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Effects of Epinephrine in the Chick Visual System

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Studies of the diffuse electroretinogram (ERG) and evoked responses in the optic lobe in the baby chick have shown that epinephrine produces changes in both. Epinephrine produces enhance-

ment of the a-wave and depression of the b- and d-waves in both intact and isolated eyes. In the optic lobe it produces enhancement of both components of the evoked response. The presence of epinephrine in the vertebrate retina and the optic lobe of chicks suggests that these changes may reflect the involvement of epinephrine in synaptic transmission.

INDEX DESCRIPTORS: Chick; Electroretinogram; Epinephrine; Nor-epinephrine; Visual System.

The purpose of this study was to determine the effects of epinephrine on the visual system of the unanesthetized 4-6 day old chick. The electrical responses of the retina to photic stimulation and evoked potentials elicited photically and electrically in the optic pathway were investigated.

An investigation of epinephrine as a possible transmitter agent in the visual system of chicks is encouraged by the relatively large amount of this amine in chicken brain (Callingham and Class, 1965). Several enzymes that are responsible for the synthesis and metabolism of epinephrine have been found in the retina, brain and related structures in domestic fowl.

The vertebrate retina responds to photic stimulation with a complex change in electrical potential which is measured by the electroretinogram (ERG). The ERG consists of several separate components designated as the a-, b-, c- and d-waves. The differentiation of a number of separate components and the origin of individual components in specific types of retinal cells has provided the basis for many ERG studies.

Various approaches have been used in an attempt to locate the generators of ERG components within the retina. These have included the use of micro-electrodes (Brown, 1968) to compare the gross ERG with local electrical changes in various retinal layers. Precise identification of the generators of all ERG components has yet to be made.

In this study, the effects of epinephrine on the ERG and evoked responses in the optic lobe were examined in order to obtain information about the possible role of this amine in neural processes in the visual system.

MATERIALS AND METHODS

Three basic preparations were used to carry out the experiments in this study. First, one in which the visual system remained intact. Second, because of the possible centrifugal effects of the brain on the retina, the eye was isolated from the brain by transection of the optic nerve. Third, in order to determine the effects of the drug directly on the optic nerve-optic lobe transmission, the optic nerves were elec-

trically stimulated to produce an evoked response in the optic lobe.

All surgical procedures were carried out under ether anesthesia. In order to allow placement of the ERG electrodes on the cornea, the lids and nictitating membrane were sutured open. The head was fixed in a stereotaxic device. A midline incision through the skin was reflected and underlying muscles and connective tissue were scraped off the skull. A small hole was drilled through the skull at a predetermined point for introduction of the evoked response electrode into the optic lobe. Two holes were drilled on the opposite side of the skull for the introduction of a pair of electroencephalogram (EEG) electrodes.

The trachea was intubated with polyethylene tubing which could later be attached to a small animal respirator. The animals were immobilized with 0.15 mg/bird of d-tubocurarine bitartrate given intramuscularly. Paralysis of the animals necessitated artificial support of respiration and body temperature. Respiration was maintained by means of a positive pressure system which delivered air through the tracheal tube. Body temperature was maintained at $39 \pm 1^\circ \text{C}$. An estimate of the animal's cardiovascular status was obtained by monitoring the electrocardiogram.

The EEG was recorded through a pair of nickel-chromium wires placed epidurally over the right cerebral hemisphere. Photically-evoked responses were recorded through stainless steel coaxial electrodes. The ERG was recorded by means of non-polarizable saline wick electrodes placed on the temporal cornea of the right eye in such a way as to avoid occlusion of most of the pupil. The ERG indifferent electrode was placed in tissue exposed by an incision in the skin of the neck.

Electrical stimulation of the optic nerve was carried out through bipolar nickel-chromium electrodes inserted near or into the left optic nerve. The stimulus consisted of square-wave pulses with a duration of 0.1 seconds, and voltages ranged from 3 to 10 volts. Photic stimulation was provided by a shuttered tungsten filament lamp. An optimal ERG response was obtained at 1.04×10^{-3} watts/cm².

Optimal placement of the coaxial electrodes to record the evoked response was obtained by slowly lowering the electrode aimed at the optic lobe until the evoked response, observed on a monitor oscilloscope, achieved maximal amplitude. Electrical stimulation of the optic nerve was produced by lowering the stimulation electrodes through overlying brain tissue while shocks were being delivered until maxi-

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mal shock-evoked responses were recorded from the optic lobes.

Some experiments required recording the ERG in eyes separated from the brain. This was done by transection of the optic nerve while the ERG was monitored to make sure that the surgical procedure had no deleterious effects on the isolated eye. Disappearance of the evoked response was considered an indication of successful transection of the nerve.

The EEG, ERG, and evoked responses were simultaneously recorded on the 4-channel ink writing Grass polygraph. The ERG was amplified through a pre-amplifier with a 0.8-second time constant. A computer of average transients (CAT) was used to obtain averages of evoked responses and ERGs. These were plotted on chart paper by means of a Texas Instruments servo-writer.

After the animals were immobilized, and during the period required for electrode placement, the ether anesthetic was allowed to dissipate. Prior to all experimental sessions the animals were relatively dark-adapted for a period of one-half hour. The general protocol involved the following: groups of twenty-five successive evoked responses and ERGs were averaged. This sample time required about four minutes.

Sampling was repeated throughout the experiment at ten-minute intervals. The controls consisted of four samples, which involved a total period of forty minutes, after which the drug was administered and its subsequent effects were followed until recovery, which occurred about three hours later. Groups of twenty-five electrically-evoked responses were produced at a frequency of one per second so that the sample time for this procedure was 25 seconds. However, as with photic stimulation, samples were obtained at 10-minute intervals. In all experiments the drug epinephrine bitartrate (10 mg whole salt/kg of body weight) was dissolved in physiological saline and administered intraperitoneally.

A determination of the concentration of epinephrine and norepinephrine in the chick optic lobe was made according to the method of Chang (1964).

RESULTS

The contents of epinephrine and norepinephrine in the chick optic lobe are listed in Table 1. In each of the six assays performed the content of epinephrine was much greater than that of norepinephrine; the mean values being 3.063 $\mu\text{g/g}$ wet weight and 0.117 $\mu\text{g/g}$ respectively. Epinephrine was determined to be present in significantly ($p \leq 0.001$) greater quantities.

ERG responses were recorded, and the a-, b-, and d-wave voltages were determined. Percent changes of the three ERG waves during the course of approximately 160 minutes after injection of 10 mg/kg epinephrine are shown for the intact (Figure 1) and isolated (Figure 2) chick eye. In the intact eye the a-wave was significantly ($p \leq 0.001$) increased whereas the b- and d-waves were significantly ($p \leq 0.001$) decreased. In the isolated eye the a-wave was significantly ($p \leq 0.01$) increased and the b-wave was significantly ($p \leq 0.01$) decreased, but the d-wave was not significantly affected.

Optic lobe responses, evoked both photically and electrically, were recorded and the voltages of the two evoked response components were determined. Percent changes of the components during the 160 minutes of post-epinephrine recording are shown in Figure 3 for the isolated and intact chick eye. The positive component of the photic-evoked re-

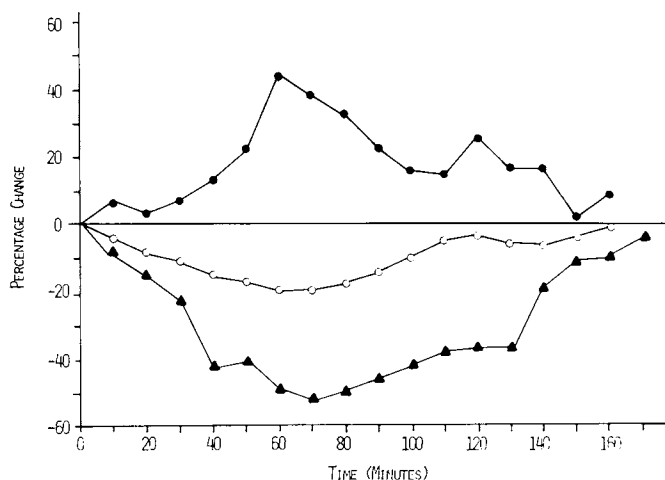


Figure 1. Percentage change of three ERG waves, intact chick eye, from mean of control trials, after injection of 10 mg epinephrine/kg of body weight. Each point represents mean of seven subjects: a-wave (●), b-wave (Δ), and d-wave (○).

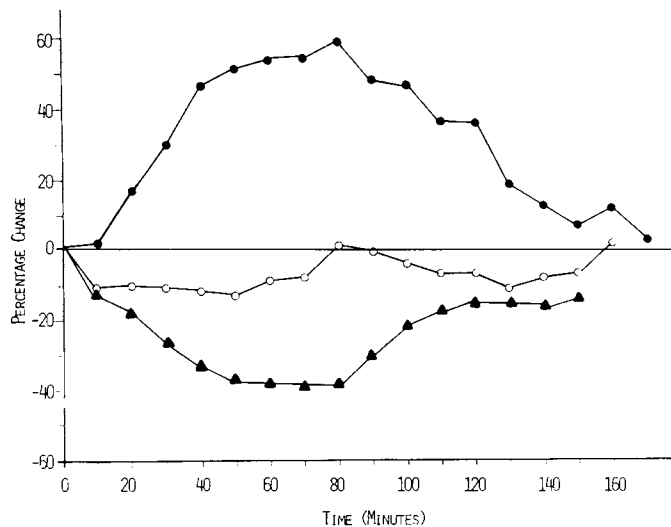


Figure 2. Percentage change of three ERG waves, isolated chick eye, from mean of control trials, after injection of 10 mg epinephrine/kg of body weight. Each point represents mean of six subjects: a-wave (●), b-wave (Δ), and d-wave (○).

sponse was not significant but the negative phase was significantly ($p \leq 0.01$) increased. Both phases of the electrically-evoked response were significantly ($p \leq 0.01$) increased during the course of the experiment.

Since peak changes in the ERG waves and evoked response components occurred at approximately seventy minutes post-epinephrine injection, this time period was used as a time constant in comparing the respective waves and components of the various preparations. At seventy minutes post-

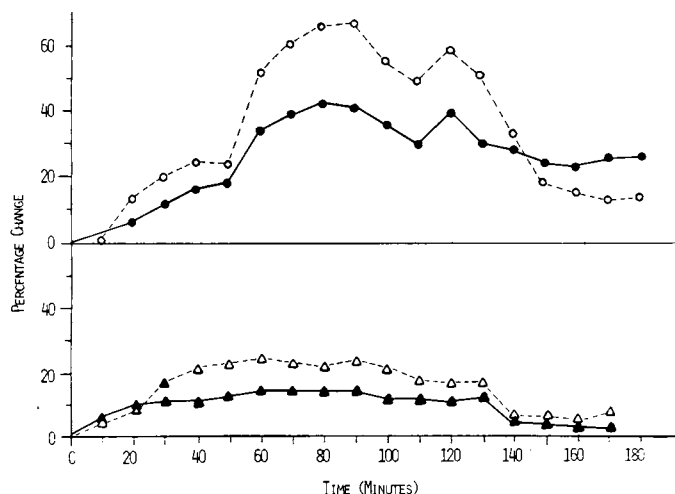


Figure 3. Percentage change of two components of response evoked electrically (top) and photically (bottom) from mean of control trials, after injection of 10 mg epinephrine/kg of body weight. Each point represents mean of five subjects: positive phase (●, △), negative phase (○, △).

TABLE I. COMPARATIVE CONTENTS OF NOREPINEPHRINE (NE) AND EPINEPHRINE (E) IN THE OPTIC LOBE OF FIVE-DAY-OLD COCKERELS; EPINEPHRINE WAS DETERMINED TO BE PRESENT IN SIGNIFICANTLY ($p \leq 0.001$) GREATER QUANTITIES

Norepinephrine		Epinephrine	
$\mu\text{g/g-tissue}$		$\mu\text{g/g-tissue}$	
	0.081		3.108
	0.056		3.119
	0.165		2.987
	0.112		3.072
	0.163		3.074
	0.126		3.074
Mean	0.117		3.063
S.E.M.	0.018		0.019

epinephrine injection, the mean percentage change for the a-wave was +38.7 for the intact eye and +54.9 for the isolated eye.

The isolated eye a-wave values were increased significantly ($p=0.051$) more than the intact eye a-wave values. The mean percentage change for the b-wave at seventy minutes was -51.9 for the intact eye and -31.2 for the isolated eye. The intact eye b-wave values were decreased more than the isolated eye b-wave values, but the level of significance ($p=0.117$) was low. The d-wave values for the intact eye were decreased significantly ($p=0.017$) more than the isolated eye d-wave values; the mean percentage changes at seventy minutes being -19.1 and -8.0 respectively.

Examination of the evoked responses shows that for both positive and negative components, the responses from electrically stimulated optic lobes (mean percentage changes +38.9 and +62.1 respectively) were increased more than

those photically stimulated (+21.9 and +13.2 respectively). The statistical test yielded a level of significance for the positive component of the electrically-evoked response of $p=0.053$ and for the negative phase of $p=0.003$ when tested for significant differences from the respective components of the photic-evoked response.

Although the isolated chick eye produced increased positive percentage change values for the a-wave when compared to a-wave values in the intact eye, the drug effects on the b- and d-waves were actually decreased in the isolated eye preparation. The isolated eye preparation, however, produced an increase in all three ERG waves recorded when compared to the intact eye ERG recordings. Electrical stimulation of the optic nerve elicited a greater positive increase in both phases of the optic lobe evoked response, and hence a greater drug effect, relative to the photic response emanating from retinal photoreceptors.

DISCUSSION

The effects of epinephrine on the ERG of the intact and isolated eyes are qualitatively similar. The possibility that these effects result from cardiovascular changes are minimal because the peak effects on the ERG occur approximately 60-70 minutes after the peak change in blood pressure.

Apparent enhancement of the a-wave may be produced by a concurrent increase in b-wave latency. An alternative explanation is that there is a direct effect of epinephrine on conductance through the distal segments of the photoreceptors. The positive going voltage responsible for the ERG a-wave has been explained on the basis of the light-induced changes in an ongoing dark current (Hagins, et al., 1970). It is possible that epinephrine has a direct effect on generation of the dark current either through changes in membrane conductance or by interaction with the active transport of sodium (Orlov, 1961, and Born and Bulbring, 1956).

Because the avian retina is innervated by afferent fibers it is possible that in the intact eye the drug may produce changes in the ERG indirectly through the afferent system. In the pigeon the afferent system appears to synapse mainly with amacrine cells (Maturana and Frenk, 1965). It is difficult to relate changes in amacrine cells to a-wave changes. It is possible that parts of the afferent system terminate in more distal regions of the retina and thereby affect changes in the a-wave amplitude. If this is true, it would appear that an epinephrine-activated afferent system is inhibitory on the a-wave.

Depression of the b-wave by epinephrine may be due to a direct effect of the drug on generators of the b-wave. There is presently some controversy concerning the origin of the b-wave. Miller and Dowling (1971) have concluded that the voltage changes manifested in the b-wave result from indirect depolarization of the Müller cells. Other work (Ogden and Wylie, 1971) has located the origin of the b-wave in the inner nuclear layer without specification of the cell type of origin. The presence of epinephrine in the vertebrate retina (Waltman and Sears, 1964) suggests the possibility that adrenergic retinal elements may inhibit the direct generators of the b-wave. Depression of the b-wave is greater in the intact system than in the isolated eye. Although the difference is not statistically significant ($p=0.117$), it suggests

the possibility that epinephrine may have an indirect effect on the ERG b-wave by acting on the brain via the afferent visual pathway.

In some retinas, particularly those which are cone dominated, termination of the stimulus produces a positive deflection followed by a negative deflection. This has been called the d-wave. In rod dominated retinas termination of the light stimulus produces a negative deflection which has been called the off-effect.

According to Brown (1968) the positive limb of the d-wave is produced by the passive return of the a-wave, whereas the negative limb of the d-wave is produced by the return of the P III component. According to Ogden and Wylie (1971) the pigeon ERG does not contain a DC component and hence has no d-wave. Termination of a light stimulus produces a positive deflection but no negative one. Because the chick ERG shows a distinct d-wave, the presence of the DC component is implied.

Depression of the d-wave by epinephrine can be interpreted in either of two ways. Since epinephrine may increase the a-wave voltage, this should produce an increase in the positive limb of the d-wave if the DC component is unaffected. However, since the d-wave is depressed, a decrease in the latency of termination of the DC component is implied. It is also possible that the Brown model of the origin of the d-wave does not apply to the chick ERG and that depression is produced by an effect of epinephrine on some independent generator of the d-wave. Because depression of the d-wave is significantly ($p=0.017$) greater in the intact eye compared to the isolated eye, it is possible that part of the depression results from an effect of epinephrine on the brain which indirectly effects the retina through an afferent pathway.

The initial (positive) component of evoked responses has been interpreted to represent post-synaptic electrogenesis. The second (negative) component has been attributed to a spread of depolarization along apical dendrites on through other neurons, toward the recording electrode. Epinephrine enhanced both components of the evoked response. Augmentation of the evoked response produced by photic stimulation was considerably less than the evoked response produced by electric stimulation. Since photic stimulation must include retinal pathways, it is possible that the reduced

epinephrine effect under these conditions is due to the depressant effects of the epinephrine on the retina (as evidenced by the reduced b-wave in the isolated eye) which opposes the augmenting effect of epinephrine in the optic lobe itself.

It is therefore likely that epinephrine has independent and opposite effects on transmission of signals in the retina and the optic lobe. The abundant occurrence of epinephrine in the chick optic lobe and its effects on evoked responses recorded there raises the possibility either that retinal ganglion cells are adrenergic or that they synapse with adrenergic neurons in the optic lobe whose action enhances transmission to the neurons in the optic lobe which receive direct projections from optic nerve fibers.

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