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LIGHT-INDUCED CHANGES IN cAMP LEVELS IN LIMULUS PHOTORECEPTORS

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SUMMARY

Illumination reduces cAMP levels about 3-fold in Limulus photoreceptors. Cyclic GMP levels are not significantly changed under identical conditions. In addition, the cAMP content in dark-adapted photoreceptors is about 4-fold the content of cGMP. It is proposed that cAMP may be involved in the regulation of the metabolism or function of photoreceptor systems which contain the photopigment in the transducing surface membrane.

Particular emphasis has been placed on cyclic nucleotides as mediators of the light-induced permeability changes of the vertebrate photoreceptor membrane (for reviews, see Bitensky, et al. (1) Goridis and Weller, (2) and Hubbell and Bownds (3). Recent studies have demonstrated that endogeneous levels of cyclic guanosine monophosphate (cGMP) in vertebrate rod photoreceptors are significantly reduced by light (4-9). The light sensitivity, speed, gain, specificity, and energy requirements are considered sufficient for the cGMP system to play a central role as a metabolic and/or transmitter link between photon capture and the decreased Na permeability of the rod photoreceptor membrane (4-16).

Previous studies by Schmidt and Fein (17) and Corson et al. (18) indicate that cyclic nucleotide metabolism may influence the dark- and light-activated currents in an invertebrate photoreceptor preparation, the ventral eyes of Limulus, but the specific cyclic nucleotide that affects these currents is unknown. Therefore, it was of interest to examine the changes in cAMP and cGMP levels induced by light. Our findings demonstrate that light causes a

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relatively large reduction in cAMP levels, whereas the light-induced change in cGMP levels is not significant. In addition, the data show that cGMP content of dark-adapted photoreceptors is approximately 4-fold lower than that of cAMP.

MATERIALS AND METHODS

Limulus polyphemus (carapace diameter 6 - 8 inches) were obtained from the Marine Biological Laobratory, Woods Hole, Mass. *Limulus* ventral photoreceptors are large photosensitive cells distributed along the lateral olfactory nerve trunk and concentrated as a cluster at the nerve terminal (19). Pairs of olfactory nerves were removed from the animal, carefully desheathed and the nerve terminal containing the photoreceptor cluster dissected from each trunk which served as control (see Figure 1).

Lengths of unstretched nerves were measured (±mm) so that the protein, cyclic nucleotide concentration and photoreceptor number could be expressed per unit length. Before adaptation to light and dark, the dissected samples were stored in cold filtered sea water. When a sufficient number of photoreceptors was obtained, the samples were warmed to room temperature (22°C) and divided; one sample of the nerve pair was exposed to 1 hour of light (2.6 W m^{-2}), the matching sample to 1 hour of complete darkness. The same procedure was done with the nerve sections that were used as controls. These stimuli conditions and dissection procedures provided photoreceptors that were in steady states of both light and dark adaptation and electrophysiologically viable (JS, personal observation). All samples were then frozen in darkness or light in an ethanol-dry-ice bath (-75°C) and lyophilized. After extraction of the cyclic nucleotides with 0.1 N HCl, the samples were boiled for 5 min and centrifuged at 3,500 g for 15 min. The supernatants were lyophilized, resuspended in 50 mM sodium acetate, pH 6.2, and aliquots were serially diluted with the same buffer. Cyclic AMP and cyclic GMP were acetylated before measuring their concentrations by radioimmunoassay, according to the technique of Harper and Brooker (20). Protein was measured in the homogenates by the method of Lowry *et al.* (21); bovine serum albumin served as standard.

RESULTS AND DISCUSSION

The initial finding by Schmidt and Fein (17) that several phosphodiesterase inhibitors, whose putative role is to increase intracellular levels of cyclic nucleotides, suppressed both the voltage-dependent dark current and light-induced current in *Limulus* (18) prompted the present biochemical determination of the changes in levels of cAMP and cGMP that are induced by light in this system. *Limulus* photoreceptors are clustered at the nerve terminal (Fig. 1) and with careful dissection a sufficient quantity of photoreceptors can be obtained for the radioimmunoassay of cAMP and cGMP levels. Table I shows that following light adaptation there is a 2.8-fold decrease in cAMP concentration when the results are expressed in pmol per mg protein or a 5.2-fold decrease when expressed in femtomoles per photoreceptor.

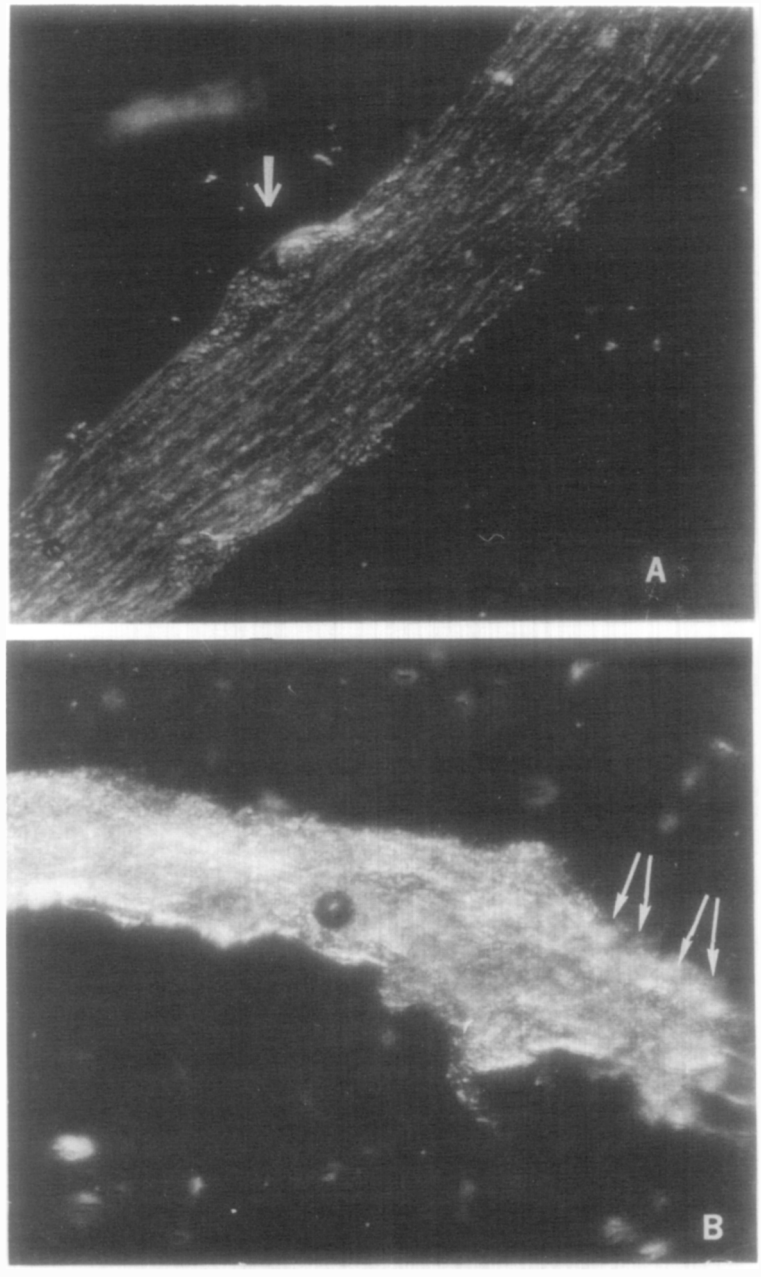

Figure 1: Light micrograph of Limulus ventral eye preparation. Arrows indicate ventral photoreceptor cells along the ventral eye nerve trunk (A) and terminal (B). Terminal was partially teased apart to show photoreceptors. The mean distribution of photoreceptors per unit length at the nerve terminal was 11-fold the number distributed along the nerve trunk (N = 360). Nerves have been desheathed, 100 x.

Table I: Cyclic nucleotide concentration of Limulus ventral eye following light and dark adaptation.

	cAMP		cGMP	
Exposure	pmol/ mg protein	fmol/ photoreceptor	pmol/ mg protein	fmol/ photoreceptor
Dark	10.2±2.7	2.7±0.8	2.4±0.8	0.9±0.6
Light	3.6±1.0	0.5±0.3	2.0±0.5	0.5±0.2
Dark/Light	2.8 ($p<0.1$)	5.2 ($p<0.1$)	1.2 (n.s)	1.8 (n.s)

Values were obtained by averaging the cyclic nucleotide per mg protein of each nerve terminal after subtraction of the corresponding levels in the nerve trunk (control). The control values were calculated as cyclic nucleotide per mg protein in nerve trunk length equivalent to those of the nerve terminal. To express the results per photoreceptor, the cyclic nucleotide content per mm nerve trunk was subtracted from that of the nerve terminal and the ratio between this value and number of photoreceptors per mm in nerve terminal minus nerve trunk was calculated. Values represent the mean ± standard error of 4 separate experiments (see text for further details). n.s = not significant.

In contrast, the light-induced change in cGMP levels under identical conditions is not significant independently of the way the data is expressed. Furthermore, the cAMP content of the invertebrate dark-adapted photoreceptor is about four-fold the content of cGMP.

The levels of cyclic nucleotides reported here represent a minimum estimate of cAMP and cGMP concentrations in the photoreceptors since all measurements were carried out with samples that had been maintained and lypholized in cold filtered sea water. This medium has a Na concentration of about 460 mM, which partially interfers with the radioimmunoassay. In control experiments with nerve sections only, we determined that when the salt concentration was reduced to 20 mM, the levels of cAMP increased by about 60% and those of cGMP by about 15%. Prolonged exposure to such low ionic strength will not maintain photoreceptor viability and therefore could not be used in this study.

Thus, Limulus represents a photoreceptor system which has cyclic AMP as the primary cyclic nucleotide; where light modulates the levels of cyclic AMP without affecting the levels of cyclic GMP. This situation is similar to that described by Farber and Lolley (22) for the cone-dominant retinas of ground squirrels and is in contrast to what is observed with rod-dominant retinas, where cyclic

GMP is the main cyclic nucleotide, probably the modulator of rod visual cell metabolism or function. In addition, vertebrate cones and invertebrate photoreceptors of Limulus have another common feature. Both systems have light-absorbing membranes confluent with the cell surface. This is again in contrast to rod photoreceptors, where most of the photopigment is contained in discs which are separated from the plasma membrane. Together, these observations raise the possibility that cyclic AMP may be involved in the metabolism or function of Limulus visual cells and, more in general, of photoreceptor systems where the photopigment molecules reside in the surface membrane

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