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The Activity of Lysosomal Enzymes of the Wall of the Pulmonary Trunk in Rabbits

Aktywność enzymów lizosomalnych ściany pnia płucnego u królika

Lysosomes are cellular organelles containing acid hydrolases participating in degradation of elements of connective tissue found in arterial wall and therefore they became of interest to many researchers in recent years (1, 3, 4, 5—16). Most often the study of their activity was concerned with the interior membrane and medial aorta and myocytes isolated from it both in healthy animals affected with e.g. atherosclerosis, hypertension, diabetes (4—8, 10—12, 15, 16). Only a few studies inform about lysosomal enzymes of peripheral arterial walls (9, 13, 14). Therefore, we have undertaken the estimation of activity of acid phosphatase, glycosydase, lipase and sulphatase of the interior and middle coat of the pulmonary trunk in rabbit, which is a model experimental animal in studies of diseases of vascular system.

MATERIAL AND METHODS

Studies were carried out on 64 adult, 140-day-old rabbits obtained from a breeding center of the Department of Genetics and Methods of Animal Improvement, The Agricultural Academy, Cracow. Both males and females of New Zealand white breed were used (NZ — 18 animals), black bay (CzP — 19 animals), and genetic cross-breeds: females NZ x males CzP (NZX — 14) and females CzP x males NZ (CzPX — 13). Rabbits were killed in a traditional way and bled. Pulmonary trunk after washing in saline solution was frozen at the temp. -20°C . After adventitia had been prepared the vessel was homogenized in 2 ml 0.1 M phosphate buffer (pH 6.0) containing 0.1% Tritone x-1000 acting as an agent breaking lysosome membranes. The obtained homogenate was centrifuged for 20 minutes at 12.000 r.p.m. at the temp. $+4^{\circ}\text{C}$, and the obtained supernatant was used for further studies. The principle of determination of activity of lysosomal enzymes consisted in breaking suitable substrates by them and releasing 4-methyloumbelliferol (2). The activity of enzymes was determined in nanomoles per 1 mg of protein and 1 h of incubation. The statistical analysis was made at the Department of Genetics and Methods of Animal Improvement, The Agricultural Academy in Cracow using the analysis of multifactorial variance (mixed model least-squares and maximum likelihood computer program PC-1, W. Harvey, 1987, USA).

RESULTS AND DISCUSSION

Means of least squares of activity of lysosomal enzymes of the internal and middle coat of the pulmonary trunk are presented in Table 1. It can be seen that lipase is the most active, N-acetylo- β -D-glucosaminidase is slightly less active, acid phosphatase showed distinctly lower, and sulphatase and β -galactosidase the lowest activity. These results are close to those obtained by Wilk and Wójtowicz for aorta and iliac arteries (9, 13), which are elastic vessels, similarly to pulmonary trunk, but quite differently from data describing basilar artery, which is a muscular type of vessel (13, 14).

Table 1. Means of least squares of activity of lysosomal enzymes of internal and middle coat of the rabbit's pulmonary trunk

Enzyme	Mean S.E.		Sex		Race Breed			
			δ	♀	NZ	CzP	NZX	CzPX
	64	—	31	37	18	19	14	13
Acid phosphatase	7.09	0.362	8.14 A	6.05 A	6.07	7.59	8.58	6.14
β -galactosidase	1.97	0.137	2.43 G	1.51 G	2.82 ABE	1.79 AC	1.93 BD	1.34 CDE
NAGL	18.66	1.01	21.52 A	15.79 A	18.69	18.84	21.70	15.40
Lipase	20.22	1.375	19.28	21.16	17.18	18.72	24.96	20.03
Sulphatase	2.32	0.266	2.82 a	1.81 a	2.42	2.56	2.19	2.10

The numbers with the same letters are significantly different ($p \leq 0.05$) — small letters or highly significantly different ($p \leq 0.01$) — capital letters.

The activity of studied enzymes, apart from lipase, presented crucial differences: acid phosphatase, β -galactosidase and N-acetylo- β -D-glucosaminidase or fundamental differences: sulphatase depending on the sex of animals. In comparison with the results obtained by Wilk for iliac arteries, the activity of lysosomal enzymes in the pulmonary trunk was higher in males than in females.

The activity of acid phosphatase, lipase, sulphatase and N-acetylo- β -D-glucosaminidase of pulmonary trunk in animals of particular breeds was very similar but only the activity of β -galactosidase revealed statistically very important differences.

Table 2 presents phenotype correlation between the activities of the studied lysosomal enzymes in the wall of the pulmonary trunk after the variabilities due to experimental factors have been eliminated. It can be seen from the table that there are highly important correlations of the activity of acid phosphatase with N-acetylo- β -D-glucosaminidase, and β -galactosidase with sulphatase and

Table 2. Phenotype correlations between activities of lysosomal enzymes of the internal and middle coat of the rabbit's pulmonary trunk

	Acid phosphatase	Lipase	β -galactosidase	Sulphatase	NAGL
Acid phosphatase	—	-0.0757	0.2645*	-0.0894	0.3567**
Lipase		—	-0.0149	0.0535	0.0086
β -galactosidase			—	0.4243**	0.7238**
Sulphatase				—	0.4303**
NAGL					—

** Highly significant differences ($p \leq 0.01$).

* Significant differences ($p \leq 0.05$).

N-acetylo- β -D-glucosaminidase, and also important, acid phosphatase with β -galactosidase. These results are different from those obtained by Wilk and Wójtowicz both for elastic and muscular arteries (9, 13).

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STRESZCZENIE

Badania przeprowadzono na 64 dojrzałych płciowo królikach różnych ras i obu płci. Oznaczono ilościowo aktywność enzymów lizosomalnych błony wewnętrznej i środkowej pnia płucnego. Stwierdzono, że największą aktywność wykazywała lipaza, nieznacznie mniejszą — N-acetylo- β -D-glukozaminidaza, wyraźnie mniejszą — kwaśna fosfataza, a najmniejszą — sulfataza i β -galaktozydaza. Działanie większości badanych enzymów, poza lipazą, wykazywało istotne różnice związane z płcią królików. Aktywność tych enzymów, poza β -galaktozydazą, była zbliżona u przedstawicieli poszczególnych ras.