Retinal function in an isolated, perfused mammalian eye

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Retinal function has been examined by identifying the activity of both rod and cone receptor systems in the electroretinogram (ERG) and the response of the optic nerve in the isolated cat eye, perfused through its ophthalmic artery with a solution devoid of hemoglobin. This preparation can maintain an ERG of normal configuration and of relatively large amplitude, dark-adapts after strong adapting lights, shows a Purkinje shift, and has synaptic transmission from the receptors up to and including the ganglion cell layer of the retina. In this preparation, rod function is more vulnerable to damage than cone function, and this vulnerability appears to occur somewhere between the rod receptor cell and second-order neurons in the retina.

Key words: physiology of cones, physiology of rods, isolated eye, electroretinogram, optic nerve, electrical activity, retinal threshold, retinal ganglion cells, visual evoked response.

An organ culture of the mammalian retina could provide a useful preparation for studying the metabolic and biochemical events which are associated with the electrophysiology of this structure.

A number of reports have demonstrated that retinal function can be well maintained in isolated pieces of retina removed from the eye. Although such preparations are extremely useful, they have several disadvantages. The neural retina is separated from the pigment epithelium layer, which plays an important role in photoreceptor function. For example, rhodopsin regeneration either fails to occur or occurs only under unusual circumstances, in the absence of pigment epithelium. In addition, the retina must be nourished by diffusion of materials from its surface to deeper layers, preventing easy separation of inflow from outflow and producing an uneven and undoubtedly abnormal distribution of metabolites in thicker areas of this actively metabolizing tissue. Also, a certain amount of damage must inevitably occur whenever the entire retina or a fragment of it is removed from the eye.

In the isolated, perfused cat eye, the entire retina is perfused through its own arterial circulation, thus establishing a nutritional status which is essentially physiological. Since the venous outflow can be collected and analyzed, nutritional requirements and biochemical characteristics of the preparation can be determined by arteriovenous differences. Since the anatomy within the perfused eye is undisturbed, the photoreceptors are left adjacent to the pigment epithelium, and the
Fig. 1. Schematic diagram of the perfusion, testing, and recording system for studying the isolated cat eye. The letters signify the following: R = ribbon filament light source; L = lenses; S = flag shutter; P = photocell; N = neutral density filters; C = wavelength selective filters; A = recording system for the ERG; B = recording system for optic nerve responses; H = heater for eye chamber; F = drop-flow meter; T = thermometer; H2O = water solution with pressure head of 160 mm. Hg.

neural connections of the retina remain intact up to the ganglion cells, which have their axons severed approximately 1 cm. from the globe.

This report examines retinal function in the isolated, perfused cat eye, by identifying and studying the activity of both the rod and cone receptor systems in the electroretinogram (ERG) and the response of the optic nerve. An abstract mentioning some of the results has already been published.8

Methods

Adult cats were anesthetized with sodium pentobarbital (35 mg per kilogram) intraperitoneally, and anticoagulated with heparin (300 Toronto units per kilogram) by intracardiac puncture. Eyes were removed with as long a segment of optic nerve as possible (approximately 1 cm.), and together with its ophthalmic artery,9,10 it was placed in an insulated chamber at 37° C. (Fig. 1). Within 3 to 10 minutes this artery was cannulated (No. 10 polyethylene tubing) under a dissecting microscope. The eye was perfused with a solution of Eagle’s basal medium,11 (without glutamine) containing 10 per cent newborn calf serum, equilibrated with a gas mixture of 95 per cent oxygen and 5 per cent carbon dioxide and maintained 160 mm. of mercury above atmospheric pressure. Under these conditions, 2 to 4 c.c. of perfusate, having a concentration of approximately 0.03 c.c. of oxygen per cubic centimeter, entered the eye each minute. Flow was measured by a calibrated drop-flow meter. We have profited considerably from the work of others, using similar techniques to study intraocular pressure12 and peripheral nerve activity13 in the isolated perfused cat eye.

A chlorided silver electrode was placed on the cornea and another on the back of the globe, in order to record the ERG; similar electrodes were placed on the optic nerve, which had been sucked into a tube (not shown in Fig. 1) in order to record the isolated electrical response of this structure. In some experiments, glass micropipette electrodes, filled with 3M KCl, have been inserted into the retina to detect the intraretinal ERG, S-potentials, and single ganglion cell responses. The ERG and optic nerve responses have been recorded within a frequency band of 0.2 to 1,000 cycles per second; the responses, detected with microelectrodes, were directly coupled to the recording oscilloscope.

The light source was a ribbon filament energized by a 6 volt wet cell battery. This light was brought to a focus on a flag shutter driven by a galvanometer (Fig. 1). After this point the light was collimated and refocused on a small glass ball placed just in front of the cornea, in order to illuminate a large portion of the retina.
directly. The energy and wavelength of the test light could be altered by neutral or narrow band absorption or interference filters. A small portion of the test beam was deflected to a vacuum tube photocell, in order to monitor continuously the wave shape of the light stimulus. These stimuli were rectangular pulses of light ranging from 10 to 100 msec in duration and presented to the eye every 1 or 2 seconds for brief periods. In order to determine spectral sensitivity functions, the relative energies of 9 different wave bands of light were measured with a thermopile and galvanometer after all of the infrared emission in the beam had been removed by appropriate filtering.

For histology, retinas were removed within 3 minutes from eyes perfused for 6 to 8 hours and fixed in a 2 per cent solution of glutaraldehyde, with a phosphate buffer at pH 7.4 at 5 to 10° C. for 12 hours and postfixed in 1 per cent osmium tetroxide solution for 1 hour. The retinas were dehydrated in cool alcohol, transferred to propylene oxide at room temperature, and embedded in epon which was cured overnight at 50° C. The specimens were sectioned at a thickness of 7 μ, examined by light microscopy with phase optics, and compared with normal retinas processed identically.

Results

Fig. 2 shows the ERG and optic nerve response of the isolated, perfused cat eye. The ERG has an initial negative a-wave followed by an oscillatory positive b-wave, and resembles ERG’s which we have recorded from the intact animal. The optic nerve response appears as a positive potential, beginning after the start of the a-wave but before the b-wave of the ERG.

Fig. 3 shows the relationships between the logarithm of the energy of a short and a long wavelength light stimulus and the amplitude of both the a-wave and the b-wave of the ERG and the optic nerve response for a dark-adapted preparation. The amplitude of all of these responses increases continuously with the logarithm of light intensity resembling their behavior in the intact animal. These two spectrally different lights have almost identical effects on both components of the ERG, but affect the optic nerve differently, the red light being much more effective than the blue. This difference is much more apparent with stronger than with weaker stimuli and will be shown subsequently to be due to the relatively strong effect which the cone receptor system exerts on the optic nerve response.

Fig. 4 shows how thresholds for eliciting a- and b-waves of the ERG behave during perfusion and then after a period in which the retina has been strongly light-adapted. Immediately after enucleation, the threshold of the ERG is relatively high, more so for the b-wave than the a-wave. Within about an hour, thresholds decrease and the preparation stabilizes, during which time the suprathreshold responses illustrated in Figs. 2 and 3 are obtainable. Such responses can remain stable for at least 6 to 8 hours. In the case of Fig. 4, after 3½ hours of perfusion, the retina has been exposed to a strong adapting light, sufficient to raise ERG thresholds more than 10,000-fold. Nevertheless, the ERG recovers completely in about 90 minutes, somewhat more rapidly for the a- than
Fig. 3. Relationship between the amplitude of the a-wave (-----○----- and ○-----) and b-wave (-----○-----) of the ERG, the optic nerve response (-----□----- and □-----) and the logarithm of the relative energy of the light stimulus at 405 μm (open symbols) and 640 μm (closed symbols) in the dark-adapted, perfused cat eye.

the b-wave component of the response.

Fig. 5 shows action spectra based on threshold ERG and optic nerve response in the dark- and strongly light-adapted state. In the dark-adapted state, the action spectra of both responses closely parallel the human Commission Internationale d'Eclairage scotopic luminosity function, except at the very long wavelength end of the spectrum. This departure from a rod action spectrum is considered to be due to the intrusion of a cone mechanism in the cat retina.

In the strongly light-adapted state, the action spectra of both responses become more sensitive to long wavelengths, typical of the Purkinje shift from rod to cone vision. These light-adapted action spectra resemble the one which Dodt and Walther obtained from the intact cat eye, using flicker stimuli above the fusion frequency of the rods. More recently Hrachovina has also found a similar cone action spectrum in the ERG of the intact cat using strong light-adaptation, as we have done in the perfused eye, to separate
cone from rod function. We, therefore, conclude that both rod and cone mechanisms characteristic of the normal eye are well represented in both the ERG and optic nerve responses of the isolated, perfused cat eye.

The difference between the action spectra of the ERG and optic nerve response observed with suprathreshold stimuli (Fig. 3) reveals an interesting phenomenon in the cat retina. Fig. 6 attempts to demonstrate what may be responsible for this by showing pairs of ERG and optic nerve responses to short and long wavelength stimuli selected to have equal effects on the rods. With the weakest stimuli, the ERG's elicited by both lights are the same; the optic nerve responses are not. This disparity increases with stronger stimulation. With the two strongest stimuli, the ERG's begin to show slight differences in their rising limbs, indicated by arrows, which must be due to the intrusion of cones since the stimuli are equivalent for rods. This early and just discernible cone ERG is associated with a relatively large cone signal in the optic nerve. A similar phenomenon is observed in single ganglion cells of monkey retina, where it has been shown that the latency of cone signals is much shorter than those of rods, so that whenever stimuli are sufficient to activate both receptor systems, cone signals arrive first to excite the cell and depress its excitability for subsequent rod signals. Such a mechanism may also be responsible for the relatively large cone signal in the cat's optic nerve.

Stopping perfusion causes immediate changes in the retinal responses to light. Fig. 7 shows that these changes are different for the a- and b-waves of the ERG.

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Fig. 4. Time course of the changes in ERG thresholds for the a-wave (●) and the b-wave (○) during perfusion and after a bright adapting light presented for 5 minutes (vertical bar). The symbols below indicate how the temperature (△) and flow rate (▲) change during this period. The ordinate on the lower left is in degrees centigrade and the one on the right in milliliters of perfusate per minute.

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Fig. 5. The relationship between the logarithm of the light energy required to elicit a threshold ERG b-wave (●, ○) and optic nerve response (■, □) in the dark- (closed symbols) and light- (open symbols) adapted perfused cat eye. The continuous line is the human scotopic luminosity curve (Commission Internationale d'Éclairage, C.I.E.); the hatched line is the action spectrum found by Dodt and Walther for the cone ERG in the intact cat eye.

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and the optic nerve response. Within seconds after perfusion is stopped, the amplitude of the b-wave (arrows) decreases, while the response of the optic nerve remains unaffected. The optic nerve response begins to diminish only after the b-wave has become considerably reduced. The last response to disappear is the a-wave. These changes can be reversed if the perfusion is restarted soon enough.

Fig. 8 shows how stopping perfusion affects ERG and optic nerve responses to long and short wavelength stimuli matched to have equal effect on the rods. This reduces the amplitude of the large rod b-wave (arrows), indicating that the rod system is more sensitive to this insult than the cone system. The greater tolerance of the optic nerve response to cessation of perfusion, in comparison to the b-wave of the ERG, must presumably be due to the fact that the optic nerve response reflects cone much more than rod activity.

This hypothesis receives further support from intraretinal recordings from perfused eyes which, because of damage or reduced...
Fig. 6. Pairs of ERG (above) and optic nerve (below) responses to suprathreshold stimuli at 405 nm (left) and 640 nm (right) matched to have equal effects on the rods. The energies of the stimuli are increased from below upwards equally for both wavelengths. Two responses to the same stimulus are superimposed. The vertical hatched lines indicate the beginning of the light stimulus. The calibration, lower right, indicates 0.12 mV vertically and 70 msec horizontally. Positivity is upward.

flow rates, have large a-waves and extremely reduced b-waves, and this was the case during many of the initial experiments. Fig. 9 shows intraretinal responses obtained under such circumstances. The left column shows intraretinal ERGs, positive in polarity because they are recorded in the photoreceptor layer of the retina. Their behavior to the spectrally different stimuli points out the strong rod influence on the a-wave. The middle column shows S-potentials elicited by the same stimuli and in the same region of the retina from which the intraretinal ERG's had been recorded. There are two distinct components in the S-potential, one more rapid and more sensitive to long wavelengths, the other slower and more sensitive to short wavelengths. It appears that for these S-potentials rod and cone responses are both present, but the rod signal is much less conspicuous and the cone signal more conspicuous than in the a-wave of the ERG. The right column shows the impulses of a single ganglion cell in this preparation and demonstrates that, at the ganglion cell level as in the optic nerve, there are only cone responses to these suprathreshold stimuli. In such preparations with large a-waves and few or
no detectable b-waves, ganglion cell responses even at threshold show no evidence of rod function.8

Fig. 10 shows photomicrographs of different retinal layers. The over-all appearance of the sections resembles that of unperfused retinas. The pigment epithelium appears normal and closely attached to the outer segments of the photoreceptors. Both rod and cone photoreceptor cells can be clearly identified. The more inner layers of the retina also appear normal.

Discussion

The results indicate that retinal function, as measured by the ERG and the optic nerve response, can be maintained within a normal range for many hours in
Fig. 9. Intraretinal ERG's (left column), S-potentials (middle column), and single ganglion cell responses (right column) obtained from a microelectrode inserted into different depths of the same region of the retina in the isolated perfused eye. The numbers on the left indicate the wavelength of stimulation in nanometers (nm). Each horizontal row of responses has been obtained with the same stimuli. The lowest trace shows the response of a photocell monitoring the light stimulus. The vertical hatched lines indicate the beginning of this light stimulus on each response. The calibration, lower right, signifies vertically, 0.2 mv. for the ERG's, 5 mv. for the S-potentials, and 0.5 mv. for the ganglion cell responses and horizontally, 50 msec. for ERG and 25 msec. for the other responses. Positivity is upward.

the isolated, perfused cat eye. Normal function, in this case, includes ERG's of normal configuration and relatively large amplitude, retinal adaptation, a Purkinje shift, and evidence for the presence of synaptic transmission from the visual receptors to the ganglion cell layer of the retina. As far as we know, there is no other organized cellular structure in the mammalian central nervous system that has exhibited this kind of normal function in organ culture with a perfusate devoid of hemoglobin.19

The advantages of this preparation are that the retina can be excited easily and physiologically by light, so that function can be evaluated rapidly and continuously during perfusion. These light-evoked responses, detectable at the cornea in the form of the ERG, especially its b-wave component, are extremely sensitive to retinal damage and can be directly compared with the same responses recorded from the intact animal, providing a good check on the normalcy of the perfused preparation. The ERG monitors activity, including that of the photoreceptor cells, at the beginning of the visual system in the retina; the optic nerve response provides a measurement of the activity which finally leaves the retina, so that this part of the central nervous system can be monitored electrophysiologically at both its input and output. A nonpulsatile perfusion system eliminates vascular pulsations, a major problem associated with recording from...
within nerve cells in the intact animal. At the same time, one can accurately control the solutions which enter and measure what leaves the retina in a manner that is not easy to do in other parts of the brain. These advantages may make the isolated, perfused eye a useful preparation for linking neuropharmacology with neurophysiology in the mammalian central nervous system.

Ames and Gurian have reported that the optic nerve response fails more rapidly than the retinal (ERG) response, when the superfused rabbit retina is deprived of either glucose or oxygen and concluded that the most critical events occurring dur-

Fig. 10. Photomicrographs obtained with phase optics of the photoreceptor and pigment epithelium (A) and the inner nuclear and ganglion cell (B) layers of a perfused cat retina. The arrows point to rod and cone receptor cells.
ing deprivation took place in the neural portion of this tissue. Our results are in agreement with their conclusion that neural in contrast to receptor events in the retina are more sensitive to metabolic deprivation, but we do not agree with their finding that the optic nerve response fails more rapidly than the ERG. The most sensitive response to deprivation of the perfused cat retina is the b-wave of the ERG and, in particular, its rod component. Exactly where in the retina this occurs is not certain. It must be a channel used mainly by rods and not cones, since cone signals are transmitted to ganglion cells in the absence of the rod b-wave. In primate retinas there is anatomic and physiologic evidence for a separate set of bipolar cells for rods and cones. A similar situation may exist for horizontal cells. If such is the case in cat retina, then a likely site for early injury following deprivation would appear to be mechanisms involved in transmitting signals from the inner terminals of rod receptors to rod bipolar or horizontal cells. The detection of both rod and cone responses in S-potentials suggested in this study and recently clearly demonstrated in the intact cat eye makes the possibility of separate rod and cone horizontal cells in cat retina less likely and strengthens the hypothesis that mechanisms involved in rod receptor-rod bipolar transmission are the retinal events most vulnerable to deprivation injury. Such a defect can explain the absence of rod but the presence of cone signals in ganglion cells of retinas without rod b-waves.

During the course of this project, we noticed an abstract of Jazawa and Seaman describing success in recording ERG's from an isolated bovine eye, maintained by pulsatile perfusion of blood. It will be interesting to compare their electrophysiologic results with ours, which have been obtained in the absence of hemoglobin. In our system rather high and presumably unphysiologic flow rates were essential to maintain large b-wave responses in the ERG. Perhaps these rates could be reduced in the presence of erythrocytes, hemoglobin, or hemoglobin substitutes, which would bring the oxygen concentration in the perfusate closer to that of blood.

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Space limitation

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