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CYCLIC NUCLEOTIDES IN EXPERIMENTAL GLAUCOMA

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There is evidence in the literature that the sympathetic nervous system is involved in the pathogenesis of glaucoma [2, 3, 10, 13]. However, these data are largely contradictory.

The concept of the role of chronic activation of the β-receptor apparatus of the eye as an important pathogenetic mechanism of glaucoma development was put forward by Kryzhanovskii et al. [4]. Their investigations showed that blockade of the sympathetic effect with lithium hydroxybutyrate [7], like β-receptor blockade, can prevent the development of experimental glaucoma, whereas α-receptor blockade may facilitate its development [4, 5]. It is an interesting fact that noradrenalin, which activates primarily α-receptors, can weaken the galucomogenic effect of adrenalin, which activates mainly β-receptors [6].

We know that the hormonal and neurotransmitted signal from β-adrenoreceptors is realized by cyclic AMP (cAMP). The state of β-reception can therefore be judged to some extent by the intracellular cAMP level. Changes in cyclic nucleotide (cAMP and cGMP) concentrations have been found in the aqueous humor of glaucoma patients. However, determination of the cyclic nucleotide levels in the aqueous humor is not sufficient to allow the state of this system to be judged in the eye tissues.

The aim of this investigation was to study concentrations of cyclic nucleotides cAMP and cGMP in different parts of the eyes of rabbits with experimental glaucoma.

A model of experimental glaucoma developed in the Academician V. P. Filatov Odessa Research of Eye Diseases [9], induced by chronic intravenous injection of adrenalin, was used.

EXPERIMENTAL METHOD

Experiments were carried out on "White Giant" rabbits about 2 years old. The control animals were of the same age. Glaucoma was induced by intravenous injection of 0.1 ml of 1:1000 adrenalin solution on alternate days for 3 months [9]. The intraocular pressure (IOP) was measured tonometrically daily. Material was taken for biochemical study 1.5 months after the beginning of adrenalin injections, when the initial changes in IOP had appeared, and 1.5 years after cessation of the 3-month periods of adrenalin injections, when the animals had developed persistent and advanced glaucoma. Weighed samples of eye tissues, put into test tubes with 2 ml of ethanol, purified with silver nitrate and converted into the absolute form, were homogenized with quartz sand and centrifuged at 2000 rpm for 15 min. The residue was washed with 1 ml of alcohol and recentrifuged under the same conditions. The pooled supernatent — an alcoholic tissue extract — was evaporated at 55°C. Before determination of cyclic nucleotides the evaporated extracts were diluted with Tris-EDTA buffer, pH 7.5 [11].

The cAMP and cGMP concentrations were determined in tissues of the retina, the vascular coat of the eye, ciliary body, and iris by radioimmunoassay, using commercial kits ("Amersham International," England). Radioactivity was counted on a RackBeta scintillation counter (LKB, Sweden). The results were subjected to statistical analysis.

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TABLE 1. cAMP Concentration (in pmoles/g tissue) in Different Eye Tissues of Rabbits with Experimental Glaucoma (M ± m)

Eye tissue	cAMP concentration (n = 8)		
	in normal animals (control)	after adrenalin injections for 1.5 months	18 months after end of adrenalin injections
Iris	992,3±120,3	1434,8±99,4 $p<0,02$	3116,2±161,6 $p<0,001$
Ciliary body	614,4±97,9	1082,0±59,9 $p<0,001$	1655,8±107,0 $p<0,001$
Retina	2428,6±318,8	1424,7±98,6 $p<0,01$	2395,8±248,0 $p>0,5$
Vascular coat	1488,9±122,6	1661,1±79,6 $p<0,2$	2171,5±173,6 $p<0,01$

TABLE 2. cGMP Concentration (in pmoles/g tissue) in Various Eye Tissues of Rabbits with Experimental Glaucoma

Eye tissue	cGMP concentration (n = 8)		
	in normal animals	after adrenalin injections for 1.5 months	18 months after end of adrenalin injections
Iris	152,1±21,9	154,8±21,8 $p>0,5$	168,0±20,9 $p>0,5$
Ciliary body	87,1±12,6	105,5±16,8 $p<0,2$	84,1±13,3 $p>0,5$
Retina	1457,2±181,6	1331,4±129,2 $p>0,5$	776,3±146,7 $p<0,02$
Vascular coat	739,0±67,9	652,4±28,3 $p<0,2$	915,0±101,2 $p<0,1$

EXPERIMENTAL RESULTS

During the development of experimental glaucoma the cAMP concentration in the eye tissues rose significantly (Table 1).

During the period of adrenalin injections (1.5 months) the cAMP concentration in the retina rose by 45% compared with the control, in the ciliary body it increased by 76%, and in the vascular coat by 12%. The cAMP concentration of the iris of animals with developed glaucoma (18 months after the end of injections) was 3.1 times higher than in the control and 2.2 times higher than during adrenalin injections, i.e., than in the initial stage of experimental glaucoma. The cAMP concentration in the ciliary body was 2.2 times higher than in the control and 53% higher than in the initial stage of glaucoma; the corresponding figures for the vascular coat were 46% and 31%.

In the late stages of glaucoma the cAMP concentration in the retina was virtually equal to its initial level, but during the period of adrenalin injections the cAMP level fell by 71% compared with the control.

Meanwhile no distinct change was found in the cGMP concentration in the eye tissues tested (Table 2).

The cGMP level in the iris and ciliary body was virtually unchanged. In the vascular coat a small but not significant fall in the cGMP concentration (13.3%) was observed during the period of adrenalin injections, whereas in the late stages of observation its concentration was increased, though not significantly, by 24% compared with the control. In the retina the cGMP concentration fell, but not significantly, during the period of adrenalin injections, but fell significantly by 87.7% in the late stages of observation compared with the control.

Calculation of the ratio between the cAMP and cGMP concentrations (Table 3) revealed changes compared with the control. This parameter was increased particularly in the iris (by 3 times) and in the ciliary body (by 2.5 times).

TABLE 3. Ratio of cAMP/cGMP Concentrations in Different Eye Tissues of Rabbits with Experimental Glaucoma

Eye tissue	In normal animals (control)	After adrenalin injections for 1.5 months	18 months after end of adrenalin injections
Iris	6,5	9,2	18,5
Ciliary body	7,0	9,3	19,6
Retina	1,6	1,0	3,0
Vascular coat	2,0	2,5	2,3

Since the effects of β-adrenoreceptors are mediated through changes in the cAMP concentration [8], the results point to enhanced β-adrenoreceptor function in the eye, especially in its anterior portion (the iris and ciliary body) during the development of experimental glaucoma. Increased sensitivity to catecholamines may perhaps develop under these circumstances, either through a change in the number of β-adrenoreceptors in the membranes or a change in the level of adenylate cyclase function [14].

A different picture is observed in the retina. The cAMP level falls during the stage of adrenalin injections, and in the stages of advanced glaucoma it returns to normal. The cAMP level in the vertebrate retina is known not to be under β-adrenoreceptor control, but it is linked with activity of photoreceptors transmitting signals from dopaminergic neuroregulators, and which also take part in certain other metabolic processes [15]. Possibly under the influence of the injected adrenalin the activity of these processes in the retina declines.

Cyclic GMP is unconnected with β-adrenoreceptor activation, and for that reason synthesis of this nucleotide is not increased in the eye tissues of rabbits with experimental glaucoma. The cGMP level in the cells is controlled by cholinergic, α-adrenergic, and histamine-like substances and by certain prostaglandins [12].

The investigations thus showed that the development of experimental glaucoma in rabbits is accompanied by a rise of the cAMP level in the tissues, its synthesis being initiated by β-adrenoreceptors (especially in the iris and ciliary body). This effect can be explained by intensification of β-adrenergic control in these particular eye tissues. Elevation of the cGMP level, unconnected with β-adrenoreceptor activation, does not take place. The results are in agreement with the previous concept that β-adrenoreceptor activation in the eye is a factor in the pathogenesis of glaucoma [4-6].

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