Preretinal oxygen changes in the rabbit under conditions of light and dark

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Preretinal oxygen measurements were made in pigmented rabbits under conditions of light and dark. The avascular rabbit retina was chosen to eliminate the effects of autoregulation by the retinal vasculature, thus more clearly defining the role of the photoreceptors on preretinal measurements of oxygen delivery from the choroid. Measurements were made 50–100 μm away from the retina using oxygen microelectrodes. An average preretinal oxygen value of 9.8 ± 1.3 SE mm Hg (n = 12) was measured in room light under normoxic conditions. A change from light to dark conditions always resulted in a measured decrease in preretinal oxygen levels. During the first 30 min of dark adaptation, a 25.8% (±5.5% SD) decrease was obtained. This oxygen decrease is reversible during sequential light adaptation, reaching plateau in approximately 15–20 min. These results indicate that the photoreceptors have a significant effect on choroidal oxygen transmission across the retina. Invest Ophthalmol Vis Sci 29:988–991, 1988

Under normal conditions, the underlying principles that determine oxygen distribution throughout the retina are diffusion of oxygen from the choroidal and retinal vasculatures and its subsequent use by retinal tissue. Previous studies have shown the photoreceptor-retinal pigment epithelium (RPE) complex to be the major oxygen consumer in the retina. In vitro, photoreceptor oxygen consumption has been shown to vary under light and dark conditions. In vivo studies have confirmed the existence of light-dependent retinal oxygen consumption.

One of the problems in determining the effect of the photoreceptors on preretinal oxygen levels has been compensatory changes (autoregulation) by the retinal vasculature, as shown by Feke et al. Therefore, minimization of the effects of retinal vasculature autoregulation is essential to better define the role of the photoreceptors in modulating oxygen delivery from the choroid to the inner retina. The avascular rabbit retina offers the opportunity to study the influence of choroidal oxygen on the inner retina without the confounding effects of the retinal vasculature. This report is the first attempt to determine the effects of light and dark conditions on preretinal oxygen tension in the rabbit.

Materials and Methods. Electrode: The electrode used for these experiments was a polarographic, PO2-sensitive needle electrode (Diamond Electro-tech oxygen microelectrode #760, M1, Ann Arbor, MI). It had the following specifications: length, 2.5 cm; tip diameter, 0.7 mm; typical current, 50.0 nanoamps (@ PO2 = 150 mm Hg); time constant, (0–90%) <4.0 seconds, membrane thickness, 50.0 μm; and temperature coefficient, ±5.0% per degree Celsius. Electrode oxygen consumption at 10 nA (PO2 = approx. 30 mm Hg) is 1 × 10^{-12} moles/min, or about 1 × 10^{-5} that of 1 mm² of retina. Thus, the polarographic electrode does not represent a significant oxygen sink compared to the immediately adjacent retina.

The electrode was polarized for 24 hr at −0.750 volts in a normal saline solution before each experiment. Just prior to its use and immediately after each experiment, the electrode was calibrated (Model 1251 calibration cell; Transidyne General Co., Ann Arbor, MI) in a normal saline solution at 37.0°C equilibrated with analyzed gases of approximately 0, 5.0, 10.0 and 21.0% O2 (Med-Tech Gases, Medford, MA). Data were excluded if more than 5.0% drift was noted. Oxygen percentage was converted to partial pressure as follows: PO2 = (760-47) × % O2. Calibration at 0% O2 assumed 0.5% residual O2 in the equilibrated saline and the chemical microsensor was set to read 3 mm Hg.

Anaesthesia: Pigmented rabbits (male and female) weighing 2–3 kg were anesthetized with an initial i.m. injection 0.75 ml of ketamine HCl (100 mg/ml) and 0.5 ml of xylazine (20 mg/ml). An ear vein was catheterized with a heparin-treated butterfly needle to maintain I.V. anesthesia with a 1:1 solution of sodium pentobarbital (50 mg/ml) and sterile normal saline. This was given in 0.1–0.2 ml increments as needed. Alternatively, urethane 1.5 g/kg was administered I.V. and anesthesia maintained by i.m. injection of ketamine 10 mg/kg. Mean arterial blood pressure (MAP) and heart rate were monitored by means of a cannula inserted into the femoral artery. While these parameters were more stable in animals anesthetized with urethane, no difference was found in
the preretinal PO$_2$ readings compared to those obtained under ketamine and pentobarbital anesthesia. To facilitate insertion of the microelectrode into the eye the head of the rabbit was mounted in a stereotaxic restraint device (Harvard Apparatus, S. Natick, MA). One drop of 1.0% atropine and 0.5% mydriacyl were placed in the rabbit's eye to dilate the pupil. Temperature was monitored by a digital thermometer (Model 49TA, YSI, Co., Inc., Yellow Springs, OH) and rectal probe (YSI, series 400, probe #15-176-24). Temperature was maintained between 37.0 and 38.5°C with a heating pad when required. Xylocaine (1.0%, 10 mg/ml) was used for topical anesthesia and sterile saline for irrigation.

Electrode placement: A hole was made superiorly in the sclera 5-6 mm behind the limbus using an 18 gauge needle. Immediately a polarographic needle electrode was inserted and attached to a hydraulic microdrive system (Model 607WCP; DKI, Tujunga, CA). A reference electrode (#344, Diamond Electrotech) was placed under the eyelid of the other eye. Both electrodes were attached to a chemical microsensor (Model 1201, Transidyne General Co.). IOP was maintained by a hydrostatic pressure head applied to the anterior chamber through a 25 gauge needle attached to a saline reservoir placed at a height of 27 cm (=20 mm Hg) above the level of the rabbit's eye.

Using the microdrive, the needle electrode was advanced toward the retina. The needle was observed during this procedure through a zero-power contact lens on the cornea and a Zeiss binocular operating microscope. Once the dimpling of the retina was seen, indicating contact, the needle was withdrawn 50-100 µm to its preretinal position. Care was taken to place the electrode where there were no blood vessels (which are found primarily in the visual streak in rabbits), approximately 7-10 mm below center of optic disc. To record the values measured by the electrode, the microsensor output was connected to a chart recorder (Model 17401 A, Hewlett Packard, Palo Alto, CA, paper—#9280-258). A simultaneous tape recording of the microsensor output was also made (HP 3968A instrumental recorder).

Experimental procedure: Once the needle electrode was in place, the lights were turned off. The only remaining light source was dim red illumination. The rabbit was then allowed to dark-adapt for at least 30 min. On occasion, more time was allowed if it was felt that the PO$_2$ was still changing significantly. An intense (16.7 µWcm$^{-2}$ sec$^{-1}$) tungsten source was directed into the eye following adaptation, and changes in the PO$_2$ were recorded for an average of 30 min. This process of alternate dark and light adaptation continued anywhere from two to four cycles, depending on the stability of the animal. Inspection of the retina for damage was made continuously throughout the experiment. Afterwards, the rabbit was sacrificed with an overdose of pentobarbital, I.V. or i.c.

All rabbits used in this study were housed and handled according to the guidelines of the ARVO Resolution on the Use of Animals in Research.

Results. In order to determine the appropriate preretinal area that is not affected by oxygen gradients from the visual streak vasculature, the preretinal oxygen topography was measured (Fig. 1). Under room light conditions, the preretinal oxygen profile perpendicular to the visual streak showed a decreasing oxygen gradient from the optic disc towards the periphery. Between 7 and 10 mm from the optic disc there was a plateau region which was used for our light and dark measurements. In this region an average preretinal oxygen value of 9.8 ± 1.3 SE mm Hg (n = 12) was measured in room light under normoxic conditions.

A change in the preretinal oxygen level was always noted with a change in light conditions: increasing in the light and decreasing in the dark. Under dark conditions, a 25.8% (±5.5% SD, n = 12) decrease in preretinal oxygen levels (PO$_2$) was measured. The results, shown in Figure 2, from a single representative experiment demonstrate the reversible changes in
Effect of Sequential Light/Dark Cycles on Preretinal Oxygen Levels

**Fig. 2.** Continuous recording of preretinal PO$_2$ during repeated light/dark cycles in a single experiment. Although amplitudes show some variability, the directionality of changes were consistent throughout (increase in light and decrease in dark). PO$_2$ values are expressed as partial pressure of oxygen in mmHg.

Preretinal oxygen levels in response to light and dark. These changes consisted of slow graded increases or decreases in preretinal PO$_2$. The light and dark changes in preretinal oxygen levels could be measured without significant decrease in amplitude of response for 3-4 hr following initial anesthesia of the rabbit.

Upon initiating dark conditions, preretinal oxygen levels gradually decreased until a stable plateau was reached at approximately 30 min (Fig. 3A). Under light conditions, preretinal oxygen levels increased until a plateau was reached at approximately 15 to 20 min (Fig. 3B). Consistently, the response to light was slightly faster than the response to dark. For both light and dark changes the time course was nonlinear and approximated an exponential curve. Once a plateau was reached under either light or dark conditions, preretinal oxygen levels remained stable for at least 30 min.

While changes in the readings were in the same direction as would be expected from a temperature effect on the electrode, change in electrode temperature did not appear to be contributing factor. Imposing an IR cut off filter (0% transmission @ >850 nM) into the illumination path had no effect on the micro-electrode reading. Additionally, there was no significant difference between PO$_2$ measurements obtained under high intensity illumination plus room light and room light alone.

**Discussion.** We have previously shown that the neurosensory retina has an extremely high oxygen consumption which is primarily accounted for by the photoreceptor dark current.$^3$ Furthermore, light decreases this photoreceptor oxygen use by suppressing the dark current. This finding suggests that the photoreceptors are important in modulating the oxygen transport from the choroidal blood to the inner retina.$^1$ If this hypothesis is correct then inner retinal oxygen levels should vary between conditions of light and dark. Measurement of this effect is difficult due to the capability of the retinal vasculature to autoregulate. A previous study has shown that oxygen satu...
ration increases in the retinal veins of humans when the lights are turned on, indicating an increase in inner retinal oxygenation. The only study to directly measure light/dark oxygen changes in the inner retina resorted to hyperoxic conditions in order to negate the effects of autoregulation by the retinal vasculature. We were able to measure the light/dark difference in preretinal oxygen levels under physiological conditions by using the avascular rabbit retina.

As was found in the previous in vivo light/dark oxygen studies, we always observed an increase in preretinal oxygen levels when going from the dark to light (Fig. 2). The 26% change in preretinal oxygen levels that we observed in rabbits was less than half the indirectly estimated change noted in humans. This difference is most likely related to differences in retinal metabolism between species with and without inner retinal vasculatures. Species without inner retinal vasculatures appear to have a greater capacity for anaerobic metabolism based on greater retinal glycogen stores.

In the absence of a retinal vasculature this oxygen difference can only be accounted for by a variation in oxygen flux across the retina. This is unambiguous support for our hypothesis that the photoreceptors gate oxygen transmission between the choroid and inner retina. Indeed, we believe that the observed 10-second delay between the initiation of a light change and the measurement of an oxygen change represents transretinal diffusion time. Furthermore, the time course of the change in oxygen resembles the kinetics of photoreceptor adaptation (Fig. 3). The results of this study will be useful in our future efforts to develop a comprehensive model of oxygen delivery and use in the retina.

Key words: oxygen, rabbit, retina, light and dark, microelectrode


References