It can be postulated that contrycal, by inhibiting protease activity in the early stage of granulation tissue formation, thereby stimulated the incorporation of labeled precursors into certain biological polymers, including into collagen proteins. The simultaneous reduction in the quantity of breakdown products — the natural stimulators of repair processes — evidently was responsible for some delay in the maturation of the granulation tissue formed around the coil implanted under the skin.

LITERATURE CITED

1. A. Ts. Anasashvili, Vopr. Med. Khim., No. 5, 489 (1963).
2. T. L. Zaets et al., Éksp. Khir., No. 5, 49 (1974).
3. A. L. Zaides et al., Biokhimiya, No. 1, 5 (1964).
4. V. I. Mazurov and V. N. Orekhovich, Biokhimiya, No. 1, 33 (1959).
5. V. I. Struchkov et al., in: Proceedings of the Second All-Union Symposium on Medical Enzymology [in Russian], Dushanbe (1974), p. 9.
6. I. Todorov, Clinical Laboratory Investigations in Pediatrics [in Russian], Sofia (1960).
7. Yu. A. Yurkov and V. V. Alatyrtsev, Lab. Delo, No. 12, 705 (1966).
8. D. Fischer et al., Med. Welt (Stuttgart), No. 40, 1542 (1973).
9. S. M. Fitch et al., Nature, 176, 163 (1955).
10. F. W. Gierhake et al., Chirurg, 42, 360 (1971).
11. L. Warren, J. Biol. Chem., 234, 1971 (1959).
12. F. Wroblewski and J. S. LaDue, Proc. Soc. Exp. Biol. (New York), 90, 210 (1955).

EFFECT OF PROSTAGLANDIN $F_{2\alpha}$ ON LYSOSOMAL MEMBRANES OF THE EYE TISSUES

B. S. Kasavina and T. V. Ukhina

UDC 617.7:612.015.1

Both in experiments *in vitro* and after intravenous injection of prostaglandin (PG) $F_{2\alpha}$ labilization of the lysosomal membranes takes place in the sclera and ciliary body but not in the cornea of the rabbit eye. Under the influence of PG, glycosidase activity appears in the vitreous body, where it cannot be found normally.

KEY WORDS: prostaglandin $F_{2\alpha}$; lysosomal membranes; rabbit eye tissues.

Prostaglandins (PG) are present in nearly all mammalian and human tissues and have a broad spectrum of action. Recently two types of PG ($F_{2\alpha}$ and E_1) have been discovered in the eye tissues [9, 11], and their localization [4, 5, 11] and their effect on certain tissues of the eye [6, 7, 14] have been demonstrated. Prostaglandins can act on membranes and on cell metabolism both through a change in the ionic permeability of the membranes and through their action on the cyclic AMP system and on certain hormones [8, 15]. However, the effect of PG on the membranes of the eye tissues has not been studied, with the exception of the ciliary body, for which an increase in the permeability of the cell structures under the influence of PG has been demonstrated [6, 13].

In this investigation the effect of PG $F_{2\alpha}$ on the lysosomal membranes of the eye tissues was studied. Changes in the activity of enzymes hydrolyzing glycoside bonds in mucopolysaccharides (β-galactosidase, β-glucosidase, hyaluronidase) were investigated in the sclera, ciliary body, cornea, aqueous humor, and vitreous body.

Moscow Helmholtz Research Institute of Eye Diseases.

(Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 6, pp. 679-681, June, 1976. Original article submitted December 15, 1975.

This material is protected by copyright registered in the name of Plenum Publishing Corporation, 227 West 17th Street, New York, N.Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission of the publisher. A copy of this article is available from the publisher for $7.50.

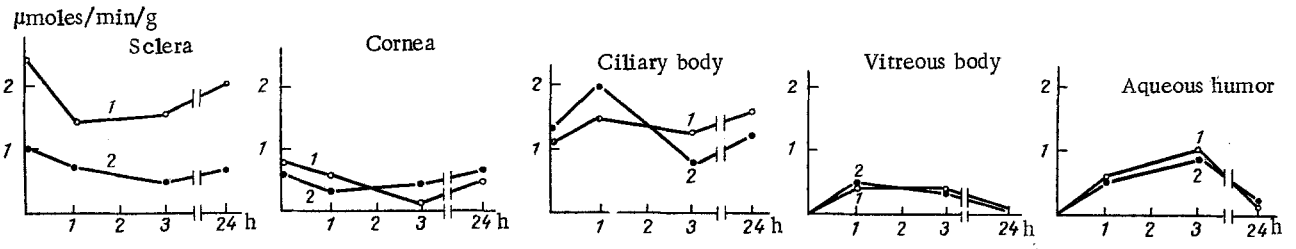

Fig. 1. Dynamics of changes in glycosidase activity in the eye tissues under the influence of PG $F_{2\alpha}$. Ordinate, activity of β-galactosidase (1) or β-glucosidase (2) (in μmoles/min/g protein); abscissa, time after administration of PG $F_{2\alpha}$ (in h).

TABLE 1. Effect of Prostaglandin $F_{2\alpha}$ (0.1 μg/ml) *in vitro* on Activity of Lysosomal Glycosidases of the Sclera, Cornea, and Ciliary Body (M ± m)

Fraction	Sclera				Cornea			
	β-galactosidase		β-glucosidase		β-galactosidase		β-glucosidase	
	total	free	total	free	total	free	total	free
Homogenate (control)	2,4± 0,03	2,4± 0,06	0,98± 0,01	0,3± 0,01	0,79± 0,05	0,72± 0,01	0,68± 0,01	0,40± 0,05
Mitochondrial-lysosomal (control)	1,3± 0,05	1,2± 0,06	0,53± 0,01	0,16± 0,01	0,42± 0,03	0,39± 0,01	0,42± 0,03	0,25± 0,02
Homogenate (PG $F_{2\alpha}$)	1,35± 0,03	1,35± 0,05	0,65± 0,04	0,35± 0,05	0,54± 0,04	0,47± 0,03	0,32± 0,01	0,20± 0,01
Mitochondrial-lysosomal (PG $F_{2\alpha}$)	0,73± 0,04	0,73± 0,04	0,55± 0,06	0,20± 0,02	0,33± 0,02	0,17± 0,03	0,23± 0,01	0,14± 0,01

Legend. Mean results of 6-8 experiments shown.

EXPERIMENTAL METHOD

Experiments were carried out on 60 male chinchilla rabbits weighing 2-2.5 kg. In the experiments *in vivo* PG $F_{2\alpha}$ was injected intravenously into the rabbits in a dose of 20 μg/kg (the optimal dose was chosen on the basis of data in the literature for the clinical use of PG). In the experiments *in vitro* PG was added to the incubation medium in doses of 0.1, 0.15, and 0.2 μg/ml. In other experiments, hydrocortisone or deoxycorticosterone acetate (DOCA) were given (intraperitoneally, 10 mg/kg) simultaneously with PG. The effect of these hormones on the lysosomal membranes of eye tissues was demonstrated by the writers previously [2].

The rabbits were killed 1, 3, and 24 h after receiving the injection of PG. The tissues were isolated at 4°C, carefully freed from impurities and homogenized in 0.3 M sucrose made up in 0.01 M Tris-HCl, pH 7.4, and 0.001 M EDTA. Homogenates of the sclera, ciliary body, and cornea were centrifuged at 900g for 20 min. The supernatant was again centrifuged at 6000g for 30 min. The resulting residue was washed and resuspended in sucrose (mitochondrial-lysosomal fraction). The supernatant thus obtained was centrifuged (VAC-601) at 105,000g for 1 h. The cytosol fraction was obtained in the supernatant. Methods of determination of enzyme activity were described previously [1, 10].

EXPERIMENTAL RESULTS AND DISCUSSION

As is clear from Figs. 1 and 2 and from Table 1, 1 h after injection of PG $F_{2\alpha}$ *in vivo* the activity of the enzymes in the various eye tissues studied showed considerable changes. For instance, in the sclera the absolute values of total and free β-galactosidase activity, of total β-glucosidase activity in the homogenate and the mitochondrial-lysosomal fraction, and also of hyaluronidase activity in the homogenates were reduced. However, the free activity expressed as a fraction of the total activity was increased, especially so in the mitochondrial-lysosomal fraction, suggesting that labilization of the lysosomal membranes of the sclera may have taken place through the action of PG $F_{2\alpha}$. Analysis of the experimental results of the ciliary body and cornea in the same way, taking into account the localization of the enzymes studied in the lysosomes, suggests that PG $F_{2\alpha}$ labilizes principally the lysosomal membranes of the sclera and ciliary body but stabilizes them in the cornea. This may also probably explain the appearance of β-galactosidase and β-glucosidase activity (up to 0.6

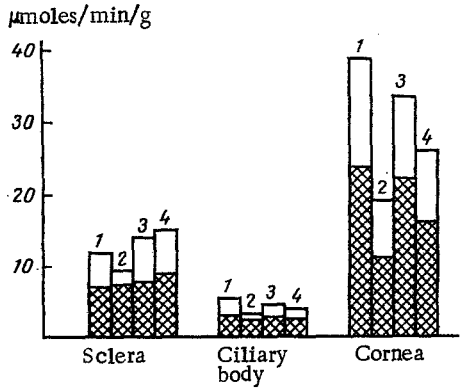

Fig. 2. Effect of PG $F_{2\alpha}$ and hormones *in vivo* on hyaluronidase activity of the eye tissues. Shaded areas represent activity in mitochondrial-lysosomal fraction; unshaded areas) activity in homogenate. 1) Control; 2) PG $F_{2\alpha}$; 3) PG + DOCA; 4) PG + hydrocortisone. Ordinate, enzyme activity (in μmoles/min/g protein).

μmole/min/g protein) in the vitreous body and aqueous humor, where they are not normally found. This hypothesis is confirmed by the very small increase in the yield of these enzymes in the cytosol fraction in the ciliary body and the decrease in their yield in the cornea.

The results are in agreement with data in the literature on the increase in permeability of the cell membranes of the ciliary body and vessels under the influence of PG [12].

In experiments *in vitro* the same pattern of changes was observed in enzyme activity but it was much more marked. The changes in activity increased with an increase in the dose of PG $F_{2\alpha}$.

Following simultaneous administration of hydrocortisone and PG (or DOCA and PG) the hyaluronidase activity differed from normal by a lesser degree than after administration of the hormone alone. This was evidently because of antagonism between the hormones and PG with respect to their action on the lysosomal membranes of the eye tissues, in agreement with data in the literature on relations between PG and steroid hormones [15].

When given by intravenous injection, PG $F_{2\alpha}$ thus causes considerable changes in the enzyme systems of the eye tissues, evidently through its action on the lysosomal membranes.

LITERATURE CITED

1. B. S. Kasavina, P. V. Sergeev, and N. B. Chesnokova, Dokl. Akad. Nauk SSSR, 204, 149 (1972).
2. B. S. Kasavina, T. V. Ukhina, and T. K. Demina, Dokl. Akad. Nauk SSSR, 222, 236 (1975).
3. S. Bergström, R. Ryhage, and B. Samuelsson, Acta Chem. Scand., 16, 501 (1962).
4. P. Bhattachergee, Exp. Eye Res., 18, 181 (1974).
5. B. Ehinger, Exp. Eye Res., 17, 43 (1973).
6. K. Green, Invest. Ophthal., 12, 752 (1973).
7. K. Green and K. Kim, Invest. Ophthal., 14, 36 (1975).
8. E. W. Horton, Physiol. Rev., 49, 122 (1969).
9. E. Karim, The Prostaglandins, Oxford (1972), p. 29.
10. B. S. Kasavina and N. B. Chesnokova, Exp. Eye Res., 15, 1 (1973).
11. K. Masuda, Y. Izawa, and S. Mishima, Jap. J. Ophthal., 17, 167 (1973).
12. H. S. Perkins, Brit. J. Ophthal., 58, 462 (1974).
13. T. Vegge, A. H. Neufeld, and M. B. Sears, Invest. Ophthal., 14, 33 (1975).
14. M. B. Wattzman, Exp. Eye Res., 16, 307 (1973).
15. C. Werning and W. Siegenthaler, Dtsch. Med. Wschr., 95, 2345 (1970).