ROD LATE RECEPTOR POTENTIAL AND RHODOPSIN CONCENTRATION OF AN ISOLATED FROG RETINA

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Abstract The relation of the threshold of the late receptor potential (late RP) and ERG b-wave with the rhodopsin concentration in the rod outer segments of an isolated bullfrog retina were studied by simultaneous recordings of electrical responses and spectral change. The curve indicating log threshold rise of the late RP with the decrease of rhodopsin showed two phases separated by a kink, which was found at 50% rhodopsin concentration; the b-wave threshold, however, was linearly related to rhodopsin concentration. From the intensity response curves (IR curves) at various rhodopsin concentrations, the amplitude decrease of both waves following bleaching light adaptation was compared and the mode of the conversion from the late RP to the b-wave was discussed.

There have been several attempts to reveal the relation of rhodopsin concentration in the retina to retinal photosensitivity and function. To examine retinal sensitivity, the measurement of electroretinogram (ERG) threshold has been ordinarily used by several workers, for instance, in rat (WEINSTEIN *et al.*, 1967), in frog (BAUMANN, 1967) and in skate (DOWLING and RIPPES, 1970). All such studies showed that the logarithm of the scotopic b-wave threshold is linearly related to the rhodopsin concentration in the retina. From their results, however, it is difficult to obtain an accurate estimation of the relationship between rhodopsin concentration and visual cell sensitivity because the amplitude of the b-wave is influenced by other cells having synaptical or lateral interactions, and moreover it is still uncertain which is the original cell eliciting the b-wave for frog retina, although MILLER (1973) has recently indicated the glial Müller cell to be responsible.

In the present study, the amplitude or threshold of the late receptor potential (late RP) of the isolated frog retina was compared with the rhodopsin concentration in the same retina by simultaneous recordings of electrical response and spectral change. To separate the late RP from the ERG, sodium aspartate was

Received for publication August 22, 1974

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used, which is known as an excellent agent in isolating the late RP by virtue of its suppressive action upon the b-wave (FURUKAWA and HANAWA, 1955; SILLMAN *et al.*, 1969a, b; HANAWA and TATEISHI, 1970; DOWLING and RIPPS, 1972). Present studies showed that the b-wave activity does not provide a valid index of visual cell function.

METHODS AND MATERIALS

The dark-adapted bullfrog (*Rana catesbiana*) retina, whose pigment epithelium was removed during isolation, was sandwiched between two acryl resin compartments each having an opening (6 mm in diameter) in the middle of their separating planes in the same manner as described previously (HANAWA *et al.*, 1967). The area of the exposed retinal surface of 28.3 mm² was immersed in Conway solution for the ERG, or in Conway solution containing 5 mM D-sodium aspartate for the late RP which was bubbled with 98% O₂ and 2% CO₂ gas mixture throughout the course of the experiment. The pH of each solution was maintained at 7.8 and the experiment was carried out at room temperature (22–24°C).

The acryl resin chambers, with one side was replaced with glass slide to allow a spectrophotometric beam to pass through each chamber and the retina, was set in the dark sample compartment of a spectrophotometer (Shimazu, MPS-50L). The chamber with the retina was kept in the dark sample compartment for at least 1 hr and the absorbance measurement of the retina was performed at a wavelength of 350–650 nm.

To record the transretinal electrical response, two saturated KCl-calomel electrodes were set inside the sample compartment, each of them connected to the solution in each compartment of the chamber through a narrow agar bridge (Fig. 1). The KCl-calomel electrodes were then connected to a chopper-type direct writing DC amplifier or an oscilloscope with RC coupled pre-amplifier (time constant, 2.0 sec).

To stimulate the retina, the flashing light from a xenon lamp (20 joule) was guided by a fiberoptics tube from outside into the sample compartment, and its intensity was varied by inserting neutral filters in the light path. The log relative intensity of the light necessary to evoke 20 μ V response of either late RP or b-wave was determined as the threshold, and the response amplitude as a function of stimulus intensity was successively recorded. For the purpose of partial bleach or complete bleach of rhodopsin, a 150W white tungsten lamp was used instead of the xenon flash lamp. The retina was illuminated by these lights in the physiological direction.

RESULTS

The rod late RP threshold and rhodopsin concentration

Oscilloscope recordings of either ERG or aspartate-isolated late RP from

Fig. 1. Simultaneous recording procedure for the retinal electrical response and spectral change. C, acryl resin compartment; P, isolated preparation; O, oxygen bubbling; A, agar bridge; E, calomel electrode; K, saturated KCl solution; SAM, sample compartment of spectrophotometer. The experimental chamber with two sides of glass to allow the recording beam of the spectrophotometer to pass through, was set in SAM.

different retinas are shown in Fig. 2. The intensity 0 was determined from the fully bleached retina at the end of each experiment. The fully bleached retina contained no rhodopsin in the rods, since the pigment epithelium was removed during the isolation of the retina and therefore the isolated frog retina lost the ability to regenerate rhodopsin at a physiologically significant rate under these experimental conditions. In reality, the frog cone visual pigment can be regenerated completely without the pigment epithelium within a few minutes (TAYLOR, 1969) or within 10 to 15 min (GOLDSTEIN, 1967, 1970) at room temperature. Hence, at a fully bleached stage, the late RP, when elicited, arose only from the cones (cone late RP); and in this series of experiments, the light intensity necessary to evoke $20 \mu\text{V}$ criterion response at the fully bleached state was standardized as 0.

Fig. 2. Oscilloscope recordings of the ERG and late RP from different retinas (time constant 2.0 sec). Both retinas were at partially bleached stage (78.8% rhodopsin in the case of ERG; 41.0% in the case of late RP). Light intensity was expressed as log relative intensity. 20 μ V amplitude of both waves was regarded as the criterion response for the threshold.

The amplitude of either the ERG b-wave or the late RP increases as a function of stimulus intensity. As for the amplitudes of both waves, the distances from the negative to the positive peak for the b-wave and from the base line to the negative peak for the late RP were measured. It has been known that both the rise and fall of the rod late RP within the scotopic range occur slowly and smoothly, and the rise and decay of the cone late RP is much faster (BROWN *et al.*, 1965; TOYODA *et al.*, 1970; WHITTEN and BROWN, 1973; HANAWA and MATSUURA, 1975). It was also obvious in the present experiment that the rapid cone phase was superimposed on the slow rod phase when evoked by an intense light stimulus (+3.5).

In order to examine the electrical changes in a retina following rhodopsin decomposition, the thresholds and intensity response curves (IR curves) of both waves were recorded at various rhodopsin concentrations.

A typical experimental result for the late RP threshold changes with the changes of rhodopsin amount is shown in Fig. 3. Among all the data for the late RP, only the data obtained by DC recording were used, since the rod late RP has a prolonged time course.

The spectrum of the retina, which was kept for at least 1 hr in the dark sample compartment of the spectrophotometer to attain equilibrium, was first recorded (Fig. 3, left: A). After having recorded the threshold and IR curve of the retina with this spectrum A, the retina was then exposed to a 150 W white tungsten lamp for 7 sec to decompose a partial amount of rhodopsin. With this exposure, the rhodopsin photoproducts (metarhodopsin II, metarhodopsin III, and so on) were formed and decayed. Since this fading of the photoproducts continues for 30 to

Fig. 3. Left: Absorption spectra of isolated frog retina. A, dark-adapted stage; 1-7, partially bleached stages; B, fully bleached stage. 150 W white tungsten lamp was used for the aim of partial or full bleach of rhodopsin. Rhodopsin amount at each stage was calculated as difference absorbance from each spectrum to the spectrum B at 500 nm. Right: Relation of the late RP threshold with rhodopsin content (%). Threshold change was expressed as I/I_0 (I_0 , log threshold at dark-adapted stage; I , log threshold at bleached stage).

50 min in the dark after illumination (HANAWA and MATSUURA, 1975), the second spectrum recording was performed after 1 hr from the cessation of the exposure (Fig. 3, left: 1). This was to keep these photoproducts from directly disturbing the spectrum itself or to desensitize the electrical activity of the retina (DONNER and REUTER, 1967, 1968; BAUMANN and SCHEIBNER, 1968; ERNST and KEMP, 1972; HANAWA and MATSUURA, 1975). The threshold was determined and the IR curve was recorded again at this stage. Next partial bleach was performed by white lamp exposure for 10 sec. The longer exposure was necessary for a lesser rhodopsin content in the retina, to maintain the almost same bleached amount of rhodopsin at every stage. In this case, the exposure duration for the stepwise bleaching was 15 sec from spectrum 2 to spectrum 3, 20 sec from 3 to 4, 25 sec from 4 to 5, 35 sec from 5 to 6, and 60 sec from 6 to 7. The spectrum marked B was obtained by 30-min illumination of the retina from spectrum 7 by white lamp. Since rhodopsin can not regenerate significantly without the pigment epithelium, it can be assumed that retina having spectrum B contains no rhodopsin. The rhodopsin amount was calculated as the difference absorbance from each spectrum to spectrum B at 500 nm. The rhodopsin amount at the fully dark-adapted stage of the retina (A-B) was 0.574; at the next stage (1-B), it was 0.508, with 88.5% rhodopsin still remaining. At subsequent stages, the rhodopsin amounts were 0.437 (76.1%), 0.344 (59.9%), 0.225 (39.2%), 0.135 (23.5%), 0.075 (13.1%), and 0.030 (5.2%), respectively. The total absorbance change from the fully dark-

adapted to fully bleached retina was 0.5–0.9 in a good preparation.

The threshold measurements for the late RP were performed at each stage of rhodopsin content. The intensity of the light stimulus to elicit the criterion response was determined as the threshold value after having waited for 1 or 2 min after each spectrum recording. This was to eliminate the effect of the recording beam of the spectrophotometer, as this scanning from 350 to 650 nm also evokes a small late RP which decays within 30 sec. The light intensity varied in steps of 0.1 log unit near the criterion response to collect exact data for the threshold and the result was also confirmed by the extrapolation of the successively recorded IR curve.

The threshold of the late RP rose as the rhodopsin concentration decreased. The late RP at the fully dark-adapted stage (spectrum A) had its threshold at -6.45 log unit and at the next stage (spectrum 1), it was -4.5 . In the following stages, values were -3.9 , -3.4 , -3.05 , -2.7 , -2.2 , -1.3 , and 0, respectively. The range of the threshold in the span from 100 to 0% rhodopsin was 5.5–6.5 log unit throughout all experiments.

These data were plotted in Fig. 3 (right), to show the direct relation of the rod late RP threshold with rhodopsin concentration. As can be seen in the figure, the threshold is not completely linearly related to the amount of rhodopsin remaining in the retina, but the curve shows two phases separated by a kink, which is found at 50% rhodopsin concentration.

All other experimental data showed the same tendency, although generalization concerning all the data is difficult, since it was impossible to decompose rhodopsin at a constant or a fixed rate.

The late RP amplitude and rhodopsin concentration

The IR curves of the late RP at each stage of rhodopsin concentration were recorded after each threshold determination by increasing the light intensity in steps of 0.5 or 1.0 log unit up to the photopic level (Fig. 4, left). The successive light stimulus was given to the retina after the response by its preceding stimulus had decayed completely on DC recording. The marks A, B and numberings on the right of the IR curve respectively coincide with those in Fig. 3 (left), which shows the spectra of this retina, the values in parentheses indicating the percent rhodopsin concentration. Each IR curve was sigmoid in shape, although the saturation points were not determined. Following bleaching light adaptation, the curve shifted to the right.

From these IR curves at various light adaptation stages, the relation of the late RP amplitude with the rhodopsin concentration at a given intensity of the stimulus could be easily determined (Fig. 4, right). As indicated in this figure, it is clear that the mode of the amplitude changes following rhodopsin decomposition differs in terms of the stimulus intensity. Using a weaker light stimulus (e.g., -2.9), the late RP amplitude decreases in a hyperbolic way with the decrease of

Fig. 4. Left: IR curves of the late RP at various stages of rhodopsin concentration. Marks A, B and numberings on the right of IR curves respectively coincide with those in Fig. 3 (left) which shows the absorption spectra of this retina with values in parentheses indicating rhodopsin percent concentration. Right: Amplitude decrease of the late RP with the decrease of rhodopsin content. Values on the right indicate the log relative light intensities. It is clear that the mode of amplitude decrease differs in terms of the stimulus intensity.

rhodopsin; and as the stimulus becomes stronger, it tends to be linearly related (-1.9), turning into a parabolic decrease (-0.4).

The b-wave and rhodopsin concentration

Using the same experimental procedure as for the late RP, the threshold values and IR curves for the b-wave at various rhodopsin concentrations were recorded by oscilloscope with RC coupled pre-amplifier (time constant, 2.0 sec). The scotopic b-wave threshold is linearly related to the rhodopsin concentration (Fig. 5, left). These plots were obtained from four different retinas, since it was difficult to maintain the b-wave in a steady state for a long time. This linear relationship finding coincides with other authors' data on the relation of the b-wave threshold with rhodopsin concentration (WEINSTEIN *et al.*, 1967; BAUMANN, 1967; DOWLING and RIPPES, 1970).

The amplitude changes of the b-wave following rhodopsin decomposition are also shown in Fig. 5 (right), in the same manner as for the late RP. Although only a few points to a given flash intensity could be obtained, it can be said that the b-wave amplitude varies roughly in proportion to the amount of rhodopsin remaining in the retina.

Fig. 5. left: Relation of the b-wave threshold with rhodopsin content (%). I_0 , log threshold at dark-adapted stage; I , log threshold at bleached stage. Plots were obtained from four different retinas. right: b-wave amplitude decrease of a retina with the decrease of rhodopsin. Values on the right indicate the log relative light intensities.

DISCUSSION

The threshold of the late RP. The present study serves to demonstrate that the threshold of the late RP is not linearly related to the rhodopsin concentration in the rods. The curve indicating the log threshold rise following rhodopsin decomposition shows two phases separated by a kink, which is found at 50% rhodopsin content. There is a discrepancy between the present results and theoretical values which are simply predicted from the probability change of quantal absorption. The present results indicate that much more rhodopsin molecules than were calculated, were decomposed to evoke the threshold response.

In any case, the fact that findings for the b-wave threshold, obtained by the same experimental procedure employed for the late RP, coincide with other authors' data, excludes the possibility that the result for the late RP would be distorted by various experimental factors, including optical systems.

Recently, HOOD *et al.* (1973) showed in their study of aspartate-isolated frog late RP that the permanent loss in log sensitivity is roughly proportional to the percent of pigment bleached. But, they also pointed out the discrepancy between their results and theoretical values.

PENN and HAGINS (1969, 1972) described a steady electric current (the dark current) which flows in the interstitial space of the receptor layer of the retina and enters the rod outer segments in darkness. The same authors considered the late RP to be the result of the reduction of this dark current on illumination. They also proposed that this control of the current is likely to depend on control of plasma membrane permeability by an agent released from the discs. One possible explanation for the discrepancy between experimental and theoretical findings

involves this active agent accumulation released from the discs. In other words, if the efficiency of said accumulation decreases with the decrease of rhodopsin in the discs, the decomposition of much more rhodopsin molecules than were calculated would be necessary to evoke a constant amplitude of potential.

In fact, our previous report (HANAWA and MATSUURA, 1975) showed that the amplitude of the late RP in the time course of response decay is proportional to the log of the 380 nm absorbing photoproduct amount, and that decreasing rhodopsin content requires much more 380 nm absorbing product, namely the decomposition of much more rhodopsin molecules, to produce a definite amplitude of response. Other findings (HELLER *et al.*, 1970, 1971) have indicated that the disc membrane (not the enveloping plasma membrane) permeability changes on illumination in such a way that the passive ionic flux is enhanced. Hence, it is likely that the efficiency to reduce the dark current would be influenced by the amount of rhodopsin remaining in the discs.

Comparison of the amplitude changes of the late RP and b-wave following bleaching light adaptation. As shown in Fig. 4 (right), the late RP amplitude evoked by a weak light stimulus (-2.4 or -2.9 , for instance) decreases in a hyperbolic way with the decrease of rhodopsin content, and by a moderate light (-1.9), it has a linear relation; moreover, in the case of a stronger light (-0.4), it shows a parabolic decrease. On the other hand, it is supposed that the b-wave amplitude

Fig. 6. Rough comparison of the amplitude decrease of the late RP and b-wave following bleaching light adaptation. Both amplitudes were expressed as percent of response at dark-adapted stage and compared with each other at the same rhodopsin concentration. The curve differs in terms of the stimulus intensity. Dashed line; in the case of a weaker light stimulus (-2.4). Continuous line; in the case of a stronger light (-0.4).

evoked by any light intensity within the scotopic range has a linear relationship with rhodopsin content, although it was difficult to maintain the b-wave in a stable state for a long time to obtain sufficient corroborative data.

It is possible to compare the amplitude decrease of both waves following rhodopsin decomposition. Both amplitudes were expressed as percent of response at a fully dark-adapted stage and compared with each other at the same rhodopsin concentration (Fig. 6). The curve indicating the relation of both waves differs in terms of the stimulus intensity. When the retina is illuminated by a weaker light, the relation is shown as the dashed line in the figure which closely resembles that between the pre-synaptic and post-synaptic potential in the giant axon of the squid (KATZ and MILEDI, 1967). It is striking that the same correlation holds in the retina too (late RP; pre-synaptic, b-wave; post-synaptic). But, when the light stimulus is intense, the relation is quite the reverse (continuous line), suggesting some inhibitory effect on the conversion from the late RP to the b-wave.

Although this result is very suggestive of some synaptical interactions in the retina, it is impossible to discuss more in detail at present.

The author wishes to thank Prof. I. Hanawa for his advice and encouragement throughout the course of this work.

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