Slow PIII Component of the Carp Electroretinogram

PAUL WITKOVSKY, F. E. DUDEK, and HARRIS RIPPS

From the Departments of Physiology and Ophthalmology, Columbia University, New York 10032 and the Departments of Physiology and Ophthalmology, New York University School of Medicine, New York 10016

ABSTRACT The slow PIII component of the electroretinogram (ERG) was studied in the isolated, aspartate-treated carp retina. Although the latter is richly populated with cones, slow PIII appeared to reflect almost exclusively the activity of rods; e.g. the spectral sensitivity of the potential paralleled closely the rod pigment curve, its operating range (i.e. the V-log I curve) was limited to 3 log units above absolute threshold, and raising background intensities to photopic levels produced saturation of the increment threshold function without evidence of a cone-mediated segment. Only after bleaching away a significant fraction of the porphyropsin was it possible to unmask a small photopic contribution to slow PIII, as evidenced by a displacement in the action spectrum to longer wavelengths. The spatial distribution of the slow PIII voltage within the retina (Faber, D. S. 1969. Ph.D. Thesis. State University of New York. Buffalo, N. Y.; Witkovsky, P. J. Nelson, and H. Ripps. 1973. *J. Gen. Physiol.* **61**:401) and its ability to survive aspartate treatment indicate that this potential arises in the Müller (glial) fiber. Additional support for this conclusion is provided by the slow rise time (several seconds) and long temporal integration (up to 40 s) of the response. In many respects the properties of slow PIII resemble those of the *c*-wave, a pigment epithelial response also subserved by rod activity. On the other hand, the receptoral (fast PIII) and the *b*-wave components of the ERG behave quite differently. Unlike slow PIII, response saturation could not be induced, since both potentials are subserved by cones when the stimulus conditions exceed the limits of the scotopic range. Receptors appear to govern light adaptation at photopic background levels; both fast PIII and *b*-wave manifest identical incremental threshold values over this range of intensities. However, under scotopic conditions, the sensitivity of the *b*-wave is affected by luminous backgrounds too weak to alter fast PIII threshold, indicating a postreceptoral stage of adaptation.

INTRODUCTION

The electroretinogram (ERG) recorded across the vertebrate retina is a complex response, representing presumably the algebraic sum of the light-evoked potentials arising from different populations of cells (Granit, 1933).

While there is less than complete agreement as regards the relative contributions of each cell type to this composite waveform (cf. Granit, 1947; Brown, 1968; Rodieck, 1972), a variety of experimental methods have been successfully employed in identifying the origins of the principal ERG components (Noell, 1953; Brown, 1968; Faber, 1969; Penn and Hagins, 1969; Miller and Dowling, 1970; Witkovsky et al., 1973). Perhaps the most unexpected finding to have emerged from these studies is that, except for the *a*-wave, the transretinal voltages are generated almost exclusively by glial and pigment epithelial cells, i.e. non-neuronal elements (Faber, 1969; Miller and Dowling, 1970; Steinberg et al., 1970).

In an earlier study of the carp ERG (Witkovsky et al., 1973), we described the response properties of the late receptor potential (PIII in Granit's nomenclature), a vitreous-negative response that forms the leading edge of the electroretinographic *a*-wave (Brown, 1968; Penn and Hagins, 1969). Although it provided a good index of gross receptoral activity, we found that both rods and cones contributed to the recorded voltage, even in the dark-adapted preparation. Because of this, it proved difficult to separate fully the responses of either type of photoreceptor by selective chromatic adaptation or to characterize the behavior of either type over its entire operating range.

However, in addition to the receptoral component, a slow potential of the same polarity was detected both by differential-depth recording and in the transretinal response obtained after bathing the isolated retina in sodium aspartate (Na Asp). The slow rise and fall times of the response suggest that it arises from the glial (Müller) cells, and its resistance to Na Asp, that it reflects the activity of the photoreceptors (the only neural elements to retain their functional integrity in Na Asp). Faber (1969) has dubbed this negative potential "slow PIII" to distinguish it from the faster, shorter latency receptoral component which is referred to hereafter as "fast PIII."

The present experiments were undertaken with two objectives. The first was to furnish a more detailed account of the slow PIII potential: its spectral sensitivity, response to light adaptation, and temporal summation, and to compare these functions with those derived from measurements of other ERG components. The second was to determine whether slow PIII, because of its dependence upon receptoral activity, provides an alternative metric by which to assess the functional properties of rods and cones.

METHODS

The preparation and recording conditions were similar to those described previously in greater detail (Witkovsky et al., 1973). Experiments were performed on the freshwater carp (*Cyprinus carpio*), dark adapted for at least 3 h prior to ocular enucleation. For most studies, the retina was removed from the hemisected globe, placed receptor-

side up on Ringer-soaked filter paper, and maintained in a Lucite chamber beneath a continuous flow of moist gas (95% O_2, 5% CO_2). The chamber was flooded for 5 min with Ringer solution (Witkovsky, 1965) containing 100 mM sodium aspartate in place of the equivalent concentration of NaCl, and then drained of excess fluid. The sodium salt of aspartic acid has proven effective in abolishing the ERG *b*-wave as well as the light-evoked responses of neurons situated proximal to the photoreceptors (Sillman et al., 1969; Ames and Pollen, 1969; Dowling and Ripps, 1971). In other experiments (those involving *b*- or *c*-wave recordings), the retina remained *in situ* and was excised together with the posterior ocular layers to form an eyecup preparation 10–13 mm in diameter; no fluid was added to the preparation.

The ERG was recorded between two chlorided-silver electrodes: one located in the floor of the chamber, the other in a drawn glass capillary and in contact with the retina through a Ringer-agar bridge. The electrodes were connected to a cathode follower (W-P Instruments, Inc., Hamden, Conn.), the output of which was direct-coupled to the amplifier of a Grass penwriter (bandpass = 0–35 Hz; Grass Instrument Co., Quincy, Mass.). Photic stimuli were provided by a dual-beam optical system that utilized a DC-run 150 W Xenon arc lamp (Xenon Corp., Medford, Mass.) to provide both test and adapting fields. The exposure duration, intensity, and spectral composition of each field was controlled independently, the latter by means of narrow-band interference filters (Balzer, Lichtenstein) or a monochromator (Canalco, Inc., Rockville, Md.). The two beams were subsequently combined in a half-silvered mirror and reflected onto the preparation at an angle of 30° to the normal by a prism; both fields illuminated the entire retina uniformly. Flux densities were measured in the plane of the retina with a spectrally calibrated photomultiplier, and the fraction of rod pigment bleached was determined directly by transmission densitometry (Dowling and Ripps, 1970).

Measurements of fast and slow PIII voltages were made from penwriter traces taken at high gain. In these traces we observed that even in the response to a dim flash, a fast initial phase and a later slower phase were evident. A line was drawn tangent to the initial slope of the response. Where this line departed from the record was taken as the peak of the fast PIII voltage. Its value was subtracted from the total voltage swing to give the amplitude of the slow PIII response.

In Figs. 1–3, 5, and 8, I_t (500 nm) refers to the stimulus and I_b (504 nm) to the background field. Intensities are given as quanta incident s^{-1} μm^{-2} of retina. The corresponding number of quanta absorbed per rod per second can be determined by reference to the absorption spectrum of carp porphyropsin corrected for its *in situ* density (0.39 at λ_{max} = 525 nm) as previously determined (Witkovsky et al., 1973) and the cross-sectional area (3 μm^2) of carp rod outer segments. Thus, in Figs. 1–3 log I_t = 0.0 is equivalent to 1.70 quanta absorbed per rod per second and in Figs. 5 and 8 log I_b = 0.0 is equivalent to 1.72 quanta absorbed per rod per second.

RESULTS

PIII Complex

Fig. 1 shows responses to stimuli of increasing intensity as recorded across the isolated retina after it had been immersed in Na Asp. The isolated retina

was used for this phase of the study, since it was essential to eliminate the *c*-wave (of the pigment epithelium) which is of opposite polarity but similar time-course to that of slow PIII. Under these experimental conditions, only the negative components of the ERG were elicited by photic stimuli.

Although differential depth recording within the retina is required to separate fully the fast- and slow-PIII potentials (Witkovsky et al., 1973), it was possible to distinguish between them in the transretinal responses of Fig. 1 by virtue of the marked differences in time-course. Fast PIII rises to its peak amplitude within 1 s after stimulus onset, irrespective of intensity. This is true also for the responses of vertebrate rods, recorded intracellularly, where it has been shown that the voltage maximum is reached in less than 1 s for the full range of stimulus intensities (Baylor and Hodgkin, 1973; Fain and Dowling, 1973; Kleinschmidt, 1973). Slow PIII, on the other hand, can grow in amplitude for times up to 30 s, depending on stimulus parameters (compare left and right sides of Fig. 1). Thus with long duration (~30 s), moderately bright stimuli, slow PIII is seen usually to arise from the leading edge of fast PIII, and to grow in amplitude for the duration of the stimulus (Fig. 1, left column). In fact, because of its ability to integrate temporally the effects of prolonged illumination (see below), slow PIII invariably comprised 80% or more of the maximum voltage swing.

Fig. 2 shows the voltage vs. intensity relation for slow PIII responses elicited by 30-s stimuli; amplitude is expressed as the percentage of its maximal value so as to weight appropriately the results obtained from six preparations. Plotted on log-linear coordinates (open circles), it is clear that the relationship can be described by the nonlinear expression: $V/V_{max} = I^a/(I^a + \sigma)$, where σ is the intensity I required to give a voltage $V = 0.5\ V_{max}$, and a is the constant that determines the slope of the curve (Naka and Rushton, 1966). The line drawn through the data is a plot of this function for $a = 1$, a value in good agreement with those obtained from *receptor* potential measurements in a variety of vertebrate retinae (Baylor et al., 1971; Grabowski et al., 1972; Fain and Dowling, 1973). Transforming the data to log-log coordinates (filled circles) shows that slow PIII amplitude is a linear function of intensity for about the first 1.3 log units of its dynamic range, but the response increases at a progressively slower rate (up to V_{max}) as intensity is increased further.

The scale of abscissae in Fig. 2 has been expressed in quantal flux, but based on measurements of the *in situ* density of porphyropsin and the dimensions of carp rods (cf. Witkovsky et al., 1973), it is possible to estimate the rate of quantal absorption that gives rise to a half-maximal potential (i.e. $V/V_{max} = 0.5$). It turns out that for stimuli of 30-s duration, σ corresponds to a rate of absorption of 34 quanta per rod per second, and the voltage maximum is reached when quanta are absorbed at the rate of several hundred

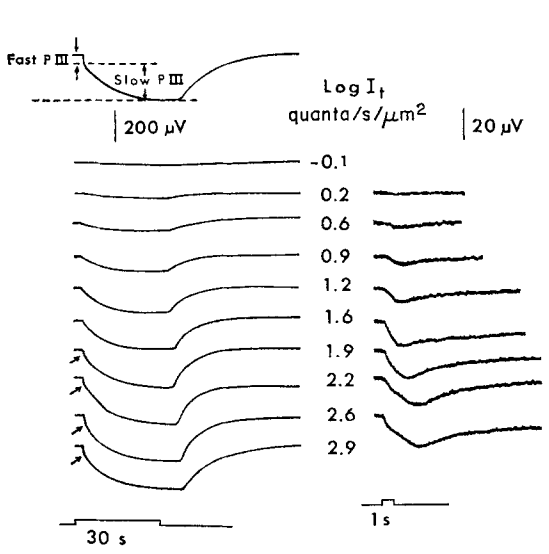

FIGURE 1

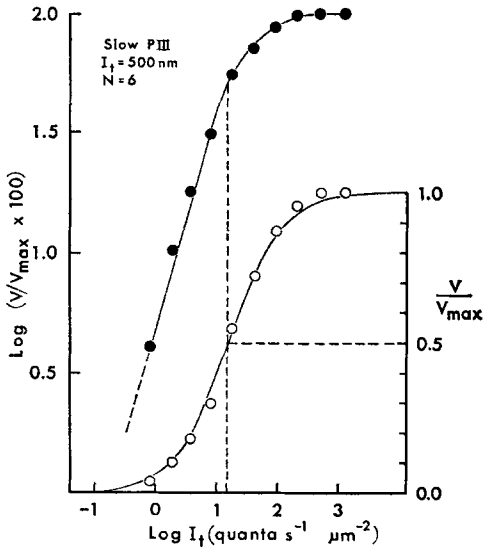

FIGURE 2

FIGURE 1. (Left) A series of ERG recordings from the aspartate-treated, dark-adapted carp retina in response to 30-s, 500-nm flashes of increasing intensity. Increasing corneal negativity is downward. Note that an early fast deflection (fast PIII) is discernible in the responses to bright flashes (arrows) followed by a slowly increasing potential (slow PIII). However, at any stimulus intensity, all the fast PIII voltage is generated within 1 s after response onset. (Right) An ERG series in response to 1-s 500-nm test flashes. Note that no voltage is produced by a 1-s flash of log I_t = 0.2 whereas a measurable response is elicited by a 30-s flash of the same intensity. Both fast and slow PIII components are apparent in the response to a bright 1-s flash, e.g. log I_t = 2.6, but only slow PIII is elicited by less intense stimuli. The amplitudes of fast and slow PIII waves were measured as shown in the schematic drawing above.

FIGURE 2. The averaged amplitudes in percent maximum slow PIII voltage (right-hand ordinate) as a function of flash intensity (500 nm, 30 s) is given by the open circles. The line through the points is the equation $V/V_{max} = I^a/I^a + \sigma$ where a = 1. The values of the open circles are translated to log-log coordinates (filled circles) and referred to the left-hand set of ordinates. A straight portion of the line drawn through these data has a slope of +1 indicating the linear relation between voltage and intensity. The half saturation value (V/V_{max} = 0.5) is given by the vertical dashed line intercept with the scale of abscissae and corresponds to an incident flux of 16 quanta s^{-1} μm^{-2}.

per rod per second. The latter value is particularly significant; when steady backgrounds deliver quanta at this rate, the incremental threshold approaches infinity (see below).

Another interesting feature of the slow PIII voltage is the dependence of response amplitude upon stimulus duration (Fig. 1, right column). As shown by the voltage-intensity curves of Fig. 3, there is for each value of log I, a progressive increase of amplitude as stimulus duration is lengthened from

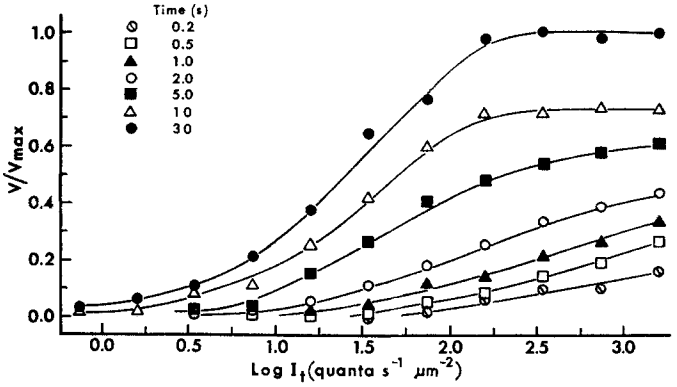

FIGURE 3. A family of V-log I curves for slow PIII responses elicited by 500-nm stimuli of different durations. V_{max} refers to the maximum voltage elicited by 30-s stimuli.

0.2 to 30 s. Note that with brief flashes, the maximum response elicited is only a small fraction of the voltage obtained with long duration, intense stimuli. To determine whether the Bunsen-Roscoe law ($I \cdot t$ = constant) applies to these data, the energy needed to elicit a criterion voltage (i.e. 0.2 V_{max}) with each stimulus duration was read from the graphs. The results are compared in Fig. 4 with those obtained for fast PIII by a comparable procedure. With regard to fast PIII, reciprocity (indicated by the slope of the dashed line) holds for stimulus durations ≤ 1 s; for longer durations, intensity alone determines response amplitude. However, from the data points of Fig. 4 it appears that although slow PIII exhibited linear summation for exposure times ≥ 5 s ($\log t = 0.3$), disproportionately high intensities were required to generate the criterion response with briefer flashes. Indeed, the asymptotic voltage levels of Fig. 3 together with the departure from the Bunsen-Roscoe relation (Fig. 4) indicate that long stimulus durations are essential for the full expression of the slow PIII voltage. Although not shown on the graph, we observed in three control experiments that stimulus durations exceeding 40 s produced no further increment in PIII voltage. These findings, together with those obtained by differential depth recording (Witkovsky et al., 1973), are consistent with the notion that fast and slow PIII arise from different retinal sources, which develop electrical potentials having very different temporal characteristics.

Light Adaptation

The incremental threshold (ΔI) provides a measure of the change in sensitivity that accompanies adaptation to steady ambient illumination. Our procedure was first to obtain the V-log I relation of the dark-adapted retina, and then to repeat the sequence with test stimuli superimposed upon back-

ground fields (I_b) of increasing intensity. Before the start of an experimental run, each new background was exposed for 2 min, a period sufficient to attain a "steady state" as determined by response stability. In general, the effect of background illumination was to shift the V-log I curve to the right on the scale of intensities, and to reduce the voltage that could be elicited by a test stimulus. From the family of curves generated in this fashion, it was possible to read off the stimulus intensity required to yield a criterion response at each level of I_b. Essentially the same protocol was followed in determining the increment threshold functions for other components of the ERG studied.

SLOW PIII Fig. 5 shows the increment threshold data for slow PIII obtained in 12 separate experiments; the values of ΔI are plotted with respect

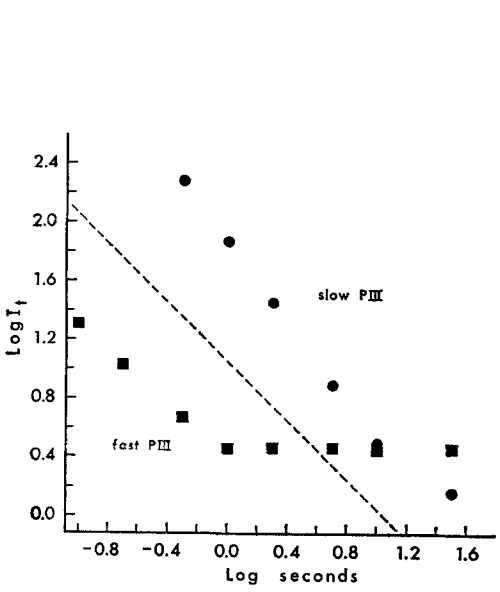

FIGURE 4

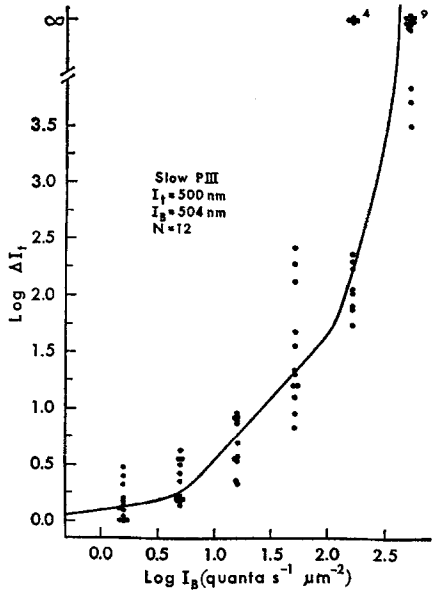

FIGURE 5

FIGURE 4. The intensity-time relation of fast PIII (squares) and slow PIII (circles) voltages. The scale of ordinates indicates the intensity needed to generate a criterion (0.2 V_{max}) response as a function of stimulus duration (scale of abscissae). The dashed line of slope −1 indicates perfect reciprocity (Bunsen-Roscoe law).

FIGURE 5. The increment-threshold function of the slow PIII response for 30-s stimuli. The data points represent the stimulus intensities to generate 0.2 V_{max} in 12 separate experimental runs. The incremental thresholds (ΔI_t) obtained in the presence of steady background fields (I_b) are given relative to the dark-adapted threshold set arbitrarily at log ΔI_t = 0.0. An arbitrary line is drawn through the data to suggest the similarity with the data of Aguilar and Stiles (1954). Note that at the two brightest background intensities, the incremental threshold was driven towards infinity, i.e. no response could be elicited with stimuli 5.5 log units above absolute threshold. See text for further details.

to dark-adapted threshold, arbitrarily set at log $\Delta I_t = 0$. The results resemble in several important respects those obtained by Aguilar and Stiles (1954) for the rod mechanism of human vision, although the range of background intensities over which ΔI varies is appreciably smaller. Nevertheless, there is an initial section wherein thresholds are only slightly elevated above the absolute level, a region where thresholds increase with approximately unit slope, and a section that rises steeply as ΔI approaches infinity (i.e., saturation). Missing, however, is any evidence of the transition to a second, non-saturating mechanism seen in human vision. With the brighter backgrounds (log I_b = 1.5–2.5), the scatter in the data is very pronounced, since some preparations reached saturation with weaker backgrounds than others. In none, however, was it possible to detect an incremental response when I_b exceeded 2.5 log units. A computation similar to that described earlier reveals that for the intensities at which saturation first occurs, quanta are absorbed at the rate of 170–460 per rod per second. Interestingly, the human rod mechanism begins to saturate when each rod absorbs about 220 quanta per second (Aguilar and Stiles, 1954).

The previous results suggest that rods alone contribute to the slow PIII response in both the dark- and light-adapted preparations. But a more definitive means by which to identify the photochemical mechanisms subserving a retinal response is to determine its action spectrum. The data of Fig. 6 show the results of these determinations for three conditions of adaptation: (*a*) in complete darkness, (*b*) during exposure to a background field only 0.5 log unit weaker than that which produced response saturation, and (*c*) after bleaching away ~15% of the rod photopigment, a maneuver that was shown previously to eliminate the rod contribution to the receptor potential of carp (Witkovsky et al., 1973). The continuous curve which fits so well the data for conditions *a* and *b* is the absorption spectrum of carp porphyropsin corrected for its *in situ* density (cf. Bridges, 1967). There seems little doubt, therefore, that the whole of the increment threshold function of Fig. 5 reflects the behavior of carp rods. However, the results obtained for condition *c* show that suppression of the rod response unmasks a small cone-mediated contribution to slow PIII, for these data match almost exactly the photopic spectral sensitivity curve of the fast PIII response obtained after a comparable form of light adaptation (Witkovsky et al., 1973).

A further question with regard to the increment threshold function concerns the mechanism responsible for producing saturation of slow PIII; i.e., is the sharp increase in ΔI due to quantal absorption by rods or by cones? To answer this, we repeated the ΔI measurements for a 500-nm test field superimposed in turn upon scotopically equated backgrounds of green (504 nm) and orange (594 nm) light. The results in each case were identical, i.e., the two incremental threshold functions were superimposed across the entire

range of intensities over which measurements could be obtained. Thus, all of the rise in PIII threshold is the result of quantal capture by rods.

THE *c*-WAVE Our analysis of slow PIII has shown that it reflects almost exclusively the activity of the rod receptors. Since a similar observation has been made in connection with *c*-wave responses in the cat (Steinberg, 1971; Schmidt and Steinberg, 1971), it seemed appropriate to compare these two potentials in the carp retina. We find that in regard to its spectral sensitivity (Fig. 7) and increment threshold (Fig. 8) functions, the behavior of the *c*-wave parallels almost exactly that of slow PIII. The *c*-wave is, in fact, even less responsive to cone-mediated activity, since it could not be recorded from light-adapted preparations. Thus, two extrareceptoral, non-neuronal potentials appear to arise as a direct consequence of photically induced rod activity; one is known to originate in the pigment epithelium, the other is most likely of glial origin.

FAST PIII The fast PIII voltage is presumably the receptoral component of the PIII complex (Sillman et al., 1969; Witkovsky et al., 1973). The incremental thresholds for fast PIII were studied previously (Witkovsky et al., 1973), and the squares of Fig. 8 confirm the earlier results; ΔI is at first slightly affected by I_b and then increases as a linear function of I_b for the range of intensities tested. Note that with weak backgrounds the data follow the same

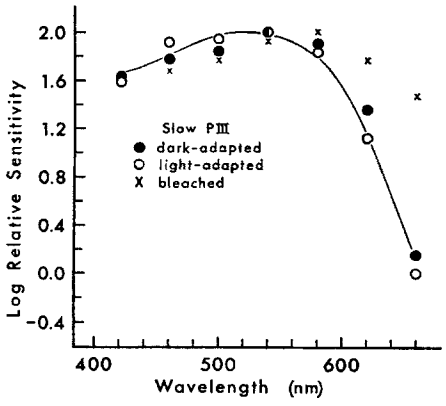

FIGURE 6

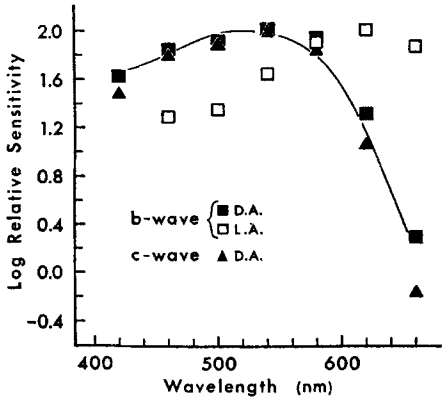

FIGURE 7

FIGURE 6. The spectral sensitivity of slow PIII under three conditions of adaptation in relation to the *in situ* absorption spectrum of carp porphyropsin (continuous line). A criterion voltage of 0.5 V_{max} was selected to generate the curves.

FIGURE 7. The spectral sensitivity of the *b*-wave and *c*-wave components of the ERG to 30-s stimuli; criterion voltages were 0.5 V_{max}. Light-adapted measurements were obtained on a white background field sufficiently intense to saturate the rod mechanism; the *c*-wave could not be elicited from the light-adapted preparation. The continuous line gives the *in situ* absorption spectrum of carp porphyropsin.

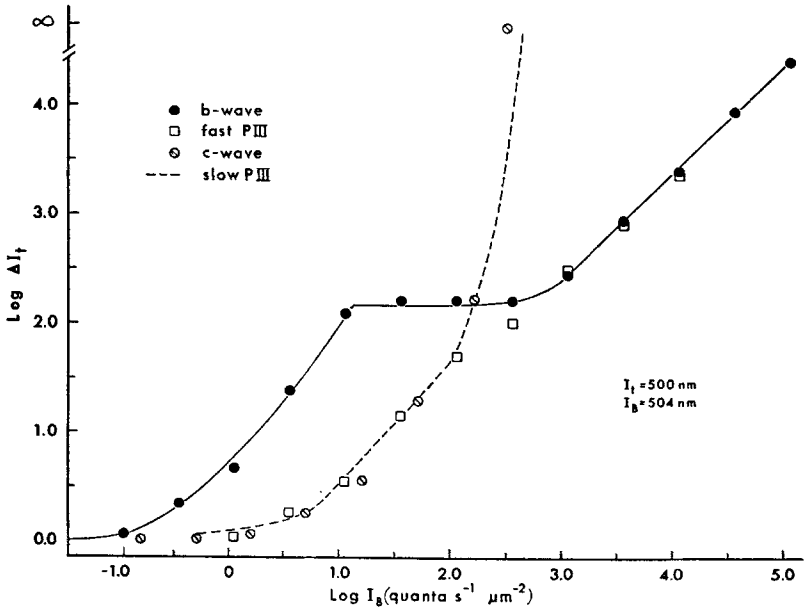

FIGURE 8. The incremental-threshold functions of various components of the carp ERG. The dashed line curve for slow PIII was taken from Fig. 5. Note that the lower portions of the increment threshold functions of fast PIII, slow PIII, and *c*-wave coincide in spite of differences in their absolute thresholds of up to 1.0 log unit. Further details in text.

course as for the *c*-wave and slow PIII, but when the latter begin the steep climb to saturation, the thresholds for fast PIII continue along the Weber line ($\Delta I/I$ = constant). The spectral sensitivity data of the former study showed that, although the transition is practically imperceptible, the photopic mechanism subserves threshold when the incident intensity I_b exceeds 300 quanta/s/μm^2.

THE *b*-WAVE Another interesting facet of our component analysis is seen in the data for the ERG *b*-wave. Although it is well known that in the carp (as in other species having rods and cones) the *b*-wave reflects both scotopic and photopic mechanisms (Witkovsky, 1968), separation of the two in the increment threshold function is surprisingly complete. The *b*-wave data of Fig. 8 (filled circles) fall upon a distinctly biphasic curve, consisting of a low intensity rod branch, and a high intensity cone branch. The shape of the function is, in fact, very similar to that obtained by Stiles (1949) for human peripheral vision. In addition, Stiles established by spectral measurements that for all values of I_b to the right of the rod-cone kink, the more sensitive cone mechanisms determine threshold. An analogous situation obtains for the *b*-wave spectral sensitivity of the carp retina (Fig. 7). Between

dark- and light-adapted preparations there is a marked shift in λ_{max} from 540 to ~620 nm; in carp these wavelengths approximate closely the spectral peaks of the rod and the red-sensitive cone photopigments, respectively (Marks, 1965; Tomita, 1965; Witkovsky et al., 1973).

In addition to the foregoing, comparing the increment threshold functions for the various ERG components reveals several noteworthy features. In Fig. 8 the values of ΔI measured upon background fields I_b are plotted for each component as the change in threshold above the dark-adapted value for that component. Thus, the fact that the results for fast- and slow-PIII and the *c*-wave fall upon the same curve for values of log $I_b \leq 2.1$ suggests that the sensitivities of these components are governed by the same process, namely the effect of I_b on the sensitivity of the photoreceptors.

For the *b*-wave, on the other hand, threshold is raised at levels of I_b about 1 log unit below that which induces a change in any of the other components. This finding clearly points to a stage of adaptation proximal to the receptors for the rod-mediated segment of the increment-threshold function. Although the rise in *b*-wave thresholds parallels that occurring in the PIII complex, the former do not saturate. Instead, the photopic mechanism comes into play, and an entirely new increment-threshold function is generated. Initially there is an extensive range of field intensities over which incremental thresholds remain constant, but when I_b exceeds 300 quanta/s/μm^2, the *b*-wave threshold rises along the Weber line precisely in register with the values obtained for the cone-mediated fast PIII. Thus in the photopic range, the data suggest that all of the rise in *b*-wave threshold effected by the background field can be accounted for by desensitization of the cones.

DISCUSSION

Relation of Slow PIII to Rod Activity

Our results indicate that in the carp retina, the slow PIII response depends almost exclusively on quantal absorption by rods. Both the dark- and the light-adapted spectral response curves of slow PIII match closely the *in situ* absorption spectrum of the rod photopigment (Witkovsky et al., 1973). In addition, the slow PIII response in carp retina and the rod-dependent *c*-wave of the cat retina (Schmidt and Steinberg, 1971) generate *V*-log *I* curves which are well fit by the hyperbolic tangent function (Naka and Rushton, 1966), which also describes the voltage-intensity function of individual rods (Grabowski et al., 1972; Kleinschmidt, 1973). These findings suggest that both slow PIII and *c*-wave are connected functionally to a single receptor mechanism (rod) in spite of the presence of cones in both carp and cat retinas. It is noteworthy that the rod-connected horizontal cell of the fish retina (Stell, 1967) behaves comparably (Kaneko and Yamada, 1972). The

spectral sensitivity of this cell matched the rod pigment curve when the retina was either dark- or moderately light-adapted. However, with strong steady backgrounds, the incremental threshold reached saturation and there was no evidence of a cone-mediated response.

There are further indications that slow PIII reflects rod function. For the slow PIII (or the *c*-wave) of carp retina, the increment threshold function begins to saturate when each rod absorbs quanta at the rate of 170–460/s. This agrees well with the value of 220 quanta absorbed rod^{-1} s^{-1} obtained from psychophysical determinations of saturation of the rod system (Aguilar and Stiles, 1954) and with analogous measures upon single neurons in the cat lateral geniculate nucleus (Daw and Pearlman, 1971). The mechanism of rod saturation is still poorly understood. In his study of the *c*-wave Steinberg (1971) found that the effect of a steady background was to *compress* the response range to superimposed stimuli between the DC level imposed by the background and V_{max}. And in the present work, we observed that the intensity of the stimulus which elicits a maximal slow PIII voltage (cf. Fig. 2) is the same as that which saturates the response when applied as a steady background (cf. Fig. 5). Thus, rod saturation at the receptoral level in carp may be due simply to the imposition of a steady hyperpolarization equal to V_{max}, which precludes any incremental response.

Adaptive Properties of other ERG Components

Unlike the slow PIII response and the *c*-wave, the spectral curves of the carp ERG *b*-wave indicate that it is subserved by the rod system under scotopic conditions and by the cone system in the photopic state (Witkovsky, 1968; this report). In addition, the *b*-wave increment threshold function has well-defined rod and cone branches, analogous to those obtained by Stiles (1949) in the human peripheral retina.

A significant feature of the rod branch of the *b*-wave incremental threshold in carp retina is its sensitivity to background illumination. As shown in Fig. 8, the rise in *b*-wave threshold exceeds that of any other ERG component. In this regard, it is of particular interest to compare the relative changes of *b*-wave and the fast PIII potential. Since fast PIII is presumed to reflect the behavior of rod receptors, the difference suggests that there is an additional stage of adaptation located between the sites of generation of photoreceptor and *b*-wave voltages. A similar conclusion was reached by Brown and Watanabe (1965) based on results obtained in the cynomolgus monkey.

In the mesopic range of background intensities, ΔI remains constant for the *b*-wave while it increases sharply for the rods. Here, presumably, the cone system which triggers the *b*-wave generator is insensitive to background intensities in the range $\log I_b = 1.2 - 2.5$. With photopic backgrounds, both *b*-wave and cone responses rise along the Weber line, giving no evidence

of saturation over the range of intensities tested. Thus in the light-adapted state, all the apparent desensitization produced by the background field seems to occur at the receptoral level. A similar phenomenon is observed in the all-cone retina of the ground squirrel (Green and Dowling, 1975).

Origin of the Slow PIII Response

The very slow time-course of the slow PIII response, its ability to integrate temporally the effects of quantal absorption over many seconds, and its resistance to the effects of Na Asp point to a non-neuronal origin for this potential. Only two non-neuronal elements are present in the retina: the pigment epithelium and the Müller fiber. Since our data were obtained from retinas devoid of epithelium, the obvious candidate for the retinal locus of slow PIII is the Müller cell, but confirmation by intracellular recording is still lacking. However, Faber (1969) performed a voltage profile analysis of the components of the rabbit ERG in which he determined that the source of slow PIII is located in the outer plexiform layer whereas its sink is distributed throughout the remaining retina. He concluded that slow PIII must arise in the Müller fiber, since only that cell had the appropriate spatial distribution. The results of our previous study utilizing differential depth recording (Witkovsky et al., 1973) also demonstrated that the bulk of the slow PIII voltage was generated between the terminals of the photoreceptors and the vitreal surface of the retina. This finding and all the data of the present report are consistent with Faber's conclusions as regards the origins of slow PIII.

Ionic Basis of the Slow PIII Response

An interesting general property of glial cell membranes is their great sensitivity to changes in extracellular potassium concentrations (Kuffler et al. 1966; Baylor and Nicholls, 1969; Ransom and Goldring, 1973). It is found that K^+ efflux follows neuronal spike firing and transiently increases extracellular $[K^+]$ (Keynes and Ritchie, 1965). This change presumably depolarizes the glial cell membrane (cf. Kuffler, 1967). But if slow PIII is a glial potential, the cornea-negative polarity of the transretinal recording indicates a *hyperpolarization* of the Müller cell membrane. While there is no direct evidence linking such a change with the movement of K^+ ions, studies of the ionic basis of photoreceptor potentials show that the dark voltage across the receptor membrane is dependent on both sodium and potassium currents (Penn and Hagins, 1969; Sillman et al., 1969; Korenbrot and Cone, 1972). Furthermore, it has been hypothesized that there is in the dark-adapted retina an outward K^+ current from the inner regions of the photoreceptor which is attenuated by light. Zuckerman (1973) has proposed that this change may be responsible for the generation of the slow PIII voltage.

However, in view of the untested assumptions involved, further work is needed to establish the relation between potassium fluxes and Müller fiber potentials (Miller, 1973; Winkler, 1973).

The Müller Fiber as Possible Source of Both Slow PIII and b-Wave Components of the ERG

Miller and Dowling (1970) recorded intracellularly from a cell subsequently identified by dye injection as the Müller fiber, and from which they obtained light-induced waveforms similar in time-course and sensitivity to the *b*-wave of the ERG. But if both slow PIII and the *b*-wave have a common source, they undoubtedly are triggered by activity of different classes of retinal neurons. The slow PIII component is initiated directly by rod activity, whereas many lines of evidence indicate that the *b*-wave is elicited by responses of neurons situated postsynaptic to the receptor. For example, Tomita et al. (1960) localized the *b*-wave to the inner nuclear layer through intraretinal microelectrode recording. Brown caused the disappearance of the *b*-wave through interruption of the retinal circulation that nourishes the inner retinal layers, while the receptoral component of the ERG remained (reviewed in Brown, 1968). The same result obtains after application of Na Asp to the retina, and as already discussed, the adaptational properties of the *b*-wave are clearly distinct from those of the photoreceptor layer. Also of importance is the fact that the *b*-wave and slow PIII, being of opposite polarity, must interact, and therefore some of the response characteristics attributed to the *b*-wave may be a consequence of this interaction.

In view of the large amplitude of slow PIII and its obvious presence in many ERG recordings in the literature, e.g. Sillman et al. (1969) and Miller and Dowling (1970), it is surprising that this component has received so little attention. The fact that the ERG is a polyphasic waveform comprising components of different time-course and polarity was appreciated very early in the century (Piper, 1911). Granit and co-workers succeeded in fractionating the ERG by the use of chemical agents and anoxia and one may observe that in his analysis both fast and slow PIII's are combined in a single PIII component (Granit, 1947). More recent analyses based principally upon intraretinal microelectrode recordings, still fail to discriminate clearly fast and slow PIII waveforms, e.g. Bornschein et al., 1966; Brown, 1968; Rodieck, 1972).

The preceding discussion documents the striking fact that the greatest proportion of the ERG voltage (slow PIII, *b*-wave, and *c*-wave) derives from non-neuronal sources. Glial cells play a comparable role in the production of the evoked potential recorded from the surface of the amphibian brain (Cohen, 1970). In consideration of the evident importance of glial cell potentials in ERG generation, and the utility of the ERG both as a measure of

retinal function and in the diagnosis of retinal dysfunction, further studies of glial cell potentials in the retina hold promise for an increased understanding of the electroretinogram.

We thank Mr. H. Rosskothen for construction of equipment, Miss J. Zakevicius for preparing the drawings, and Drs. G. B. Arden and R. H. Steinberg for helpful criticism.
Supported by grants EY01063 to Dr. Witkovsky and EY00285 to Dr. Ripps.

Received for publication 25 May 1974.

REFERENCES

AGUILAR, M., and W. S. STILES. 1954. Saturation of the rod mechanism of the retina at high levels of stimulation. *Opt. Acta.* **1**:59.

AMES, A., III, and D. A. POLLEN. 1969. Neurotransmission in central nervous tissue: a study of isolated rabbit retina. *J. Neurophysiol.* **32**:424.

BAYLOR, D. A., M. G. F. FUORTES, and P. M. O'BRYAN. 1971. Receptive fields of cones in the retina of the turtle. *J. Physiol.* (*Lond.*). **214**:265.

BAYLOR, D. A., and A. L. HODGKIN. 1973. Detection and resolution of visual stimuli by turtle photoreceptors. *J. Physiol.* (*Lond.*). **234**:163.

BAYLOR, D. A., and J. G. NICHOLLS. 1969. After-effects of nerve impulses on signalling in the central nervous system of the leech. *J. Physiol.* (*Lond.*). **203**:571.

BORNSCHEIN, H., R. HANITZSCH, and A. LÜTZOW. 1966. Off-effekt und negative ERG-Komponente des enukleierten Bulbus und der isolierten Retina des Kaninchens. I. Einfluss der Reizparameter. *Vision Res.* **6**:251.

BRIDGES, C. D. B. 1967. Spectroscopic properties of porphyropsin. *Vision Res.* **7**:349.

BROWN, K. T. 1968. The electroretinogram: its components and their origins. *Vision Res.* **8**:633.

BROWN, K. T., AND K. WATANABE. 1965. Neural stage of adaptation between the receptors and inner nuclear layer of monkey retina. *Science* (*Wash. D. C.*). **148**:1113.

COHEN, M. W. 1970. The contribution by glial cells to surface recordings from the optic nerve of an amphibian. *J. Physiol.* (*Lond.*). **210**:565.

DAW, N. W., and A. L. PEARLMAN. 1971. Rod saturation in the cat. *Vision Res.* **11**:1361.

DOWLING, J. E., and H. RIPPS. 1970. Visual adaptation in the retina of the skate. *J. Gen. Physiol.* **56**:491.

DOWLING, J. E., and H. RIPPS. 1971. Aspartate isolation of receptor potentials in the skate retina. *Biol. Bull.* (*Woods Hole*). **141**:384.

FABER, D. S. 1969. Analysis of the slow trans-retinal potentials in response to light. Ph.D. Thesis. State University of New York. Buffalo, N. Y.

FAIN, G. L., and J. E. DOWLING. 1973. Intracellular recordings from single rods and cones in the mudpuppy retina. *Science* (*Wash. D. C.*). **180**:1178.

GRABOWSKI, S. R., L. H. PINTO, and W. L. PAK. 1972. Adaptation in retinal rods of Axolotl: Intracellular recordings. *Science* (*Wash. D. C.*). **176**:1240.

GRANIT, R. 1933. The components of the retinal action potential in mammals and their relation to the discharge in the optic nerve. *J. Physiol.* (*Lond.*). **77**:207.

GRANIT, R. 1947. Sensory Mechanisms of the Retina. Oxford University Press, London.

GREEN, D. G., and J. E. DOWLING. 1975. Electrophysiological evidence for rod-like receptors in the gray squirrel, ground squirrel and prairie dog retinas. *J. Comp. Neurol.* In press.

KANEKO, A., and M. YAMADA. 1972. S-potentials in the dark-adapted retina of the carp. *J. Physiol.* (*Lond.*). **227**:261.

KEYNES, R. D., and J. M. RITCHIE. 1965. The movement of labelled ions in mammalian non-myelinated nerve fibres. *J. Physiol.* (*Lond.*). **179**:333.

KLEINSCHMIDT, J. 1973. Adaptation properties of intracellularly recorded Gekko photore-

ceptor potentials. *In* Biochemistry and Physiology of Visual Pigments. H. Langer, editor. Springer-Verlag New York Inc., New York. 219–224.

KORENBROT, J. I., and R. CONE. 1972. Dark ionic flux and the effects of light in isolated rod outer segments. *J. Gen. Physiol.* **60**:20.

KUFFLER, S. W. 1967. Neuroglial cells: Physiological properties and a potassium mediated effect of neuronal activity on the glial membrane potential. *Proc. R. Soc. Lond. B Biol. Sci.* **168**:1.

KUFFLER, S. W., J. G. NICHOLLS, and R. K. ORKAND. 1966. Physiological properties of glial cells in the central nervous system of amphibia. *J. Neurophysiol.* **29**:768.

MARKS, W. B. 1965. Visual pigments of single goldfish cones. *J. Physiol. (Lond.)*. **178**:14.

MILLER, R. F. 1973. Role of K^+ in generation of *b*-wave of electroretinogram. *J. Neurophysiol.* **36**:28.

MILLER, R. F., and J. E. DOWLING. 1970. Intracellular responses of the Müller (glial) cells of mudpuppy retina: their relation to *b*-wave of the electroretinogram. *J. Neurophysiol.* **33**:323.

NAKA, K. I., and W. A. H. RUSHTON. 1966. *S*-potentials from colour units in the retina of fish (*Cyprinidae*). *J. Physiol. (Lond.)*. **185**:536.

NOELL, W. K. 1953. Studies on the electrophysiological and the metabolism of the retina. U.S.A.F. School Aviation Med. Proj. No. 21-1201-0004, Report no. 1.

PENN, R. D., and W. A. HAGINS. 1969. Signal transmission along retinal rods and the origin of the electroretinographic *a*-wave. *Nature (Lond.)*. **223**:201.

PIPER, H. 1911. Über die Netzhautströme. *Arch. Anat. Physiol. Abt.* 85–132.

RANSOM, B. R., and S. GOLDRING. 1973. Ionic determinants of membrane potentials presumed to be glia in cerebral cortex of cat. *J. Neurophysiol.* **36**:855.

RODIECK, R. W. 1972. Components of the electroretinogram—a reappraisal. *Vision Res.* **12**:773.

SCHMIDT, R., and R. H. STEINBERG. 1971. Rod-dependent intracellular responses to light from the pigment epithelium of the cat retina. *J. Physiol. (Lond.)*. **217**:71.

SILLMAN, A. J., H. ITO, and T. TOMITA. 1969. Studies on the mass receptor potential of the isolated frog retina. I. General properties of the response. *Vision Res.* **9**:1435.

STEINBERG, R. H. 1971. Incremental responses to light recorded from pigment epithelial cells and horizontal cells of the cat retina. *J. Physiol. (Lond.)*. **217**:93.

STEINBERG, R. H., R. SCHMIDT, and K. T. BROWN. 1970. Intracellular responses to light from the cat's pigment epithelium: origin of the *c*-wave of the electroretinogram. *Nature (Lond.)*. **227**:728.

STELL, W. K. 1967. The structure and relationship of horizontal cells and photoreceptor-bipolar synaptic complexes in goldfish retina. *Am. J. Anat.* **121**:401.

STILES, W. S. 1949. Increment thresholds and the mechanisms of colour vision. *Docum. Ophthalmol.* **3**:138.

TOMITA, T. 1965. Electrophysiological study of the mechanisms subserving color coding in the fish retina. *Cold Spring Harbor Symp. Quant. Biol.* **30**:559.

TOMITA, T., M. MURAKAMI, and Y. HASHIMOTO. 1960. On the *R* membrane in the frog's eye: its localization and relation to the retinal action potential. *J. Gen. Physiol.* **43**(6, Pt. 2):81.

WINKLER, B. S. 1973. Dependence of fast components of the electroretinogram of the isolated rat retina on the ionic environment. *Vision Res.* **13**:457.

WITKOVSKY, P. 1965. The spectral sensitivity of retinal ganglion cells in the carp. *Vision Res.* **5**:603.

WITKOVSKY, P. 1968. The effect of chromatic adaptation on color sensitivity of the carp electroretinogram. *Vision Res.* **8**:823.

WITKOVSKY, P., J. NELSON, and H. RIPPS. 1973. Action spectra and adaptation properties of carp photoreceptors. *J. Gen. Physiol.* **61**:401.

ZUCKERMAN, R. 1973. Ionic analysis of photoreceptor membrane currents. *J. Physiol. (Lond.)*. **235**:333.