Response of Inner Retinal Oxygen Extraction Fraction to Light Flicker Under Normoxia and Hypoxia in Rat

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Purpose. Oxygen extraction fraction (OEF), defined by the ratio of oxygen metabolism (MO_2) to delivery (DO_2), determines the level of compensation of MO_2 by DO_2. In the current study, we tested the hypothesis that inner retinal OEF remains unchanged during light flicker under systemic normoxia and hypoxia in rats due to the matching of MO_2 and DO_2.

Methods. Retinal vascular oxygen tension (PO_2) measurements were obtained in 10 rats by phosphorescence lifetime imaging. Inner retinal OEF was derived from vascular PO_2 based on Fick's principle. Measurements were obtained before and during light flicker under systemic normoxia and hypoxia. The effects of light flicker and systemic oxygenation on retinal vascular PO_2 and OEF were determined by ANOVA.

Results. During light flicker, retinal venous PO_2 decreased ($P < 0.01$, $N = 10$), while inner retinal OEF increased ($P = 0.02$). Under hypoxia, retinal arterial and venous PO_2 decreased ($P < 0.01$), while OEF increased ($P < 0.01$). The interaction effect was not significant on OEF ($P = 0.52$), indicating the responses of OEF to light flicker were similar under normoxia and hypoxia. During light flicker, OEF increased from 0.46 ± 0.13 to 0.50 ± 0.11 under normoxia, while under hypoxia, OEF increased from 0.67 ± 0.16 to 0.74 ± 0.14.

Conclusions. Inner retinal OEF increased during light flicker, indicating the relative change in DO_2 is less than that in MO_2 in rats under systemic normoxia and hypoxia. Inner retinal OEF is a potentially useful parameter for assessment of the relative changes of MO_2 and DO_2 under physiologic and pathologic conditions.

Keywords: retina, oxygen extraction fraction, vascular oxygen tension, light flicker, hypoxia

Stimulation of the retinal tissue with light flicker increases the inner retinal energy metabolism.[1,2] This increase in energy metabolism is compensated by an augmented supply of oxygen and glucose due to increased blood flow in a process known as functional hyperemia.[3,4] Impaired functional hyperemia, as demonstrated by attenuated vasodilation during light flicker, has been reported in diabetic retinopathy[5–8] and glaucoma,[9,10] suggesting an incomplete compensatory blood flow response to increased energy metabolism. However, since to our knowledge no clinical methods are available for measurements of inner retinal energy metabolism, it is unknown if changes in oxygen metabolism and delivery (product of blood flow and arterial oxygen content) are matched in health and disease.

The relative changes of inner retinal oxygen metabolism (MO_2) and oxygen delivery (DO_2) during light flicker can be quantified by measurement of inner retinal oxygen extraction fraction (OEF), which is defined by the ratio of MO_2 to DO_2.[11] Without direct measurements of either MO_2 or DO_2, inner retinal OEF can be derived from measurements of arterial and venous oxygen levels based on Fick's principle.[11,12] During light flicker, if OEF remains unchanged, then changes in MO_2 and DO_2 are matched, while an increase or decrease in OEF with light flicker would indicate either under- or overcompensation of MO_2 by DO_2, respectively.

In the current study, we formulated the hypothesis that inner retinal OEF remains unchanged with light flicker, under systemic normoxia and hypoxia. Under normal physiology (normoxia), the increase in MO_2 with light flicker should be matched by an increase in DO_2 due to vasodilation, thus resulting in no change in inner retinal OEF. Under a severe hypoxic challenge, even without light flicker, MO_2 and DO_2 are significantly reduced.[13] With light flicker, no increase in DO_2 is expected due to hypoxia-induced maximized vasodilation. Additionally, since the tissue under hypoxia is already deficient of oxygen, MO_2 is unlikely to increase. Consequently, inner retinal OEF is not expected to change with light flicker under hypoxia. To test this hypothesis, we measured the response of OEF to light flicker in rats under systemic normoxia and hypoxia by vascular oxygen tension (PO_2) imaging.

Methods

Animals

Ten Long Evans pigmented rats (weight 444 ± 99 g, mean ± SD; $N = 10$) were used in this study. Rats were cared for in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Anesthesia was induced with intraperitoneal injections of ketamine (100 mg·kg^{-1}) and xylazine (5 mg·kg^{-1}), and maintained with supplemental doses of ketamine (20 mg·kg^{-1}) and xylazine (1 mg·kg^{-1}). The body temperatures of rats were maintained at 37°C using a heated animal holder. Blood pressure and heart rate were monitored continuously with a data acquisition system (Biopac Systems,

Goleta, CA, USA) linked to a pressure transducer connected to a catheter placed in a femoral artery.

Rats were ventilated first with room air (21% oxygen, normoxia) and then with 10% oxygen (hypoxia) through an endotracheal tube connected to a small animal ventilator (Harvard Apparatus, Inc., South Natick, MA, USA). Mechanical ventilation prevented any anesthetic depressant effect on respiration. Blood was drawn from the femoral arterial catheter to measure systemic arterial oxygen tension (P_aO_2), carbon dioxide tension (P_aCO_2), and pH with a blood gas analyzer (Radiometer, Westlake, OH, USA) and determine hemoglobin (Hgb) concentration with the use of a hematology system (Siemens, Tarrytown, NY, USA). Rats were maintained normocapnic by adjusting the respiratory minute volume and performing blood gas analysis 5 minutes after each adjustment until P_aCO_2 was within the range of 35 to 45 mm Hg.

An oxygen sensitive molecular probe, Pd-porphine (Frontier Scientific, Logan, UT, USA), was dissolved (12 mg·mL^{-1}) in bovine serum albumin solution (60 mg·mL^{-1}) and administered (20 mg·kg^{-1}) through the femoral arterial catheter, typically 10 minutes before imaging. The pupil was dilated with 2.5% phenylephrine and 1% tropicamide. During imaging, a glass cover slip and 1% hydroxypropyl methylcellulose were applied to the cornea to minimize the refractive power and prevent dehydration.

Retinal Vascular PO_2 Imaging

Retinal vascular PO_2 was measured with our previously established optical section phosphorescence lifetime imaging system.[14] In brief, a laser line was projected on the retina at an angle with respect to the imaging path. This allowed depth-resolved imaging of phosphorescence emission within the major retinal arteries and veins. Phosphorescence lifetimes were measured by a frequency-domain approach as described previously.[15] The lifetimes then were used to calculate the PO_2 values using the Stern-Volmer relationship, defined as $PO_2 = (1/\kappa_Q)\cdot(1/\tau - 1/\tau_0)$, where κ_Q (mm Hg^{-1}·μs^{-1}) is the quenching constant for the triplet-state of Pd-porphine, τ (μs) is the measured phosphorescence lifetime, and τ_0 (μs) is the lifetime in a zero-oxygen environment.[15] A red-free retinal image was acquired for documenting the locations of PO_2 measurements. Vascular PO_2 images were obtained at a nasal and temporal sector relative to the optic disc. Each sector was bounded by 2 major arteries with a major vein between them. In each sector, 3 repeated images were acquired. For each image, the mean PO_2 of the 2 major arteries (PO_{2A}) and the PO_2 of the vein (PO_{2V}) between the arteries were used for calculation of inner retinal OEF. The laser power at the cornea was approximately 40 μW, which is safe for 1 hour of continuous viewing according to the American National Standard Institute for Safety Standards.[16]

OEF Calculation

According to our previously established method for quantifying inner retinal OEF,[12] vascular PO_2 measurements (PO_{2A} and PO_{2V}) in retinal sectors were used to calculate OEF based on the following equation,

$$OEF = \frac{MO_2}{DO_2} = \frac{Blood\ Flow \cdot (O_{2A} - O_{2V})}{Blood\ Flow \cdot O_{2A}} = \frac{O_{2A} - O_{2V}}{O_{2A}}$$

where O_{2A} and O_{2V} are the arterial and venous O_2 contents, respectively. The O_2 contents were calculated as O_{2max}* Hgb * $SO_2 + k$ * PO_2, where O_{2max} is the maximum oxygen carrying capacity of Hgb (1.39 mLO_2·g^{-1}),[17] k is the oxygen solubility in blood (0.003 mLO_2·dL^{-1}·mm Hg^{-1}),[17] and SO_2 is the vascular oxygen saturation. Vascular SO_2 was calculated using the rat oxygen dissociation curve: $SO_2 = (PO_2/P_{50})^n/[1+ (PO_2/P_{50})^n]$, where n is an empirical constant taken to be 2.6,[18] and P_{50} is the PO_2 when SO_2 is 50% at the experimentally measured blood pH value.[18,19] Because O_{2V} cannot exceed O_{2A}, inner retinal OEF ranges from 0 to 1, following the equation above. The repeatability of OEF measurements was assessed previously by calculating the standard deviation of repeated measurements and was on average 0.08.[12]

Retinal Stimulation by Light Flicker

Using our previously established protocol for stimulating the retina with light flicker,[20,21] imaging was performed first under continuous light illumination (before flicker) and then under flickering light (during flicker) for each systemic oxygenation condition (normoxia and hypoxia). For light flicker stimulation, a filter with a transmission wavelength of 568 ± 5 nm was placed in front of the illumination housing of a slit-lamp biomicroscope. A shutter attached to a solenoid was placed in the light path to flicker light at a frequency of 10 Hz. The light wavelength and flickering frequency were selected based on previous studies that showed a maximal or near maximal vascular response under these conditions.[20,21] The time-averaged light powers before and during flicker were matched by doubling the light intensity during flicker. Imaging was performed before and 2 minutes after the initiation of light flicker.

Statistical Analysis

Three repeated measurements of retinal oxygenation parameters (PO_{2A}, PO_{2V}, and OEF) were averaged for each sector. Values in nasal and temporal sectors then were averaged in each rat. A 2-way repeated measures ANOVA was used to determine the effects of light flicker (before and during) and systemic oxygenation condition (normoxia and hypoxia) on each retinal oxygenation parameter. Statistical significance was accepted at $P < 0.05$.

Results

Systemic Physiological Status

The systemic physiological status of rats under normoxia and hypoxia have been reported previously.[12] As expected, the systemic P_aO_2 under hypoxia (34 ± 4 mm Hg) was significantly lower compared to that under normoxia (93 ± 8 mm Hg; $P < 0.01$, $N = 10$). Since the ventilation was controlled, systemic P_aCO_2 was not different under the two systemic oxygenation conditions ($P = 0.76$). However, under hypoxia, arterial blood pH, blood pressure, and heart rate decreased significantly ($P < 0.01$).

Retinal Oxygenation Parameters

Retinal oxygenation parameters before and during light flicker, and their differences (during minus before) under normoxia and hypoxia are listed in the Table. There was a significant effect of light flicker on PO_{2V} ($P < 0.01$, $N = 10$), but not on PO_{2A} ($P = 0.71$). During light flicker, PO_{2V} decreased on average by 1.3 and 1.2 mm Hg under normoxia and hypoxia, respectively. As expected, there were significant effects of systemic oxygenation on retinal PO_{2A} and PO_{2V} ($P < 0.01$), as we reported previously.[12]

Measurements of inner retinal OEF in individual rats before and during light flicker under normoxia and hypoxia are plotted in the Figure. During light flicker, OEF increased in 8 of 10 and 9 of 10 rats under normoxia and hypoxia, respectively. There were significant main effects of light flicker and systemic oxygenation on OEF ($P \leq 0.02$, $N = 10$). However, the interaction effect was not significant ($P = 0.52$), indicating

Table. Retinal Oxygenation Parameters (Mean ± SD, $N = 10$) Before and During Light Flicker, and Their Difference (During Minus Before) Under Two Systemic Oxygenation Conditions (SOC), Normoxia (N) and Hypoxia (H)

					ANOVA *P* Value		
	SOC	Before Flicker*	During Flicker	Difference	Flicker	SOC	Flicker × SOC
PO_{2A}, mm Hg	N	44 ± 4	44 ± 4	0.1 ± 1.9	0.71	<0.01	0.92
	H	19 ± 4	20 ± 3	0.1 ± 0.7			
PO_{2V}, mm Hg	N	28 ± 5	27 ± 4	−1.3 ± 1.0	<0.01	<0.01	0.80
	H	11 ± 4	10 ± 5	−1.2 ± 1.8			
OEF	N	0.46 ± 0.13	0.50 ± 0.11	0.05 ± 0.04	0.02	<0.01	0.52
	H	0.67 ± 0.16	0.74 ± 0.14	0.07 ± 0.11			

* Retinal oxygenation parameters before light flicker have been reported previously.[12]

similar responses of OEF to light flicker under the two systemic oxygenation conditions. Before light flicker, mean inner retinal OEF measurements were 0.46 and 0.67 under normoxia and hypoxia, respectively. During light flicker, inner retinal OEF increased to 0.50 (11%) and 0.74 (10%) under normoxia and hypoxia, respectively.

Discussion

Measurement of inner retinal OEF provides information about the matching of oxygen metabolism and delivery. In the current study, we demonstrated that inner retinal OEF increased during light flicker under normoxia and hypoxia. This increase in OEF was due to a smaller relative change in DO_2 compared to MO_2, indicating MO_2 was undercompensated by DO_2 during light flicker.

Under normoxia, the response of inner retinal OEF to light flicker indicated the increase in MO_2 was not completely matched by an increase in DO_2. In agreement with findings of the present study, undercompensation of MO_2 by DO_2 during light flicker has also been shown by decreases in inner retinal or optic nerve head tissue PO_2 in rats and cats (1–7 mm Hg),[22–25] and an increase in arteriovenous PO_2 difference in rats (4 mm Hg).[20] Conversely, in humans, light flicker caused an increase in retinal venous SO_2 (4%), while arterial SO_2 did not change,[26] thereby suggesting overcompensation of MO_2 by DO_2. The different findings of these studies are likely attributable to variations between species and experimental protocols. Nevertheless, the complimentary findings of only slight alterations in retinal OEF and tissue PO_2 with light flicker in rats suggest that DO_2 almost fully compensated for MO_2 and, thus, the energy-dependent neural activity in the retina was unlikely to be limited by oxygen availability. Additionally, our finding of increased OEF coupled with the known increase in blood flow (and DO_2) confirms MO_2 increases with light flicker.

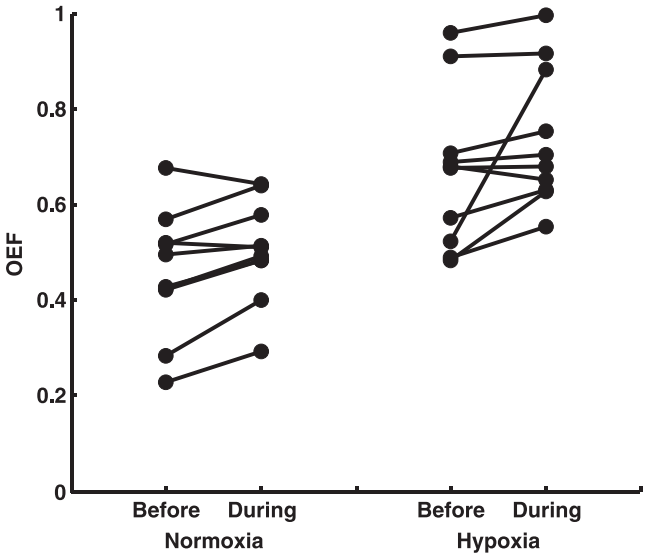

Figure. Measurements of inner retinal OEF in individual rats before and during light flicker under systemic normoxia and hypoxia. During flicker, OEF increased in 8 of 10 and 9 of 10 rats under normoxia and hypoxia, respectively. Inner retinal OEF measurements before light flicker have been reported previously.[12]

Under hypoxia, inner retinal OEF increased with light flicker, contrary to our hypothesis that OEF would remain unchanged, which was based on the following line of reasoning. Under this severe hypoxic challenge, inner retinal MO_2 was found previously to be reduced to approximately one-third of that under normoxia[13]; thus, the tissue was already deficient in oxygen and MO_2 would not be able to increase with light flicker. Furthermore, vasodilation was likely maximized due to hypoxia; thus, precluding an increase in DO_2. As a result, with no change in MO_2 and DO_2, OEF was expected to remain unchanged. Nonetheless, we found inner retinal OEF increased with light flicker, which is most likely attributed to an increase in MO_2, since DO_2 is unlikely to decrease with light flicker. The question then arises as to how oxygen extraction and MO_2 could increase with light flicker, given the existing oxygen deficiency in the retina under hypoxia. We offer the following explanation for our observation. Even under systemic hypoxia, tissue near blood vessels still may be adequately supplied with oxygen and, thus, could respond to light flicker with increased MO_2, whereas tissue farther away from blood vessels is unable to respond to light flicker because of hypoxia. According to Fick's laws of diffusion, with an increase in MO_2, the oxygen gradient from the vessel wall into the tissue would steepen, thereby increasing the rate of oxygen loss from blood and OEF.[27] Simultaneously, the lower oxygen content of blood under hypoxia coupled with the steeper oxygen gradient with light flicker will cause less tissue to be oxygenated, and, thus, exacerbate overall tissue hypoxia.

Although the responses of inner retinal OEF to light flicker were similar under normoxia and hypoxia, we speculated that the corresponding increases in MO_2 may have been different. Under hypoxia, compensation by DO_2 was likely lower due to maximized vasodilation; hence, a given increase in OEF would result from a smaller change in MO_2. The light flicker-induced increase in MO_2 can be estimated based on Fick's principle (MO_2 = OEF*O_{2A}*blood flow).[11] Under normoxia, the measured increase in OEF (from 0.46 to 0.50) and unchanged O_{2A} (unchanged PO_{2A}), along with a previously reported increase in blood flow (from 9.9 to 13.5 μL/min),[28] yielded an estimated light flicker-induced MO_2 increase of 48%. This value is in agreement with an estimated 37% increase in MO_2 based on

PO_2 and blood flow measurements at the optic nerve head in cats.[24] Under hypoxia, based on the measured increase in OEF (from 0.67 to 0.74), unchanged O_{2A} (unchanged PO_{2A}), and a presumed negligible change in blood flow, the light flicker-induced MO_2 increase was estimated to be 10%. The smaller increase in MO_2 under hypoxia compared to that under normoxia indicates systemic hypoxia suppressed the ability of MO_2 and, presumably, energy-dependent neural activity, to respond to light flicker. Our estimation of a reduced MO_2 response to light flicker agrees with a previous report of attenuated electrical activity in the cat retina under a similar systemic hypoxic condition.[29] The estimated MO_2 changes due to light flicker were limited by a lack of direct blood flow measurements in the current study. Future studies are needed to establish the magnitude of MO_2 increase with light flicker by simultaneous measurements of retinal blood flow and vascular oxygen contents.

In conclusion, the finding of increased inner retinal OEF substantiates that inner retinal MO_2 is increased with light flicker, and that this increase may be attenuated due to limited oxygen availability under hypoxia. With impaired functional hyperemia, changes in OEF are expected to be dominated by alterations in MO_2 in response to light flicker. Overall, inner retinal OEF can be used for quantitative assessment of functional hyperemia with respect to the relative changes of oxygen delivery and metabolism under physiologic and pathologic conditions.

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