Retinal Venous Oxygen Saturation Increases by Flicker Light Stimulation

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PURPOSE. Luminance flicker stimulation of the photoreceptors is known to increase retinal blood flow. Elevated blood velocity was determined using laser Doppler velocimetry, and increased vascular diameters during flicker were observed by measurements with a retinal vessel analyzer. Oxygen supply may be the target of the regulation of retinal blood flow. Thus, the oxygen saturation (SO2) in retinal arterioles and venules was investigated along with their diameters.

METHODS. Dual-wavelength (548 nm and 610 nm) fundus images were taken in 19 healthy volunteers (mean age, 26 ± 2.5 years) before (baseline) and during luminance flicker stimulation (12.5 Hz; modulation depth, 1:25). Retinal vessel SO2 (dual-wavelength optical oximetry) and diameters (central retinal arterial and venous equivalents [CRAE and CRVE]) were determined.

RESULTS. CRAEs and CRVEs of 193 ± 20 μm and 228 ± 20 μm at baseline increased statistically significant to a maximum of 202 ± 19 μm (P < 0.0005) and 242 ± 17 μm (P < 0.0005), respectively, under flicker stimulation. Although the arterial SO2 remained unchanged at 98%–99%, an increase of the venous saturation from 60% ± 5.7% to 64% ± 5.9% (P < 0.0005) was found.

CONCLUSIONS. In agreement with earlier investigations, the vessel dilation found here indicates an elevation of retinal blood flow by luminance flicker stimulation. This increase of the flow should meet the enhanced metabolic need of the neural retina under a physiological stimulus. The augmentation of venous oxygenation may indicate a higher capillary oxygen concentration, necessary to provide a sufficient diffusion rate of oxygen from the capillaries to the inner retinal tissue. (Invest Ophthalmol Vis Sci. 2011;52:274–277) DOI:10.1167/iovs.10-5537

Functional deficiencies in diseases affecting the retinal perfusion, such as diabetic retinopathy and glaucoma, may impair oxygen supply to the inner retina. This may be investigated by a newly developed method combining retinal vessel oximetry with flicker light stimulation of neuronal activity. Illumination of the eye with flicker light is known to increase the metabolic demand of the inner retina. 1-4 The augmented neuronal as well as metabolic activity of neurons and glial cells is associated with increased oxygen consumption. 1 As is known from studies of the brain, an enhanced local blood flow may compensate for the increased oxygen demand in stimulated neural tissue. An increased blood flow in the retina secondary to flicker light stimulation, measured in monkeys using labeled microspheres, was first reported by Bill and Sperber. 7 A way to noninvasively study the retinal blood flow changes could be very useful in understanding the etiology of diseases in which blood flow changes are known to occur (e.g., diabetes and glaucoma). Garhofer et al. 6 measured the response of blood flow parameters in human retinal arterioles and venules along with changes of retinal vessel diameters.

The primary aim of retinal blood flow regulation is to provide a stable oxygen supply to the retina. Direct measurements of the retinal oxygen partial pressure (PO2) would be the best measure to determine whether a supply deficit existed. This may contribute to the understanding of diseases such as diabetes mellitus, glaucoma, vascular disease, or endothelial dysfunction secondary to systemic diseases. Intraretinal measurement of tissue PO2 with a polarographic electrode, however, is an invasive procedure and, thus, restricted to animal experiments. 10 Human investigations have been performed so far in the vitreous during vitrectomy. 11-13 Shahidi et al. 14 observed the intravascular PO2 in the rat retina and choroid by imaging the oxygen-sensitive phosphorescence quenching of palladium-porphyrin. Because the molecular oxygen probe is not approved for human investigations, unfortunately, PO2 imaging is limited to animal experiments too.

The measurement of retinal vessel hemoglobin oxygen saturation (SO2), 15-21 on the other hand, is a noninvasive optical method suitable for clinical use. In this article we describe the response of arterial and venous SO2 as well as vessel diameters to a retinal luminance flicker light stimulus. This stimulus increases only inner retinal metabolism. Under light adaptation, oxygen from the retinal circulation is used solely by the inner retina.

This is, to the best of our knowledge, the first study performing oximetry during flicker illumination.

METHODS

Subjects

The study adhered to the tenets of the Declaration of Helsinki and was approved by the local institutional review board. Informed consent was provided by all subjects before performing any study procedures. Measurements were made in 19 healthy subjects (9 male, 10 female) 26.0 ± 2.5 years of age before and during light flicker stimulation. The pupil of one eye was dilated with tropicamide (Pharma Stulln GmbH, Stulln, Germany) before the investigation. Oximetry readings were taken before as well as 10, 20, 30, 40, 50, 60, 90, 120, 150, and 180 seconds after the onset of the flicker. All measurements were performed under light-adapted conditions. The irradiance at the fundus was 17.5 μW/mm².

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Accepted for publication March 16, 2010; revised May 20 and July 8, 2010; accepted July 9, 2010.

Disclosure: M. Hammer, Imedos Systems UG (C); P. W. Vilser, Imedos Systems UG (D, E); P. T. Riemer, Imedos Systems UG (E); F. Lient, None; S. Jentsch, None; J. Dawczynski, None; D. Schweitzer, None.

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Retinal Vessel Oximetry and Flicker Stimulation

SO₂ measurements were performed using the ‘oxygen tool’ of the vessel map system (Imedos UG, Jena, Germany) described elsewhere. Briefly, fundus images (fundus camera FF 450; Carl Zeiss Meditec AG, Jena, Germany; and digital camera KY-F75; JVC Inc., Yokohama, Japan) were taken in a 30° field using a customized dual-bandpass filter (transmission bands at 548 nm and 610 nm, bandwidth 10 nm each, fitting the green and the red camera channels). Optical densities of the vessels were measured as the logarithmic ratio of the fundus reflection at the vessel and beside the vessel. To exclude specular reflex from the vessel, pixels with a reflection above 20% over the mean value were excluded. The ratio of the optical densities at 610 nm to that at the isosbestic wavelength 548 nm is proportional to the vessel hemoglobin SO₂ after compensation for vessel diameter and fundus pigmentation. A linear relationship between the optical density ratio and a relative SO₂ measure was established by calibration. Using this technique, the SO₂ was measured in all vessels in a peripapillary annulus with an inner radius of 1 and an outer radius of 1.5 disc diameters (Fig. 1) and averaged over all arterioles and venules. Typically, approximately 20 single measurements in each of six to eight arterioles and venules were averaged. Vessel diameters were measured along with the SO₂ values from the images as described by Vilser et al. From these measurements, the central arterial and venous diameter equivalents (CRAEs and CRVEs) were calculated according to Hubbard et al. Statistical analyses were performed with a paired t-test (SPSS 15.0.1; SPSS Inc., Chicago, IL).

Full-field (30°) luminance flicker stimulation of the retina was induced by inserting an electro-optical chopper into the illumination path of the fundus camera. This provided a rectangular wave irradiation with a frequency of 12.5 Hz and a bright-to-dark contrast >25:1.

RESULTS

An example of oximetry readings is shown in Figure 1. Diameter measurements were performed for the same vessels as the oxymetry readings. Mean values of central arterial and venous diameter equivalents as well as SO₂ values are given in Table 1. Comparing the data of left and right eyes, there were no statistically significant differences.

To eliminate interindividual differences, the changes of CRAEs and CRVEs were calculated (Fig. 2). These increased to a maximum of 10 ± 5.7 μm or 5.6% ± 4.9% (CRAEs) and 17 ± 7.9 μm or 7.6% ± 3.4% (CRVEs). At all time points during flicker, the equivalents were statistically significant greater (t-test, P < 0.0005 for CRVEs; 0.0005 < P < 0.008 for CRAEs) than at baseline.

To eliminate interindividul variance from the SO₂ data, the difference of the baseline measurements from that under flicker stimulation was calculated (Fig. 3). Because the venous SO₂ values did not reach steady state after 60 seconds in seven subjects, the flicker episode was extended to 180 seconds in a second cohort of 12 subjects. For analysis, both cohorts were pooled. A maximal increase of the venous SO₂ of 5.2% ± 3.2% (Fig. 3) was found after 120 seconds of flicker. The increase over baseline was statistically significant for all oximetry readings collected 10 seconds or more into the flicker stimulus (t-test, 0.0005 < P < 0.018).

DISCUSSION

Flicker light stimulation increases the diameters of retinal arterioles and venules. The relative increase of 5.6% and 7.6% for

| TABLE 1. CRAEs and CRVEs as Well as Oxygen Saturations before and during Flicker Stimulation |
|--------------------|-----------------|-------------|-----------|-----------|
|                   | CRAE (μm)   | CRVE (μm)  | Arterial SO₂ (%) | Venous SO₂ (%) |
| Before flicker     | 193 ± 20    | 228 ± 20   | 99 ± 3.0         | 60 ± 5.7      |
| During flicker (max)| 202 ± 19   | 242 ± 17   | 99 ± 3.4         | 64 ± 5.9      |
the CRAEs and CRVEs, respectively, found in this study is in good agreement with findings from measurements in single vessels.\textsuperscript{25–27} Because, by the Hagen-Poiseuille law, the flow is proportional to the fourth power of the vessel diameter, this increase indicates an augmentation of the retinal blood flow by approximately 20%. At least in part, this increase might be necessary to fulfill the elevated metabolic demand of the inner retina during neuronal activity. Measurements by Robert Linsemeier (personal communication, 2009) revealed a slight decrease of retinal PO\textsubscript{2} during neuronal activity evoked by flicker light. This drop of oxygen concentration may trigger vasodilation to maintain sufficient supply to the tissue. Because the oxygen consumption by the tissue might be higher during activation, a higher diffusion rate from the vessel to the tissue is needed. As diffusion is driven by the concentration gradient, an increased intravascular PO\textsubscript{2} is the consequence of metabolic activity. This may explain the increase in venous SO\textsubscript{2}, which is linked to the PO\textsubscript{2} by the hemoglobin oxygen binding curve, found in this study. Similar results were found in rats measuring the PO\textsubscript{2} by an oxygen-quenchable phosphorescent porphyrin probe.\textsuperscript{28,29} During flicker, the arterial PO\textsubscript{2} increased, whereas the venous PO\textsubscript{2} remained constant, indicating an augmented oxygen supply. In our experiments, we may have an arterial PO\textsubscript{2} increase too, which is not observable with the measurement of hemoglobin SO\textsubscript{2}, which cannot exceed 100%. The constant venous PO\textsubscript{2} readings in previous rat experiments conflict with our results; however, in these experiments, venous PO\textsubscript{2} was unphysiologically low because of the anesthetics used.\textsuperscript{28} Under these conditions, blood flow may have been increased to its maximum but failed to meet the metabolic needs of the tissue.

With the data presented here, we hope to better understand retinal blood flow autoregulation. Our results do not exclude anaerobic glucose consumption by the Müller cells as the driving force of blood flow regulation. As astrocytes in the brain, Müller cells are essential for neurotransmitter recycling. However, in the optic nerve head of cats, depleted of synaptic junctions and lacking Müller cells, Buerk et al.\textsuperscript{30} found an increase of blood flow as well as a decrease of the PO\textsubscript{2} on flicker stimulation. Thus, an adequate oxygen supply to neuronal ocular tissue may be the major goal of the regulatory vascular response.

Another theory is based on the fact that all energy metabolism needs electron transport by NADH. As the ratio of free cytosolic NADH to NAD\textsuperscript{+} is tightly coupled to that of lactate and pyruvate, the increase of blood flow on lactate infusion\textsuperscript{31,32} may be triggered by the NADH concentration. Ido et al.\textsuperscript{33,34} hypothesized that the electrons, carried by NADH, oxidize substrates of enzymes in metabolic pathways that activate the NO synthase. The subsequent release of NO results in vasodilation and the observed augmentation of blood flow. This mechanism, however, needs further evidence.

In a recent paper, Hardardon et al.\textsuperscript{35} found elevated retinal vessel SO\textsubscript{2} in the dark compared to that of the same subjects under light adaptation. This was explained by increased oxygen diffusion from the retinal vasculature to the outer retina in the dark-adapted eye, which triggers an increase of retinal blood flow. This theory is supported by PO\textsubscript{2} measurements in cat retinas showing that light-adapted photoreceptor outer segments (ROSs) are completely supplied by the choroid with some oxygen reaching the inner retina. Under dark adaptation, however, the retinal vasculature contributes 10% of the ROS oxygen supply.\textsuperscript{34} In our experiments, however, flicker stimulation initiates firing of retinal neurons. This enhances the
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thus, allows partial dark adaptation. However, SO2 values measured during flicker are referenced to those under light adaptation. The flicker reduces the light exposure to 50%. This is far from the conditions for dark adaptation. Furthermore, the flicker frequency of 12.5 Hz is known to increase retinal blood flow.35 In our measurements, this is reflected by the arterial as well as venous dilatation.

A limitation of the present study is the large variation of the baseline CRAEs, CRVEs, and venous SO2, which may be attributed to interindividual differences. Thus, we restricted our investigation to changes of retinal vessel oxygenation, so that each subject served as his or her own control.

In conclusion, the combination of retinal vessel oximetry with flicker light stimulation of neuronal activity is a novel technique for the investigation of retinal metabolic demand. Oxygen supply as well as consumption can be estimated from the vascular hemoglobin saturation. Further investigations are needed to show whether this method is capable of revealing functional deficiencies in diseases affecting retinal perfusion, such as diabetic retinopathy and glaucoma.

References