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CYCLIC GUANOSINE 3'5' PHOSPHATE, GUANYLATE CYCLASE AND CYCLIC GUANOSINE PHOSPHODIESTERASE IN THE EYE LENS

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While cGMP levels of rat lenses are in the range of those of other tissues, in calf lenses their values are much lower. Guanylate cyclase activities are rather high in proliferating epithelial cells of the lens and decrease strongly with cell differentiation and aging. cGMP phosphodiesterase activities are also reduced with aging in lens epithelial cells. A slight increase seems present in differentiated cortical fibers.

INTRODUCTION

cGMP is widely distributed in tissues and its level may change under various physiological and experimental conditions (1). It becomes increasingly evident that cGMP linked signals might be received, processed and integrated into regulatory biological processes quite differently from signals connected with cAMP (1). There is little information on lens cGMP. Bonomi et al. (2) suggest that the low cGMP content of the lens is related to the small number of cells and the lack of innervation. According to Polychroniadis et al. (3), cGMP in the eye is formed in the retina and choroid. However, cGMP seems to be involved in cell proliferation (4) and since there is a continuous division of epithelial cells in the lens, one would expect the presence of cGMP and a regulatory mechanism for this cyclic nucleotide. For those reasons we investigated the cGMP content and the activity of the enzymes involved in cGMP metabolism, guanylate cyclase and phosphodiesterase, as well as the effect of aging on these enzymes.

MATERIALS AND METHODS

Whole lenses, lens layers and cyclic nucleotide extracts were obtained as described in a previous paper (6). For enzyme assays tissues were homogenized in a solution of 10 mM Tris HCl in a glass Potter homogenizer in ice. Both guanylate cyclase and phosphodiesterase determinations were carried out

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at 37°C for 10 min. Final volumes of the total reaction mixture were 130 µl and 200 µl, respectively. Tissue homogenates boiled in water for 3 min were used as controls.

Guanylate cyclase activity was measured by a modification of the method described by White and Zenser (8). The guanylate cyclase reaction mixture contained 740 µM [α-^{32}P] GTP of approximately 500,000 cpm, 7.7 mM $McCl_2$, 75 µg creatine kinase (Boehringer), 15 mM creatine phosphate in 100 mM Tris HCl pH 7.5 (Boehringer), 6 mM 3-isobutyl, 1-methylxanthine (Sigma), 3.5 mM [^{3}H] cGMP of approximately 350,000 cpm and homogenate (80-200 µg protein). The reaction was initiated by adding 20 µl of substrate and terminated by adding 50 µl of a solution containing 10 mM GTP, 4 mM EDTA and 60 mM Tris HCl (pH 7.5), boiled for 3 min and cooled on ice. Next, 100 µl 60 mM Tris HCl (pH 7.5) was added and 200 µl from each tube was applied to a 7.5 x 0.5 cm neutral Woelm-N-super I (W 200) alumina column. This column was washed with 2 ml of the latter buffer and eluted with 2 ml 600 mM Tris HCl (pH 7.5). The recovered material was transferred onto a 0.5 x 3.5 cm Dowex 1 x 2 column previously washed with 2 ml H_2O. The column was further washed with 2 ml H_2O and 8 ml 0.5 N formic acid, then eluted with 3 ml 8 N formic acid and received directly into counting vials. Fractions collected were counted for ^{3}H and ^{32}P. Column and possible phosphodiesterase losses were corrected by the amount of [^{3}H] cGMP recovered.

Phosphodiesterase activity was determined by a modification of the method described by Thompson et al. (9). The cGMP phosphodiesterase reaction mixture contained 2 mM $MgCl_2$ in 80 mM Tris HCl (pH 8) and 125 µM [^{3}H] cGMP containing 100,000 cpm. The reaction was initiated by adding the homogenate (65-200 µg protein) and terminated by boiling for 3 min and cooling on ice. A second reaction was started by adding to each tube 50 µl snake venom (crotalus atrox, Sigma) 0.5 g/l 5'-nucleotidase and incubating for 20 min at 30°C. Then, 750 µl of 0.1 mM guanosine in 20 mM formate buffer (pH 7.4) was added and approximately 1 ml was applied to a 0.5 x 3 cm formate form QAE Sephadex- A25 column previously washed with 2 ml of 2 mM ammonium formate buffer (pH 7.4) and 10 ml H_2O. Guanosine was eluted with 4 ml of the same buffer (pH 7.4) directly into counting vials. Losses of cGMP were corrected for by the amount of [^{3}H] guanosine recovered in 2 independent tubes.

cGMP and GTP radioactive nucleotides were purchased from Boehringer and NEN, respectively.

RESULTS AND DISCUSSION

The possible effect of the sacrifice method as well as the post-mortem effects on cGMP levels were tested (Table 1). We found that cGMP of rat lens obtained either by decapitation or by microwave irradiation followed by perchloric acid extraction produced the same values of cGMP per mg of proteins. There was no significant decrease of cGMP in rat lenses within a 2-3 min post-mortem period while after 5 hrs a significant decrease was noted. However, in bovine lenses no decrease could be observed 5 hrs post-mortem (Table 1).

The amount of cGMP in whole calf lenses is about 1/2000 of the content of the retina (10) and 1/3 of that in the trachea (11). The amount of cGMP

TABLE 1
cGMP levels : sacrifice effect in rat lens and post-mortem effect in whole rat and bovine lens

Species	Experimental conditions	pmoles cGMP/mg prot.
Rat	1) Microwave irradiation	$0.229^{a} \pm 0.077$ (4)
	2) Decapitation and 2-3 min post-mortem	$0.223^{b} \pm 0.045$ (4)
	3) 1 hour post-mortem	$0.162^{c} \pm 0.038$ (4)
	4) 5 hours post-mortem	$0.127^{d} \pm 0.027$ (4)
Bovine	1) 2-3 min post-mortem	$0.039^{e} \pm 0.017$ (4)
	2) 5 hours post-mortem	$0.038^{f} \pm 0.006$ (12)

ab, bc, cd, ef, not significantly different, $p > 0.05$
ac, significantly different, $p < 0.05$
One group of rats were killed by microwave irradiation (1.25 KW focused on the head of the animal during 15 seconds). A second group of rats were killed as bovines by decapitation. In all cases lenses were removed and either frozen within 2-3 minutes or 1 and 5 hours post-mortem. Frozen materials were processed as described in a previous paper (6).

in rat lens approximately equals that in brain tissue (12) and is about 1.5 times greater than that in liver (12). Compared to bovine lenses levels of cGMP are 6 times higher in rat lenses.

In order to investigate changes in lens epithelial cGMP as a function of age, bovine lenses of different ages were examined. As shown by Hockwin (13) there is a significant correlation between lens weight and age of the animals.

The amount of cGMP per mg of proteins in lens epithelium is about 10 times higher than in whole lens and such a difference could be expected (Table 2). A decrease of lens epithelium cGMP is observed only in very old animals. There are strikingly lower values of cGMP in lens cortex 0.06 ± 0.03 pmol/mg protein (11 determinations) and nucleus 0.05 ± 0.03 pmol/mg protein (9 determinations) as compared to lens epithelium 0.35 ± 0.15 pmol/mg protein (14 determinations).

Guanylate cyclase activity in bovine lens epithelium is about 10 times lower than in bovine brain (14) and 100 times lower than in rod outer segments (15) ; this activity decreases considerably with age. In calf lens cortex, the activity is less than 1/3 of that in the epithelium. In lenses of adult and old

TABLE 2

Effect of aging on cGMP levels in whole bovine lens and in its epithelium

Age	Lens weight (g)	Whole lens pmoles cGMP/mg prot.	Epithelium pmoles cGMP/mg prot.
Calf	1.2 ± 0.08 (6)	0.037^{a} ± 0.008 (6)	0.33^{e} ± 0.15 (7)
Adult I	1.76 ± 0.07 (4)	0.030^{b} ± 0.028 (4)	0.38^{f} ± 0.14 (8)
Adult II	2.27 ± 0.08 (8)	0.032^{c} ± 0.013 (8)	0.32^{g} ± 0.09 (8)
Old	2.84 ± 0.05 (6)	0.033^{d} ± 0.016 (6)	0.20^{h} ± 0.04 (8)

ab, bc, cd, ef, fg, not significantly different, $p > 0.05$
eh, fh, gh, significantly different, $p < 0.05$
Age is represented by lens average weight with ± SE and in parentheses the number of lenses used.
cGMP levels are given with SE and in parentheses the number of extracts analysed in duplicate.

animals, guanylate cyclase activity of the cortex and the nucleus is not measurable (Table 3).

cGMP phosphodiesterase activities are the higher in the epithelium than in the cortex and the nucleus (Table 3). The phosphodiesterase activity in lens epithelium decreases by 60% in adult and by 90% in old animals as compared to the activity in calf lenses. In the lens cortex where the values are much lower than in the epithelium a slight increase of phosphodiesterase activity was observed in adult and in aged animals.

Lower values for guanylate cyclase activity found in the cortical calf lens area and the absence of detectable guanylate cyclase activity in adult

TABLE 3

Effect of aging on guanylate cyclase and cGMP phosphodiesterase in various bovine lens layers

Age	lens weight (g)	Epithelium		Cortex		Nucleus	
		Guanylate Cyclase*	Phospho-diesterase**	Guanylate Cyclase*	Phospho-diesterase**	Guanylate Cyclase*	Phospho-diesterase**
Calf	1.2 ± 0.08 (10)	9.89^{a} ± 3.70	3.31^{d} ± 0.7	2.51^{g} ± 1.55	0.77^{h} ± 0.08	N.D.	0.64^{k} ± 0.18
Adult	2.08 ± 0.14 (17)	1.76^{b} ± 0.95	1.22^{e} ± 0.36	N.D.	1.24^{i} ± 0.13	N.D.	0.98^{l} ± 0.07
Old	2.70 ± 0.16 (14)	0.71^{c} ± 0.26	0.29^{f} ± 0.04	N.D.	0.81^{j} ± 0.07	N.D.	0.90 ± 0.59

ab, ac, ag, dk, il, fj, de, ef, df, hi, ij, hk, significantly different, $p < 0.05$
Assays in triplicate. N.D. : not detectable.
* Guanylate cyclase : activity in pmoles, cGMP formed mg prot./min.
**cGMP phosphodiesterase activity in nmoles cGMP hydrolysed mg prot./min.

and old lenses is one of the aspects of differentiation of lens epithelial cells into cortical fibers. Moreover, guanylate cyclase activity decreases strikingly in the epithelium with age. Hence, one might expect a decrease in the response of cGMP biosynthesis to external stimuli which might utilize cGMP as a second messenger. Since it is generally considered that cGMP is involved in cell proliferation (4), the decrease of guanylate cyclase in the lens epithelium with age may be related to the decrease in the epithelial cell proliferation in the lenses of old animals. The increase of cGMP phosphodiesterase in the cortical fibers may contribute to the very low levels of cGMP in this area.

In conclusion, for bovine lenses where easy separation of the area containing epithelial cells is possible, we find a ten fold higher content of cGMP in this layer of the lens, and twelve fold higher levels of guanylate cyclase activity in proliferating epithelial cells of calf lenses, as compared to those of old animals. The decrease of lens epithelium cGMP as well as guanylate cyclase activity in aged animals can be considered as a major phenomenon of lens aging.

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