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Retinal Oxygen Tension Is Higher in Light than Dark

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ABSTRACT. The oxygen tension at the inner retinal surface in rabbits was measured with intraocular polarographic electrodes. In the air breathing rabbit, the oxygen tension is 21 ± 9 mm Hg (mean ± 1 SD, $n = 6$) in 12 foot-candles white light at the cornea. The oxygen tension falls 6 ± 2 mm Hg (mean ± 1 SD, $n = 4$, $p < 0.02$) in darkness. In bright white light (800 foot-candles), the retinal oxygen tension is about 6 mm Hg higher than at 12 foot-candles. The *in vivo* retinal oxygen consumption was calculated to be 2.4 ml O_2/100 g/min in light and 2.6 ml O_2/100 g/min in dark. The higher oxygen consumption is probably responsible for the lower oxygen tension of the retina in dark as compared to light. Breathing 100% oxygen elevates the preretinal oxygen tension to 190 ± 72 mm Hg (mean ± 1 SD, $n = 4$) in light. (*Pediatr Res* 23: 5–8, 1988)

Abbreviations

P(x), oxygen tension at distance x from Bruch's membrane
Q, retinal oxygen consumption
Q_i, inner retinal oxygen consumption
Q_o, outer retinal oxygen consumption
D, diffusion constant for oxygen
k, solubility constant for oxygen
x_L, thickness of retina
x_a, thickness of outer retina
P, oxygen tension
D, diffusion constant for oxygen

The physiological effects of light and oxygen on the retina are of considerable general interest and specifically play a role in the pathogenesis of retinopathy of prematurity. Retinopathy of prematurity has long been known to be related to oxygen breathing (1). Recently, Glass *et al.* (2) reported that bright lights also contribute to the development of retinopathy of prematurity. This report shows that light elevates the retinal oxygen tension in rabbits. Therefore, illumination may not be an independent risk factor for the development of retinopathy of prematurity, but rather acts through raising the retinal oxygen tension. The report also shows that preretinal oxygen levels rise to levels above the hemoglobin saturation level when the rabbits breathe 100% oxygen. The extraordinarily high oxygen tension of the retina in hyperoxia may help explain the sensitivity of the immature retina to oxygen breathing. Also a method to calculate the retinal oxygen consumption *in vivo* is shown, and the oxygen consumption calculated in light and dark.

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MATERIALS AND METHODS

Six New Zealand Red rabbits were anesthetized with ketamine hydrochloride (20–30 mg/kg intramuscularly) and anesthesia was maintained with alphachloralose (80 mg/kg intravenously). The pupils were dilated with 2.5% phenylephrine and 1% tropicamide on the cornea. The rabbits were intubated and mechanically ventilated. A femoral artery was cannulated for continuous arterial blood pressure measurements and arterial blood oxygen and carbon dioxide tension, pH (blood gas analyzer, Instrumentation Laboratory Inc., Lexington, MA, model 713), and hematocrit determinations (Table 1). The electrocardiogram and rectal temperature were continuously monitored and body temperature maintained close to 38° C with a heating pad. The animal was placed in a stereotactic head holder. A lateral canthotomy and a conjunctival peritomy were performed. Holding ties were placed under two recti muscles. The sclera was punctured with a cannula in the area of the pars plana of the ciliary body and the polarographic oxygen electrode (Diamond Electrotech Inc., MI, model 760) placed in the vitreous gel approximately 0.1 mm in front of the avascular retina as described previously (3, 4). The preretinal oxygen tension was measured continuously while the light levels varied between darkness when the room was darkened and the animal's head covered with a thick black cloth and 12 foot-candles of white light (General Electric Inc., Fairfield, CT, "Watt Miser 35") measured at the corneal level with the pupil fully dilated. In one experiment (two eyes), the light level was varied between dark, room light (12 foot-candles), and the Zeiss operating microscope light with the illuminance at the cornea measuring 800 foot-candles and the measurement performed on the illuminated area of retina. A plano-concave corneal contact lens was in place during the experiments. This eliminates the refractive power of the cornea and reduces the refractive power of the eye to the degree that little focusing of the diffuse lights takes place and insignificant magnification of the probe in the vitreous cavity is seen. The illuminance measured at the corneal level should be close to the illuminance at the retinal surface. However, a more detailed analysis of the optics of the rabbit eye is necessary to exactly quantitate the illuminance at the retina.

Oxygen consumption calculation. The avascular portion of the rabbit retina is a 0.18-mm thick layer of tissue that receives oxygen from the choroid. Alm and Bill (5) showed that the oxygen tension in venous choroidal blood in the cat is almost the same as arterial blood due to the relatively great blood flow rate. The oxygen extraction in the uvea is only 1.02 ± 0.16 volume percent. Elgin (6) found the arteriovenous oxygen content difference in the dog to be 0.4–0.9 volume percent and the difference in oxygen tension between arterial blood and uveal venous blood to be only 16 mm Hg when the dogs breathed atmospheric air. We consider the choroid a constant tension oxygen source with oxygen tension equal to the arterial blood oxygen tension. The oxygen diffuses from the choroid, through the retina, where it is consumed, and to the vitreous where the oxygen tension is measured. The oxygen tension (P) drop from

Table 1. *Mean arterial blood pressure, MAP (mm Hg), arterial blood oxygen tension, PaO_2 (mm Hg), carbon dioxide partial pressure, $PaCO_2$ (mm Hg), and pH, hematocrit (%), and rectal temperature (°C) (mean ± 1 SD, n = 6)*

MAP	76 ± 10
PaO_2 (on 21% O_2)	125 ± 23
PaO_2 (on 100% O_2)	343 ± 77
$PaCO_2$	40 ± 5
pH	7.40 ± 0.04
Hematocrit	35 ± 6
Temperature	38.0 ± 0.8

the choroid to the preretinal vitreous is determined by the distance (x) from the choroid, the diffusion constant (D), oxygen solubility (k), and the oxygen consumption (Q), as derived from Fick's law of diffusion. The oxygen consumption is the only variable where the same retinal area is measured over time and the thickness, diffusion, and temperature stay constant.

The oxygen flux across the retina is determined by Dk dP/dx. In a steady state, the oxygen consumption Q is related to the second derivative of the oxygen tension, *i.e.*

$$Q = Dk \frac{d^2P}{dx^2} \quad (1)$$

Equation (1) can be solved to give the retinal oxygen tension at distance x from the choroid (P[x]).

$$P(x) = \frac{x^2Q}{2Dk} + Bx + P(0) \quad (2)$$

where P(O) is the oxygen tension at the chorioretinal interface. We assume that at the inner retinal surface (x_L) the flux from the retina into the vitreous is zero in the steady state (7). This is reasonable due to the very low oxygen consumption of the vitreous.

$$\frac{dP}{dx} = 0 = \frac{Qx_L}{Dk} + B \quad (3)$$

$$B = -\frac{Qx_L}{Dk} \quad (4)$$

By inserting the value for B in equation (2) we find:

$$P(x_L) = \frac{Qx_L^2}{2\ Dk} - \frac{Qx^2_L}{Dk} + P(0) \quad (5)$$

or

$$P(0) - P(x_L) = \frac{Qx_L^2}{2Dk} \quad (6)$$

Alm and Bill (8) demonstrated that 80% of the oxygen consumption in the cat retina occurs in the outer retina, where the photoreceptors have a high density of mitochondria. For the purposes of calculating the retinal oxygen consumption, we divide the retina into the outer retina with oxygen consumption Q_o that equals 1.6 Q and the oxygen consumption in the inner retina Q_i equals 0.4 Q, where Q is the oxygen consumption of the retina as a whole (mlO_2 [min g]$^{-1}$).

Equation (6) can be amended for a 2 layer retina to give:

$$P(0) - P(x_L) = Q\left[\frac{Q_{0x}x^2a}{2QDk} + \frac{Q_i(x^2_L - {}^{x2}{}_a)}{2QDk}\right] \quad (7)$$

where x_a = 0.009 cm is the border between the outer and inner retina (4). The thickness (x_L) of the rabbit retina varies with location and the avascular retina averages 0.018 cm. The diffusion constant and solubility, Dk, for oxygen in rabbit cerebral cortex is 2×10^{-5} ml O_2 atm^{-1} cm^{-1} min^{-1} (9, 10).

Inserting numbers for Q_o/Q (1.6), Q_i/Q (0.4), x_a and x_L = 0.018 cm (inner border of retina) gives the retinal oxygen consumption Q as:

$$Q = [P(0) - P(x_L)]\ 17.6\ mlO_2\ min^{-1}\ atm^{-1}\ (100\ g)^{-1} \quad (8)$$

We measure $P(x_L)$ as preretinal oxygen tension and P(O) as arterial blood oxygen tension and insert in units of atmospheres into equation (8) to calculate the retinal oxygen consumption Q.

RESULTS

The preretinal oxygen tension in air breathing rabbits in 12 foot-candles white fluorescent light is 21 ± 9 mm Hg (mean ± 1 SD, n = 6). When the lights are turned off the preretinal oxygen tension falls 6 ± 2 mm Hg (mean ± 1 SD, n = 4, p = 0.018) or 24 ± 9% (mean ± SD, n = 4) (Fig. 1). The oxygen tension difference between the arterial blood and the preretinal vitreous is 105 ± 26 mm Hg (mean ± SD, n = 4) in light and this difference is increased by 6 ± 2 mm Hg (mean ± SD, n = 4, p = 0.018, two-tailed paired t test) when the lights are turned off. This corresponds to retinal oxygen consumption of 2.4 ml O_2 min^{-1} 100 g^{-1} in light (12 foot-candles) and 2.6 ml O_2 min^{-1} 100 g^{-1} in dark. In one experiment the preretinal oxygen tension was 17 mm Hg in dark, 23 mm Hg in room light, and 29 mm Hg in 800 foot-candles white light while the arterial blood oxygen tension stayed at 123 mm Hg (Fig. 2).

Breathing 100% oxygen raised the preretinal oxygen tension to 190 ± 72 mm Hg (mean ± SD, n = 4) in 12 foot-candles of light (Fig. 3).

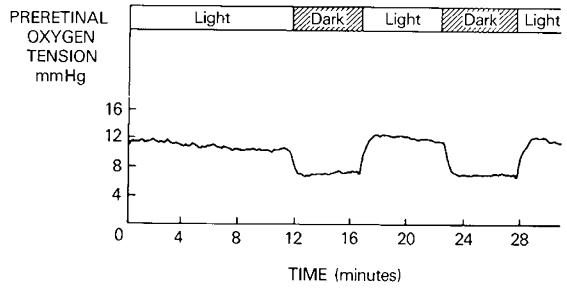

Fig. 1. Preretinal oxygen tension in the rabbit. The oxygen tension is higher in light than dark. *Light* is 12 foot-candles of white light (General Electric Watt Miser) measured at the level of the cornea with the pupil fully dilated.

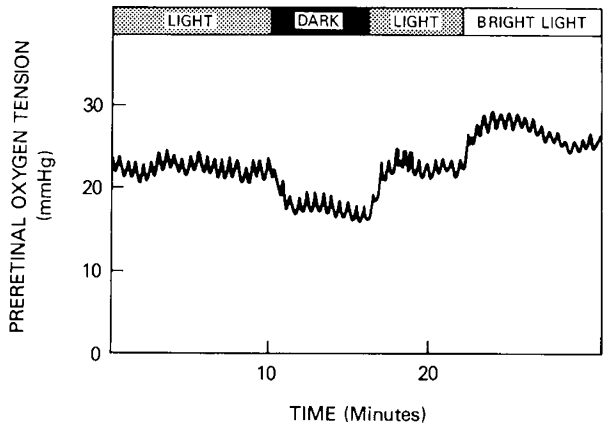

Fig. 2. Preretinal oxygen tension in air breathing rabbit. The rabbit was initially in room light (12 foot-candles), then the lights were turned off (*dark*). The room lights were turned on again (*light*) and subsequently a bright light (800 foot-candles at the cornea) was aimed at the eye. The preretinal oxygen tension shows a close relationship to the light levels.

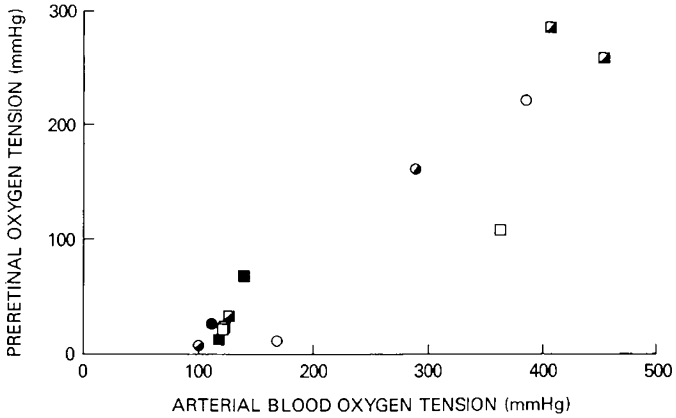

Fig. 3. Preretinal oxygen tension as a function of arterial blood oxygen tension in 12 foot-candles illuminance. Different labels are used for each experimental animal.

DISCUSSION

The oxygen tension at the retinal surface is lowest in the dark and rises with increased illumination. This corresponds to, and is probably caused by, lower oxygen consumption of the retina in light compared to the retina in the dark. The finding of decreased retinal oxygen tension in the dark is in agreement with our previous report on hyperoxic cats (11), rhesus monkeys (3), and recent reports by Tillis *et al.* (12) and Linsenmeier (13).

In humans, the increased retinal oxygen tension in light should lead to autoregulatory vasoconstriction (14) in the retina and reduced retinal blood flow in vascularized retinas in light as has been observed in the human retina (15, 16).

The calculated oxygen consumption of 2.4 (light) − 2.6 (dark) ml O_2 min^{-1} 100 g^{-1} is in good agreement with *in vitro* studies. Cohen and Noell (17) found the retinal oxygen uptake in adult New Zealand White rabbit retina to be 2.8–3.3 ml O_2/min/100 g and considerably less in young rabbit retinas. *In vitro* studies of retinal oxygen consumption in frogs have shown increased oxygen uptake in dark compared to light (18, 19). Our data shows this effect *in vivo* in a mammalian retina.

When the rabbits breathed 100% oxygen at one atmosphere, the preretinal oxygen tension rises to 190 mm Hg. The high rate of blood flow in the choroid allows oxygenation of the retina and choroid from dissolved oxygen alone (5). The choroidal and retinal oxygen tension can stay above the dissociation pressure of oxyhemoglobin and still supply the oxygen needs.

The calculation of retinal oxygen consumption *in vivo* is based on several assumptions, notably that the arteriovenous oxygen tension fall in the choroid is small enough to be ignored and that the oxygen flux across the internal limiting membrane of the retina is negligible. These assumptions are reasonable in the air breathing rabbit. O'Day *et al.* (20) have shown the choroidal blood flow in the rabbit to be about 0.84 ml/min. Hemoglobin carries 1.34 ml oxygen on each g of oxyhemoglobin (21) and in a rabbit with a hematocrit of 35% the choroidal blood has 0.135 ml oxygen available per min if the oxyhemoglobin is fully saturated. We have measured the retinal oxygen consumption of the rabbit *in vitro* to be 5.6 μl of oxygen per min (22) and the current data indicated lower oxygen consumption *in vivo*. To supply the retina with oxygen, the arteriovenous oxygen tension difference in the choroid only needs to be 14 mm Hg or less and our assumption that the choroidal oxygen tension equals the arterial blood oxygen tension is reasonable. However, if the oxygen tension rises above the dissociation pressure of oxyhemoglobin, this assumption is no longer valid. When the rabbit breathes 100% oxygen, the arterial oxygen tension rises to 300–500 mm Hg. The oxygen requirements of the retina now come from oxygen dissolved in blood. Blood only carries 30 nl O_2/ml mm Hg of dissolved oxygen and a large arteriovenous oxygen tension difference is needed to supply the retina with oxygen (21). This will cause the choroidal oxygen tension to be markedly lower than the arterial blood oxygen tension and the difference between the arterial blood oxygen tension and the preretinal oxygen tension cannot be used to calculate the retinal oxygen consumption. Additionally, the assumption that the oxygen flux across the internal limiting lamina of the retina is negligible may not be realistic when the rabbit is breathing 100% oxygen. Therefore, we have calculated the oxygen consumption of the retina solely based on the observation in rabbits breathing 21% oxygen.

The sensitivity of the developing (avascular) retina to the oxygen breathing may be related to the retinal oxygen tension being extraordinarily high in hyperoxic conditions, especially if the subject is also in bright light such as in a neonatal intensive care unit. Glass *et al.* (2) recently reported that premature infants have a higher risk of retinopathy of prematurity (retrolental fibroplasia) if they are kept in bright light (60 foot-candles). They suggest that light may be a risk factor for the development of retinopathy of prematurity. Oxygen is a well-established risk factor in retinopathy of prematurity (1) and bright illumination may increase the risk of retinopathy of prematurity by raising the retinal oxygen tension, as the present report demonstrates.

While increased retinal oxygen consumption in dark is most probably responsible for the decreased retinal oxygen tension in dark, an alternate explanation is possible. Parver *et al.* (23, 24) have shown scleral temperature elevations that indicate increased choroidal blood flow in response to light shone in the eye being measured or the fellow eye in man and monkey. They used a very bright light source (indirect ophthalmoscope at 7.5 V setting) possibly similar to our operating microscope light. Increased choroidal blood flow may be a factor in raising chorioretinal oxygen tension in very bright light.

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