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Light-Mediated Cyclic GMP Hydrolysis Controls Important Aspects of Kinetics of Retinal Rod Voltage Response*

W. H. Miller and S. B. Laughlin[1]

Department of Ophthalmology and Visual Science, Yale Medical School, New Haven, CT 06510, USA

Abstract. Pulsatile injections of cyclic GMP into rod outer segments of the isolated toad retina cause transient depolarizations that are reduced in amplitude in proportion with the receptor potential by low Na^+ Ringer's. This reduction in the amplitude of the cyclic GMP depolarization may be due to the direct effect of external Na^+ concentration on dark current and an indirect effect resulting from the inactivation of a sodium-calcium exchange mechanism raising the intracellular Ca^{2+} concentration. By comparison the reduction in cyclic GMP response amplitude effected by illumination is accompanied by faster kinetics. This difference suggests that the reduced amplitude and speedier response reflect a light induced increase in phosphodiesterase (PDE) activity rather than the effects of Ca^{2+}. Large doses of cyclic GMP can distort the kinetics of both the light response and the recovery from a depolarization caused by a pulse of cyclic GMP by similarly slowing both types of responses. This similarity in the kinetics of the cyclic GMP response and the initial hyperpolarizing phase of the receptor potential suggests that the kinetics of the initial phase of the receptor potential are controlled by light-mediated cyclic GMP hydrolysis.

Key words: Phototransduction – Cyclic GMP – Rods – Response kinetics

Introduction

Phototransduction in vertebrate retinal rods is suspected to be regulated by light-mediated hydrolysis of cyclic GMP (reviewed in Miller 1983). Support for this theory is derived in part from evidence suggesting that light-mediated hydrolysis of cyclic GMP in rod outer segments (ROS) provides the

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1 Present address: Dept. of Neurobiology, Australian National University, Canberra, Australia

amplification, power, speed, linkage with photolyzed rhodopsin, and necessary physiological properties to exert that regulation (Yee and Liebman 1978; Nicol and Miller 1978; Woodruff and Bownds 1979; Fung and Stryer 1980; Stryer et al. 1981; Miller 1982; Kuhn et al. 1981; Bennett 1982). Here we present additional evidence that pulsatile iontophoretic injections of cyclic GMP into ROS provide an index of phosphodiesterase (PDE) activity and that light-mediated cyclic GMP hydrolysis controls the kinetics of the initial hyperpolarizing phase of the ROS membrane voltage response.

Methods

Pulses of cyclic GMP are iontophoretically injected into ROS of isolated retina preparations of the toad, *Bufo marinus* (as in Miller 1982), using eyes that are enucleated from guillotined and double pithed animals. The retinas are superfused with Ringer's: 105 mM NaCl, 2.5 mM KCl, 1.6 mM $MgCl_2$, 1.6 mM $CaCl_2$, 8 mM glucose, and 3 mM HEPES. Low Na^+ Ringer's is identical except for the substitution of 78 mM choline chloride for NaCl to make a final concentration of 27 mM Na^+. Intracellular recordings are made with glass micropipettes filled with 2 M KCl or 25 mM cyclic GMP (K salt, P-L Biochemicals). One nA current pulses are delivered through the recording pipette for 1–40 ms to deliver pulses of 1–40 picocoulomb (pC). Six million ions per pC are passed assuming a transferance number of 1.

Results

1. Effects of Low Na^+ Ringer's on Transient Depolarizations Caused by Injections of Cyclic GMP

We previously showed that brief pulses of cyclic GMP injected into ROS cause transient depolarizations that become prolonged in darkness and are antagonized by illumination (Miller 1982). This is illustrated in Fig. 1. Down on the signal trace indicates the iontophoretic injection of 5 pC of cyclic GMP and up indicates a 0.1 s, −4 log units light flash. On the top record the hyperpolarizing receptor potential caused by the first flash is followed by two cyclic GMP injections. Note that the depolarization (filled arrow) caused by the second injection has a greater amplitude and longer recovery phase than the first injection even though the amount of cyclic GMP injected is the same, as would be expected if PDE became slowly inactivated following the light flash. The negative spike (open arrow) is the negative-current injection artifact. The transient depolarization to cyclic GMP following the second light flash is smaller in amplitude and the recovery is greatly accelerated, corresponding to the biochemically demonstrated light activation of PDE. The portion of the record with the horizontal line over it is expanded in the lower trace to show this better, and in addition, the transient depolarization following the light flash is normalized and traced near the previous depolarization (solid arrow) so that the

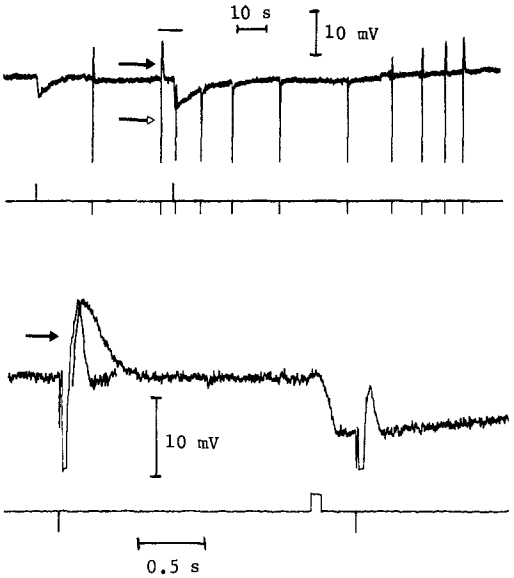

Fig. 1. Top inset from Fig. 3 is reproduced here to demonstrate effect of light on transient depolarizations caused by injections of pulses of cyclic GMP. Up on signal trace indicates 0.1 s, −4 log units light flash (about 2,000 photons/rod). Down on signal trace and downwards spikes on records indicate 5 pC iontophoretic injection of cyclic GMP. Horizontal line above record indicates portion of record that is amplified and expanded on lower traces. At filled arrow on lower trace, cyclic GMP depolarization subsequent to light flash is normalized to previous depolarization and superimposed on it to illustrate that illumination accelerates the recovery from the cyclic GMP depolarization

time courses of the two events can be more easily compared. The pulsatile cyclic GMP injections are postulated to provide an index of PDE activity, or more properly, of the net ability of ROS PDE to hydrolyze small increments of cyclic GMP considering local synthetic and hydrolytic activity. The objective of the experiments described here is to test our hypothesis that the transient depolarization to cyclic GMP represents PDE activity, not responses to injected current. We can rule out the possibility that the antagonism of the cyclic GMP transient depolarization is caused by the light-induced hyperpolarization per se since the antagonism is greater for injections subsequent to the light flash when the hyperpolarization has decreased. The same type of reasoning can rule out the Cs-sensitive increased Na^+ permeability as the cause of the antagonism; following a bright flash, the cyclic GMP dependent depolarization is completely suppressed at the peak of the receptor potential and before the increased Na^+ permeability occurs. Nevertheless, because the cyclic GMP induced depolarizations are suspected to increase ROS Na^+ permeability (Miller and Nicol 1981) and because illumination causes ROS ionic changes, it is important to determine to what extent the properties of the cyclic GMP depolarizations are a function of Ringer's Na^+ concentration as distinguished from illumination.

The effects on both the cyclic GMP depolarization and the receptor potential caused by reducing the Ringer's Na^+ from 105 mM to 27 mM are shown on Fig. 2. Both the receptor potential and the cyclic GMP depolarization are reversibly and proportionally reduced in amplitude, though the receptor potential returns more rapidly than the cyclic GMP depolarization. Although the cyclic GMP depolarization is antagonized by low Na^+ Ringer's, the recovery from the depolarization is not accelerated in contrast with the effect of light. This is shown more clearly on the inset of Fig. 2, where the portions of the records under the

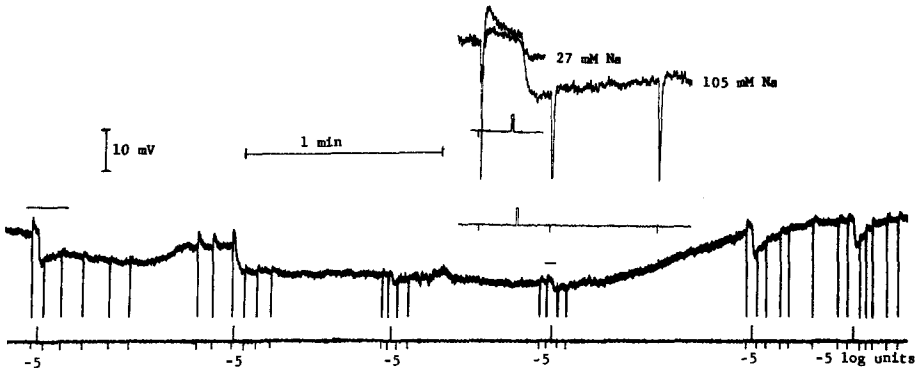

Fig. 2. ROS intracellular recordings of membrane voltage. Abrupt downward vertical lines on both signal traces and intracellular recordings indicate negative iontophoretic pulses of 10 pC of cyclic GMP. Upward displacement of the signal traces indicates a 0.1 light flash in all cases; intensity of −5 log units about 200 photons/rod. Just before the first flash, Ringer's superfusion was changed from normal to low Na^+ and switched back again where the baseline becomes wavy after the third flash. Because of dead space in superfusion system it takes almost a minute for a changed solution to have an effect. Horizontal lines just above record indicate portions of records that are expanded and superimposed on inset. Injections of cyclic GMP^- cause transient depolarizations that are antagonized by light and by low Na^+. Suppression of cyclic GMP depolarizations by low Na^+ Ringer's, in contrast to light, does not speed kinetics

horizontal lines above the record trace are amplified, expanded and superimposed in order to facilitate the comparison of the control and low Na^+ cyclic GMP depolarizations. The antagonism of the cyclic GMP depolarizations by light uniquely acts by accelerating the recovery from the depolarization. This is additional evidence that the hyperpolarizing recovery phase from the cyclic GMP depolarization reflects PDE activity.

2. Cyclic GMP Pulses Can Slow Kinetics of Light Response and Cyclic GMP Transient Depolarization Similarly

Saturating pulses of cyclic GMP injected into a fresh ROS increase the latencies of the receptor potentials in proportion to the amounts injected without greatly affecting the receptor potentials' kinetics (Nicol and Miller 1978; Miller and Nicol 1979). These properties have sometimes been observed to change after the injection of cyclic GMP when the ROS sometimes becomes depolarized (Brown and Waloga 1981) for unknown reasons. Under this condition saturating pulses of cyclic GMP not only increase the latency of the receptor potential but also slow the kinetics of both the receptor potential and the recovery from the cyclic GMP depolarization (Miller 1982). Because the recovery from a cyclic GMP pulse is postulated to be controlled by PDE activity, it is interesting to compare that recovery phase to the initial phase of the receptor potential under normal conditions and when the kinetics of the receptor potential are slowed by the injections of cyclic GMP after the cell is depolarized as described by Brown and

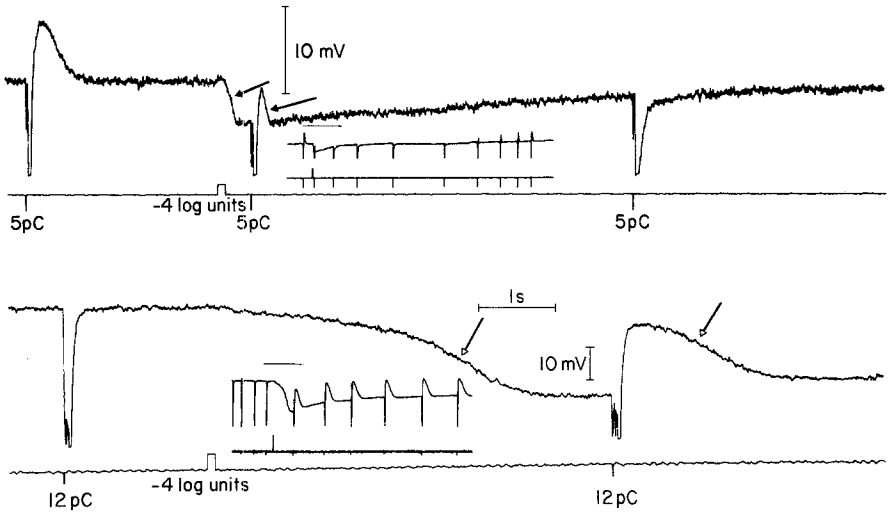

Fig. 3. Effect of cyclic GMP loading on the kinetics of the initial phase of light response and recovery from cyclic GMP induced transient depolarizations are similar. Signal trace description as in Fig. 2. Top records: Depolarizing responses to 5 pC injections of cyclic GMP, Recovery from pulse just following initial hyperpolarizing phase of receptor potential has similar kinetics to that of initial phase (solid arrows). Bottom records: Similar to top records except that ROS was depolarized by previous cyclic GMP injections (Brown and Waloga 1981) and then overloaded by repeated 12 pC injections of cyclic GMP which slow kinetics of the light response and the subsequent cyclic GMP injection. The fact that the kinetics of both light responses and the recoveries from cyclic GMP injections are affected similarly suggests they are both controlled by light-mediated cyclic GMP hydrolysis. (From Miller 1982, with permission)

Waloga, 1981. The top records of Fig. 3 show the normal light response as well as cyclic GMP responses. The horizontal line just above the trace on the inset marks the portion of the record that has been amplified and expanded. Note the similarity in the kinetics of the recovery phase of the cyclic GMP depolarization and the initial phase of the receptor potential (filled arrows). The expanded part of the lower trace has the same time scale as the expanded part of the upper trace and the light flashes in both cases are the same intensity and duration so that both expanded traces can be directly compared. The cyclic GMP pulse at the start of the lower trace contrasts with that at the start of the upper trace in that it does not cause a depolarization. This is because the ROS is already depolarized as described by Brown and Waloga, 1981, evidently reducing the driving force for the cyclic GMP depolarization. The effect of the cyclic GMP on ROS in this condition is to increase the latency and slow the receptor potential as shown. Then, with the cell hyperpolarized by light the next injection of cyclic GMP depolarizes and the recovery from that depolarization is similar to the initial phase of the receptor potential (open headed arrows). The fact that the recoveries from cyclic GMP pulses and the initial phases of the receptor potentials in these different conditions display similar kinetics suggests they have the same underlying mechanism: light-mediated cyclic GMP hydrolysis.

Discussion

1. Na^+-Ca^{2+} Exchange Affects Interpretation of Low Na^+ Experiments

We have shown that low Na^+ Ringer's reduces the amplitude of the depolarization caused by the injection of cyclic GMP in proportion to the reduction of the receptor potential. Even though Na^+ is the only ROS permeability affected by light, it is not certain that the reduction in the receptor potential and the cyclic GMP dependent depolarization is caused by reduction of the Na^+ current because internal Ca^{2+} is suspected to rise as a result of lowering the driving force of the Na^+ gradient for Na^+-Ca^{2+} exchange (reviewed in Fain and Lisman 1981; Owen and Torre 1981). Evidence for this comes from Schnetkamp (1980) who observed a rapid efflux of Ca^{2+} from ROS in the presence of Na^+ but very little Ca^{2+} efflux when Na^+ was substituted by other cations, as well as from Gold and Korenbrot (1981) who observed a light dependent ROS Ca^{2+} efflux that is partially inhibited by low Na^+ Ringer's. However, the low Na^+ effect on the cyclic GMP depolarization is important because it provides additional evidence that the antagonism of the cyclic GMP depolarization by illumination is a measure of PDE activity. Both low Na^+ Ringer's and illumination would be expected to reduce the Na^+ current and elevate internal ROS Ca^{2+}, but only illumination speeds the kinetics of the recovery from cyclic GMP depolarizations. The only known property of the ROS system that could account for the speeding of the kinetics of the recovery from the depolarization caused by the injection of cyclic GMP is the light-mediated activation of PDE. The suppression of the cyclic GMP dependent depolarizations by low Na^+ Ringer's therefore provides additional evidence that the physiological effects of brief cyclic GMP injections into ROS measure ROS PDE activity.

2. Cyclic GMP May Depolarize and its Hydrolysis Hyperpolarize by Different Mechanisms

The means by which cyclic GMP controls the light-sensitive sodium conductance either directly or indirectly are unknown, but several mutually compatible alternatives have been proposed. Cyclic GMP could act via a kinasing reaction (Polans et al. 1979); it could act indirectly by determining the level of free calcium (Cavaggioni and Sorbi 1981); or protons, a product of light-induced cyclic GMP hydrolysis, could release calcium to shut off the dark current (Pugh et al. 1982). Our attempts to test this latter hypothesis have failed for, although intracellular proton injections in pC quantities can transiently hyperpolarize the cell, this hyperpolarization does not show a sufficient dependence on illumination or external sodium to associate it exclusively with the light-sensitive conductance. Nevertheless, these preliminary experiments do not rule out the attractive possibility that protons mediate the light-induced decrease in dark current. Since the production of protons by itself cannot explain the depolarizing response to cyclic GMP, it is tempting to speculate that the gain of transduction

is doubled by allowing cyclic GMP to open channels by one pathway and its hydrolysis product to close them by another.

3. *Conclusion*

In conclusion we have shown that transient depolarizations caused by the injections of cyclic GMP into ROS are suppressed by low Na^+ Ringer's in proportion to the receptor potential but without accelerating the kinetics as light does. This is additional evidence that the antagonism by light of the transient depolarization caused by the injection of cyclic GMP is caused by light-mediated cyclic GMP hydrolysis. Further, the kinetics of this physiological sign of cyclic GMP hydrolysis are affected similarly to the kinetics of the initial hyperpolarizing phase of the receptor potential by cyclic GMP loading which suggests that the initial phase of the receptor potential is controlled by light-mediated hydrolysis of cyclic GMP.

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