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Chorioretinal Vascular Oxygen Tension Changes in Response to Light Flicker

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Abstract

PURPOSE—To investigate oxygen tension (P_{O_2}) changes in the retinal and choroidal vasculatures in response to visual stimulation by light flicker.

METHODS—A previously developed optical section phosphorescence imaging system was used to measure P_{O_2} separately in the retinal veins, arteries, and capillaries and in the choroid before and during light flicker. Imaging was performed in rats during light flicker at frequencies between 0 and 14 Hz. Light flicker–induced changes in the chorioretinal vasculature P_{O_2} and arteriovenous P_{O_2} differences were determined. Retinal arterial and venous P_{O_2} were measured along blood vessels as a function of the distance from the optic nerve head.

RESULTS—Retinal arterial P_{O_2} and arteriovenous P_{O_2} differences increased with increasing light flicker at frequencies up to 10 Hz, after which no further increase was observed. Significant increases in retinal arterial P_{O_2} ($P = 0.009$; $n = 10$) and in retinal capillary P_{O_2} ($P = 0.04$, $n = 10$) were measured in response to light flicker at 10 Hz. Retinal arteriovenous P_{O_2} differences during light flicker were significantly greater than differences before light flicker ($P = 0.01$; $n = 10$). Retinal arterial P_{O_2} decreased significantly with increased distance from the optic nerve head ($P \leq 0.004$), whereas retinal venous P_{O_2} remained relatively unchanged ($P \geq 0.4$).

CONCLUSIONS—Measurement of changes in the chorioretinal vasculature P_{O_2} can potentially advance the understanding of oxygen dynamics in challenged physiological states and in animal models of human retinal diseases.

Oxygen consumption is a major factor in retinal metabolism. Oxygen is delivered to the retinal tissue by the choroidal and retinal circulations. The supply of oxygen is adapted according to the metabolic activity of the retinal tissue. Visual stimulation by light flicker increases the metabolic demand of the inner retina.^{1–5} Therefore, changes in the P_{O_2} of the chorioretinal vascular beds are likely to be induced by light flicker, particularly in the retinal circulation, which supplies the inner retina. Light flicker–induced changes in the intravascular P_{O_2} have been reported based on the blood oxygen level–dependent signals on magnetic resonance imaging, which only provide a combined measurement in all the chorioretinal vasculatures.⁶ Although optic nerve head P_{O_2} increases from light flicker with phosphorescence imaging,⁷ P_{O_2} changes in the retinal arteries, veins, capillaries, and the choroid have not been investigated.

Increased retinal blood vessel diameter and blood flow in response to light flicker, under normal physiological conditions, have been demonstrated,^{8–10} and reduced retinal hemodynamic

response to flicker has been reported in patients with hypertension, glaucoma, and diabetes.^{11–13} According to the Fick principle, retinal tissue oxygen consumption equals the product of retinal blood flow and arteriovenous oxygen content difference.¹⁴ Therefore, measuring retinal intravascular P_{O_2} separately in the artery and in the vein, coupled with the knowledge of blood flow, may provide a better means for assessing retinal oxygen consumption than blood flow measurement alone.

In the retina, a measurable efflux of oxygen from the arterial vasculature has been reported.^{15,16} Moreover a reduction in the arterial P_{O_2} gradient along the vessel caused by increased blood flow has been demonstrated in isolated small arterial preparations.¹⁷ Therefore, arterial P_{O_2} changes resulting from altered blood flow must be considered in interpreting the retinal intravascular P_{O_2} changes in response to light flicker.

We have previously developed a technique for measuring P_{O_2} separately in the retinal and choroidal vasculatures.^{18–20} The purpose of the present study was to measure P_{O_2} changes in the chorioretinal vasculatures and to determine the retinal arteriovenous P_{O_2} difference in response to light flicker. Given that arterial P_{O_2} is influenced by oxygen diffusion,^{15,17} variations in retinal arterial P_{O_2} along blood vessels in the absence of visual stimulation were also investigated.

MATERIALS AND METHODS

Animals

Seventeen male Long Evans pigmented rats (each weighing 450–650 g) were used for this study. The animals were treated in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were anesthetized using ketamine (85 mg/kg intraperitoneally) and xylazine (3.5 mg/kg intraperitoneally). Anesthesia was maintained by intraperitoneal infusion of ketamine and xylazine at the rate of 0.5 mg/kg per minute and 0.02 mg/kg per minute, respectively. The pupils were dilated with 2.5% phenylephrine and 1% tropicamide. A molecular probe (Pd-porphyrin; Frontier Scientific, Logan, UT) was dissolved (12 mg/mL) in bovine serum albumin solution (60 mg/mL) and physiological saline buffered to a pH of 7.4, and injected intravenously (20 mg/kg) through a tongue vein. Before imaging, 1% hydroxypropyl methylcellulose was applied to the cornea, and a glass coverslip was placed on the cornea to eliminate its refractive power and to prevent corneal dehydration.

Imaging

We have previously developed an optical section phosphorescence imaging system for quantitative measurement of P_{O_2} in the chorioretinal vasculatures.²⁰ Briefly, a laser beam ($\lambda = 532$ nm) was projected at an oblique angle on the retina after intravenous injection of porphyrin (an oxygen-sensitive molecular probe), and phosphorescence emission was imaged. The incident laser beam was not coaxial with the viewing axis; hence, choroidal and retinal vasculatures appeared laterally displaced according to their depth location on the phosphorescence optical section image. Because of the quenching of the probe phosphorescence emission by the surrounding oxygen, P_{O_2} is related to phosphorescence lifetime according to the Stern-Volmer expression: $P_{O_2} = (\tau_0 / \tau) / [1 + (\kappa_Q)(\tau_0)]$, where P_{O_2} (mm Hg) is the oxygen tension, τ (μ sec) is the phosphorescence lifetime, κ_Q (1/mm Hg μ sec) is the quenching constant for the triplet-state phosphorescence probe, and τ_0 is the lifetime in zero oxygen environment. Phosphorescence lifetime was measured with a frequency-domain approach by varying the phase relationship between the modulated excitation source and the sensitivity of the camera detector, as previously described.^{20,21}

For visual stimulation, the illumination light from a slit lamp biomicroscope was used as the source for light flicker. A filter that transmitted light at a wavelength of 568 ± 5 nm (Edmund Industrial Optics, Barrington NJ) was placed in the slit lamp illumination housing to eliminate overlap with the phosphorescence emission and thus to permit imaging during light flicker. The light power was $110 \mu\text{W}$ at the pupil. A shutter was attached to a solenoid and placed in front of the illumination housing of the slit lamp biomicroscope to alter the flicker frequency. The solenoid was externally driven by a square wave generator (Tenma, Springboro, OH) to vary the flicker frequency between 2 and 14 Hz.

Each rat was placed in front of the imaging instrument. Laser power was adjusted to $100 \mu\text{W}$, which is safe for viewing according to the American National Standard Institute for Safety Standards.²² Imaging was performed at single locations within 1 disk diameter from the edge of the optic nerve head, before and during light flicker at a frequency of 10 Hz ($n = 10$) and at varying frequencies ($n = 3$). Three sets of phase-delayed phosphorescence optical section images were acquired before light flicker was initiated, and three sets of images were acquired at the same location during light flicker, after 2 minutes of visual stimulation. In the absence of visual stimulation, imaging was performed at 20 spatially consecutive locations along a retinal artery and vein, spanning a distance of 2 disk diameters, beginning from a location near the edge of the optic nerve head ($n = 4$). Images were acquired at 6-second intervals, permitting resolution of physiological events that occurred within a factor of this time scale.

The set of phase-delayed phosphorescence optical section images was analyzed by relating the phosphorescence intensity and the experimentally varied phase delay between the modulated laser and camera sensitivity.²⁰ Measurement of P_{O_2} was based on the parameters that described the best-fit sinusoidal curve to the data points.²¹ The mean and SD of P_{O_2} in the retinal arteries, veins, and capillaries and in the choroid were calculated. Analysis of variance (ANOVA; repeated measures) was performed to determine the effect of flicker frequency on retinal arterial and venous P_{O_2} measurements. Paired Student *t* test was performed to compare measurements obtained before and during light flicker. Flicker-induced change in P_{O_2} was determined by the following expression: $(\text{P}_{\text{O}_2\text{during flicker}} - \text{P}_{\text{O}_2\text{before flicker}}) \times 100 / \text{P}_{\text{O}_2\text{before flicker}}$. Linear regression analysis was performed to determine the significance of the association between retinal arterial (or venous) P_{O_2} measurements and the distance from the optic nerve head. Statistical significance was accepted at $P < 0.05$.

RESULTS

The optimum light flicker frequency for altering P_{O_2} in the retinal vasculatures was determined based on data obtained in three rats during light flicker at frequencies of 2, 6, 10, and 14 Hz. The relationship between retinal arterial (and venous) P_{O_2} and light flicker frequency is depicted in Figure 1. Retinal arterial P_{O_2} increased with increasing flicker frequencies up to 10 Hz, after which no further increase in P_{O_2} was observed ($P = 0.004$). Retinal venous P_{O_2} remained relatively constant at all frequencies ($P = 0.5$).

Before light flicker, the average P_{O_2} readings in the retinal veins, arteries, and capillaries and in the choroid were 31 ± 5 , 41 ± 5 , 39 ± 5 , and 55 ± 7 mm Hg (mean $\pm$ SD; $n = 10$), respectively. During light flicker at 10 Hz, the average P_{O_2} readings in the retinal veins, arteries, and capillaries and in the choroid were 32 ± 5 , 46 ± 8 , 41 ± 7 , and 54 ± 7 mmHg ($n = 10$), respectively. A significant increase in the retinal arterial P_{O_2} ($P = 0.009$; $n = 10$) and the retinal capillary P_{O_2} ($P = 0.04$; $n = 10$) was measured in response to light flicker. Retinal venous and choroidal P_{O_2} did not vary significantly during light flicker ($P > 0.05$; $n = 10$). The percentage P_{O_2} changes in the retinal veins, arteries, capillaries and in the choroid are shown in Figure 2. Flicker-induced P_{O_2} changes were greatest in the retinal artery (13%) and capillaries (5%).

Retinal arteriovenous P_{O_2} difference was measured within 1 disk diameter from the edge of the optic nerve head before and during light flicker, as shown in Figure 3. The retinal arteriovenous P_{O_2} difference during flicker (14 ± 6 mm Hg) was significantly greater than the difference before flicker (9 ± 2 mm Hg; $P = 0.01$; $n = 10$).

Retinal arterial (and venous) P_{O_2} was measured at 20 locations along the retinal arteries and veins over a distance spanning 2 disk diameters (approximately 600 μ m).²³ Results obtained in one rat are shown in Figure 4. Arterial P_{O_2} decreased linearly with increased distance (slope, -0.04 ; y-intercept, 63), whereas venous P_{O_2} remained relatively unchanged (slope, -0.004 ; y-intercept, 33). The significance of the association between P_{O_2} in the retinal vasculature and distance from the optic nerve head was determined (Table 1). Retinal arterial P_{O_2} decreased significantly with increased distance from the optic nerve head ($P \leq 0.004$; $n = 20$), but retinal venous P_{O_2} did not vary significantly with increased distance from the optic nerve head ($P \geq 0.4$; $n = 20$).

DISCUSSION

In the present study, retinal arterial P_{O_2} and capillary P_{O_2} were significantly greater during light flicker than before flicker. No significant change in retinal venous P_{O_2} or choroidal P_{O_2} was observed. The arteriovenous P_{O_2} difference measured before light flicker was consistent with previously reported values in rats²¹ and increased significantly in response to light flicker. Animals in the present study were allowed to breathe spontaneously under anesthesia and, hence, were likely to be hypoxemic, as reported in our previous studies.²⁰ Although this might have altered the magnitude of the compensatory response, our results showed that the rats were capable of substantial retinal vascular compensation in response to light flicker.

Retinal arterial P_{O_2} increased with each successive increment in flicker frequency until it reached a maximum at 10 Hz. No further increase was observed at the subsequent flicker frequency of 14 Hz. In mice²⁴ and in rats (Qian H, personal communication, February 2006), the electrophysiological response to variable temporal frequencies of flicker stimulation has been shown to be maximal at a frequency similar to that at which the retinal arterial P_{O_2} change was greatest. The corresponding flicker frequency at which maximal electrophysiological and intravascular P_{O_2} responses have been observed in rats may be indicative of a relationship between retinal oxygenation and neuronal activity.

In the optic nerve head, a decrease in the optic nerve head tissue P_{O_2} concurrent with an increase in blood flow has been demonstrated with flicker stimulation.^{25,26} Contrary to our results, venous P_{O_2} measured by phosphorescence imaging was reported to increase at the optic nerve head during flicker.⁷ The change in the venous P_{O_2} may be different because the optic nerve head veins receive blood from the retina, choroid, and the optic nerve head microvasculature and because oxygen consumption in response to flicker may differ between the retina and the optic nerve head tissues.

In the retina, a measurable efflux of oxygen from the pre-capillary vasculature has been demonstrated.¹⁵ The occurrence of diffusion of oxygen from the retinal arteries is further substantiated by the presence of periarterial capillary-free zones.²⁷ Studies in isolated arterial preparations have shown that increased blood flow in small arteries and in arterioles results in a decrease in the efflux of oxygen per unit of blood volume and, consequently, a smaller longitudinal oxygen tension gradient.¹⁷ In the present study, a decrease in the intravascular P_{O_2} along the length of the retinal arteries, from proximal to distal, was measured, substantiating the presence of oxygen loss from the retinal arterial lumen. The presence of an increase in retinal blood flow in response to flicker stimulation^{8–10} and the flow-dependent

gradient in the P_{O_2} along the artery apparently explain the finding of increased retinal arterial P_{O_2} in the present study.

Retinal tissue oxygen consumption is related to the product of the blood flow and the arteriovenous oxygen content difference.¹⁴ The observation of increased arteriovenous P_{O_2} difference coupled with the previously reported increase in blood flow^{8–10} implies increased inner retinal oxygen consumption during visual stimulation by light flicker. Moreover, the finding that the increased arteriovenous P_{O_2} difference in the present study was caused by a lack of change in the retinal venous P_{O_2} and an increase in the arterial oxygen P_{O_2} indicates that the effect of increased flow on retinal arterial P_{O_2} is dominant in the regulation of oxygen supply to the inner retinal tissue during light flicker. Furthermore, this finding suggests at least a partial match between the increased oxygen consumption and supply. Retinal vascular P_{O_2} , as with the vascular P_{O_2} of other tissue, depends on the balance between the amount of oxygen delivered and the amount consumed by tissue. During light flicker, inner retinal metabolic rates are increased,^{1,2} which causes reductions in retinal venous P_{O_2} . However, the vasodilatory response to light flicker increases retinal blood flow,^{8,25,28} resulting in increased retinal arterial P_{O_2} that counteracts the effects of increased oxygen consumption on the retinal venous P_{O_2} . The arteriovenous P_{O_2} difference may vary depending on the distance from the optic nerve head because the volume of measured tissue that is consuming oxygen changes. Therefore, the findings of the present study may be limited to locations 1 disk diameter from the optic nerve head edge.

Similar to findings of a previous study that reported no change in choroidal blood flow with flicker,²⁸ the choroidal P_{O_2} remained relatively unchanged during light flicker, supporting the selective effect of the light flicker on the inner retinal metabolic activity. This finding corroborates previous studies that have reported light flicker-induced changes in the electrophysiological response^{4,5} and glucose uptake³ of the inner retina, along with a minimal change in the outer retina.

In summary, increased retinal arterial and capillary P_{O_2} caused by light flicker were demonstrated in this study. Measuring changes in the chorioretinal vasculature P_{O_2} may advance the understanding of oxygen dynamics in challenged physiological states and in animal models of human retinal diseases.

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References

1. Ames A 3rd, Li YY, Heher EC, Kimble CR. Energy metabolism of rabbit retina as related to function: high cost of Na^+ transport. *J Neurosci* 1992;12:840–853. [PubMed: 1312136]
2. Bill A, Sperber GO. Aspects of oxygen and glucose consumption in the retina: effects of high intraocular pressure and light. *Graefes Arch Clin Exp Ophthalmol* 1990;228:124–127. [PubMed: 2338249]
3. Bill A, Sperber GO. Control of retinal and choroidal blood flow. *Eye* 1990;4(pt 2):319–325. [PubMed: 2199239]
4. Falsini B, Riva CE, Logean E. Flicker-evoked changes in human optic nerve blood flow: relationship with retinal neural activity. *Invest Ophthalmol Vis Sci* 2002;43:2309–2316. [PubMed: 12091432]
5. Falsini B, Riva CE, Logean E. Relationship of blood flow changes of the human optic nerve with neural retinal activity: a new approach to the study of neuro-ophthalmic disorders. *Klin Monatsbl Augenheilkd* 2002;219:296–298. [PubMed: 12022022]

6. Duong TQ, Ngan SC, Ugurbil K, Kim SG. Functional magnetic resonance imaging of the retina. *Invest Ophthalmol Vis Sci* 2002;43:1176–1181. [PubMed: 11923263]
7. Ferrez PW, Chamot SR, Petrig BL, Pournaras CJ, Riva CR. Effect of visual stimulation on blood oxygenation in the optic nerve head of miniature pigs: a pilot study. *Klin Monatsbl Augenheilkd* 2004;221:364–366. [PubMed: 15162281]
8. Riva CE, Logean E, Falsini B. Visually evoked hemodynamical response and assessment of neurovascular coupling in the optic nerve and retina. *Prog Retin Eye Res* 2005;24:183–215. [PubMed: 15610973]
9. Garhofer G, Zawinka C, Resch H, Huemer KH, Dorner GT, Schmetterer L. Diffuse luminance flicker increases blood flow in major retinal arteries and veins. *Vision Res* 2004;44:833–838. [PubMed: 14967208]
10. Formaz F, Riva CE, Geiser M. Diffuse luminance flicker increases retinal vessel diameter in humans. *Curr Eye Res* 1997;16:1252–1257. [PubMed: 9426960]
11. Garhofer G, Zawinka C, Resch H, Huemer KH, Schmetterer L, Dorner GT. Response of retinal vessel diameters to flicker stimulation in patients with early open angle glaucoma. *J Glaucoma* 2004;13:340–344. [PubMed: 15226664]
12. Garhofer G, Zawinka C, Resch H, Kothly P, Schmetterer L, Dorner GT. Reduced response of retinal vessel diameters to flicker stimulation in patients with diabetes. *Br J Ophthalmol* 2004;88:887–891. [PubMed: 15205231]
13. Nagel E, Vilser W, Lanzl I. Age, blood pressure, and vessel diameter as factors influencing the arterial retinal flicker response. *Invest Ophthalmol Vis Sci* 2004;45:1486–1492. [PubMed: 15111606]
14. Berne, RM.; Levy, MN. *Physiology*. 2nd ed.. St. Louis, MO: CV Mosby; 1988.
15. Riva CE, Pournaras CJ, Tsacopoulos M. Regulation of local oxygen tension and blood flow in the inner retina during hyperoxia. *J Appl Physiol* 1986;61:592–598. [PubMed: 3745049]
16. Buerk DG, Shonat RD, Riva CE, Cranstoun SD. O₂ gradients and countercurrent exchange in the cat vitreous humor near retinal arterioles and venules. *Microvasc Res* 1993;45:134–148. [PubMed: 8361397]
17. Duling BR, Berne RM. Longitudinal gradients in periarteriolar oxygen tension: a possible mechanism for the participation of oxygen in local regulation of blood flow. *Circ Res* 1970;27:669–678. [PubMed: 5486243]
18. Shahidi M, Blair NP, Mori M, Zelkha R. Feasibility of noninvasive imaging of chorioretinal oxygenation. *Ophthalmic Surg Lasers Imaging* 2004;35:415–422. [PubMed: 15497552]
19. Shakoor A, Shahidi M, Blair NP, Mori M. Noninvasive assessment of chorioretinal oxygenation changes in experimental carotid occlusion. *Curr Eye Res* 2005;30:763–771. [PubMed: 16146922]
20. Shahidi M, Shakoor A, Shonat RD, Blair NP, Mori M. A method for chorioretinal oxygen tension measurement. *Curr Eye Res* 2006;31:357–366. [PubMed: 16603469]
21. Shonat RD, Kight AC. Oxygen tension imaging in the mouse retina. *Ann Biomed Eng* 2003;31:1084–1096. [PubMed: 14582611]
22. ANSI. Orlando, FL: The Laser Institute of America; 1993. American National Standard for Safe Use of Lasers—ANSI Z136.1–1993.
23. Cohan BE, Peach AC, Jokelainen PT, Bohr DF. Optic disc imaging in conscious rats and mice. *Invest Ophthalmol Vis Sci* 2003;44:160–163. [PubMed: 12506069]
24. Krishna VR, Alexander KR, Peachey NS. Temporal properties of the mouse cone electroretinogram. *J Neurophysiol* 2002;87:42–48. [PubMed: 11784728]
25. Buerk DG, Riva CE. Adenosine enhances functional activation of blood flow in cat optic nerve head during photic stimulation independently from nitric oxide. *Microvasc Res* 2002;64:254–264. [PubMed: 12204650]
26. Riva CE, Harino S, Shonat RD, Petrig BL. Flicker evoked increase in optic nerve head blood flow in anesthetized cats. *Neurosci Lett* 1991;128:291–296. [PubMed: 1945050]
27. Claxton S, Fruttiger M. Role of arteries in oxygen induced vasoobliteration. *Exp Eye Res* 2003;77:305–311. [PubMed: 12907163]

28. Garhofer G, Huemer KH, Zawinka C, Schmetterer L, Dorner GT. Influence of diffuse luminance flicker on choroidal and optic nerve head blood flow. *Curr Eye Res* 2002;24:109–113. [PubMed: 12187482]

FIGURE 1.

Oxygen tension measurements in the retinal arteries and veins obtained in three rats during light flicker at varying frequencies of 2, 6, 10, and 14 Hz. Error bars represent the SD of measurements. Measurements before light flicker are indicated by data points at 0 Hz frequency.

FIGURE 2.

Oxygen tension changes caused by light flicker at 10 Hz in the choroid and in the retinal arteries, capillaries, and veins obtained in 10 rats. Error bars represent the SE of the means of measurements.

FIGURE 3.

Mean retinal arteriovenous oxygen tension difference before and during light flicker at 10 Hz. Error bars represent the SE of the means. The difference during flicker was significantly greater than the difference before flicker ($P = 0.01$; $n = 10$).

FIGURE 4. Relationship between retinal arterial (and venous) oxygen tension and relative distance from the edge of the optic disk.

TABLE 1

Results of Linear Regression Analysis

Rat	Retinal Artery <i>n</i> = 20		Retinal Vein <i>n</i> = 20	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
1	0.82	0.004	0.20	0.6
2	0.61	0.009	0.14	0.6
3	0.79	<0.001	0.18	0.5
4	0.93	<0.001	0.24	0.4

Linear regression analysis was performed on 20 measurements taken along the lengths of the retinal blood vessels of four rats.