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LIGHT ACTIVATION OF PHOSPHODIESTERASE ACTIVITY IN RETINAL ROD OUTER SEGMENTS

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SUMMARY

Light "activates" phosphodiesterase activity of bovine rod outer segments in the presence of 0.1 mM ATP. In contrast, no difference in phosphodiesterase activity can be observed between dark-adapted and light-bleached outer segments in the absence of ATP.

Cyclic AMP may play a central role in the visual process since the activity of the enzyme adenylate cyclase in isolated, dark-adapted rod outer segments appears to be decreased upon illumination [1]. This is possibly a most exciting observation since a rapid drop in cyclic nucleotide level could mediate the conversion of the photic stimulus into a neural response or also be involved in the process of dark-adaptation through changes in membrane permeability, cation flux [2], etc. Recently, we have confirmed, in part, these observations and, in assays for adenylate cyclase, have found a decreased recovery of cyclic AMP in light-bleached outer segments in comparison to those in the dark-adapted state [3]. This indicated that the primary site of action of light might be on cyclic nucleotide degradation rather than on synthesis. We have also found, in contrast to the work of Goridis et al. [4], that the activity of guanylate cyclase of bovine rod outer segments appears to be decreased by light in a manner similar to that of adenylate cyclase [5]. Extremely high phosphodiesterase activity is also apparent in isolated outer segment preparations with considerably higher hydrolysis of cyclic GMP than cyclic AMP [6, 7]. Although no direct effect of light on phosphodiesterase activity has been observed [6, 7], it is the object of this communication to describe a "light activation" of phosphodiesterase activity in the presence of ATP which could account for the results previously ascribed to the effect of light on the cyclase enzyme.

Radiolabelled cyclic AMP (22–24 Ci/mmole) and cyclic GMP (3.5–4.0 Ci/mmole) were obtained from New England Nuclear Corp., Boston, Mass. Unlabelled nucleotides were purchased from Sigma Chem. Co., St. Louis, Mo. Rod outer segments from bovine retinas (dark-adapted overnight) were isolated by discontinuous sucrose density gradient centrifugation in buffer containing 67 mM phosphate,

TABLE I

EFFECT OF LIGHT ON PHOSPHODIESTERASE ACTIVITY IN THE PRESENCE OF ATP

Control values for cyclic AMP hydrolysis (4.1 nmoles/mg/min) and cyclic GMP hydrolysis (21.3 nmoles/mg/min) were arbitrarily set at 100 %. Values given are averages of triplicates from 4 experiments; replicate agreement was within 12 %.

Treatment	Cyclic AMP phosphodiesterase activity (% of control)			Cyclic GMP phosphodiesterase activity (% of control)		
	Light	Dark	Light/dark ratio	Light	Dark	Light/dark ratio
Control (no ATP)	100	98	1.0	100	103	1.0
+0.1 mM ATP	179	94	1.9	152	96	1.6

1.5 mM $MgCl_2$, pH 7.6 as described previously [7]. The percentage of unbleached rhodopsin in these preparations was at least 80 % as assessed by comparing the 498-nm absorption before and after the addition of 9-*cis*-retinal (P. O'Brien, personal communication). When appropriate, outer segments were bleached by exposure to normal fluorescent room lighting. Phosphodiesterase activity was determined by the method of Thompson and Applemen [8] using 40 mM Tris buffer (pH 7.6) and 5 mM $MgCl_2$. Thin-layer chromatography [9] was used to confirm the validity of this method with reference to the rod outer segments [7]. Identical results were obtained with the two techniques. Substrate concentrations were 5 μM, concentrations of cyclic nucleotide which approach physiological levels and where significant differences in specificity of cyclic nucleotide hydrolysis in subcellular fractions has been demonstrated [10]. Substrate utilization was 15–20 % under these conditions [10]. In each experiment, triplicate samples of isolated outer segments (or boiled outer segment controls) were preincubated with or without ATP at an appropriate concentration for 10 min at 25 °C followed by incubation with cyclic nucleotide at 25 °C for 10 min. The reaction was stopped by boiling for 5 min.

Table I demonstrates the effect of light on phosphodiesterase activity of dark-adapted rod outer segments. No difference in enzyme activity between dark-adapted and light-bleached rods was observed in the absence of ATP. In contrast, the effect of light in the presence of 0.1 mM ATP resulted in increased hydrolysis of both cyclic AMP and cyclic GMP. The light/dark ratio of enzyme activities was between 1.5 and 2.0 for the two cyclic nucleotides. In preliminary studies, it appears that GTP may act in a similar manner.

In the presence of concentrations of ATP which might normally be found in the retina in vivo, hydrolysis of both cyclic AMP and cyclic GMP appears to be stimulated in photoreceptor units by light-bleaching. Thus, in assays for adenylate cyclase [1, 3], the stimulatory effect of ATP on cyclic AMP hydrolysis in light-bleached outer segments could easily be misinterpreted as an "inhibition" of adenylate cyclase activity. It is not known at present if this effect is of physiological significance or merely represents non-specific, light-induced changes in vitro in permeability, substrate availability, etc., in the photoreceptor membrane. It is interesting to consider, however, that the differential effect of ATP in light and dark might well be used in vivo as a unique control of phosphodiesterase activity and thus of cyclic nucleotide concentration.

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