The electroretinogram of the living extracorporeal bovine eye. The influence of anoxia and hypothermia

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A technique is presented for recording the bovine ERG (electroretinogram) from an isolated perfused preparation, the living extracorporeal bovine eye. By using this method, properties of the bovine ERG response were investigated under anoxic and hypothermic conditions. Retinal anoxia resulted in reduction of ERG waves in the following order: b-wave, slow positive component, and a-wave. The b-wave was most sensitive to the anoxia and diminished at 37° C. in 5 minutes while the a-wave showed only 60 per cent decrease during 6 minutes of anoxia. In recovery phase from the anoxia, ERG waves showed enhancement in the following order: a-wave, slow positive component, and b-wave. With anoxia of 60 minutes, complete recovery of the ERG never occurred upon reperfusion. When temperature was reduced to 30° C. a remarkable decline of oscillatory potential was observed. Slight decreases of amplitude and prolongation of peaking times of a- and b-waves were also observed. The ERG responses were moderately well preserved at 30° C. for a period of hours, while lower temperature resulted in a deterioration increasing with time.

Key words: living extracorporeal eye, perfusion of isolated mammalian eye, electroretinogram in bovine eye, electroretinogram in anoxia, electroretinogram under hypothermia.

The living extracorporeal eye1-4, 19 is an experimental preparation developed in our laboratory for the observation of microcirculation and the events of intravascular coagulation. Viability and metabolic activity of the tissue of the perfusing eye have been confirmed to be in near physiologic state, as previously described,4 by the evidences including oxygen utilization, carbon dioxide elaboration, glucose consumption, the blockade of these activities by cyanide, and by electroretinographic activity during perfusion.

A number of electrophysiologic studies of the isolated retina incubated in circulating media have been reported.5-12 However, as far as we know, there are few articles reporting perfusion studies of enucleated mammalian eyes as an intact organ. O'Rourke and Berghoffer13 perfused canine
eyes with whole blood to study arteriovenous differences in hemoglobin oxygen saturation and retinal respiration. Gouras and Hoff20 perfused the isolated cat eye in an effort to identify the activity of rod and cone receptor systems in the electroretinogram (ERG). This paper introduces a preparation for recording the ERG in perfused extracorporeal bovine eye. Properties of this ERG and changes resulting from deliberate induction of anoxia or hypothermia are detailed.

Methods

Eye. A bovine eye was enucleated from the orbit immediately after slaughter of the animal. When the eye was enucleated as long a segment of the optic nerve as possible was cut so that the ciliary artery which parallels the optic nerve was also cut long. The ciliary artery of the eye was catheterized promptly by 20 gauge polyethylene tubing. Before clotting of native blood had occurred in the vessels of the eye the microcirculation was flushed clear with heparinized, warmed modified Kreb’s solution injected gently into the arterial catheter with a syringe. Clearing of blood from the vessels was confirmed by observation of the retina through a contact lens shaped to fit the bovine cornea. Since the ciliary artery supplies the whole tissue of the bovine eye,14 it is sufficient to catheterize only this vessel. In the bovine eye as in the human eye, it is known that the outer layer of the retinal tissue is dependent on the choroidal vessels for nutrition and the inner layer on the central retinal artery.15

Perfusate. Fresh bovine blood was collected beforehand in a plastic bottle containing 10,000 u. of heparin per 1 L. of blood as an anticoagulant and with 0.4 ml of 2 per cent atropine per liter. Filtration of the blood through a Dacron filter, as used by Swank,16 prevented in large measure the formation of platelet aggregates that can interfere with microcirculatory flow. All inner surfaces of the perfusion system were siliconized to avoid contact activation of coagulation proteins and formation of platelet aggregation. The blood was put in a siliconized flask overlaid with oxygen, and was swirled gently for about 10 minutes until the color of the blood turned to arterial red. This oxygenated blood in its reservoir was hung about 1 meter above the eye level and allowed via a plastic tube to perfuse the eye by gravity flow through the ciliary artery catheter. With the perfusion continuing, the eye was transported to the laboratory with the exterior of the eye bathed with saline in a glass container surrounded by warm water.

Perfusion (Fig. 1). In the laboratory the catheterized eye was supported by a ring, cornea upwards, and placed just above the beaker surrounded by a temperature-regulated water bath. The catheter to the eye looped through the thermostatically controlled water bath to keep blood at a constant temperature which was varied experimentally as noted. The heparinized oxygenated bovine blood was delivered through the catheter by gravity flow with a rate of approximately 1.0 ml per minute. The venous outflow was not recirculated.

ERG recording. The perfusing eye preparation was set in a shielded dark box. To record the ERG two nonpolarized zinc-zinc sulfate electrodes were placed, one upon the cornea and the other on the sclera near the optic nerve. These were connected to a high-grain differential amplifier (Tektronix Type 1A7). Retinal responses were displayed on the screen on an oscilloscope (Tektronix Type 532) and recorded with a Polaroid camera. In some cases, when recording the slow component of the ERG, a DC (direct current) amplifier and a pen recorder (Grass Polygraph) were employed. This slow positive component, which followed the b-wave, would seem to coincide with the c-wave at least with respect to its peaking time.
Two varieties of the light stimulus were utilized. For observing the fast components of the ERG (a-, b-wave, and oscillatory potentials) a xenon stroboscope (Nihon Kohden Kogyo Co. MSR-2R) was employed. The intensities of the stimuli were indicated in joules which represented consumed energy at the firing. Six different intensities, 0.3, 0.6, 2, 20, 40, and 80 joule-, could be obtained from this stimulator. These joule values corresponded, respectively, with 27.7, 55.5, 173, 1,730, 3,470, and 6,940 candela. An intensity of 20 joules was usually used in the present study. The light stimulus was transmitted by fiber optics (American Optics Co. LG-5-24) from the source to cornea. The attenuation of the transmission by the light guide was estimated at 50 per cent. To record the slow component, a 15 second duration of light from a 6 volt flashlight was used. During experiments the light stimulus was fired every minute. This was long enough to allow recovery without distorting the shape and amplitude of the succeeding response. The ERG recording was performed after more than 20 minutes' dark adaptation, and experiments began after reproducible responses were obtained.

Anoxia of the tissues of the eye was induced by clamping the plastic tube delivering blood to the ciliary artery catheter. After a 6 minute interruption of blood supply, circulation was re-established. During this period of anoxia and the subsequent recovery phase during reperfusion, the ERG was determined at one minute intervals.

The influence of temperature upon the wave form and amplitude of the ERG was undertaken by systematically altering the temperature of perfusing blood from 37 to 30, to 20, and finally to 4° C.

Results

The ERG of the perfused bovine extracorporeal eye. Fig. 2, A shows a typical response recorded from the dark-adapted eye in good condition during perfusion. Preparations which had partial occlusion of the vessels secondary to clotting or obstruction of flow by large platelet aggregates showed altered response, especially noted in the b-waves which decreased in amplitude or disappeared completely. Therefore, only eyes which showed normal b-wave were used for these experiments.

In properly perfused preparations, the ERG exhibited constant response over a period of 5 to 6 hours. After this time, the potentials tended to attenuate gradually, as shown in Fig. 2, B. A small negative deflection, however, usually could be recorded for more than 10 hours.

Fig. 3 shows variation of ERG due to the stimulus intensities. On increasing the stimulus intensity, augmentation and steepening of a-wave, magnification of oscillatory potentials, and enhancement of b-wave were observed as commonly seen in other animals.

Fig. 4 illustrates the a- and b-wave potential change recorded under the condition of dark and light adaptation. In dark adaptation, which followed 15 minutes of
100 lux light adaptation, potential of the waves showed marked recovery by 15 minutes.

Anoxia. All electroretinographic responses attenuated upon induction of anoxia. However, the individual components were altered in differing degrees as shown in Figs. 5 and 6. The b-wave decreased first and was almost abolished after 5 minutes of anoxia. In contrast to this, the a-wave showed a gradual decrease with amplitude reduced finally to 40 per cent of control value. This amplitude was then maintained without further diminution during the 6 minutes of anoxia. The slow positive component was depressed at the same rate as the a-wave for the first 3 minutes but continued to decrease linearly to 15 per cent of its initial value after 6 minutes of anoxia. When circulation was restarted, the ERG waves showed their recovery in the following order: a-wave, slow positive component, then b-wave. It took 1, 2, and 3 minutes, respectively, for the a-wave, slow positive component, and b-wave to recover to 80 per cent of the control initial amplitudes. The a-waves alone showed overshoot phenomena during recovery. If interruption of circulation was continued for a longer period, for example 60 minutes, only a small negative response was recorded and in most instances even though circulation was recommenced after this period of time, complete recovery of the ERG never occurred.

Hypothermia. At 30°C, amplitude of a-wave decreased and in its peak time became prolonged compared to its behavior at 37°C. The most remarkable changes were those of oscillatory potentials as shown in Fig. 7. At 37°C the oscillatory potentials rose sharply and prominently and could be seen separately from the major part of the b-wave. At 30°C, however, the speed and amplitude of the potentials decreased considerably. They then became superimposed on the major portion of the b-wave. Delayed peaking of the oscillatory potentials contributed to this superimposition. At 20°C the amplitudes, especially of the b-wave, were reduced even further. Peak time of both waves and of oscillatory potential became even more delayed than they had been at 30°C. Continued perfusion with 20°C blood led to deterioration of the electroretinographic phenomena. Continued perfusion with 30°C blood, on the other hand, preserved the
reproducibility of response for hours. Fig. 7, D shows the findings after ten minutes of perfusion with 4° C. blood. The b-wave had diminished almost to the point of absence and only slow a-wave component was observed.

Discussion

In this experiment every attempt was made to maintain continuous blood flow through the eye's microcirculation at an optimum rate since oxygen supply is essential to the retinal tissues. Perfusion of this mammalian nervous system organ, however, is a difficult procedure technically since platelet aggregates can interrupt smooth flow through the microcirculation and blood clotting can obstruct perfusion permanently. Complete and immediate flushing of native blood from the microcirculation of the excised eye was effected. Heparin was used as an anticoagulant and dacron filtration of blood used for perfusion carried out to remove platelet aggregates. Care was taken in siliconizing inner surfaces of containers and tubing contacting this blood as an essential precaution in facilitating continuous microcirculatory flow.

Best results in recording the ERG wave form and measuring its size were obtained when blood flow rates of 0.8 to 1.0 ml per minute were maintained. Faster perfusion rates may cause retinal edema or hemorrhages. O'Rourke and Berghoffer used 0.2 ml per minute flow rate when they perfused isolated canine eyes for study of arteriovenous oxygen differences.

Gouras and Hoff perfused the enucleated cat eye with a solution of Eagle's basal medium, containing 10 per cent newborn calf serum, at a flow rate of 2 to 4 ml per minute. In our preparation, using oxygenated bovine whole blood, we could reduce the flow rate and maintain the eye tissues in near physiologic state. This also prolonged survival time of the eye tissues.

Comparing the respective reduction in amplitudes of each component during anoxia, O'Rourke and Berghoffer noted a time course as follows: the b-wave decreased rapidly and almost disappeared while a-wave showed only gradual depression to a final maintained level of about 40 per cent of initial deflection. The slow positive component behaved as a middle pattern between the b- and a-wave. During recovery phase from the anoxia, the a-wave recovered rapidly while the b-wave showed late enhancement and the slow positive component again showed an increase about midway in the time between the a- and b-wave. The immediate depression and disappearance of the b-wave in the bovine ERG indicates that the origin of the b-wave is more sensitive to anoxia and more severely affected by decreased blood flow through the retina. The a-wave of the bovine ERG seems more resistant to this effect of anoxia. This is in common with the response in the retinas of other animals studied in situ.

Tazawa and associates reported in situ experiments where the order of reduction of
ERG during anoxia differed between cat and dog, on the one hand, and rabbit and guinea pig, on the other. The former showed diminution in the order: b-wave, c, a-wave; the latter: c, b, a-wave. They felt that this difference might be attributable to nutritional differences in the retinas of these species. In the present results it appears that the ERG pattern of cows corresponds more to that of cats and dogs.

With anoxia of greater than 60 minutes, we were unable to find complete electroretinographic recovery. Ames and Gurian reported complete recovery 60 minutes after reoxygenation of isolated incubated rabbit retinas deprived of oxygen for a one-hour period. They interpreted this surprising reversibility to the absence of increase in carbon dioxide, lactic acid, and other products of metabolism in the in vitro experimental setting. In vivo, these metabolic products would be present and thus would increase the probability of irreversible damage.

There are differences between articles with respect to optimum temperature for maintaining ERG in isolated preparation.

Sickel reported the greatest prolongation of viability of isolated incubated rabbit retina when experiments were carried out at room temperature.

Bock and his colleague predicted that if, after enucleation of the rabbit eye, the bulbus was not kept at body temperature but was exposed freely to a room temperature of 20°C, the survival time could be twice as long. Their experimental results offered support for this hypothesis in that there was an increase of survival duration, a secondary increase of a-wave potential, and an increased oscillatory potential observed when the eye was at room temperature. They suggested from this fact that the various ERG components seemed to differ in temperature resistance.

reported that optimum temperature of the b-wave in the isolated rabbit retina was between 30°C and 35°C, and that the implicit time shortened with increasing temperature. Their results seemed to agree with our experiments.

Furthermore, Hanitzsch and associates indicated an increase in steepness and potential of a-wave with increasing temperature (temperature range: 10 to 34°C) in the isolated rabbit retina.

Ames and Gurian described optimum recovery rate at 30°C of isolated perfused retina after oxygen and glucose deprivation as contrasted with findings at 37°C. Differences in methods and in animal species rendered comparison with the present experimental results difficult. In our experiments the ERG was relatively well pre-
Fig. 7. Bovine a- and b-wave recorded under hypothermia. D was recorded 10 minutes after 4°C hypothermia. Two vertical dotted lines on the ERG indicate the peak time of a- and b-wave recorded at 37°C.

We wish to express our thanks to Pacific Meat Company for allowing us to collect bovine eyes for these experiments. We thank Dr. Kenneth C. Swan, Head, Department of Ophthalmology, Dr. Robert V. Hill, Assistant Clinical Professor of Ophthalmology, and Dr. Makoto Sato, Associate Professor of Neurosurgery, for generous loans of equipment. We are indebted to the late Mr. Jerold Jansen for help in making a photostimulating apparatus.

REFERENCES


