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

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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 Ca2+ 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 Ca2+. 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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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 MgCl₂, 1.6 mM CaCl₂, 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 transference 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