Effects of Ca\textsuperscript{2+} on the Decay of Rhodopsin Photoproducts and Photoreceptor Adaptation in the Isolated Bullfrog Retina

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Abstract

Relationships between the change in threshold of the fast PIII response and the rhodopsin photoproduct content were studied in the isolated bullfrog retina treated with Ba\textsuperscript{2+} and aspartate. A reduction in the extracellular Ca\textsuperscript{2+} concentration from 1.0 to 0.01 mM caused an increase in the decay rate of 380 nm absorbing photoproducts (metarhodopsin II and retinal) and metarhodopsin III. In both normal and low Ca\textsuperscript{2+} solutions, the threshold change after bleaching of about 50% of rhodopsin was closely related to the 380 nm absorbing photoproducts. The results suggest that the intermediate adaptation process of rods may be regulated by the 380 nm absorbing photoproducts.

Key words: bullfrog retina, adaptation of photoreceptor, rhodopsin photoproduct, Ca\textsuperscript{2+}.

Visual excitation involves photolysis of the visual pigment. On exposure to light, rhodopsin in the rod outer segments begins to degenerate into the final products (all-trans retinal and opsin), passing through several intermediates; i.e., bathorhodopsin, lumirhodopsin, metarhodopsin I, metarhodopsin II, and metarhodopsin III.

The decrease of sensitivity after an intense flash and subsequent partial recovery of sensitivity in the dark are common phenomena in vertebrate photoreceptors, even under conditions where practically no regeneration of rhodopsin occurs. The mechanism underlying photoreceptor adaptation remains unknown. The temporal course of dark adaptation is affected by the amount of bleached rhodopsin; the greater the amount, the slower the recovery of its sensitivity to light (Frank, 1971; Hood et al., 1973; Donner and Hemlå, 1979; Hanawa and Matsuura, 1980). Donner et al. (1979) have shown that in the aspartate-treated frog retina the threshold of PIII response after small bleaches of rhodopsin can
be described by \( \log \frac{I}{I_0} = a \times x \), where \( I_0 \) is the threshold intensity in the fully dark-adapted state, \( I \) is the threshold intensity at various time intervals after a bleach, \( x \) is the 380 nm intermediate concentration, and \( a \) is a constant. In agreement with the findings of Hood et al. (1973) and Hood and Hock (1975), Toba and Hanawa (1985) demonstrated that in the isolated bullfrog retina the increase in threshold of the fast PIII response after partial bleaching of rhodopsin was due to a loss in sensitivity of the red rods. Toba and Hanawa (1985) also indicated that changes in the extracellular Ca\(^{2+}\) concentration altered the adaptive state of the red rods.

A reduction in the extracellular Ca\(^{2+}\) concentration leads to a remarkable acceleration in the recovery of rod sensitivity after a flash bleaching of a substantial amount of rhodopsin (Lipton et al., 1977; Alban et al., 1980). Consequently, it would seem that if the rod sensitivity is influenced by rhodopsin intermediates, changes in the extracellular Ca\(^{2+}\) concentration should affect the decay rate of the intermediates.

The experiments described in this paper were designed to test the correlation between rod sensitivity and photochemical events in the rod. In the first section, we show that a reduction in the extracellular Ca\(^{2+}\) concentration leads to an increase of the decay rate of 380 nm absorbing photoproducts (metarhodopsin II and retinal) and metarhodopsin III. In the second section, we show that the threshold change of the fast PIII response in the dark after partial bleaching of rhodopsin is closely related to the decay of 380 nm absorbing photoproducts, even though the retina is immersed in a low Ca\(^{2+}\) solution.

**METHODS**

Throughout this study dark-adapted bullfrogs (Rana catesbeiana) were used. The techniques involved in mounting and stimulating the isolated retina were similar to those described previously (Hanawa and Matsuura, 1975). The absorption spectra were recorded over a wavelength range of 350–700 nm at a scanning speed of 5.0 nm/sec. The total absorbance change at 500 nm from the fully dark-adapted retina to fully bleached retina was 0.4–0.8. To observe the rhodopsin photoproduct decay, the dark-adapted retina was exposed to an intense light from a 500 W xenon arc lamp for 30 sec. A color filter (Fuji Film, SP-5) having a broad half-band width of about 170 nm with peak transmission at 490 nm was inserted in the light pathway. To stimulate the retina, a 500 nm interference filter was used instead of the color filter and the fast PIII responses to 500 nm light flashes of 0.1 sec in duration were recorded before and after a partial bleach of rhodopsin.

The solution of the following composition was used as the normal solution (in mM): NaCl, 67.0; KCl, 2.5; MgCl\(_2\), 1.2; NaHCO\(_3\), 25.0; Ca gluconate, 1.0; BaCl\(_2\), 0.5; sodium aspartate, 5.0; glucose, 26.0. The 0.01 mM Ca\(^{2+}\) solution was prepared simply by reducing the amount of Ca gluconate. All solutions were
stirred throughout the experiments by a stream of gas containing 98% O₂ and 2% CO₂. Under these conditions, a constant pH of 7.8 could be maintained in the bathing solution. The experiments were conducted at a room temperature of approximately 25°C.

RESULTS

Effects of Ca²⁺ on the decay of rhodopsin intermediates

An estimate of the decay rate of rhodopsin intermediates can be made by recording transretinal absorbance spectra after a bleach (HANAWA and Matsuura, 1975). An objective of this experiment was to examine whether or not the decay rate of rhodopsin intermediates is affected by reducing Ca²⁺ concentration. A 30 sec exposure of an intense light, which bleached about 50% of the original rhodopsin content, was applied to the dark-adapted retina. The concentrations of intermediates during the subsequent dark adaptation were measured at various time intervals by the absorbance changes at 380 nm (metarhodopsin II and all-trans retinal) and at 480 nm (metarhodopsin III).

The absorbance changes recorded in two retinas, one of which was immersed in the normal solution and the other in a low Ca²⁺ solution, are shown in Fig. 1. The concentrations of intermediates were expressed by the absorbance difference.
at 380 or 480 nm. The 380 nm absorbance increased at 1 min after the exposure as metarhodopsin II was formed, and then it decreased as metarhodopsin II decayed to metarhodopsin III and all-trans retinal (Fig. 1A). The thermal decay rate of metarhodopsin II could not be calculated with sufficient precision, since continuous 30 sec exposure to bleaching light was used. There was, however, a noticeable difference in the time course of the 480 nm absorbance change; the decay rate of metarhodopsin III was increased by the reduction in the extracellular Ca$^{2+}$ concentration (Fig. 1B). The decay rate of metarhodopsin III at 25°C was $1.31 \pm 0.22$ (S.D.) $\times 10^{-3}$ sec$^{-1}$ (6 retinas) in the normal solution, which was almost the same as Bauman's findings (BAUMAN, 1972), and $2.05 \pm 0.20$ (S.D.) $\times 10^{-3}$ sec$^{-1}$ (6 retinas) in the low Ca$^{2+}$ solution. As would be expected from the faster decay rate of metarhodopsin III in the low Ca$^{2+}$ solution, the absorbance at 380 nm in the low Ca$^{2+}$ solution reached a final steady level faster than that in the normal solution (Fig. 1A). In the isolated frog retina, the presence of 0.5 mM Ba$^{2+}$ had no effect on the thermal decay rates at 380 and 480 nm.

The fast PIII response threshold as a function of rhodopsin intermediate concentration

To examine whether the decay rate of 380 nm absorbing photoproducts affects the adaptational behavior of the receptors, both the threshold of the fast PIII

![Graph showing changes in absorbance and threshold](image-url)
When the frog retina was immersed in the normal solution, the absorption at 380 nm immediately increased after a bleach, and then decreased slowly (Fig. 2). On the other hand, the threshold response, which was evoked at −3.5 log units in a dark-adapted state, was immediately elevated above 0 log unit and re-
covered to 0 log unit at 2 min after the bleach. The threshold then gradually decreased, and stabilized at $-2.1 \log$ units at 41 min, when the 380 nm photoproducts had faded completely. In the low Ca$^{2+}$ solution, changes in the spectra and thresholds showed a similar behavior to those observed in the normal solution, but the recovery rate of the threshold was faster. This would be expected because of the faster decay of photoproducts.

The correlation between the threshold of the fast PIII response and the 380 nm photoproduct concentration in the normal solution (Fig. 3A) and in the low Ca$^{2+}$ solution (Fig. 3B) are shown. Though the threshold and rhodopsin content in the dark-adapted state varied in each retina, the threshold was linearly related to the amount of the 380 nm absorbing photoproducts. It should be emphasized that the linear relation observed in the normal solution remained so in the low Ca$^{2+}$ solution as well, where the decay rate of the 380 nm photoproducts was increased. The correlation between the threshold and the concentration of metarhodopsin III was poor, since the threshold still continued to decrease after metarhodopsin III had completely decayed (Fig. 2).

DISCUSSION

Immersing the isolated retina in the low (0.01 mM) Ca$^{2+}$ solution, we have demonstrated that the decay rates of metarhodopsin III and 380 nm absorbing photoproducts were accelerated. The outer segment of the bullfrog rod contains about 0.2 Ca$^{2+}$ per rhodopsin molecule and about two thirds of the endogeneous calcium is localized within rod discs (SZUTS and CONE, 1977). In the bovine rod outer segment, external calcium is exchanged with endogeneous calcium across the plasma membrane (SCHNETKAMP, 1979). If calcium flux across the plasma membrane of the frog and bovine is similar, Ca$^{2+}$ activity in the cytoplasmic space would be decreased when extracellular Ca$^{2+}$ concentration decreased from 1.0 to 0.01 mM. Although the precise mechanisms responsible for the acceleration of decay rate observed in this study are uncertain, it could be due to conformational changes of the disc membrane produced by a lowering of cytoplasmic Ca$^{2+}$ activity.

The present study has also indicated that the threshold change of fast PIII response after bleaching of about 50% of rhodopsin is closely related to the decay of 380 nm absorbing photoproducts. More interestingly, this relation still holds even when the decay rate of photoproducts is increased by immersing the isolated retina in the low Ca$^{2+}$ solution. Since the formation of metarhodopsin II in the bovine rod outer segment does not depend on the extracellular Ca$^{2+}$ concentration (NÖLL and STIEVE, 1979), the results obtained in this study suggest that an intermediate or a process of decay of metarhodopsin II has a significant role in the dark adaptation process of visual cells.

DONNER and HEMILÄ (1979) have suggested that the dark adaptation process
in the isolated amphibian retina can be divided into two phases: first, there is a rapid change which takes place within 1–2 min if the adapting light bleaches less than about 0.5% of rhodopsin (background adaptation); second, there is a persistent after-effect of light adaptation, and more than 30 min is required to recover from the effect if the adapting light is sufficiently strong to bleach a noticeable fraction of rhodopsin (intermediate adaptation). Showing the parallel time course between the dark adaptation and the decay of retinal, Donner and Hemilä suggested that retinal may be a significant factor in controlling the intermediate adaptation.

The 380 nm absorbance is composed of metarhodopsin II and retinal (Cone and Cobb, 1969). Based on computer analysis, Brin and Rips (1977) and Ernst et al. (1978) indicated a rapid decay of metarhodopsin II. Our present results, therefore, support the suggestion by Donner and Hemilä (1979), since we showed that the threshold of fast PIII response after a bleach was closely related to the 380 nm absorbing photoproducts, even when the decay rate of the photoproducts was increased in low Ca" solution.

REFERENCES


