EFFECT OF DEOXYCORTICOSTERONE ON LYSOSOMAL HYDROLASE ACTIVITY OF THE EYE TISSUES

B. S. Kasavina, P. V. Sergeev, N. B. Chesnokova, and L. M. Konstantinova

UDC 612.84.015.12.014.46: 615.357.453

After intraperitoneal injection of deoxycorticosterone acetate (DOCA), acid phosphatase is increased, glycosidase activity is reduced, and hyaluronidase activity appears in the vitreous body of the rabbit eye where it is not normally found. DOCA causes labilization of the lysosomal membranes of the ciliary body. It is postulated that these enzymes may participate in the mechanism of the increased intraocular pressure produced by the action of deoxycorticosterone.

In recent years the participation of adrenocortical hormones in the regulation of the intraocular pressure has been established [1, 3, 4, 6, 11]. The view is held [5, 9] that the increase in ophthalmotonus induced by corticosteroids is connected with a decrease in mucopolysaccharide breakdown. Lysosomes are mainly responsible for the hydrolysis of mucopolysaccharides. Meanwhile the lysosomes are a target for the action of steroid hormones under the influence of which cell metabolism may change [13].

This paper describes a study of the action of deoxycorticosterone acetate (DOCA) on the lysosomal hydrolases of the eye tissues: the ciliary body, the aqueous, and the vitreous body. Acid phosphatase and enzymes hydrolyzing glycoside bonds in mucopolysaccharides – β-glucosidase, β-galactosidase, and hyaluronidase – were investigated.

EXPERIMENTAL METHOD

Experiments were carried out on male chinchilla rabbits weighing 2.0.-2.5 kg. Free and total activity of the enzymes was determined [2].

A homogenate of the ciliary body was separated into subcellular fractions: nuclei, mitochondria, microsomes, and supernatant. The homogenate of the ciliary body, made up in 0.25 M sucrose with 0.01 M tris-HCl buffer (pH 7.4) and 0.001 M EDTA was centrifuged at 900 g for 20 min. The residue (nuclei + pigment granules) was washed twice and rehomogenized. The pooled supernatants were centrifuged at 6000 g for 30 min. The residue was washed (fraction of mitochondria) and resuspended in sucrose. The pooled supernatants were centrifuged at 105,000 g for 60 min. Detergent (Triton X-100) was added to the incubation medium to disintegrate the subcellular structures.

EXPERIMENTAL RESULTS AND DISCUSSION

Total acid phosphatase activity in the ciliary body, aqueous, and vitreous body was increased (Fig. 1a, b, c) 1 h after intraperitoneal injection of DOCA (10 mg/kg), the increase affecting the percentages of both free and total activity of the enzyme. These changes were still further increased after 4 h, but by 12 h the acid phosphatase activity was back to normal. The increase in acid phosphatase activity may evidently affect phagocytosis and, in the ciliary body, it may also perhaps influence the secretion of the aqueous humor.

Moscow Helmholtz Research Institute of Eye Diseases. Department of Molecular Pharmacology and Radiobiology, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR, S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 76, No. 10, pp. 48-51, October, 1973. Original article submitted June 23, 1972.

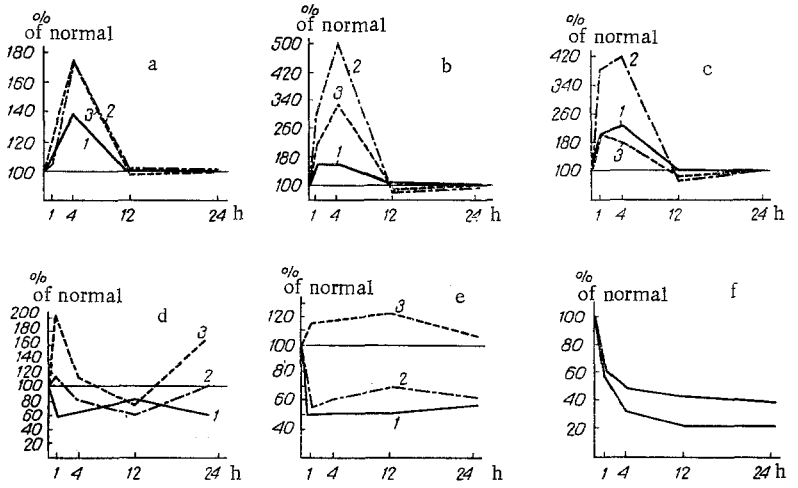

Fig. 1. Effect of intraperitoneal injection of DOCA on acid phosphatase activity in the ciliary body (a), aqueous (b), and vitreous body (c); on β-glucosidase (d) and β-galactosidase activity (e) of the ciliary body, and on hyaluronidase activity (f). In a, b, c, d, e: 1) total enzyme activity; 2) free activity; 3) relative percentage of free and total activity. In f: 1) ciliary body; 2) aqueous.

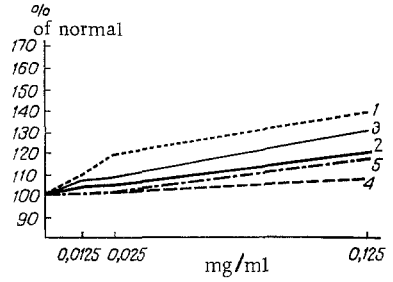

Fig. 2. Effect of DOCA in vitro on relative percentage of free and total acid phosphatase activity in ciliary body (1), aqueous (2), and vitreous body (3) and on relative percentage of free and total activity of β-glucosidase (4) and β-galactosidase (5) in the ciliary body. Abscissa, concentration of DOCA (in mg/ml); ordinate, free enzyme activity as percentage of total; values before DOCA treatment taken as 100%.

After intraperitoneal injection of DOCA the activity of β-galactosidase and β-glucosidase was reduced (Fig. 1d), e) throughout the period of observation (24 h), and (just as in the case of acid phosphatase) the relative percentages of both free and total activity of both enzymes increased. In the study of β-glucosidase biphasic changes were found in the ratio between the free and total activity of the enzyme, whereas for β-galactosidase the ratio was increased for 24 h.

DOCA had a substantial effect on hyaluronidase activity (Fig. 1, f). Activity of the enzyme was sharply reduced for 24 h in the ciliary body and aqueous, while in the vitreous, hyaluronidase activity which is not normally present was detected.

During the study of the action of intraperitoneally injected DOCA on the distribution of acid hydrolases in the subcellular fractions of the ciliary body (Table 1) an increase in the relative percentage of activity in the supernatant and of the total activity ($P < 0.05$) was observed for all enzymes, showing labilization of the lysosomes and liberation of enzymes into the cytoplasm. These results showing the labilizing action of DOCA on the lysosomes are in agreement with results obtained [8, 12] for liver lysosomes in experiments in vitro.

As a result of the direct action of DOCA on the eye tissues an increase in the relative percentage of free and total enzyme activity was observed (Fig. 2). For the acid phosphatase of the ciliary body and vitreous body the increase in this ratio took place at low concentrations of the hormone, but for acid phosphatase of the vitreous and for β-glucosidase and β-galactosidase of the ciliary body it took place only at a concentration of 0.125 mg/ml. This fact demonstrates differences in the strength of the bond between the enzymes and membranes and also, perhaps, differences in the sensitivity of the eye tissues to deoxycorticosterone.

In experiments in vitro DOCA considerably reduced the hyaluronidase activity in the ciliary body and aqueous; the degree of the decrease in enzyme activity grew with an increase in dose of the hormone. In the vitreous the direct action of DOCA caused no appearance of hyaluronidase activity. The possibility cannot be ruled out that the appearance of hyaluronidase activity in the vitreous in the experiments in vivo was due to penetration on this enzyme from the neighboring tissues and, in particular, from the retina.

TABLE 1. Effect of Intraperitoneal Injection of DOCA on Cellular Distribution of Acid Hydrolases of the Ciliary Body

Enzyme	Control					1 h after injection of hormone				
	nuclei	mitochondria	microsomes	supernatant	total activity	nuclei	mitochondria	microsomes	supernatant	total activity
Acid phosphatase	0,9±0,04 (2,9)	14,1±0,48 (53,3)	10,0±0,32 (34,9)	2,5±0,09 (8,8)	27,6±0,53 (100,0)	1,1±0,04 (3,4)	15,1±0,39 (48,9)	11,8±0,34 (38,1)	2,9±0,12 (9,5)	30,9±0,48 (100,0)
β-glucosidase	0,08±0,002 (3,4)	1,39±0,041 (60,8)	0,66±0,012 (29,0)	0,15±0,005 (6,8)	2,30±0,063 (100,0)	0,05±0,001 (2,9)	0,96±0,031 (58,7)	0,48±0,009 (29,4)	0,14±0,003 (9,0)	1,63±0,052 (100,0)
β-galactosidase	0,13±0,004 (3,9)	2,14±0,061 (67,0)	0,55±0,009 (18,3)	0,32±0,013 (10,8)	3,14±0,072 (100,0)	0,07±0,002 (3,5)	1,25±0,033 (62,5)	0,42±0,008 (21,0)	0,26±0,009 (13,0)	2,00±0,083 (100,0)
Hyaluronidase	1,6±0,12 (2,1)	40,8±0,98 (57,2)	23,0±0,53 (32,7)	5,6±0,84 ((8,0)	70,1±2,5 (100,0)	1,1±2,2 (10,4)	4,3±0,32 (45,1)	2,1±0,26 (22,0)	2,2±0,23 (22,5)	9,6±0,78 (100,0)

When injected intraperitoneally DOCA thus produced significant changes in the enzyme systems of the eye tissues, not only those functioning actively but also those with a comparatively low level of metabolism (the vitreous). Presumably the hormone either penetrates directly into the eye or the eye tissues react to the general response of the body to the hormone. The first explanation is supported by the high permeability of the blood-aqueous barrier for lipid-soluble substances [7] and also by the presence of steroid hormones in the aqueous [10]. Hydrocortisone, which also increases intraocular pressure, has a similar but shorter action on the total hydrolase activity of the eye tissues [2]. The increase in ophthalmotonus observed as a result of the action of deoxycorticosterone and hydrocortisone is presumably connected with the ability of these hormones to reduce glycosidase activity in the ciliary body and aqueous, which may cause mucopolysaccharides to accumulate in these tissues and may activate hyaluronidase in the vitreous.

LITERATURE CITED

1. A. Ya. Bunin, V. M. Pantieleva, and G. Ya. Chernyavskii, in: Proceedings of a Symposium on the Pathogenesis of Primary Glaucoma [in Russian], Moscow (1970), p. 53.
2. B. S. Kasavina, P. V. Sergeev, and N. B. Chesnokova, Dokl. Akad. Nauk SSSR, 204, 1479 (1972).
3. A. A. Filina and V. M. Pantieleva, Vestn. Oftal'mol., No. 4, 8 (1971).
4. A. E. Shevalev and O. P. Kopp, in: Glaucoma [in Russian], Moscow (1967), p. 5.
5. H. Bernstein and B. Schwarz, Arch. Ophthal. (Chicago), 68, 742 (1962).
6. T. A. S. Boyd and L. E. McLeod, Ann. New York Acad. Sci., 117, 597 (1964).
7. H. Davson, in: The Eye, Vol. 1, New York (1962), p. 167.
8. C. De Duve, R. Wattiaux, and M. Wibo, Biochem. Pharmacol., 9, 97 (1961).
9. J. Francois, Arch. Ophthal. (Paris), 25, 117 (1965).
10. H. Green, H. S. Kroman, and L. H. Leopold, Am. J. Ophthal., 44, 91 (1957).
11. E. U. Linner and P. J. Wistrand, Exp. Eye Res., 2, 148 (1963).
12. G. Weissmann, Biochem. Pharmacol., 14, 525 (1945).
13. G. Weissmann, in: A Symposium on the Interaction of Drugs and Subcellular Components in Animal Cells (1968), p. 203.