GUANYL CYCLASE IN A MAMMALIAN PHOTORECEPTOR

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1. Introduction

The formation of guanosine cyclic 3′, 5′-monophosphate (cyclic GMP) from GTP in cell free systems is catalyzed by guanyl cyclase and this reaction appears to occur in a variety of animal tissues [1, 2]. The highest amount of guanine nucleotides noted in any mammalian tissue was found in rat and bovine retina [3]. One of the possible functions of the high retinal GTP level could be to serve as precursor for cyclic GMP. This paper describes the occurrence of an unusual high activity of guanyl cyclase in rod outer segments of the bovine retina.

2. Materials and methods

Young bovine eyes were kept in the dark at 0° for 2 hr after slaughtering. All subsequent operations were carried out in dim red light, except if stated otherwise. Rod outer segments were prepared as described elsewhere [4]. Following the first wash in phosphate buffer, the packed outer segments were stirred up in an hypotonic Tris-Cl buffer (10 mM, pH 7.5) or in either of two isotonic solutions, i.e. a "medium" containing 100 mM Tris-Cl, 145 mM KCl, 1 mM $MnCl_2$ or 0.25 M sucrose buffered with 10 mM Tris-Cl, recentrifuged (20 min at 10^5 *g*) and finally suspended in the same solution used for the washing. Sequential extraction of rod outer segments was done as follows: outer segments suspended in phosphate buffer were divided in several fractions and either kept in the dark or illuminated for different times by a 75 W bulb. The fractions were spun down and the pellets homogenized in 0.1% emulphogene, 0.066 M phosphate (pH 7.0) followed by centrifugation at 10^5 *g* for 120 min. After removal of the supernatant (giving the 0.1% extract), the procedure was repeated with 0.3% emulphogene, 0.066 M phosphate (pH 7.0).

If required, aliquots of outer segment suspensions were illuminated for 20 min by a 75 W bulb. During this time the colour of the photopigment had changed from a deep purple to a pale orange. The preparations were stored in the dark at either −10° or at +2°, less than 20% of the enzyme activity was lost over a period of 3 days.

For comparison, whole bovine retinas, taken out and processed in day light, were homogenized in the "medium" described above and centrifuged (10^5 *g*, 1 hr) to separate a soluble from a crude particulate fraction. Whole rat brain was either processed in the same way or fractionated as described elsewhere [5].

Guanyl cyclase activity was determined by a modification of the methods described by White and Zenser [6] and Schultz et al. [7]. The usual incubation mixture contained 60 mM Tris-Cl (pH 7.5), 7.7 mM $MnCl_2$, 15.4 mM creatine phosphate, 75 μg creatine kinase (Sigma), [^{3}H]cyclic GMP (tritiated cyclic GMP from Amersham diluted with unlabelled cyclic GMP from Boehringer; 0.75 μmole, 2×10^4 cpm), [^{32}P]GTP (0.185 mM, $0.5-1 \times 10^6$ cpm) and 20–50 μl enzyme in a total volume of 130 μl. Following the incubation at 37° for 10 min the reaction was stopped by adding unlabelled GTP (0.5 μmole) and boiling for 3 min. Then 100 μl Tris-Cl (0.06 M, pH 7.5) were

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Table 1
Recovery of added [^{3}H]cyclic GMP and formation of [^{32}P]GMP in different preparations of rod outer segments.

	$\frac{^3H}{^3H_o} \times 100$	$\Delta\ ^{32}P$ (cpm)
Exp. I		
– Medium	3.0	560
–Tris-Cl	92	14,300
Exp. II		
–Tris-Cl–sucrose	1.8	490
–Tris-Cl	78	38,900
Exp. III		
–Tris-Cl–sucrose	3.8	1,660
–Tris-Cl	93	40,500

Rod outer segments suspended in phosphate buffer were divided into two equal parts, recentrifuged, washed and taken up in either an isotonic medium (Tris-Cl 0.1 M, pH 7.5; KCl 0.14 M; $MnCl_2$ 0.001 M) and Tris-Cl (0.01 M, pH 7.5) or in sucrose 0.25 M–Tris-Cl (0.01 M) and 0.01 M Tris-Cl buffer. In experiments I and II, the preparations were illuminated and incubated in day light; in experiment III preparation and enzyme test were carried out in dim red light. The results are expressed as percent [^{3}H]cyclic GMP recovered after incubation and separation, the ^{3}H incubated in the presence of boiled enzyme taken as 100% (($^3H/^3H_o$) × 100). The formation of ^{32}P cyclic GMP from ^{32}P GTP is expressed as cpm ^{32}P recovered from the sample minus cpm ^{32}P of the blank ($\Delta\ ^{32}P$).

added and the cyclic GMP separated from other labelled compounds by Al_2O_3 columns essentially as described [6]. The ^{32}P- and ^{3}H-disintegration rates of the column eluates were determined by liquid scintillation counting. Incubation blanks were run as described with a boiled enzyme preparation. The overall recovery of [^{3}H]cyclic GMP incubated and processed with these blanks was 70–76%, the aliquoting being considered. The recovery of [^{3}H]cyclic GMP added to some enzyme preparations was considerably lower, presumably due to the action of cyclic nucleotide phosphodiesterase. However, this loss of cyclic GMP incubated with active fractions was usually less than 10% except when stated otherwise in the text. Without addition of unlabelled cyclic GMP the recovery of tritium from incubation blanks was unaltered, though practically neither ^{32}P- nor ^{3}H-cyclic GMP could be recovered after incubation with active samples. In

Table 2
Guanylic cyclase in various preparations.

Preparation	Enzyme activity (pmoles cyclic GMP produced/min/mg protein)
Rat brain soluble fraction[a]	132
Rat brain total homogenate[b]	137
Rat brain soluble fraction[b]	525
Bovine retina particulate fraction	286[d]
Bovine retina soluble fraction	132
Bovine rod outer segments[c]	4100, 2360, 2850

[a] Prepared in Tris-KCl–$MnCl_2$ (pH 7.5).
[b] Prepared as described elsewhere [5].
[c] Prepared in Tris-Cl 10 mM, pH 7.5.
[d] Apparent value; 35% of added [^{3}H]cyclic GMP degraded during the incubation period.

other experiments, cyclic GMP fractions obtained from the Al_2O_3 column were applied to a second alumina column with or without prior treatment with cyclic nucleotide phosphodiesterase (obtained from Sigma). In samples not treated with phosphodiesterase, over 90% of the ^{32}P- and ^{3}H-cyclic GMP was found in the cyclic GMP fraction from the second column. Less than 5% of the ^{32}P and the ^{3}H in treated samples appeared in this fraction. The efficiency of the GTP regenerating system was checked measuring the ^{32}P-GTP remaining at the end of the 10 min incubation period. The guanine nucleotides were separated by thin-layer chromatography [7] and 80–85% of the ^{32}P recovered was associated with GTP. Good proportionality existed between time of incubation and the amount of cyclic GMP formed with the exception of the cases where a considerable part of the [^{3}H]cyclic GMP was lost during incubation.

3. Results

Whereas a particulate fraction isolated in isotonic medium from whole bovine retina exhibited considerable guanyl cyclase activity (table 2), the enzyme activity of outer segments prepared in isotonic solutions was masked by the extremely low recoveries of cyclic GMP when incubated with active enzyme

Table 3
Guanyl cyclase in light and dark-adapted rod outer segments.

[GTP] (μM)	Enzyme activity* Light-adapted	Dark-adapted
185	2360	2460
185	2850	2770
738	3900	3960

* Expressed as pmoles/min/mg protein.

The outer segments were prepared in dim red light from dark-adapted retinas, finally washed and taken in Tris 10 mM, pH 7.5. For light-adaptation, an aliquot of the suspension was exposed to light (20 min by a 75 W bulb) immediately before the enzyme test, which was carried out in the dark.

(table 1). This effect may be explained by the presence of an unusually active cyclic nucleotide phosphodiesterase. When washed and taken up in hypotonic Tris-Cl buffer, suspensions of outer segments degraded only a minor fraction of added cyclic GMP and a high activity of guanyl cyclase was revealed (table 1). The supernatants of the washes did not show measurable guanyl cyclase activity. The differential effect of hypotonic and isotonic buffer was the same, whether preparation and incubation were

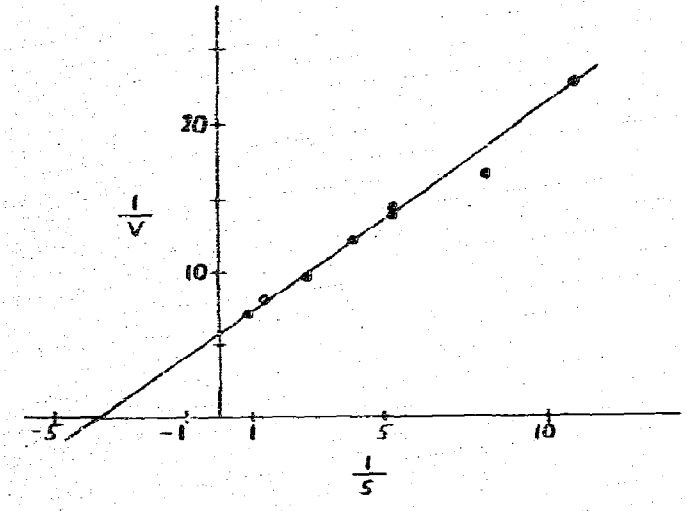

Fig. 1. Lineweaver–Burk plot of the kinetics of guanyl cyclase activity in rod outer segments. The reaction velocity v was determined at 7.7 mM Mn^{2+} and is expressed as nmoles cyclic GMP produced/min/20 μl suspension of outer segments in hypotonic Tris-Cl buffer.

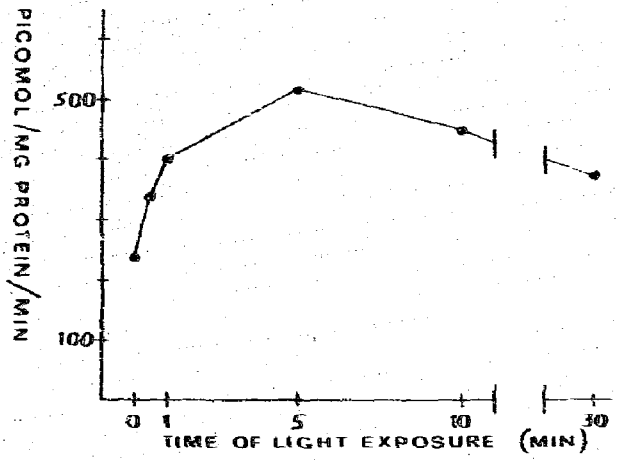

Fig. 2. Extraction of guanyl cyclase activity by emulphogene after light exposure for different times. The extraction of the outer segments was carried out as described in Methods. After the exposure to light all subsequent procedures including the enzyme test were carried out in dim red light.

carried out in dim red light (Exp. III) or illuminated suspensions were incubated in day light (Exp. I and II). In all cases, the loss of added [^{3}H]cyclic GMP was reduced but not sufficiently prevented by inclusion of papaverine (10 mM) in the incubation mixture. Theophylline (10 mM) however, was devoid of any effect.

Guanyl cyclase activity, as measured under our test conditions, was several fold higher in purified bovine rod outer segments than in a soluble or particulate fraction from whole bovine retina or in a total homogenate and soluble fractions from rat brain (table 2). There were no differences observed whether the outer segment suspensions were exposed to light or not. Also when measured under conditions near the optimal substrate concentration, no difference due to light exposure could be detected (table 3).

The apparent K_m value with respect to GTP of guanyl cyclase activity of outer segments isolated in hypotonic buffer was determined from a Lineweaver–Burk plot (0.27 mM). The maximum reaction velocity was about two times the value listed in table 2 for the same preparation and measured at 0.185 mM GTP (5750 versus 2360 pmole/min/mg protein). Also in the presence of higher GTP concentrations, increasing the Mn^{2+} was not followed by a higher reaction velocity; in contrast, raising the Mn^{2+}

concentration resulted in a gradual decrease of the formation of cyclic GMP (minus 20% at 15.4 mM $MnCl_2$ and 0.74 mM GTP).

The extraction of rhodopsin from rod outer segments by SDS is facilitated after prior illumination of the suspensions [8]. Since SDS is known to inactivate most enzymes, we used the non-ionic detergent emulphogene to test the influence of light on the solubilisation of guanyl cyclase by low concentrations of detergent. Whereas the activity in a 0.1% emulphogene extract was at the limit of detectability, considerable activity could be extracted by 0.3% emulphogene. The specific activity of guanyl cyclase in the extracts was higher, if the preparations were exposed to light prior to the extraction (fig. 2).

4. Conclusions

The data presented above demonstrate that guanyl cyclase is present in purified bovine photoreceptors, where its specific activity was several times higher than in particulate or soluble material from whole bovine retina or in rat brain homogenate. This high guanyl cyclase activity was detectable only when the outer segments were washed and taken up in hypotonic Tris-Cl, most likely because this treatment eliminated an extremely active cyclic nucleotide phosphodiesterase. The activity of guanyl cyclase in suspensions of outer segments was not changed after light exposure. Illumination, however, did influence guanyl cyclase as shown by the considerably higher specific activities in 0.3% emulphogene extracts following light exposure of the outer segments for short periods of time. At present, we are unable to give a definite explanation for these results. In the light of our previous experiments on the extractability of rhodopsin [8], we might postulate that photonic stimulation does change the physico-chemical characteristics of guanyl cyclase and facilitates the solubilization by detergent.

The precise biological importance of cyclic GMP has not yet been defined. The bovine photoreceptors, where the biosynthetic (i.e. guanyl cyclase) and the degradative (i.e. cyclic nucleotide phosphodiesterase [9]) enzymes occur in a high specific activity, may present a useful model to study the functional role of this cyclic nucleotide.

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