the Veterans Administration Hospital, San Francisco, Calif. This work was supported by a National Eye Institute Research Grant EY 01489 (to R. H. S.) and by the Research Service of the Veterans Administration (W. H. S.). Submitted for publication March 27, 1978. Reprint requests: Dr. Roy H. Steinberg, Department of Physiology, S-762, University of California, San Francisco, Calif. 94143. *Present address: School of Optometry, 101 Minor Hall, University of California, Berkeley, Calif. 94720.

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REFERENCES


Laminar separation of light-evoked $K^+$ flux and field potentials in frog retina. Chester J. KARWOSKI, MARK H. CRISWELL, AND Luis M. PHOENZA.

Light-evoked changes in [K+]c and field potentials were recorded from the retinas of grass frogs. In the proximal retina, light induced an increase in [K+]c. This increase had components at light onset and offset, was maximal with small spot stimulation, and reached greatest amplitude at the same depth as the proximal negative response (PNR). Extracellular dye marking revealed that this depth was within the inner plexiform layer. The off-components of both the $K^+$ increase and PNR occurred distal to the on-components, thus supporting recent proposals that "off" synapses lie distal to "on" synapses. Since a well-developed M-wave, having a time course nearly identical to the $K^+$ increase, was also seen in the proximal retina, this field potential appears to be a normal component of the intraretinal electroretinogram.

Light-evoked changes in extracellular potassium ion concentration ([K+]o) in the retina are of interest because they offer clues about underlying mechanisms of nearby neural activity and because changes in [K+]o have been implicated in the generation of pigment epithelial and glial cell responses and of their associated field potentials. In frog, light stimulation has been previously shown to induce a decrease of [K+]o in the distal retina and an increase proximally. In mud-puppy, the proximal $K^+$ increase has been examined in response to a wide variety of photic stimuli and shown to (1) have both on- and off-components, (2) be greatest to small-diameter stimuli, (3) have maximum amplitude at about the same depth as a field potential, the proximal negative response (PNR), and (4) have a time course nearly identical to another field potential, the M-wave.

In the present study, the proximal $K^+$ increase in the frog retina was examined in relation to slow extracellular potentials to determine whether they share characteristics comparable to those of the mudpuppy responses. In addition, given recent
Fig. 1. Simultaneous recordings in the proximal retina of light-evoked field potentials ($V_o$) and $K^+$ responses ($K^+$) to flashed spots of various diameters (given in the center). Stimulus intensity: 0.075 lm/m²; duration: 4 sec. A diffuse background of 0.05 lm/m² was also present. Resting $[K^+]_o$ was 2.9 mM. Calibration: for $V_o$, 0.2 mV; for $K^+$, 0.5 mV.

Evidence that “off” synapses are located more distal within the inner plexiform layer (IPL) than the “on” synapses, \(^8\) \(^9\) careful depth measurements of the $K^+$ increase and PNR were made to determine whether such a laminar separation in the on and off systems could be revealed with extracellular recording techniques.

**Methods.** Eyecups of grass frogs (*Rana pipiens* and *Rana berlandieri*) were placed in a small chamber through which moist gas (95% O₂, 5% CO₂) circulated. \(^7\) Responses were recorded with double-barreled micropipettes (combined tip diameter of 1 to 2 µm). One barrel, filled with a modified amphibian Ringer solution, monitored field potentials. The other barrel contained a column of potassium ion exchanger in the tip and yielded a potential approximately proportional to log $[K^+]_o$ when its output was subtracted from that of the Ringer-filled barrel. In a typical experiment, the electrode was first advanced slowly into the proximal retina. At this depth, the position of a stimulating spot (250 µm diameter) was adjusted to produce a PNR of maximum amplitude. The electrode was further advanced until the tip was beyond the retina. It was then withdrawn until a region of high noise and increasing b-wave amplitude was encountered. This level was assumed to be near the distal margin of the retina, \(^1\) \(^10\) just proximal to Bruch’s membrane, and was therefore called 100% retinal depth. The electrode was then withdrawn in 10 or 20 µm increments, and a standard series of stimuli were presented at each retinal depth. The electrode was assumed to be in the vitreous humor (0% retinal depth) when PNR amplitude decreased to zero. \(^7\)

In dye marking experiments, pipettes (tip diameter 2 to 4 µm, DC resistance 20 to 120 MΩ) were filled with an aqueous solution containing 5% Alcian blue and 0.5% Na acetate (J. A. Freeman, personal communication and ref. 11). The depth of the electrode was carefully adjusted until maximum PNR was obtained. Square current pulses (about 0.2 µA; 0.1 sec duration and presented at 5 Hz) were passed through the electrode (tip positive) for 0.5 to 2.0 min to inject dye into the preparation. The retina was then processed for light microscopy, cut into 60 µm sections, and stained lightly with eosin. The dark spots of Alcian blue were readily visible against the pink-stained background.

**Results.** The depth profile of $K^+$ flux in frog retina was found to be basically similar to that re-
Fig. 2. Depth profiles of the normalized amplitudes of the on- and off-components of the PNR and K⁺ increase. Stimulus diameter: 0.3 mm; duration: 4 sec; intensity: 7.5 lm/m². Background intensity: 1.0 lm/m². Resting [K⁺]₀ was about 2.5 to 3.5 mM throughout the proximal two thirds of the retina.

Representative field potentials and K⁺ responses from the proximal retina of frog are shown in Fig. 1. The field potentials were dominated by two responses: (1) the PNR, consisting of the initial transient components that occur at both light onset and offset and are best developed in the proximal half of the retina and (2) the M-wave, an extracellular slow potential thought to be generated by a K⁺-induced depolarization of Müller cells, consisting of the slower negative-going responses that occur at light onset and offset. M-wave amplitude was also maximum in the proximal retina—at about the same depth as the PNR. The M-wave and the K⁺ increase have been previously shown to share a nearly identical time course in the mudpuppy eyecup drained of most vitreous humor. As seen in Fig. 1, this relationship is also now established in the frog eyecup not drained of vitreous.

Fig. 1 also shows that the K⁺ increase in frog, as in mudpuppy, was maximal to small-diameter flashes and appreciably decreased with large-diameter (1.5 mm) stimuli. This relationship was always clearer with the on-component of the response. The simultaneously recorded field potentials of Fig. 3 show that, like the K⁺ increase, the PNR was maximal to small-diameter stimuli. M-wave magnitude was usually greatest with intermediate-diameter spots of 0.5 to 1.0 mm.

A difference between the field potentials and K⁺

[Diagram of depth profiles with axes labeled: Percent Retinal Depth and Percent Maximum Response.]

[Graph showing PNR and K⁺ profiles with markers for ON and OFF components.]
responses of frog and mudpuppy is that unlike mudpuppy, the on and off components of these responses in frog do not reach maximum amplitude at the same retinal depth. In Fig. 2, the amplitudes of the on and off components of the PNR and K⁺ increase were measured at 10 μm increments through the retina, and their normalized values were plotted. Three relationships are highlighted in Fig. 2. (1) The on-components of the PNR and K⁺ increase peaked at about the same level of the proximal retina. (2) The off-component of the PNR was maximal at about the same depth as the off-component of the K⁺ increase. (3) For both the PNR and K⁺ increase, the entire off-component curve was shifted about 25 to 30 μm distal to the on-component curve. It is possible that measurement of the K⁺ increase could be confounded by interaction of this response with the distal decrease in K⁺. To minimize this possibility, stimuli were used which were found to elicit a distal decrease of negligible amplitude: a high level of background illumination (see also Oakley⁶) and a small-diameter, relatively low-intensity, flashing spot.

Depth series of the PNR were taken in 30 penetrations made in 23 retinas, and K⁺ responses were simultaneously monitored in 11 penetrations. In 27 of these penetrations, the PNR off-component reached maximum amplitude distal to the on-component; they peaked at the same depth in the remaining three. The off-component of the K⁺ increase was maximum distal to the on-component in all 11 series. For both the PNR and K⁺ increase, mean percent retinal depth for maximum on- and off-components were 28% and 35%, respectively.

In previous work, the retinal depth for recording the maximum PNR and K⁺ increase has been inferred from physiological criteria.⁷ In an attempt to histologically verify these determinations, Alcian blue dye was injected at the depth where PNR amplitude was greatest. Of 27 injections made in seven retinas, 10 dye spots were recovered. Eight of these were placed at maximum off-PNR, 2 at maximum on-PNR. The recovered extracellular marks were well-demarcated and averaged about 13 μm diameter. In Fig. 3, the radial extent of all 10 recovered dye marks is indicated by the horizontal bars. Eight spots fell exclusively within the IPL, one barely encroached on the inner nuclear layer (INL), and one lay proximally in the INL. The mean location of the spots marking the off-component was at 22% retinal depth, which lies in the distal half of the IPL. This depth is proximal to the physiologically determined depth of the off-PNR (35% retinal depth, inner half of INL). We believe the dye marking is accurate but can only guess as to the presumed source of error in the physiological determinations. The hazards of determining retinal depth with physiological criteria have been discussed elsewhere.¹⁰

Discussion. All properties of the proximal K⁺ increase in mudpuppy,⁶ which were summarized in the introduction, have been replicated in the frog retina. The main difference observed between these species is that, in frog, the off-components of the K⁺ increase and PNR peak distal to the on-components, whereas in mudpuppy, on- and off-components peak at about the same depth.⁶ Since dye marking places the depth of the PNR maximum within the IPL, it seems likely that the PNR and K⁺ increase arise from activity in this layer. Recent experiments in cat and carp indicate that the IPL has a bisublaminar organization, with the off synapses lying distal to the on synapses.⁸,⁹ The laminar separation of on- and off-components of the PNR and K⁺ increase in frog would suggest that such an anatomical organization also exists in this species. In pigeon, a species which has a thick IPL like the frog, the off-PNR peaks distal to the on-PNR.¹² A similar laminar separation of the components of the K⁺ increase and PNR is not obvious in mudpuppy, probably because a relatively thin IPL in this species' makes such a separation virtually undetectable with extracellular recording.
The M-wave, a field potential recently described in mudpuppy eyecups in which the vitreous humor has been largely drained, is probably generated by K⁺-induced depolarization of Müller cells. Since M-wave amplitude is much lower in mudpuppy eyecups not drained of vitreous, it was previously uncertain to what extent its existence was dependent on vitreous drainage or whether it even occurred in undrained preparations. However, the present experiments in frogs have demonstrated a potential which is likely the M-wave since it shows several characteristic properties of this response: (1) a slow time course nearly identical to that of the K⁺ increase, (2) maximum amplitude in the proximal retina, (3) on- and off-components, and (4) reduced response amplitude when large spot stimulation is used. The frog eyecups appear to be in good condition since they are oxygenated continuously, contain at least 2 mm vitreous humor, and often produce responses with both normal threshold and unchanged amplitude for 3 hr or more. Thus the M-wave appears to be a normal component of the intraretinal electrotetrogram of frog retinas.

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Key words: retina, electrotetrogram (ERG), potassium, glial cells, PNR, K-electrode

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Light-evoked release of endogenous glycine into the perfused vitreous of the intact rat eye. BRUCE M. COULL AND ROBERT W. P. CUTLER.

Glycine, a putative neurotransmitter, is released into the perfused vitreous of anesthetized pigmented rats when the eye is stimulated by intermittent flashes of bright light. Other amino acids do not show stimulated release. This result provides further evidence for the role of glycine as a neural transmitter in the retina.

Glycine is present in high concentration in the mammalian retina. 1,2 It is located primarily in the inner nuclear, inner plexiform, and ganglion cell layers, where it may function as an inhibitory neurotransmitter. 3,4 The spontaneous and light-evoked firing of retinal ganglion cells is inhibited when the retina is bathed in a medium containing glycine. 5 Exogenous H-glycine is accumulated by amacrine cells, 6 and released upon stimulation of the incubated retina or perfused eye cup by flashes of light. 8 We now report the light-evoked release of endogenous glycine into the perfused vitreous of the intact eye.

Materials and methods. Adult male pigmented Long-Evans rats, raised in an alternating 12 hr light-and-dark cycle, were anesthetized with intraperitoneal pentobarbital (50 mg/kg) and placed in a stereotactic head holder in a spinxlike position. The rats were paralyzed with gallamine (1 mg/kg) and artificially ventilated. A 30-gauge stainless steel inflow cannula, held in a micromanipulator, was inserted into the vitreous at 12 o'clock, and a 25-gauge outflow cannula was inserted at 6 o'clock. Both cannulae were placed slightly posterior to the equatorial plane. Clear