EARLY CHANGES IN 5'-NUCLEOTIDASE ACTIVITY IN HERIDITARY DEGENERATION OF THE RETINA

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When the causes and early biochemical manifestations of hereditary degeneration of the retina in man and animals are studied, an important role is ascribed to nucleotides and their metabolic systems. Experiments on affected animals have shown early changes in activity and properties of the phosphodiesterase (PDE) of the cyclic nucleotides in the retina [7, 11], and some workers attach decisive importance to this disturbance in the development of the disease [13]. In clinical practice, observations over many years have shown the effectiveness of the preparation "Enkad," which is an RNA hydrolysate, i.e., a preparation containing nucleotides and their derivatives, in the stabilization of the disease in man [1].

The writers previously were unable to confirm data indicating a decrease in the PDE content in the retina of rats in the first stages of the disease [3]. It accordingly was decided to study the activity of other retinal enzymes connected with nucleotide conversion and, in particular, 5'nucleotidase (ND), and to compare these findings with the observed values of PDE activity. ND is particularly interesting because of the possible role of this enzyme in nucleic acid synthesis, in the regulation of intercellular interactions [12] and, in particular, in processes of metabolism between neurons and glia of the retina [10].

EXPERIMENTAL METHOD

The test objects were the retina and liver of rats with hereditary degeneration of the retina (Campbell line) and control animals (Wistar rats) of different ages. One retina was homogenized in 1 ml of 10 mM Tris-HCl buffer, pH 7.4, with 0.5 mM EDTA and 1 mM β-mercaptoethanol; liver homogenate was prepared from 0.1 g of tissue in 2 ml of the same buffer. Membrane fragments of liver were obtained in the form of residue after centrifugation of the homogenate at 12,000g for 20 min. ND activity was determined in the homogenate of the retina and of membrane fragments of the liver as inorganic phosphate formation in medium containing 40 mM Tris-HCl-buffer, pH 7.5, 6 mM $MgCl_2$, and 2 mM 5'-AMP during incubation for 20 min at 37°C. PDE activity was found in the supernatant after centrifugation of the retinal homogenate (1200·20 min) by the method in [6], using 1 mM cyclic GMP as the substrate. Activity of Na^+, K^+-ATPase in the homogenate of the retina and membrane fragments of the liver was determined as in [5]. Inorganic phosphate was estimated quantitatively by the method in [8] and protein by the method of Lowry et al. [12]. The content of rhodopsin, the marker of photoreceptor membranes in the retina, was measured spectrophotometrically [5]. PDE activity was calculated relative to retinal protein and to its rhodopsin content.

EXPERIMENTAL RESULTS

A substantial increase in PDE activity was found in the retina of the affected animals compared with that in animals aged between 14 and 30 days (Table 1). Similar results were obtained in rats of a different line with hereditary degeneration of the retina [11]. However, when PDE activity was calculated relative to rhodopsin, there was practically no difference in the values obtained in healthy and affected animals (Table 1). Differences in the character of the changes in retinal PDE activity when calculated by different methods can

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TABLE 1. Retinal PDE Activity in Affected and Healthy Rats during Postnatal Development

Age of animal, days	PDE activity in rat retina			
	Wistar	Campbell	Wistar	Campbell
	nmoles inorganic phosphorus/mg protein/min		nmoles inorganic phosphorus/mole rhodospin/min	
11—12	19,9	23,5	107	129
14—17	25	52	50	49
19—21	34	52	32	37
24—25	37	—	22	21
30—33	29	59	24	27
38—41	34	44	29	32
45—50	40	35		

Legend. Mean values of three experiments are given.

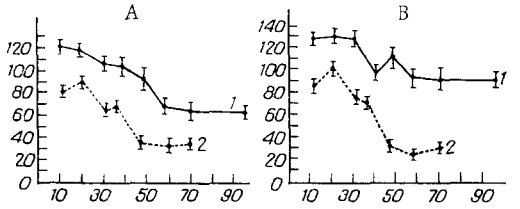

Fig. 1. Changes in 5'-nucleotidase activity in retina of Wistar (1) and Campbell (2) rats during postnatal development. A) Specific activity of enzyme; B) total activity (per retina). Abscissa, time (in days); ordinate, ND activity (A: in nmoles inorganic phosphorus/mg protein/min; B) the same, per retina).

evidently be explained by the fact that about 80% of the retinal enzyme [14] is concentrated in the photoreceptor membranes, the quantity of which varies substantially during the development of the disease: During the first month of life, parallel with formation of the outer segments of the retinal rods (OSR), containing the photoreceptor membranes, unphagocytosed fragments of OSR accumulate in the affected rats, and then they are gradually destroyed [9]. It can be tentatively suggested that the changes in specific activity of PDE, observed by the present writers and others [7, 11], are not real changes in the quantity or activity of the enzyme, but merely reflect a change in the quantity of structures containing the enzyme. Consequently, changes in retinal PDE activity in the affected animals cannot be regarded as an early biochemical trait of the disease, nor can the primary role in the mechanism of its development be ascribed to these disturbances [11].

Different results in principle were obtained when retinal ND activity was compared in affected and healthy animals. A preliminary study of the distribution of ND activity among subcellular fractions of the rat retina showed that about 20% of the total activity in the retina was contained in the layer of photoreceptor membranes, in agreement with data obtained for the bovine retina [15]. The total activity calculated per retina and the specific activity of this enzyme in rats of the Campbell strain were significantly lower, even in the early period of life (from the 10th day) than in healthy rats (Fig. 1). Recalling that the photoreceptor membranes contain only 20% of the nucleotidase activity of the whole retina and that a decrease in the activity of this enzyme in the affected rats was found before OSR formation, it can be tentatively suggested that the cause of the changes in activity of this membrane enzyme was connected with a disturbance of the membranes of the nonphotoreceptor layers of the retina. This is a fact of fundamental importance, for it is evidence of disturbances in the cells of the retina and, in particular, in its membrane structures in the early stages

TABLE 2. 5'Nucleotidase and Na^+, K^+-ATPase Activity (in nmoles inorganic phosphorus/mg protein/min) of Membrane Fractions of Rat Retina and Liver

Test object	Enzyme	Age of animal, days	Activity	
			Wistar	Campbell
Retina	Na^+,K^+-ATPase	11	42	40
"	Na^+,K^+-ATPase	29	126	134
Liver	5'-Nucleotidase	10—11	70	81
"	The same	29—32	59*	71
"	" "	60—70	61	62

Legend. Results of two experiments are given; asterisk indicates that the figure given is the result of three experiments.

(before appearance of OSR). The fact that changes in ND activity could be detected in the retina of affected animals in the early period of postnatal life means that these changes can be used for the early diagnosis of the disease.

Attention must also be drawn to the dynamics of changes in ND activity in the retina of healthy animals during postnatal development. During development of Wistar rats there was a decrease not only in the specific, but also in the total activity. This fall in activity could be due to certain regulatory mechanisms of this enzyme acting during ontogeny of normal animals not only in the retina, but also in other tissues and, in particular, in the liver [4]. Changes in ND activity in the retina of the affected rats may perhaps be associated with disturbance of the mechanisms. It is important to note that activity of another enzyme in the plasma membrane, namely Na^+, K^+-ATPase, was equal in the retinas of affected and healthy rats during the first months of postnatal life (Table 2).

When ND activity was studied in membrane fragments isolated from the liver of healthy and affected animals, no changes similar to those found in the retina were observed (Table 2).

The results suggest that changes in ND activity in the retina with rats with hereditary degeneration of that structure are specific and can be associated both with a change in the quantity of enzyme protein in the retina and with a change in its activity. The question of what causes the changes in ND activity in the retina of the affected rats and of what the significance of these changes may be to the development of retinal degeneration will be examined in future research.

LITERATURE CITED

1. A. P. Avtsyn, B. B. Fuks, M. E. Shabanova, et al., Vestn. Akad. Med. Nauk SSSR, No. 1, 71 (1977).
2. N. P. Dmitrenko, Ukr. Biokhim. Zh., 5, No. 1, 114 (1980).
3. I. A. Ostapenko and G. G. Chusova, Byull. Éksp. Biol. Med., No. 2, 211 (1980).
4. C. Agnisola, L. Foti, and I. T. Genoino, Mech. Age. Dev., 13, 227 (1980).
5. A. L. Berman, A. M. Azimova, and F. G. Gribakin, Vision Res., 17, 527 (1977).
6. W. Y. Cheung, Biochim. Biophys. Acta, 191, 303 (1969).
7. A. J. Dewar, G. Barron, and J. Richmond, Exp. Eye Res., 21, 299 (1975).
8. H. Eible and W. E. Lands, Anal. Biochem., 30, 51 (1969).
9. W. L. Herron, B. W. Riegel, and M. L. Rubin, Invest Ophthalmol., 10, 54 (1971).
10. S. T. Hussain and G. W. Kreutzburg, Neuroscience, Suppl. 5, S1 (1980).
11. R. N. Lolley and D. B. Farber, Exp. Eye Res., 20, 585 (1975).
12. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
13. H. W. Reading, Neurochemistry, 1, 477 (1980).
14. R. M. Robb, Invest. Ophthalmol., 17, 476 (1978).
15. W. F. Zimmerman, F. J. M. Daemen, and S. L. Bonting, J. Biol. Chem., 251, 4700 (1976).