O₂ consumption and CO₂ influence on the pH and electroretinogram of isolated retina of goldfish. NICOLAS M. SCHILLART AND RENE P. J. DUURSMA.

From micropolarographic O₂ measurements, it can be concluded that the isolated goldfish retina is sufficiently supplied with oxygen when 100% O₂ is blown over the retina. The retinal O₂ consumption amounts to about 60 μl of O₂ per milligram dry weight per hour. Small amounts (0.25% to 2.0%) of CO₂ added to the O₂ gas diminish the retinal pH, which is 7.0 to 7.1 in situ and after isolation. Moreover, CO₂ strongly reduces the ERG amplitude.

For more than a decade now, isolated retinas have frequently been used for electrophysiological recordings of (especially) single cell activity. Oxygen supply of isolated retinas is often provided by a flow of carbogene (95% O₂ and 5% CO₂) saturated with water vapor blown over the isolated retina. Since the high PO₂ found in the eye of fish indicates that O₂ consumption is crucial for normal retinal performance, it can therefore be doubted whether the O₂ supply is sufficient to keep the isolated fish retina in a physiological state. Furthermore, Abramov and Levine showed that CO₂ of the gas mixture reduces the amplitude of the electroretinogram (ERG) of the isolated goldfish retina. Besides its influence on the ERG, CO₂ may also affect the pH. Pure water in equilibrium with 5% CO₂ has a pH of about 4.0. Fish Ringer solution containing 2.3 mM NaHCO₃ with a pH of 7.0 is in equilibrium with 1.6% CO₂. Since at a pH of 7.0 the specific buffer capacity of bicarbonate is about 60 times that of phosphate, it is clear that under carbogene supply, even after administration of some drops of Ringer solution, the isolated retina cannot maintain a pH of 7.0 for long periods. For large CO₂ concentrations, the CO₂ of the gas mixture dominates the control of the retinal pH and consequently the functioning of retinal processes. To obtain an impression of the latter, the ERG amplitude to flickering light was used as a parameter.

Preparation of the retinas has been discussed elsewhere. In the present experiment a total of 34 isolated retinas were used. Since administration of some drops of fish Ringer solution did not improve the quality of the isolated retina, this has mostly been omitted. Generally the retina was placed receptor-side up. The PO₂ electrode penetrated the retina from above; the pH and ERG electrode just touched the retina. The electrodes were positioned within the spot of the light stimulus (mostly 2.6 mm diameter). The O₂/CO₂ gas mixture was made by mixing 100% O₂ with carbogene controlled by glass capillary flow meters (Fischer & Porter Co., Warminster, Pa.). The CO₂ concentration of the mixture had an accuracy of 0.10%. All gas mixtures were saturated with water vapor and, as well as the retina, temperature controlled (15°, 20°, or 25° within 0.1° C accuracy). The flow rate amounted to 0.4 L/min in a 27 ml chamber. The pH was measured with a semimicroglass electrode (type MI 405; Microelectrodes, Inc., Londonderry, N. H.) and the ERG with a glass-coated Pt-Ir electrode (tip 5 μm). Occasionally NaCl-filled (2.5 M), low-impedance (1 MΩ) glass pipettes were used. Intraretinal PO₂ was measured micropolarographically at ~650 mV with a glass-coated Pt microelectrode mounted in a glass pipette, the final (uncoated) tip of 2 μm being insulated with collodion (2.6% nitrocellulose in ether-alcohol). This electrode had a resistance of 200 to 500 MΩ and a linear current-concentration relation, and its performance was not affected by stirring. The characteristics of the electrode were invariant for the ranges of pH (6 to 8.4) and temperature (15° to 25° C) used. The O₂ consumption of the electrode itself is negligible (ca. 2 nl/hr), and its reaction time is ca. 5 sec. White light (tungsten band lamp, about 2 μW/mm²) was used for the PO₂ and square wave-modulated green or red light (500 or 656 nm, 20 nW/mm²) for the ERG recordings. At a stimulus frequency of 0 Hz, which was generally used, the a, b, and d waves of the ERG could not be distinguished; this flicker ERG predominantly contains the fundamental, a small second, and hardly any higher harmonics of the stimulus frequency. Therefore the amplitude of the fundamental was used as a substitute for the amplitude of the ERG response to the square wave stimulus of 3 Hz. The fundamental was measured by a two-channel phase-lock amplifier (PAR Model 129). The waveform of the ERG was inspected after averaging in order to see whether it was invariant, a condition to be fulfilled when applying this method.

Since to the 3 Hz stimulus the a, b, and d waves of the ERG are interwoven, in some experiments...
square wave-modulated flicker with a lower frequency (0.66 Hz) was used to judge whether the retina gave a regular ERG. Fig. 3, A (upper trace), shows a typical ERG with a clear a and d (off) wave. The c wave is absent due to the absence of pigment epithelium, whereas the b wave is small and sometimes practically absent. This is typical for ERGs of isolated fish retinas where the ERG is dominated by the PIII component. The waveform is fairly independent of spot size (0.1 to 2.6 mm) and intensity (0.2 to 20 nW/mm²).

In the vitreous body below the retina we measured a PO₂ of 60% or more. (For convenience sake the PCO₂ and PO₂ are expressed in volume percent related to 760 mm Hg.) Since in frog the amplitude of the b wave of the ERG is only seriously affected for a PO₂ of 10% and less, it may be expected that the recorded value of the PO₂ is sufficient for at least the distal and intermediate layers. This conclusion is in line with our observation that the peak value of the PO₂ in the retina of the intact fish is about 22% (anesthetic used, MS 222). This high value may be related to the chorioidal rete mirabile which is characteristic for teleost fish. Long-lasting anoxia (1 to 2 hr) does not irreversibly block the respiration of the isolated retina. Steady illumination and darkness gave the same PO₂, indicating that the O₂ consumption of the retina does not change with steady illumination, as has also been found for tropical fish.

The PO₂ measured as a function of depth gives basically the possibility to determine the rate of respiration (QO₂). Due to the high consumption of the receptors, the PO₂ as a function of penetration depth changes faster in the distal layers than in the proximal ones if the retina is positioned receptor-side up. A simple way to estimate the QO₂ of the whole retina is to measure the O₂ gradient in the vitreous body, with the receptor-side down. Since the vitreous body consumes hardly any O₂ for a total vitreal consumption of ca. 0.4 µl/hr is found, as calculated from the data of ref. 6), the PO₂ in the vitreous body is a linear function of electrode depth. The QO₂ is estimated according to:

\[ QO_2 = \Delta P \cdot A \cdot Dk/w, \]

in which \( \Delta P \) is the concentration gradient (atm/cm), \( A \) is the area of the retina (cm²), \( Dk \) is O₂ transmissibility (cm² • µl O₂/ml • atm • hr)

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In a vitreous layer of 0.8 mm thickness we found a gradient of 25.5%/mm (mean of four values) with 100% O₂. However, the QO₂ transmissibility (Dk) of fish vitreous body is to our knowledge unknown, and so we had to deduce a realistic value taking into account the chemical structure of the vitreous body. Since the vitreous humor, which is held together by a mesh of protein fibers, is nearly totally composed of water, the Dk should hardly deviate from the Dk of water. Hoffert et al. found (with an O₂ polarograph) for the grunt (Haemulon plumieri) in air a consumption of about 3.6 µl/mg wet weight • hr, which should be about 70 µl/mg for dry weight. In contrast Santamaria et al. found (with a Warburg apparatus) in 100% O₂, a QO₂ of 8.6 for Eugerres.
From the theoretical model of Hagins et al. for the dark current in rat rods, a $Q_{O_2}$ of 50 can be calculated. Rough estimates from the gradient of the first 0.1 mm of goldfish retina in the normal position (receptor-side up) yielded a $Q_{O_2}$ of 13, whereas from the data of Negishi et al. (their fig. 1) a value of 15 is calculated for Eugenes. For both estimates a $D_{k}$ of 1.62 $\mu l$ O$_2$/cm $\cdot$ atm $\cdot$ hr was chosen ($D_{k}$ of brain tissue). Since in the upper 0.1 mm considerable O$_2$ consumption takes place, both these estimates are lower limits. The estimate of the $Q_{O_2}$ of 60 $\mu l$ of O$_2$ per milligram dry weight per hour found for the isolated retina means that after the ophthalmic artery has been cut in order to isolate the retina, the physically dissolved oxygen ($0.63$ $\mu l$ of O$_2$) is metabolized within 1 min, a time-span about half the mean isolation time. With an O$_2$/N$_2$ gas mixture ranging between 100% and 60% O$_2$, it was found for three retinas investigated that the ERG amplitude remained constant within an accuracy of 3%. However, when a 20% O$_2$/80% N$_2$ mixture was blown over the retina instead of oxygen, both the ERG amplitude and the pH decreased strongly (Fig. 1).
minor difference is the slower decay of the d wave with the O2/CO2 mixture. The peak-peak value, the a and the d waves are all affected in the same proportion (Fig. 3, B).

On the basis of the present experiments it can be concluded that the O2 supply by diffusion through the retinal tissue appears to be sufficient for a normal physiological O2 consumption, which is roughly 60 μl of O2 per milligram dry weight per hour (20° C). In vitro immediately after isolation and in situ the pH is approximately neutral but is strongly lowered in vitro by CO2. The latter also holds for the ERG amplitude.

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REFERENCES

Similarities between the c-wave and slow PIII in the rabbit eye. Mark Lurie and Michael F. Marmor.

The c-wave of the electroretinogram was recorded from the eyes of Dutch rabbits and compared with the slow PIII response isolated by intravenous injection of sodium iodate and intravitreal injection of sodium espartate. Although opposite in polarity, the waveform of the two responses were remarkably similar over a wide range of stimulus intensities and durations. Plots of time-to-peak vs. log stimulus energy show that both responses follow the Bunsen-Roscoe law. Curves plotting the locus of all points for which the Bunsen-Roscoe law held were approximately parallel for the two responses, and the critical durations for each stimulus intensity were equivalent. The slow PIII peaked earlier than the c-wave, consistent with published observations that the time-to-peak of light-induced K+ changes is shorter near the photoreceptor inner segments than near the pigment epithelium. These data support the hypothesis that there is a common generator for the c-wave and slow PIII.

The c-wave of the electroretinogram (ERG) appears to be generated by a light-induced decrease of potassium concentration in the region of the photoreceptor outer segments. This change in potassium at the apical membrane of the pigment epithelium causes a hyperpolarization of the pigment cell, which is the origin of the c-wave.1,2,3

Faber4 demonstrated in the rabbit that there is a light-induced voltage change in the proximal retina with approximately the same time course as the c-wave, but of opposite polarity. A similar voltage change has also been demonstrated directly in the carp retina after removal of the pig-ment epithelium to eliminate the c-wave and treatment of the retina with espartate to remove synaptically mediated contributions from the inner retina.4 Faber termed this vitreal-negative voltage the "slow PIII," a term derived from Granit's nomenclature of the late receptor potential, and postulated that it arises from the Müller cells. Local hyperpolarization of these radially disposed elements in the retina would provide an origin for the voltage. Noell (cited in ref. 4) and Witskovsky et al.5 have postulated that the Müller cells probably respond to the same potassium changes noted above, which follow photoreceptor activation and induce the c-wave.

Recently the mechanism for generation of the frog c-wave has been simulated by means of an electrical analog.6 It was then demonstrated7 that a voltage resembling the slow PIII could be generated by the same electrical analog, without changing its time constant, by using the aspartate-isolated rod receptor potential as an input. We