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The Activity of Some Lysosomal Enzymes of the Thymus in Rabbits

Aktywność niektórych enzymów lizosomalnych grasicy u królików różnych ras

All the living creatures are characterized by a genetically encoded eagerness to maintain the stability of the internal environment of the organism. Among the structures affecting a homeostasis, a significant role is played by the lymphatic system. Individual organs of this system like: thymus, spleen, lymph nodes, lymph ring of the pharynx, lymph texture of alimentary and respiratory tracts play an important role in the immunological process of the organism.

Thymus, included in the group of primary lymph organs, is poorly known. It has a directing influence on the development of the so-called secondary lymph organs.

In the animals subject to the activity of different pathogenic agents, disturbances of a number of metabolic reactions have been noticed. The degree of the changes in the intensity of these processes can be reflected using different physiological indices. Among these we can have the determination of lysosomal enzyme activity (4, 7). Lysosomal interval being one of the first cellular systems responds by starting the defense mechanism in case of homeostasis disturbance (5, 9). To determine the changes taking place in individual organs, we must know their cellular physiological indices. Therefore, the aim of the present paper is to compare the activities of lysosomal thymus enzymes in rabbits of different breeds.

MATERIAL AND METHODS

The investigations have been carried out on 44 male rabbits with the weight about 2500 g, coming from the breeding of the Department of Genetics and Animals Breeding, The Academy of Agriculture, Cracow. These animals included the following breeds: New Zealand white (NZ,

black-bay (CZP), and two-directional hybrids: NZ female and CZP male (NZX) or CZP female and NZ male (CZPX).

On the 140th day of life the rabbits were killed using a traditional method and bled out. Then, thymi were collected and frozen to −20°C.

The thymi were defrosted in 0.9% NaCl and homogenized in 5 ml of 0.3 M saccharose at the temperature of melting ice 3 × 20 s. with 15 s. intervals. The homogenate obtained was rotated for 10 min at 3,200 r.p.m. The sediment was taken away, and the supernatant rotated again for 20 min at 14,000 r.p.m. in +4°C (K-24 Janetzki centrifuge). The supernatant was then poured into test tubes and used for the determination of free enzyme activity. The sediment was suspended in 5 ml of 0.3 M saccharose with Triton X-100 and left for 24 hrs at +4°C. Triton was used as the agent breaking lysosomal membranes (2). After 24 hrs the solution was rotated for 20 min at 14,000 r.p.m. at +4°C. The supernatant was used to determine the activity of bound lysosomal enzymes.

The principle of determining the activity of lysosomal enzymes: acid phosphatase, lipase, sulphatase and β-galactosidase consisted in decomposition of appropriate substrata by these enzymes, and release of free 4-methyloumbeliferol from them (1).

Sigma preparations have been used as substrates:

a) for acid phosphatase — 45 g of 4-methyloumbeliferol phosphate disoda salt dissolved in 100 ml of acetate buffer 0.1 M with *pH* 5.0;

b) for β-galactosidase — 51 mg of 4-methyloumbeliferol-β-D-galactopyranoside dissolved in 100 ml of citrate buffer 0.1 M with *pH* 3.6;

c) for lipase — 62.94 mg of 4-methyloumbeliferol palmitate dissolved in 10 ml of acetone and diluted by 10 times with acetate buffer 0.1 M with *pH* 5.0 with addition of 0.1% Triton X-100 before determination;

d) for sulphatase — 44.1 mg of 4-methyloumbeliferol sulfate potash salt dissolved in 100 ml of citrate buffer 0.1 M with *pH* 5.0.

100 µl of thymus homogenate have been incubated with 500 µl of every substrate for 18 hrs at 37°C. Then 600 µl of alcalic buffer has been added in order to stop the reaction and after 5 min the extinctions have been read on spectrophotometer Spekol 221 with the wavelength of 360 nm as contrasted with the test check where the homogenate was added only before the extinction measurement.

Quantitative determination of protein has been carried out using the Lowry's (8) method. After the determination of extinction curves for 4-methylobeliferol and protein the activity of enzymes has been calculated in nanomoles/milligram of protein per 1 h of incubation.

To determine the activity of cathepsin B the fact that this enzyme decomposes the synthetic substrate — benzoilo-DL-arginino-naphtylo-amid (BANA), releasing free naphtyloamine, has been used. Naphtyloamine, on the other hand, couples with biazonic salt. Fast Blue B (tetrazonic zinc salt of bi-orto-anizidine) giving in effect durable coloured complexes.

Cathepsin B, being a thiol protease, requires the presence of free -SH groups (cysteine), these however disturb the formation of coloured complexes. Therefore, after the enzymatic reaction is completed, a substance blocking free -SH groups, i.e. 4-chloromercurobenzoic acid (CMB), is added.

The extinctions of the samples being examined and of the naphthylamine standard have read at 520 nm against the test check. The activity has been calculated in micromoles/milligram of protein per 1 h of incubation.

The determination of D+L cathepsin activity has been carried out using the method by Langner et al. (6).

Cathepsin D at *pH* 5.0 in the presence of 3 M of urea decomposes azocasein into small azopeptids soluble in trichloroacetic acid (TCA). Azocasein, which is not decomposed, is being precipitated after incubation with 9% TCA. In such conditions azopeptids remain in the solution giving the stain, whose maximum absorption is at 366 nm.

The activity has been calculated in micromoles/mg of protein per 1 h of incubation. The results obtained have been subjected to statistical analysis.

RESULTS AND DISCUSSION

Table 1 presents the mean values of the free fractions of lysosomal thyroid enzymes: acid phosphatase (A.ph.w.), lipase (L.w.), sulphatase (Sulph.w.), β-galactosidase (β-Gal.w.), cathepsin B (Cath. B.w.) and cathepsin D + L (Cath. D + L. w.) in rabbits of NZ, CZP, NZX and CZPX breeds.

The lowest activities have been revealed by sulphatase. In the breeds being examined, it varied from 1.077 to 1.893, 1.384 nmol/mg of protein per 1 h of incubation on average. Acid phosphatase and galactosidase had similiar activity values with no substantial differences connected with animal breed. Nearly two times higher activity was reached by lipase — 10.942, also with no significant differences in the representatives of the breeds being examined. The highest values occurred in the case of proteases: Cath. B — 14.932 and Cath. D + L — 8.703 μmol/mg of protein per 1 h of incubation. The statistical analysis indicated substantial differences in the activity of these enzymes between the CZP and rabbits and the representatives of the remaining breeds.

After a similar analysis had been carried out for the activity of the bound fraction of the enzymes examined (Table 2), we can see that the highest values have been revealed by cathepsins again: B — 10.555 and D + L — 5.669 μmol/mg of protein per 1 h of incubation. However, unlike in the case of free enzymes, no substantial differences in the activity of a bound form of proteases in the rabbits of different breeds have been noticed.

Close activity values, but clearly lower than those of cathepsins, have been reached by acid phosphatase and lipase. The activity of these enzymes, as well as that of sulphatase, was higher in the bound form than in the free one. On the other hand, the activity of β-Gal.z. was lower than β-Gal.w., i.e. 3.079 ± 0.491 nmol/mg of protein per 1 h of incubation.

In all the breeds of rabbits under examination the correlation between the activity of A.ph.w. and L.w. enzymes is positive. Within the NZX and CZP breeds a strongly positive dependence between the activity of A.ph.w. and A.ph.z., and A.ph.w. and L.w. hydrolases has also been found.

The analysis of the results obtained gives the right to state that three out of the enzymes examined are distinguishable by a substantial activity and positive or negative correlation with the others. These are: A.ph.z., β-Gal.w. and Cath. D.z. The first of them — A.ph.z. shows a positive correlation with A.ph.w., L.w., L.z., β-Gal.w., Sulph.w., and Cath. D.z. enzymes in majority of breeds except for two. For NZX breed the correlation between A.ph.w. and β-Gal.w. is negative. It is similar in the case of the relation between A.ph.z. and Cath. D.z. of NZ breed. In both cases quoted above the increase of A.ph.z. activity causes the drop of β-Gal.w. and Cath. B.z. The increase of β-Gal.w. activity in NZ, NZX and CZPX rabbits causes the decrease of A.ph.w. and A.ph.z. activity (negative correlation), while β-Gal.z., Sulph.w., Cath. B.z. and Cath. D + L.w. activity grows together

Table 1. Activity of free lysosomal enzymes of thymus

Race	No. of rabbits	nmol/mg protein/1 h incubation								μmol/mg protein/1 h incubation			
		acid phosphatase		lipase		sulphatase		β-galactosidase		cathepsin B		cathepsin D+L	
		$\bar{x}$	*SE*	$\bar{x}$	*SE*	$\bar{x}$	*SE*	$\bar{x}$	*SE*	$\bar{x}$	*SE*	$\bar{x}$	*SE*
NZ	7	6.102	1.218	10.641	1.793	1.893	0.807	5.224	0.741	18.372*	2.148	7.787*	1.106
CZP	5	6.246	1.498	9.971	2.307	1.487	0.174	9.700	2.394	7.932*	3.321	16.633*	7.643
NZX	18	6.991	0.516	11.984	0.881	1.395	0.181	7.142	0.792	15.750*	1.316	7.148*	0.575
CZPX	14	5.843	0.311	10.100	0.545	1.077	0.228	8.181	0.567	14.662*	1.650	8.330*	0.494
TOTAL	44	6.400	0.343	10.942	0.550	1.384	0.164	7.458	0.470	14.932*	0.905	8.703*	0.881

* Statistically significant differences ($p \leqslant 0.05$).

Table 2. Activity of latent lysosomal enzymes of thymus

Race	No. of rabbits	nmol/mg protein/1 h incubation								μmol/mg protein/1 h incubation			
		acid phosphatase		lipase		sulphatase		β-galactosidase		cathepsin B		cathepsin D+L	
		$\bar{x}$	*SE*	$\bar{x}$	*SE*	$\bar{x}$	*SE*	$\bar{x}$	*SE*	$\bar{x}$	*SE*	$\bar{x}$	*SE*
NZ	7	14.104	2.225	14.837*	3.198	2.187	0.667	1.379	0.133	9.645	0.898	4.542	0.922
CZP	5	6.396	1.464	5.892*	1.356	1.155	0.158	5.096	2.049	8.577	1.501	5.448	1.032
NZX	18	13.333	2.387	13.230*	1.493	2.429	0.517	3.104	0.658	11.953	1.455	6.735	1.079
CZPX	14	9.542	1.544	9.417*	0.695	1.555	0.312	3.175	1.068	9.920	0.985	4.940	0.387
TOTAL	44	11.461	1.175	11.439	0.838	1.968	0.260	3.079	0.491	10.555	0.717	5.669	0.500

* Statistically significant differences ($p \leqslant 0.05$).

with the increase of β-Gal.w. activity (positive correlation). Cath. B.z. activity in NZ, NZX and CZPX rabbit breeds reveals a positive correlation with the activity of A.ph.w., A.ph.z., L.w., L.z., and Sulph.z., and only in two cases it is negative, i.e. Cath. D.z. and β-Gal.w.

The analysis of the correlation of the results of activity in the case of the remaining enzymes revealed dependence in individual cases only.

The results of the research presented above concern the physiological condition of the animals. However, it has been noticed that certain external factors influence metabolic processes of a cell, which is demonstrated by the change in the activity of numerous cellular enzymes. Some scholars proved that e.g. few hours of exposition of the entire body to X-ray irradiation causes substantial increase in the activity of acid phosphatase and β-glucuronidase (9, 10). The treatment of mice with Vinblastine also caused the increase in the activity of such lysosomal liver enzymes like cathepsin D, β-galactosidase, acid phosphatase and others (5). The values of lysosomal enzyme activities being determined quoted by different scholars show considerable divergence. Those differences may result from the use of different experimental techniques, equipment used, agents, etc. Many factors influencing the differentiation of research results have been given by Bowers et al. (3).

The present paper shows that animal breed is also of substantial importance. On the basis of the research in the activity of lysosomal thymus enzymes carried out we can state that in a physiological condition of an organism there occur substantial differences in the activity of homonymous enzymes in different rabbit breeds. It is important to determine cellular physiological indices for individual breeds, as only then these values can be used as a basis for the determination of the influence of pathogenic factors on the organism. That is why the knowledge of breed differences in the comparison of the activity of lysosomal enzymes in organisms subjected to pathogenic influences is very important.

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STRESZCZENIE

Badania przeprowadzono na królikach płci męskiej rasy nowozelandzkiej białej, czarnej podpalanej oraz ich obukierunkowych krzyżówkach. Zwierzęta uśmiercano w 140 dniu życia. Pobierano grasice, homogenizowano je i oznaczano poziomy aktywności enzymów lizosomalnych: fosfatazy kwaśnej, lipazy, sulfatazy, katepsyny B i katepsyny D+L.

Na podstawie uzyskanych wyników można stwierdzić, że w stanie fizjologicznym organizmu występują istotne różnice aktywości jednoimiennych enzymów lizosomalnych u różnych ras królików.