RAPID, LIGHT-INDUCED CHANGES OF RETINAL CYCLIC GMP LEVELS

C. GORIDIS and N. VIRMAUX
Centre de Neurochimie du CNRS, 11 Rue Humann, 67085 Strasbourg Cedex, France

and

H. L. CAILLA and M. A. DELAAGE
Centre de Biochimie et de Biologie Moléculaire du CNRS, 31 Chemin J. Aiguier, 13274 Marseille Cedex 2, France

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1. Introduction

Extremely high activities of guanylate cyclase [1] and guanosine cyclic 3′,5′ monophosphate (cGMP) phosphodiesterase [2], the enzymes of cGMP formation and degradation have been found in purified rod outer segments of vertebrate retina. Moreover, it has been shown that the activities of retinal cGMP phosphodiesterase increased considerably after illumination [3–5] or addition of bleached rhodopsin preparations [3,4]. Similary, a light-dependent inhibition of rod outer segment guanylate cyclase activity has been reported [6,7].

Isolated rod outer segments represent a preparation of highly specialized organelles, where the endogenous nucleotide metabolism cannot be studied. Only enzymatic activities can be measured in isolated rods. To assess the effect of light on cGMP levels in a more physiological system, we have studied cGMP concentrations in intact retinae. An active nucleotide metabolism has been shown to persist in isolated retinae for several hours [8,9] and retinae, incubated in a physiological medium, are capable of synthetizing [^{3}H]cGMP from tritiated hypoxanthine [4]. This report describes for the first time, that light exposure exerts a drastic effect on endogenous cGMP concentrations in retina.

2. Materials and methods

Eyes were removed from calves within 10 min after slaughter and stored on ice in a light-tight box for 2.5 hr. All subsequent operations except for the illuminations were carried out in dim red light. Calf retinae were incubated in a bicarbonate-buffered medium containing glucose [10] pre-equilibrated with 5% CO_2–95% O_2. The retinae were incubated at 37°C with continuous gassing with 5% CO_2–95% O_2. At the end of a 60 min preincubation, the retinae were placed individually into 10 ml Erlenmeyer flasks containing 3.5 ml fresh medium. After 12 min, the retinae were rapidly removed and dropped into 3.5 ml 1 M $HClO_4$. This operation was done in less than 5 sec.

After homogenisation and centrifugation to remove the protein precipitate, the extracts were neutralized with K_2CO_3 and stored at −20°C till determination of their cGMP content. Protein (18–25 mg/retina) was determined on the perchloric acid precipitates by the method of Lowry et al. [11].

Frogs (*Rana esculenta*) were dark-adapted for 12 hr before use. The retinae were incubated in a modified Frog's Ringer [8] at room temperature with continuous gassing with 5% CO_2–95% O_2. The same procedure as for the calf retinae was followed except that 3 retinae (1.8–2.2 mg protein) were incubated together and that the 12 min incubating was stopped by adding 100 µl 7 N $HClO_4$ directly to the flasks.

If the effect of light was to be tested, diffuse fluorescent room light was present for various periods during the 12 min final incubation. The retinae were incubated in the light for the times indicated immediately before the reaction was stopped. The light intensity was approximately 200 lux at the level of the incubation vessels.

The cGMP in the neutralized extracts was estimated radioimmunologically following the assay procedure described for cAMP [12]. Briefly, 200 μl of the neutralized extracts were succinylated by addition of 10 mg succinic anhydride and 20 μl triethylamine. Suitable dilutions of the succinylated extracts were mixed with [^{125}I] labelled 2′-*O*-succinyl cGMP tyrosine methyl ester and dialyzed against rabbit anti-succinyl cGPM serum. At equilibrium, the free and bound radioactivity was determined and the cGMP content computed from linear standard curves as described for the cAMP determination [12]. The samples, antigene and immune serum were diluted in 0.1 M citrate buffer (pH 6.2) containing 1 g/l bovine serum albumine and 2.5×10^{-4} M papaverine. Two to three dilutions of each sample were tested. The agreement between assays at different dilutions was within 8%. Known amounts of cGMP added to aliquots of the extracts were quantitatively recovered. At least a 10^4-fold greater concentration of cAMP was required to produce displacement of the radioactive ligand comparable to that obtained with cGMP.

The specificity of the assay was verified in some cases by measuring the cGMP concentration of the same tissue extracts before and after purification. cGMP was isolated as described [4] by successive chromatography on Al_2O_3 and Dowex 1X8 columns. The 3 ml formic acid eluate were lyophilized, the residue taken up in 3 ml 10% triethylamine (v/v) and succinylated by adding 300 mg succinic anhydride. The recovery during purification was corrected for by adding [^{3}H]cGMP. Virtually identical values were obtained before and after purification (table 1).

Table 1
Comparison of cGMP concentrations measured before and after purification of retinal extracts

Exp.	cGMP concentration*	
	Before purification	After purification
1	2.60	2.30
2	3.20	3.20
3	1.80	1.90
4	0.76	0.84

The tissue extracts of exp. 1 and 2 were prepared from dark, those of exp. 3 and 4 from light-incubated retinae.
* cGMP concentrations are expressed as $10^7 \times$ M in the perchloric acid extract.

Since the bovine eyes had been obtained from anima which had not been dark-adapted before slaughter, som rhodopsin of these retinae was present in the bleached state. Rod outer segments purified from such retinae contained no more than 10% bleached rhodopsin [14].

3. Results and discussion

Relatively high cGMP concentrations were found in intact bovine retinae incubated in a physiological medium (63.8 ± 3 pmoles/mg protein, $\bar{m}$ ± S.E.M. for N = 11). During illumination, there was a pronounced and rapid drop in retinal cGMP levels (fig. 1). When light was present for only 15 sec before stopping the final incubation, cGMP concentrations decreased 70%. Even light exposure for 5 sec caused a 30% drop of the cGMP levels. During continued incubation in the light, cGMP concentrations tended to increase again, reaching 47% of control levels after 12 min.

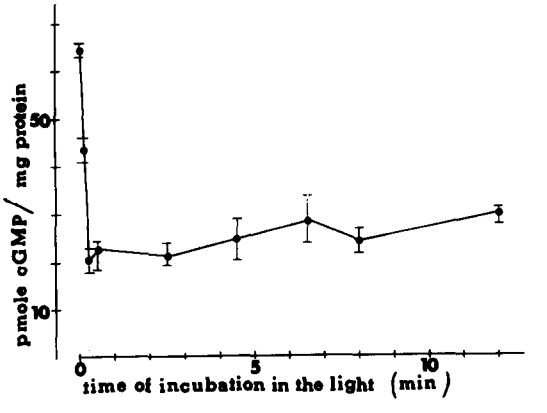

Fig. 1. The effect of light exposure on cGMP concentrations in intact calf retinae. Whole retinae were incubated as describec in Materials and methods. Diffuse fluorescent room light was present during the final 12-min incubation for the times indicated immediately before stopping the reaction. cGMP concentrations are given as the mean ± the range for 2–3 experiments. For the non-illuminated controls the mean ± S.E.M. is given fo 11 experiments. The 12-min value is significantly different fro the values after light exposure for 0.25, 0.5 and 2.5 min ($p<0.($

Table 2
Effect of illumination on cGMP concentrations in intact frog retina

Time of light exposure, min	cGMP pmole/mg protein
0	28.2
0.5	18.7
3.5	9.2

The incubated retinae were exposed to diffuse fluorescent room light for 0.5 of 3.5 min immediately before stopping the 12-min final incubation, or left in the dark. Duplicate determinations were performed and agreed within ± 9%.

Rana esculenta, which had been fully dark-adapted, showed somewhat lower retinal cGMP levels. As with bovine retinae, light exposure caused a 3-fold drop of the cGMP concentrations in incubated frog retinae (table 2).

Since whole intact retinae have been used in these experiments, the changes of cGMP levels observed could be produced elsewhere than in the photoreceptors. However, several lines of evidence suggest that the rod outer segments are the site of the light effect on cGMP metabolism. First, guanylate cyclase [1,7] and cGMP phosphodiesterase [2,4,13] activities are markedly enriched in purified rod outer segments when compared to whole retina. Second, the light-sensitivity of photoreceptor guanylate cyclase [6,7] and cGMP phosphodiesterase [3,5,13] have been described and the light-effect on cGMP hydrolysis could be mimicked by adding bleached rhodopsin preparations [3,4,13].

The role of cGMP in retina function is unclear at present. By analogy with the second messenger function of cyclic nucleotides in other systems, cGMP might have a role as intermediate between photon capture and changes in outer segment membrane permeability or as modulator of this process. There are certain time constraints, which must be met, if cGMP levels are to act as a signal in visual excitation. The swift drop of cGMP concentrations in whole retina caused by illumination is compatible with such a role. Moreover, under the conditions of illumination used in our experiments not all photoreceptors are bleached immediately. Therefore, cGMP levels could fall even more rapidly in some photoreceptors, than the estimations on whole retina suggest. However, definite proof or disproof of a specific function for cGMP in photoreception can only be obtained by studying the physiological responses of th retinal rod to the addition of exogenous cGMP, its ana ogues or of phosphodiesterase inhibitors. Such studies are now in progress in our laboratory.

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