RGC Sensitivity to Mild Systemic Hypoxia

Hélène Kergoat, Marie-Ève Hérard, and Marianne Lemay

Purpose. Systemic hyperoxia, hypercapnia, and hypoxia are known to alter retinal perfusion. The effects such experimentally induced systemic blood gas perturbations have on inner neuroretinal function in humans were examined.

Methods. Twenty healthy adults participated in each of three test sessions. The effects of breathing pure oxygen (O2), carbon dioxide, or a hypoxic gas on the pattern electroretinogram (pERG) were investigated. The stimulus consisted of high-contrast, black-and-white, 48-min arc checks reversing at 1 Hz. pERGs were recorded with a Dawson-Trick-Litzkow electrode at the end of 5 minutes of breathing room air, 5 minutes of breathing the test gas, immediately after the flow of gas was stopped, and 10 minutes after the flow of gas was stopped.

Results. Amplitudes and implicit times of the major positive (P50) and negative (N95) components of the pERG were not altered during the pure O2 and carbon dioxide breathing sessions. Although the amplitude and implicit time of P50 were not modified significantly with systemic hypoxia, they were depressed and delayed, respectively, for N95.

Conclusions. Inner neuroretinal function remained unchanged during increased blood O2 and carbon dioxide levels known to alter retinal blood flow, but it was altered during decreased blood O2 levels. Overall, these results indicate that the generators of P50 are resistant to systemic hyperoxia, hypercapnia/hypoxia, and hypoxia. They further indicate that the generators of N95, namely the retinal ganglion cells, are particularly sensitive to transient, mild systemic hypoxia. (Invest Ophthalmol Vis Sci. 2006;47:5423–5427) DOI:10.1167/iovs.06-0602

The neural retina is nourished by the central retinal artery circulation and the ciliochoroidal vascular bed. It is well known that the retinal circulation is autoregulated.1 Only recently, however, have studies demonstrated the capacity of the human choroid to regulate its blood flow in response to altered perfusion2,3 or flicker.4 Many studies have used altered blood oxygen (O2) or carbon dioxide (CO2) content to investigate the vascular regulatory capacity of the human retina. They have shown that systemic hyperoxia induces a reduction in the diameter of major retinal vessels5,6 and a parallel decrease in blood flow.5 Systemic hypercapnia has been reported to increase retinal vessel diameter and blood flow.7 Systemic hypoxia has not been investigated widely but is known to increase retinal vessel diameter.8 Little attention has been given, however, to the capacity of the human retina to sustain its normal neural function under conditions altering its blood supply. To that effect, studies have reported that systemic hyperoxia had minimal effect on the photopically elicited flash electroretinogram (fERG)9 and that it selectively altered one of the oscillatory potentials of the scotopically recorded fERG.10 It has further been demonstrated that the function of the neural generators of the photopically driven fERG b-wave was transiently depressed in the presence of short, acute, mild, systemic hypoxic stress.11 Systemic hypercapnia accompanied by systemic hypoxia has been shown to attenuate the response of the scotopically recorded fERG b-wave.10 Our objective in the present study was to investigate the effects of systemic hyperoxia, hypercapnia/hypoxia, and hypoxia on inner neuroretinal function through the recording of the pattern electroretinogram.

Materials and Methods

Subjects

Twenty healthy adults between 20 and 30 years of age participated in the pure O2, carbon dioxide, or hypoxic test sessions of this study. All subjects were prescreened to have normal systemic and ocular health and 20/15 (6/4.5) visual acuity in the test eye. They were not taking any medication that affected systemic or ocular blood flow, were nonsmokers, and were asked to abstain from beverages and food containing caffeine on the study day. All aspects of the experiments were fully explained to the subjects before participation, and each signed a consent form before testing. The research protocol had been approved by the ethics committee for human research of our institution, and it complied with the tenets of the Declaration of Helsinki.

Preparation of Subjects

Each subject was asked to sit comfortably on an ophthalmic chair located 0.75 m in front of a television monitor, where the test stimulus was displayed. An active Dawson-Trick-Litzkow-type (Desktop Linux; OSDL, San Francisco, CA) fiber electrode was draped in the inferior cul-de-sac of the right eye, from the nasal to the temporal canthus. An Ag/AgCl reference electrode was positioned on the temporal side of the test eye, approximately 1 cm from the external canthus. A second Ag/AgCl electrode was applied on the wrist to serve as ground. All necessary electrical connections between the subject and the clinical averager (CA-1000) were made. The left eye was covered with a black light-tight patch during all testing. The subject wore his or her best-corrected ophthalmic lenses during testing. Ambient light in the laboratory was reduced throughout the experiment.

Breathing the Test Gas and Recording the Physiological Variables

A soft rubber mask (7930 series; Hans-Rudolph, Kansas City, MO) was positioned tightly on the subject's mouth and nose. The mask was secured in place with a headband that was attached tightly on either side of the mask with adjustable fasteners (Velcro, Manchester, NH) and plastic clips. A nose clip ensured that the nostrils were closed and that breathing would be through the mouth only during testing. Two one-way valves located inside a 5.4-cm diameter plastic tube on either side of the mask ensured that the gas was inhaled from one side and

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exhaled from the other side. The test gas was delivered to a 10-L reservoir bag (nondiffusing gas collection bag; 6015 series; Hans-Rudolph) from a gas tank through a tube (Tygon; Piedmont Plastics, Charlotte, NC). The flow of gas was adjusted to 6 L/min for pure O2, and it was adjusted to the rate and depth of breathing of each subject for the carbogen and hypoxic gases (8–12 L/min). The air exhaled by the subject with each breathing cycle was sampled every 8 seconds through a fine cannula (no. 1606; Salter Laboratories, Arvin, CA) attached to the inside of the 3.4-cm diameter plastic tube and was located after the one-way valve. This cannula was connected to a capnograph/oximeter system (model 7100 CO2SMO, Novametrix; TruDel Medical, Montreal, Canada) to allow measurements of the end-tidal CO2 (EtCO2) and respiratory rate (RR). An oxygen sensor also connected to the CO2SMO system was attached to the middle finger of the right hand to measure arterial oxygen saturation (SaO2) and pulse rate (PR) throughout testing. SaO2, EtCO2, RR, and PR were measured continuously throughout the test session, and these data were saved on a memory card for later retrieval and analysis.

Intracocular pressure (IOP) in the right eye was measured for each subject the day before the testing session so as not to alter the corneal epithelium before testing. A brachial cuff (SpaceLabs Inc., Redmond, WA) was positioned on the left arm for blood pressure (BP) measurements. Two measurements of the systolic and diastolic pressures were taken within the last 2 minutes of each of the four phases of testing. For the third phase, however, the BP was measured twice consecutively, as soon as the flow of gas was stopped. Mean BP was calculated as: BPmean = (BPdiastolic + 2/3(BPmean - BPdiastolic)). Ocular perfusion pressure (OPP) was then derived according to the formula: OPP = 2/3(BPmean) – IOP.

A test session was divided into four phases in which the various parameters were measured: phase 1—after breathing room air for 5 minutes (baseline); phase 2—after breathing pure O2, carbogen (5% CO2 in 95% O2), or hypoxic gas (12% O2 in 88% nitrogen) for 5 minutes; phase 3—immediately at the end of breathing the test gas (gas off); phase 4—10 minutes after the end of breathing the test gas (recovery). The experiment was conducted in a quiet laboratory, at ambient temperature, and at sea level atmospheric pressure.

Electrophysiological Testing

The stimulus used was a high-contrast (98%), black-and-white checkerboard containing 48-min arc checks reversing at 1 Hz. The impedance of the electrodes was kept below 5 kΩ throughout testing. Three trials, each an average of 100 150-msec epochs, were recorded for each phase of the experiment. The signal was filtered between 1 and 30 Hz, amplified, and stored for later retrieval and analysis. All signals were processed by the CA-1000 clinical averager. Pattern electroretinogram (pERG) recording was started at the end of phases 1, 2, and 4; at the end of phase 3, pERG recording was started as soon as the flow of gas was stopped. When the first set of pERGs in an experimental phase was started, an event button was pressed on the capnograph/oximeter system so that the data for the physiological variables acquired during the pERG recordings could be identified for later analysis. During data acquisition, the subject was asked to fixate a red dot located in the center of the screen, to blink normally, and to maintain clarity of the checks.

Analyses

Amplitude and implicit time of the P50 and N95 components of the pERG for each subject were measured and averaged across the three trials obtained in each phase of the experiment. The amplitude of P50 corresponded to the voltage difference between the peak and its preceding trough, whereas the amplitude of N95 was taken as the voltage difference between its trough and the P50 peak. The implicit time of each wave was evaluated from the time of stimulus presentation to its peak and trough, respectively. Amplitude and implicit time data were further group-averaged across subjects within each phase for each of the three test gases used. SaO2, EtCO2, RR, and PR data obtained within the first 2 minutes of positioning of the event marker on the capnograph/oximeter system for each phase were averaged for each parameter and each subject within each phase of the experiment.

These data were considered representative of the steady state level for each physiological variable within each phase of the study. These data were further group-averaged across subjects within each phase for each of the three test gases used. The two measurements of BP were averaged together, and further averaged across subjects, in a similar fashion.

All data were expressed as mean ± SEM values for each test session. Analysis of variance (ANOVA) with repeated measures for an alpha level of 0.05 was used to determine whether the data differed significantly between the various phases of the experiment for each test gas used.

RESULTS

Group-averaged data for all physiological variables recorded during the pure O2, carbogen, and hypoxic test sessions are presented in Table 1. SaO2 increased (P = 0.0001), but EtCO2 (P = 0.0001) and PR (P = 0.0001) decreased during pure O2 breathing. In addition, EtCO2 (P = 0.0001), SaO2 (P = 0.0001), and BP (P = 0.0005) increased during carbogen breathing. SaO2 (P = 0.0001) and EtCO2 (P = 0.0001) decreased, but PR (P = 0.0001) and BP (P = 0.0007) increased during breathing of hypoxic gas. Group-averaged baseline measurements of IOP/OPP were 15.2 ± 0.6/44.9 ± 1.2 mm Hg, 14.7 ± 0.6/44.8 ± 1.1 mm Hg, and 14.1 ± 0.5/39.0 ± 0.8 mm Hg, respectively, during systemic hyperoxia, hypercapnia/hyperoxia, and hypoxia. These values were all within normal limits.

Group-averaged data indicated that the amplitude and implicit time of the P50 and N95 components of the pERG were not modified (P > 0.05) throughout the pure O2 and carbogen breathing test sessions. Group-averaged data indicated that the amplitude and implicit time of the P50 component of the pERG were not modified (P > 0.05) throughout the hypoxic test session, whereas the amplitude and implicit time of the N95 component were, respectively, reduced and delayed (P = 0.0001). Data for the pure oxygen, carbogen, and hypoxic test sessions are presented in Table 2.

Typical waveforms recorded with our experimental setup and highlighting the changes in the N95 component of the pERGs during the hypoxic test session are presented in Figure 1.

DISCUSSION

The increase in SaO2 level during pure O2 breathing indicates that systemic hyperoxia was readily achieved in our experimental protocol. The group-averaged PR decreased during systemic hyperoxia, a finding that has been reported earlier in humans.9,12 Systemic hyperoxia was accompanied by hypocapnia, a physiological response attributed to the decrease in the affinity of hemoglobin for CO2 in the presence of high blood O2 partial pressure.13 BP values were not altered throughout the O2 session, as also reported earlier.10

The increase in SaO2 and EtCO2 levels during carbogen breathing indicated that systemic hyperoxia and hypercapnia were achieved. No adverse effects were experienced by any of the subjects during carbogen breathing. Systemic BP increased during carbogen breathing, which was likely an effect of the CO2 rather than the O2 content of the gas because BP was not altered during pure O2 breathing in the present study and in earlier studies.14–16

The decrease in the SaO2 level during the hypoxic test session indicated that systemic hypoxia was readily achieved. The hypoxic gas mixture was apparently well tolerated by all

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subjects because none reported any discomfort. The 89% SaO₂ level achieved during systemic hypoxia would correspond to a Pao₂ of approximately 55 to 60 mm Hg, based on the oxygen-hemoglobin dissociation curve. This Pao₂ value also took into account the decrease in EtCO₂ observed during the inhalation of the hypoxic gas. This decrease in EtCO₂, which is a good indicator of the alveolar Pco₂, was not accompanied by a group-averaged increase in the RR. Considering the Henderson–Hasselbach equation and assuming a constant bicarbonate level, it is predicted that the averaged baseline arterial pH level was increased by only approximately 0.05 U, a change too small to further modify the level of Pao₂. An increase in the PR and cardiac output are physiological responses known to occur as immediate adaptations to acute altitude-induced hypoxia. The BP increased slightly during systemic hypoxia, a finding reported earlier during experimentally induced or high-altitude hypoxia. Collectively, these results indicate that not only was systemic hypoxia attained, it led to some compensatory mechanisms in attempts to maintain adequate tissue oxygenation.

P50 and N95 components of the pERG are thought to originate from preganglion and ganglion cells, respectively. Studies have concluded that retinal ganglion cells (RGCs) are particularly sensitive to conditions decreasing normal perfusion or oxygen supply to the retina. Papst et al. concluded that ocular hypertension alters RGC function because of a decrease in the oxygen supply to the retina. It has been shown that RGCs are more sensitive to transient complete ischemia than the more distal retinal cells. pERG has been used to detect retinal ischemia sufficient to lead to preproliferative

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<th>Gas Off</th>
<th>Recovery</th>
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<td>N95</td>
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Values are mean ± SEM. n = 20 subjects.
* Data were significantly different from baseline.
blood flow, whereas systemic hypercapnia, accompanied and blood flow. Furthermore, studies in humans and monkeys have shown that retinal vessels dilate and retinal blood flow increases in the presence of systemic hypoxia. This implies that the preganglion and ganglion cells were generally resistant to altered blood gas perturbations, except when the blood oxygen content was reduced. It is well known that the central retinal artery circulation has the capacity to adjust blood supply to the tissue in the presence of altered physiology. Hence, it has been shown that systemic hypoxia reduces retinal vessel diameter and blood flow, whereas systemic hypercapnia, accompanied or not by systemic hypoxia, increases retinal vessel diameter and blood flow. Furthermore, studies in humans and monkeys have shown that retinal vessels dilate and retinal blood flow increases in the presence of systemic hypoxia accompanied or not by isocapnia. In the present study, systemic hypoxia accompanied by hypocapnia (pure oxygen breathing) or hypercapnia (carbogen breathing) did not alter the function of the RGCs. Systemic hypoxia was also accompanied by hypocapnia and resulted in a decrease and a delay in the N95 component of the pERG. It is, therefore, valid to conclude that it was the decrease in arterial blood oxygen content that led to altered neural function.

The N95 component was already delayed during systemic hypoxia, but its amplitude decreased a little afterward only when the hypoxic gas was stopped. Conversely, the data show that the delay already started to recover by that time, though the SaO₂ level indicated that systemic hypoxia was still present. These results suggest that systemic hypoxia may differentially alter signaling time (depressed by longer duration of hypoxia) and amplitude (more sensitive to the early effects of systemic hypoxia) of the RGC response. Further studies using a shorter exposure to deeper levels of hypoxia or longer exposure to the same level of systemic hypoxia will be required to better elaborate the timing and amplitude responsivity of the RGCs to hypoxia.

Studies in the cat have shown that RGC function is resistant to mild systemic hypoxia but that it is altered drastically when the degree of hypoxia is more severe. In these studies, a hypoxic level that kept the arterial Po2 value greater than 35 to 45 mm Hg did not alter RGC function. Furthermore, the PR and BP were not altered either when the Po2 values remained higher than 45 mm Hg, whereas these two physiological variables were seen to increase in the present study at a PaO2 approximating 55 to 60 mm Hg. From such animal studies, it has been concluded that the inner retina is well protected against mild systemic hypoxia, a phenomenon attributed to the regulatory capacity of the central retinal artery circulation. In light of these animal studies, and considering our present results, it may be that mild systemic hypoxia induces more effects in humans and that it leads to metabolic changes that are not fully compensated by vascular regulation or that some retinal neurons in the human retina are particularly sensitive to a decrease in blood oxygen content.

Previous studies have shown that the photopically and scotopically recorded a-wave was not altered during mild systemic hypoxia but that the b-wave was attenuated. In addition, though the photopic oscillatory potentials were not depressed, the amplitude of OP3 tended to decrease with mild systemic hypoxia. Taken together, these results suggest that the innermost retinal layers are more sensitive than the outermost layers to low-level systemic hypoxia. Furthermore, because the neurogenerators of the ERG a-wave and the P50 component of the pERG were not altered but those giving rise to the ERG b-wave and the N95 component of the pERG were attenuated, it is reasonable to propose that some neurons are more sensitive to mild systemic hypoxia through a mechanism that could possibly involve biochemical or metabolic processes or a limited capacity of the retinal vasculature to regulate its blood flow and maintain adequate oxygenation to tissues in the presence of hypoxia. Earlier studies in humans, however, show that systemic hypoxia increases the standing potential of the eye and diminishes the light rise of the electro-oculogram, indicating that the outermost retina is also affected by the decreased blood oxygen content. The level of systemic hypoxia attained in those studies was slightly greater than that obtained in the present investigation. Further studies using graded levels of systemic hypoxia will be required to determine the relative susceptibility of the various retinal neurons to decreased blood oxygen content.

In conclusion, our results indicate that RGCs in humans are particularly sensitive to acute, transient, mild systemic hypoxic stress.

Acknowledgments

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References


