Oxygen Distribution and Consumption in the Developing Rat Retina

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Purpose. To determine the intraretinal oxygen distribution and oxygen consumption in the rat before eye opening and maturation of the retina.

Methods. Oxygen-sensitive microelectrodes were used to measure the oxygen tension as a function of depth through the retina in anesthetized Sprague–Dawley rats at postnatal day (P)15. Measurements were made under air-breathing conditions and at increasing levels of systemic oxygenation (20%, 40%, 60%, 80%, and 100% oxygen) under light-adapted conditions. Oxygen consumption in the outer retina and in the predominantly avascular region of the inner retina was assessed by fitting the oxygen profiles to a mathematical model of oxygen consumption. The retinas were processed for histology and compared with retinas from mature animals.

Results. Under normal conditions, the intraretinal oxygen distribution at P15 was significantly different in some respects from that in mature animals. Oxygen consumption analysis indicated an average outer retinal oxygen consumption rate of 103 ± 15 nL/min per cm² and an inner retinal oxygen consumption rate of 42.4 ± 11.7 nL/min per cm². Inner retinal oxygen consumption was significantly (P < 0.001) lower than that previously measured in mature rats, but outer retinal oxygen consumption was similar. Systemic hyperoxia increased the oxygen level throughout the retina, but choroidal Po₂ in particular remained significantly lower than in adult rats (P < 0.001). At P15 there were marked differences in the relative thickness of some retinal layers when compared with adult rats. In particular the inner and outer nuclear layers were much thicker at P15, the outer segments of the photoreceptors and the inner and outer plexiform layers were not fully developed.

Conclusions. At P15, before eye opening, the oxygen consumption of the inner retina is lower than in mature retinas, presumably reflecting the immaturity of the retina in such young animals so soon after their first exposure to light stimuli.

Invest Ophthalmol Vis Sci. 2006;47:4072–4076 DOI: 10.1167/ iovs.05-1638

Disruption of the intraretinal oxygen environment is thought to be a component of many retinal diseases. Understanding the normal oxygen environment in the retina is necessary if the influence of disease states is to be assessed. In the rat, measurements of intraretinal oxygen distribution have now been made in mature animals and in juvenile animals as young as postnatal day (P)20. In the present work, we examined the intraretinal oxygen distribution and consumption at P15, a time when the eye is about to open and retinal development is incomplete. At this age, the naturally occurring wave of photoreceptor cell death begins. Manipulation of the retinal oxygen environment at this “critical period” can modulate the rate of photoreceptor cell death, suggesting a role for oxygen in the normal development process. The intraretinal oxygen distribution at this time point is therefore of particular interest. Oxygen information from rats younger than P20 is confined to vitreous measurements using nuclear magnetic resonance (NMR) techniques in newborn rats (20 g body weight). Such measurements of vitreous oxygen tension reflect the oxygen level of the adjacent inner retina, but they tell us little about the oxygen environment within the deeper layers of the retina. This is an important limitation, since the intraretinal oxygen distribution is remarkably heterogeneous. Even in normal animals, there can be significant hypoxia within the retina that is not reflected in vitreous oxygen levels. Thus, the normal intraretinal oxygen environment in the retina of rats younger than P20 is not known. There is histologic evidence that the structure of the rat retina continues to evolve in the first few weeks after birth. Although the number of retinal ganglion cells and axons has peaked at birth, the inner plexiform layer (IPL) first appears only on the last day of gestation and the outer plexiform layer (OPL) at P5, and neither is mature at P15. It has been demonstrated that light stimuli can alter the retinal plasticity in the IPL during retinal development after eye opening.

It therefore seems likely that the intraretinal oxygen environment will also change during this period, because the intraretinal oxygen distribution reflects the combination of oxygen sources and consuming regions in the retina. The present study was designed to measure the intraretinal oxygen environment in rats before eye opening and completion of retinal development.

Methods

The experimental techniques were similar to those reported in our earlier work in adult rats and mice. Rats (body weight, 27.8 ± 0.7 g; n = 5) at postnatal day (P)15 were anesthetized with an intraperitoneal injection of 20 mg/kg inactin, and the eye was opened by incision of the lateral and temporal eyelids. A tracheal cannulation was performed to allow mechanical ventilation. The animals were then placed in a robotic stereotaxic apparatus and the eye 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 using techniques based on those described by Whalen et al. The electrode was visualized inside the eye via a plano concave contact lens and operating microscope (OPMI, Carl Zeiss Meditec, Inc., Oberkochen, Germany). The electrode was positioned so that the tip of the electrode was placed close to the surface of the inferior retina in a region free of major retinal vessels. All electrode movements during intraretinal penetrations were under computer control, with the oxygen level being recorded at 10-μm intervals.

From the Centre for Ophthalmology and Visual Science, The University of Western Australia, Nedlands, Perth, Western Australia. Supported by the National Health and Medical Research Council of Australia and the Australian Research Council Centre of Excellence in Vision Science. Submitted for publication December 23, 2005; revised May 8, 2006; accepted July 10, 2006.

Disclosure: S.J. Cringle, None; P.K. Yu, None; E.-N. Su, None; D.-Y. Yu, None

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Oxygen-Consumption Model

Models of intraretinal oxygen consumption have expanded over time to accommodate our improved understanding of the diversity of the oxygen requirements of different layers within the retina. Early focus was on the inner segments of the photoreceptors, which were shown to be the dominant oxygen consumers in the outer retina." More recently, it has been demonstrated that in species with vascularized retinas such as the rat, inner retinal oxygen consumption is also heterogeneous, and that the inner and outer plexiform layers have a particularly high oxygen consumption." To extract oxygen consumption information from the intraretinal oxygen profiles in the present study, the data were fitted to a modified form of the model we used in previous studies in the rat." A slight modification was required because the influence of the hyaloid vasculature in such young rats means that there can be a significant oxygen gradient at the retinal surface. The boundary condition that the oxygen gradient at this location is zero was therefore replaced with the PO2 at this location.11 Because the outer retina is completely avascular, the consumption analysis includes the entire outer retina. However, in the inner retina, the superficial and deep capillary layers must be excluded from the analysis; thus, the inner retinal oxygen consumption value is primarily that of the IPL.13 Oxygen consumption values have been corrected for a 30° angle of penetration through the retina.15

Statistics

All statistical analyses were performed with commercial software (SigmaStat; Systat Software Inc, Point Richmond, CA). Student’s t-test was used to compare oxygen levels and oxygen consumption rates in P15 rats with previous data from mature animals, for each ventilation condition. One-way ANOVA was used to compare the oxygen consumption rates as a function of ventilation level in P15 and mature rats. All data are presented as the mean ± SE, and all error bars on graphs are the standard error.

RESULTS

Retinal sections taken from P15 and adult rats are shown in Figure 1. The samples are from the inferior retina approximately 2 disc diameters from the disc edge. This location corresponds with the typical location of the oxygen profile measurement. The retina has a thickness comparable with that of the mature rat retina and has a clearly defined layer structure. However, the proportion of each layer in the P15 rat was remarkably different from that in the mature animal. The outer and inner nuclear layers were notably thicker and contained at least 50% more nuclei. However, the outer segments of the photoreceptors and of the IPL and OPL were relatively thin. Deep retinal capillaries were visible in the OPL, whereas the vasculature of the choroid appeared to be compacted.

An example of an intraretinal oxygen measurement in a P15 rat is shown in Figure 2. The oxygen tension decreased with retinal depth before leveling off in advance of a rapid increase in oxygen level in the outermost retina. The best-fit curve to the mathematical model is shown superimposed. In the middle retinal layers, the perturbations in the oxygen level probably reflect the presence of deep retinal capillaries. In the example shown, the oxygen tension at the retinal surface was 20.0 mm Hg, and at the inner retinal minimum (track length, 190 μm), the oxygen tension was 3.5 mm Hg. Local perturbations of the oxygen level with depth were apparent before a steep increase in oxygen level to 18.0 mm Hg at the outer retinal boundary.

The average intraretinal oxygen levels as a function of track depth for different levels of oxygen ventilation in P15 rats (n = 5) are shown in Figure 3. With 20% oxygen the average oxygen level at the retinal surface was 22.4 ± 3.9 mm Hg, which was not significantly different from that in mature animals (18.6 ± 1.3 mm Hg, P = 0.582). The average oxygen tension in the choroid was 21.9 ± 2.6 mm Hg which was significantly lower that in mature animals (42.3 ± 2.0 mm Hg, P = 0.002). With successive increases in inspired oxygen, intraretinal oxygen tension increased. With 100% oxygen ventilation, the average oxygen level at the retinal surface rose to 74.6 ± 12.6 mm Hg, and choroidal oxygen tension rose to 73.2 ± 9.5 mm Hg. Compared with previous data from mature animals, the PO2 at
the retinal surface was not significantly different ($P = 0.093$) but the choroidal Po$_2$ was lower ($P < 0.001$) in the P15 animals.

The average outer retinal oxygen consumption in P15 rats ventilated with 20% oxygen was $103 \pm 14.9$ nL/min per cm$^2$ which was not significantly different from that in previous studies in mature rats which was reported to be $148 \pm 11$ nL/min per cm$^2$ ($P = 0.074$) and $128.4 \pm 18.5$ nL/min per cm$^2$ ($P = 0.319$) respectively.$^{13,14}$ Average outer retinal oxygen consumption is shown in Figure 4 as a function of the percentage of inspired oxygen. The data for P15 rats ($n = 5$) are shown together with published data for mature rats ($n = 5$).$^{14}$ At both ages, there was no significant change in outer retinal oxygen consumption with increasing levels of inspired oxygen.

The average inner retinal oxygen consumption in P15 rats ventilated with 20% oxygen was $42.4 \pm 11.7$ nL/min per cm$^2$ compared with $184 \pm 17$ nL/min per cm$^2$ and $136.1 \pm 5.6$ nL/min per cm$^2$ in the earlier studies in mature rats.$^{13,14}$ The oxygen consumption rate of the inner retina at P15 was significantly lower than that in mature rats (both $P < 0.001$). The average inner retinal oxygen consumption is shown in Figure 5 as a function of the percentage of inspired oxygen. The data for P15 rats ($n = 5$) are shown together with published data for mature rats ($n = 5$).$^{14}$ In both P15 and mature rats, inner retinal oxygen consumption tended to increase with inspired oxygen level, and this increase was statistically significant in the mature animals ($P < 0.05$) but did not reach statistical significance in the P15 group ($P = 0.18$).
**DISCUSSION**

The retina of the rat is immature at birth when compared with humans, corresponding to that of a 4-month-old human fetus. This relative immaturity of the retina appears to be a common feature of many of the nonprimate animals used in medical research, such as mouse, rabbit, cat, and dog. At P15 in the rat, a time when the eye has not yet opened fully, our measurements of intraretinal oxygen distribution and consumption provide important information about the retinal cellular environment and metabolic activity in the immature retina. Previous measurements of oxygen tension in the immature rat eye have been confined to the vitreous compartment.

Our results show that although the intraretinal oxygen distribution at P15 has similarities to that in adults, reflecting the presence of major oxygen sources and sinks, there are significant differences in some retinal layers. Analysis reveals that inner retinal oxygen consumption is significantly lower in the P15 animals. This correlates well with the histologic appearance of the retina, which shows the IPL to be underdeveloped at this time point. In vitro studies of retinal oxygen uptake have suggested the total retinal oxygen uptake at P15 to be less than 70% of that in the mature rat. However, those studies did not discriminate between inner and outer retinal oxygen uptake. Our study suggests that the lower oxygen consumption of the retina at P15 is due to lower oxygen consumption in the inner retina.

A further difference in the intraretinal oxygen distribution at P15 is a lower choroidal PO2 than that of mature rats. It may be that the choroid is not fully developed at P15. Histologically, the choroid appeared compacted, but this may not be a reliable measure of choroidal function. The fact that the PO2 at the retinal surface was in the normal range for microelectrode or noninvasive NMR measurements makes it unlikely that general systemic hypoxia could be responsible. The lower choroidal PO2 also persisted with systemic hyperoxia. We are not aware of any choroidal blood flow measurements in young rats, but it seems reasonable to speculate that choroidal blood flow may be lower in young than in adult rats. We can be confident that the low choroidal PO2 is not due to high intracellular pressure due to displacement of vitreous by the microelectrode. Recent calculations for the mouse eye, which is even smaller than the eye of a P15 rat, demonstrated that the vitreous displacement by the electrode is negligible in terms of the spatial distribution of retinal oxygen tension and consumption in the face of a low choroidal PO2. In vitro studies of retinal oxygen uptake have suggested the total retinal oxygen uptake at P15 to be less than 70% of that in the mature rat. However, those studies did not discriminate between inner and outer retinal oxygen consumption. Our study suggests that the lower oxygen consumption of the retina at P15 is due to lower oxygen consumption in the inner retina.

In the present study, we have chosen to express oxygen-consumption rates in terms of oxygen uptake per square centimeter of retina. If we assume that the outer retina makes up 40% of the retinal thickness then we can convert the oxygen consumption to a per weight basis. Outer retinal oxygen consumption in the present study in P15 rats equates to 0.92 ± 0.13 mL/min per 100 g. Expressing an oxygen consumption rate for the inner retina on a per weight basis is not appropriate because not all the inner retinal layers were included in the analysis.

The small size of the rats at P15 and some aspects of the structure of the eye pose technical difficulties for microelectrode-based technologies to explore the intraretinal oxygen environment. The hyaloid system is still present at P15 and the electrode needs to penetrate the hyaloid membrane. This sometimes leads to breakage of the electrode tip, with the sudden change in electrode current, indicating that a replacement electrode is necessary. Visualization of the retinal surface and vasculature is also more difficult because of the influence of the hyaloid vasculature and membrane.

Monitoring systemic conditions such as blood pressure and heart rate requires a different approach than that used in mature rats. Measurement of blood pressure by direct cannulation of the femoral artery is problematic in such a small animal. Instead, we opted to use a commercially available tail-cuff system for measuring systemic blood pressure. This allows only occasional blood pressure measurements, but is sufficient to monitor blood pressure at key stages in the experiment. Exsanguination of blood samples for blood gas analysis is also problematic because, at P15, the body weight is less than 30 g, and blood volume would be rapidly depleted. There is also the risk that the surgical trauma of cannulation of a major systemic artery will adversely affect the systemic condition of such a small animal. However, to rule out the possibility that low systemic blood oxygen levels were responsible for the lower choroidal PO2 observed, we subjected a group of P15 rats to the same anesthetic protocol and extracted a sample of arterial blood from the aorta for blood gas analysis. The mean arterial PO2 was 92.6 ± 15.4 mm Hg (n = 7), which is comparable to our previous measurements in adult rats under air breathing conditions. This is consistent with other studies in young rats which showed that arterial PO2 was not suppressed in young animals.

The main findings from the present study illustrate that the oxygen environment and oxygen consumption rates change significantly during the natural process of retinal development. This is the first direct evidence that oxygen supply and consumption within the retina indeed change during retinal development. A better understanding of the role of oxygen in retinal development may be important in understanding the role of abnormal oxygen changes in diseases such as retinal degeneration or retinopathy of prematurity.

**Acknowledgments**

The authors thank Dean Darcey and Judi Granger for expert technical assistance.

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