is distance, and Dk is the product of the oxygen diffusion (D) and solubility (k) coefficients.

Integrating twice gives an equation for the oxygen tension P in each layer j as a function of x

$$P_j(x) = \frac{Q_j}{2Dk} x^2 + \alpha_j x + \beta_j \qquad (1)$$

We can determine the constants α and β in each layer by applying boundary conditions.

At $x = 0$, the oxygen tension is equal to that in the choriocapillaris (P_c), and therefore from equation 1 we get $\beta_1 = P_c$ at $x = L_8$, where L_x is the layer, the boundary with the vitreous; the oxygen gradient is zero. Thus, at $x = L_8$,

$$\frac{dP}{dx} = 0$$

so

$$\frac{Q_8}{Dk} L_8 + \alpha_8 = 0$$

therefore

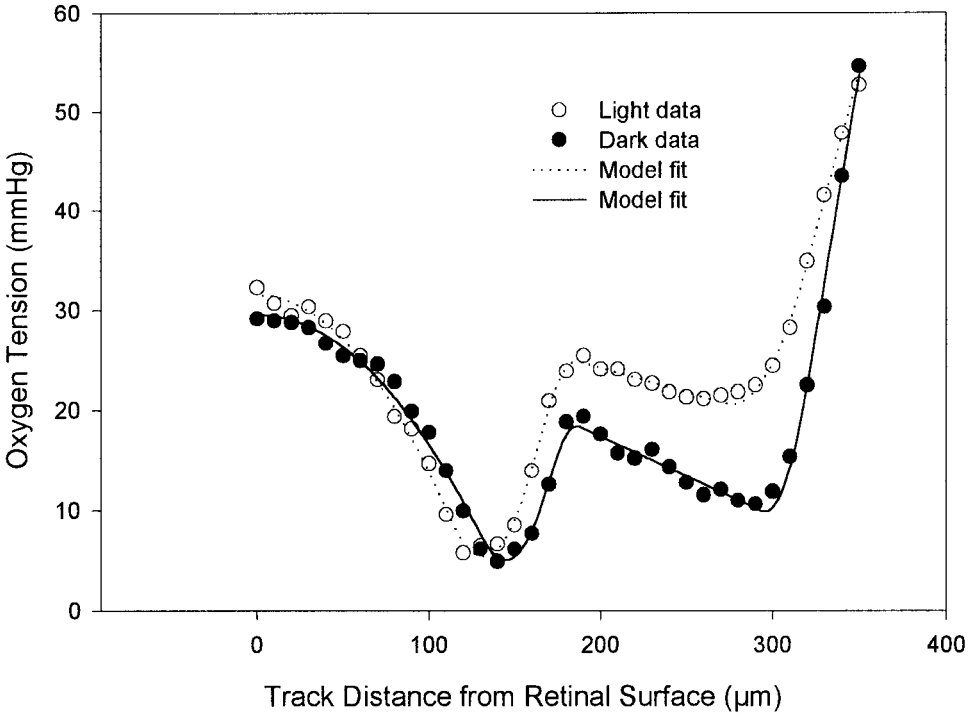

FIGURE 2. Typical intraretinal oxygen profiles in a rat under light- and dark-adapted conditions. The best fit to the model is shown superimposed for light- and dark adapted conditions.

$$\alpha_8 = -\frac{Q_8}{Dk} L_8$$

Boundary conditions at junctions between adjacent layers require that both oxygen tension and flux be continuous—for example, $P_1 = P_2$ and

$$\frac{dP_1}{dx} = \frac{dP_2}{dx}$$

at $x = L_1$. The boundary of layers 1 and 2. Thus,

$$\frac{Q_1}{2Dk} L_1^2 + \alpha_1 L_1 + \beta_1 = \frac{Q_2}{2Dk} L_1^2 + \alpha_2 L_1 + \beta_2 \qquad (2)$$

and

$$\frac{Q_1}{Dk} L_1 + \alpha_1 = \frac{Q_2}{Dk} L_1 + \alpha_2 \qquad (3)$$

Equation 2 yields

$$\beta_2 = \left(\frac{Q_1 - Q_2}{2Dk}\right) L_1^2 + (\alpha_1 - \alpha_2) L_1 + P_c$$

and equation 3 yields

$$\alpha_1 - \alpha_2 = \left(\frac{Q_2 - Q_1}{Dk}\right) L_1,$$

$$\therefore \beta_2 = \left(\frac{Q_1 - Q_2}{2Dk}\right) L_1^2 + \left(\frac{Q_2 - Q_1}{Dk}\right) L_1^2 + P_c = \left(\frac{Q_2 - Q_1}{2Dk}\right) L_1^2 + P_c$$

Applying the same analysis applied to all other boundaries determines all the integration constants. The oxygen tension at any point can then be expressed in terms of the oxygen level in the choroidal capillaries (P_c) and the oxygen consumption rate and position of each retinal layer.

Layers 1, 3, 5, and 7 were assumed to have negligible oxygen consumption. This is based on the known properties of layers 1 and 3 from outer retinal studies[2] and our desire to restrict our analysis of inner retinal oxygen consumption to the predominantly avascular layer containing the inner plexiform layer. A further restriction is that in those layers in which oxygen supply and consumption are intermingled (layers 4 and 8), we can only deal with net oxygen supply or consumption, and thus in these layers the absolute level of oxygen consumption cannot be quantified.

The equations were entered into a curve fitting routine (Sigmaplot 2000; SPSS Science) that determined the parameters producing the best fit to the experimental data by an iterative process (Marquardt-Levenberg). None of the parameters was fixed; each was allowed to vary to provide the best fit. The closeness of the fit is expressed using the resultant bivariate coefficient of determination (r^2). Data are expressed as the mean ± SE. Statistical testing was performed on computer with Student's *t*-test (Sigmastat 2000; SPSS Science).

RESULTS

Figure 2 shows a typical intraretinal oxygen profile in the rat under light-adapted and dark-adapted conditions. The symbols mark the actual data points, and the lines show the best fit to the mathematical model. The fit between the data and the model was good under both light-adapted ($r^2 = 0.996$) and dark-adapted ($r^2 = 0.992$) conditions. The closeness of the fit between the data and the model was good in all animals ($n = 20$), under both light-adapted ($r^2 = 0.987 \pm 0.002$) and dark-adapted ($r^2 = 0.983 \pm 0.006$) conditions. The mean track length through the retina was 324 ± 9.6 μm. Assuming a penetration angle of 30° from the perpendicular, this equates to a mean retinal thickness of 280 ± 8.6 μm. The same correction factor was applied to all oxygen consumption calculations. Mean oxygen consumption data of all animals are shown in Figure 3. Under light-adapted conditions the mean oxygen consumption of the outer retina was 148 ± 11 nL

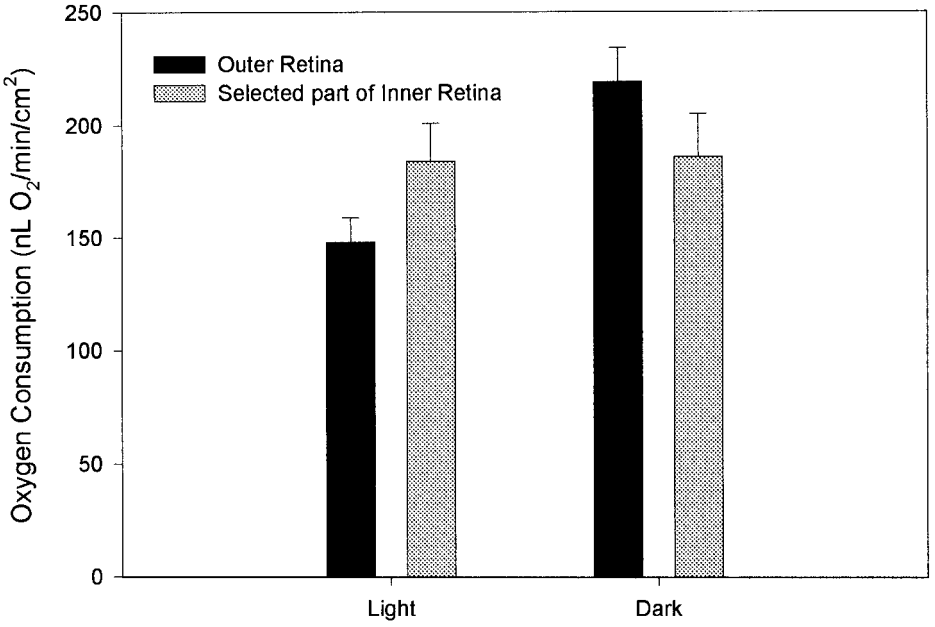

FIGURE 3. Average data for oxygen consumption in the outer retina and the selected region in the inner retina, containing the inner plexiform layer, in the light and in the dark.

O_2/min/cm^2 (n = 20) and that of the included portion of the inner retina was 184 ± 17 nL O_2/min/cm^2. The oxygen consumed by this region of the inner retina was significantly greater than that consumed by the outer retina as a whole ($P <$ 0.01). In the dark, outer retinal oxygen consumption rose to 219 ± 15 nL O_2/min/cm^2, which represented a significant increase of 47.8% ($P < 0.001$). The oxygen consumption of the included region of the inner retina was 186 +19 nL O_2/min/cm^2 in the dark, which was not significantly different from that in the light ($P = 0.899$). Transient oxygen changes in the outer retina were successfully recorded in 16 animals. An example of the change in oxygen tension as a function of time is shown in Figure 4, along with the fitted exponential curves. Average time constants and average amplitude of the PO_2 change was calculated on each animal, and the mean across all animals determined. The time constant of the half-amplitude response for the light-to-dark transition was 14.9 ± 1.8 seconds, and the magnitude of the PO_2 change was -7.7 ± 0.6 mm Hg. The dark-to-light response was slightly, but not significantly (P = 0.077), slower (time constant 19.1 ± 1.6 seconds) and of opposite polarity (7.6 ± 0.8 mm Hg). In a subset of animals (n = 7), the transient oxygen response was recorded at the retinal surface or in the region of the inner retinal minimum in the inner plexiform layer. In neither location were there consistent changes in oxygen tension after light–dark or dark–light transitions.

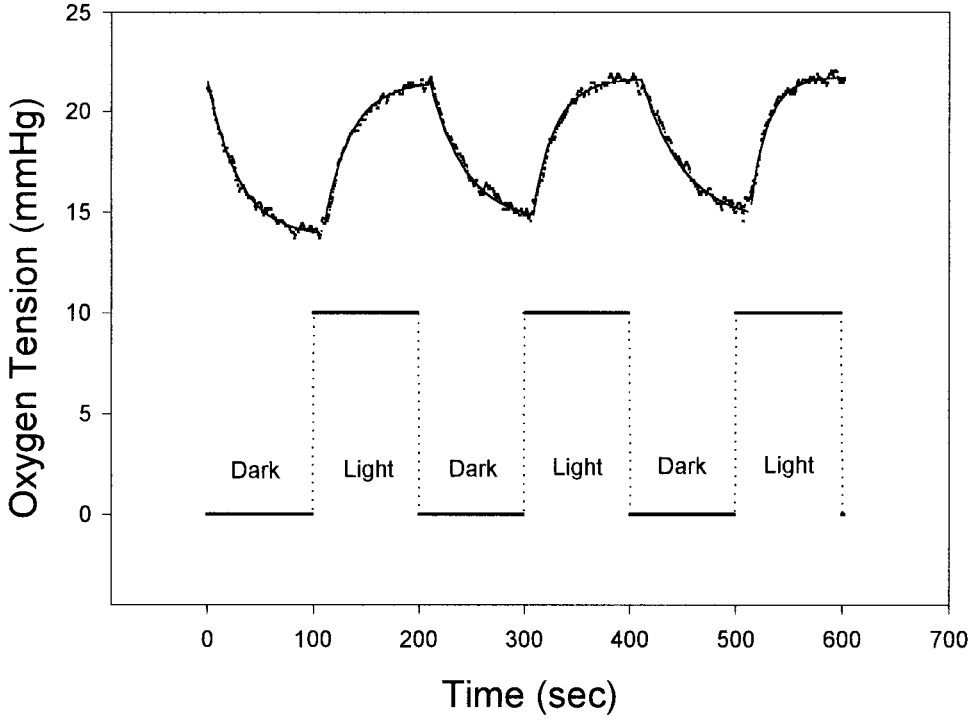

FIGURE 4. An example of the change in oxygen tension in the region of the outer retinal minimum (inner segments) during repeated light and dark transitions. The best fit to a single exponential model is shown *superimposed*.

Discussion

To our knowledge there have been no previous reports of quantitative in vivo studies of outer retinal oxygen consumption in the rat, an animal of increasing relevance to models of retinal disease. In the only previous in vivo assessment of inner retinal oxygen consumption in the rat, Zuckerman et al.[21] used an optical technique to determine the oxygen consumption of the innermost retina by examining the tangential oxygen gradients in the region of the capillary-free zone around retinal arteries. The absence of capillaries in this region allows the oxygen consumption of the tissue to be determined from a spatial analysis of the oxygen gradients emanating from the retinal artery. Their estimate of the rate of oxygen consumption in this region was 0.58 mL O_2/min per 100 mL tissue, which they noted was only approximately 25% of the published amount of outer retinal oxygen consumption reported by Linsenmeier[3] in the cat. Although it is not possible to identify exactly which retinal layers were being measured in Zuckerman et al.,[21] they argue convincingly that their measurements are dominated by the most superficial retinal layers.

Our study in the rat quantified the oxygen consumption of a deeper region of inner retina—that containing the inner plexiform layer—and demonstrated that oxygen uptake in this region of the inner retina alone exceeds that of the light-adapted outer retina. Taking into account the likelihood of significant oxygen consumption in other layers of the inner retina that could not be included in our analysis, it seems likely that, in vivo, the oxygen consumption of the rat inner retina as a whole is significantly greater than that of the outer retina. This was shown to be the case in the occluded cat retina where the consumption of the inner half of the retina was 2.6 times that of the outer half under light-adapted conditions.[8] If we assume that the outer retina constitutes 40% of the total retinal thickness, then the average oxygen consumption rate for the outer retina (Q_{or}) on a per-weight basis can be calculated. The resultant data for the in vivo rat are 1.32 ± 0.1 mL O_2/min per 100 g in the light and 1.96 ± 0.14 mL O_2/min per 100 g in the dark. These consumption levels for light-adapted Q_{or} are similar to those reported in some in vivo studies in the cat (1.4,[8] and 1.6[22] mL O_2/min per 100 g). However, other studies in the cat have reported much higher levels (2.7,[2] 3.2,[3] and 5.3[23] mL O_2/min per 100 g). Light-adapted Q_{or} measured in other species include the pig at 4.4 mL O_2/min per 100 g[24] and guinea pig at 2.07 mL O_2/min per 100 g.[5]

Human data suggest an even higher rate (9.7 mL O_2/min per 100 g), although it is not known which particular cell types were being measured by this "time-to-blackout" technique, and the measurements were made under both hyperoxic and hyperbaric conditions,[1] which are now known to increase retinal oxygen consumption in some species.

In vitro studies of retinal oxygen metabolism in isolated rat retinas provide alternative methods for comparing inner and outer retinal oxygen consumption. Medrano and Fox[11] used pharmacologic blockade to identify inner and outer retinal contributions to oxygen uptake. They reported an oxygen consumption rate of 2.1 mL O_2/min per 100 g in the light-adapted retina as a whole and attributed 46% of the oxygen uptake to the inner retina. Under dark-adapted conditions, the oxygen consumption of the outer retina increased by approximately 60%, bringing the whole retinal oxygen consumption up to 2.8 mL O_2/min per 100 g, of which only 35% was attributed to the inner retina. Earlier work in dystrophic rat retinas provided an assessment of retinal oxygen uptake, with and without a viable outer retina.[25] That study estimated whole retinal oxygen consumption in the normal adult rat to be approximately 75 μL O_2/25 mg per hour, which equates to approximately 5 mL O_2/min per 100 g.[25] In an interesting finding, they noted that retinal oxygen uptake was reduced but by no means eliminated after the degenerative loss of the outer retina—a point later verified in independent studies of isolated retinas from RCS rats[13] and later supported by nonquantitative in vivo studies in this model.[17]

Comparing oxygen consumption rates under different conditions or between in vivo and in vitro environments must be undertaken with caution. There is convincing evidence that the oxygen environment can dramatically influence oxygen metabolism. This is of course to be expected at hypoxic levels at which oxygen metabolism must inevitably be suppressed, but even excess oxygen can influence the result. In isolated retinas, a direct relationship between environmental oxygen level and retinal oxygen uptake has been reported in the rat,[13] and rabbit.[26,27] There is also evidence to suggest that retinal oxygen metabolism in vivo is significantly affected by the prevailing oxygen environment, with hyperoxia leading to a large increase in retinal oxygen uptake in the pig,[28] cat,[23] and rabbit.[29] Although these studies implicated the outer retina as the site of increased oxygen uptake, Linsenmeier and Yancey[30] found that oxygen consumption in the outer retina was unaffected by hyperoxia, and more recent studies in the rat have suggested that the inner retina in particular is capable of increasing its oxygen uptake under conditions of systemic hyperoxia.[9,10] This is also supported by an earlier study in a rat under hyperoxic conditions, in which examination of the data presented in Zuckerman et al. (see Ref. 21, Fig. 6) suggests that the rate of oxygen consumption in hyperoxia was double that in normoxia.

Outer retinal oxygen consumption in vivo has been shown to be increased by dark adaptation in many species. However, the magnitude of the effect varies considerably. The present study reports a 47.8% increase in outer retinal oxygen consumption (Q_{or}) in the dark. This is much less than reported in microelectrode-based studies in the cat (118%[3], 67%[2], 200%[22], and 178%[8]), and more in line with that reported in the monkey (16%–36%)[4] and guinea pig (30%)[31] and in oxygen-extraction–based measurements in the cat (19%)[23] and pig.[24] Our data for dark-induced increase in Q_{or} are comparable to in vitro estimates of a 60% increase reported in the rat,[11] but are greater than the modest effect seen in an earlier in vitro study in rat retinas.[13]

The time course of the reduction in outer retinal PO_2 in the dark is relatively rapid, given the time required to achieve full dark adaptation. Our reduction time in the rat (14.9 seconds) is similar to that reported for the light-to-dark transition time constant in the cat (12.2 seconds).[3] In avascular retinas or avascular regions of partially vascularized retinas, the change in outer retinal oxygen consumption flows on to the inner retina and vitreous,[32] where it can be measured with relatively large electrodes[33,34] or without the need for retinal penetration.[31] Light-induced changes in preretinal oxygen level have also been observed under hyperoxic conditions, in which the masking effect of the retinal circulation is reduced.[35,36] However, under normal conditions, such as the present study in the rat, the light-induced changes in outer retinal oxygen consumption does not produce a detectable change in preretinal oxygen level.

Having quantified the normal rates of oxygen metabolism in specific layers of the rat retina in vivo and identified the effects of increased outer retinal oxygen consumption in the dark, we are now in a better position to assess the influence of the wide range of retinal disease models that are available in the rat. In particular, it is clear that the oxygen requirements of the inner retina may exceed those of the outer retina. This may help elucidate the putative role of altered oxygen metabolism in ischemic and degenerative retinal diseases.

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