EFFECT OF HORMONES ON LIPID PEROXIDATION IN THE TISSUES OF THE EYE

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UDC 612.84.015.3:612.397.2.018.2

The effect of hormones with different spectra of action on peroxidation of unsaturated fatty acids of lipids of the eye tissues was investigated. Various hormones were shown to have both an antioxidant and a prooxidant action on the sclera, cornea, and ciliary body, depending on the dose of the hormone, the duration of its action, and other conditions. The possibility that lipid peroxidation processes may participate in the development of ophthalmopathies is discussed.

KEY WORDS: lipid peroxidation; hormones; eyes.

Stimulation of lipid peroxidation (LPO) in biological membranes is known to be one mechanism of development of pathological processes in the cell [1, 2]. Hormones have been shown to affect the intensity of this process in cellular and subcellular structures [5]. Biochemical changes in the organ of vision in certain pathological states accompanied by endocrine disturbances are perhaps also connected with the effect of hormones on LPO in the eye tissues. However, the problem of LPO in the eye has not yet been studied.

The object of this investigation was to study peroxidation of unsaturated fatty acids of lipids in intact eye tissues (sclera, cornea, and ciliary body) and also under the influence of hormones with different spectra of action: the glucocorticoid hydrocortisone, the mineralocorticoid deoxycorticosterone (DOC), the thyroid hormone thyroxine, and the pituitary thyrotrophic hormone.

EXPERIMENTAL METHOD

Experiments were carried out on male chinchilla rabbits weighing 2-2.5 kg. The intensity of spontaneous LPO in the eye tissues was judged by determining their content of malonic dialdehyde (MDA), an end product of peroxidative reactions [4]. MDA was determined in homogenate and in the lysosomal fraction of the eye tissues by the method of Tappel and Zalkin [7], modified by the present writers for eye tissues. Homogenates and lysosomes were obtained by the method described previously [3]. The incubation mixture in a volume of 1 ml contained 50 mM potassium-phosphate buffer (pH 7.4) and from 2 to 5 mg of the protein preparation. Protein was determined by Lowry's method [6]. In the experiments in vivo the hormones were injected intraperitoneally in a dose of 10 mg/kg body weight. In the experiments in vitro the hormones were added to the incubation medium in doses of: 10^{-3}, 10^{-6}, and 10^{-8}M.

EXPERIMENTAL RESULTS

LPO was studied in intact eye tissues. It will be clear from Table 1 that all the tissues studied normally contain MDA. Its highest level was found in the cornea, evidence that lipid and peroxidation takes place more intensively there than in the sclera and ciliary body. However, in the lysosomal fraction the quantity of this compound was equal in all tissues studied, possibly because of their identical content of lysosomes (in vols. %). The MDA level in the sclera showed considerable variability of the intensity of LPO compared with the cornea and ciliary body.

Since the MDA content in the tissues of the eye depends on the time elapsing after sacrifice, a control experiment was set up in which the test tissues were incubated for different times. After incubation for 30 min clear changes were observed in the MDA content in the test tissues, which increased until 60 min (compared with normal). For instance, after incubation of the sclera for 30 min the MDA content rose from 0.85 μmoles/g protein to 1.1, and after 60 min to 1.5. The same dependence of the intensity of peroxidation on time also was observed under the influence of hormones. After the action of hydrocortisone (10^{-3}M), on the sclera for 30 min the MDA level increased to 1.2, and after its action for 60 min to 1.64.

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. 89, No. 1, pp. 19-20, January, 1980. Original article submitted February 7, 1979.

0007-4888/80/8901-0022 $07.50

TABLE 1. MDA content in Eye Tissues (M ± m)

Tissue	MDA, μmoles/g protein	
	homogenates	lysosomes
Sclera	0,85±0,2	0,40±0,10
Cornea	0,98±0,01	0,44±0,03
Ciliary body	0,70±0,05	0,48±0,02

Note. Mean results of 6-10 experiments are given (in Table 2 also).

TABLE 2. Effect of Hormones on LPO in Eye Tissues (M ± m)

Hormone	Dose of hormone	MDA, μmoles/g protein		
		sclera	cornea	ciliary body
Control		1,50±0,10	1,62±0,05	1,33±0,05
Hydrocortisone	10 mg/kg	0,82±0,10†	0,93±0,07‡	0,83±0,10†
	10^{-6}M	0,97±0,06†	0,73±0,04‡	0,68±0,05‡
	10^{-3}M	1,64±0,12	1,65±0,10	1,40±0,07
Deoxycorticosterone	10 mg/kg	0,88±0,02‡	0,97±0,03‡	0,87±0,10†
	10^{-6}M	0,98±0,10†	0,93±0,05‡	0,78±0,10‡
	10^{-3}M	1,64±0,10	1,89±0,10	1,60±0,01
Thyroxine	10 mg/kg	1,68±0,05	1,85±0,10	1,48±0,10
	10^{-6}M	0,98±0,05†	1,28±0,06†	0,93±0,03‡
	10^{-3}M	1,62±0,06	1,89±0,10*	1,56±0,10
Thyrotrophic hormone	10 mg/kg	1,56±0,05	1,74±0,06	1,40±0,05
	10^{-6}M	1,40±0,10	1,36±0,05†	1,25±0,10
	10^{-3}M	1,60±0,06	1,70±0,10	1,50±0,10

*P < 0.05, †) P < 0.01, ‡) P < 0.001 levels of significance of difference from control. Incubation time 60 min.

The results of the study of the effect of the various hormones on LPO in the eye tissues are given in Table 2.

During the study of the action of hydrocortisone on the MDA content in the eye tissues in vivo a decrease was found in this LPO product in all tissues of the eye tested, and also in the lysosomes of the cornea and ciliary body.

In the experiments in vitro hydrocortisone in a dose of 10^{-8}M had virtually no effect on the MDA level. In a dose of 10^{-6}M, however, the hormone led to a statistically significant decrease in the MDA level in the cornea, but produced virtually no change in its level in the sclera or ciliary body. With an increase in the dose of hydrocortisone to 10^{-3}M the MDA content in the cornea and ciliary body remained within the previous control values. In a dose of 10^{-6}M hydrocortisone can evidently induce an antioxidant effect. This action of a relatively high dose of hydrocortisone can perhaps be attributed either to competition for initiators of free-radical oxidation between the hormone and fatty acids of the phospholipids, or to embedding of the hydrocortisone into the membranes, with a change in their structural properties, affecting the process of peroxidation. Another possibility is that this hormone or its metabolites possess antiradical properties.

On administration of DOC in experiments in vivo a statistically significant fall in the MDA level was observed in all tissues tested.

In the experiments in vitro DOC behaved as an antioxidant or as a prooxidant depending on the dose used. For instance, in doses of 10^{-8} and 10^{-6}M the hormone caused a decrease in MDA both in homogenates and in lysosomes of the sclera and cornea. In a dose of 10^{-3}M, on the other hand, the hormone led to an increase in the intensity of peroxidative reactions in the eye tissues tested. This action of the hormone may be the result of inactivation of sulfhydryl groups of cellular antioxidants or the result of some other interaction with true antioxidants normally present in the tissues.

A similar effect on the eye tissues was found as a result of the action of thyroxine both in vivo and in vitro in a dose of 10^{-3}M.

Thyrotrophic hormone caused changes in the test tissues similar to those produced by thyroxine, although they were less marked.

These results showing a high intensity of lipid peroxidation in the intact tissues of the eye may be evidence that LPO reactions participate in the normal metabolic activity of the eye. The different levels of lipid

peroxidation in the different eye tissues may evidently determine differences in the resistance of the sclera, cornea, and ciliary body to pathological factors. This could be one explanation of the different response of cellular organelles to hormonal and other influences [3]. The effect of hormones on peroxidation of unsaturated fatty acids of lipids in cellular and lysosomal membranes of the eye tissues and also its dependence on the dose of the preparation and the type of tissue must be taken into account when endocrine preparations are used in ophthalmology.

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ROLE OF ANTIOXIDANTS IN THE REGULATION OF LIPID PEROXIDATION OF PRESERVED TISSUES DURING PROLONGED STORAGE

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UDC 612.015.32:547.87

The effect of natural and artificial antioxidant on accumulation of lipid peroxidation (LPO) products in preserved tissues during prolonged storage was studied. At the end of the characteristic period for each method of preservation the intensity of LPO was shown to switch from a low level to a self-accelerating regime. When antioxidants were used this critical transition appeared much later. Of the antioxidants tested, vitamin E and propyl gallate were the most effective.

KEY WORDS: preservation of tissues; lipid peroxidation; antioxidants.

Bioantioxidants are one of the most important factors in the maintenance of lipid oxidation reactions at a definite, stationary level [1]. The presence of antioxidants is an essential condition for the structural integrity of membranes, and their content in a biosystem characterizes its adaptive possibilities – its ability to function normally under the influence of unfavorable factors also. In the living organism changes in the intensity of lipid peroxidation (LPO) in the tissues are compensated by adequate changes in the content of antioxidants [2]. By contrast, in isolated surviving tissues the content of endogenous antioxidants is limited to the reserves actually present, and for that reason activation of LPO leads to rapid utilization of the antioxidants [7].

It was shown previously that preserved tissues remain capable of survival only as long as the free-radical oxidation reaction is maintained in the steady state [4]. Transition of oxidative processes into an unsteady state leads to undesirable consequences for the cell such as increased permeability of biomembranes, inhibition of activity of many enzymes, depression of synthetic processes, disintegration of cell metabolism, and ultimately necrosis [4, 3].

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(Presented by Academician of the Academy of Medical Sciences of the USSR G. G. Gasanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, pp. 21-23, January, 1980. Original article submitted June 15, 1979.

0007-4888/80/8901-0024$07.50