ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. LIII, 18 SECTIO D 1998

Katedra i Zakład Anatomii Prawidłowej Człowieka Akademii Medycznej w Lublinie
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Lysosomal enzyme activity of iliac arterial walls in rabbits

Aktywność enzymów lizosomalnych ścian tętnic biodrowych u królika

The discovery of lysosomes made by De Duve in 1959 initiated the studies concerning the structure and function of those cell organelles. They were found to contain approximately 100 various types of enzymatic proteins, mainly acid hydrolases involved in the metabolism of proteins, carbohydrates and lipids (1). Moreover, the lysosomal enzymes take part in the degradation of the connective tissue composing the arterial walls. With the increasing frequency of vascular diseases this resulted in the recent interest of researchers in these enzymes.

The activity of these enzymes was determined by means of histo-chemical and quantitative methods using the specific substrates. The studies concerned mainly the internal and middle layers of aorta or its isolated myocytes in healthy animals and those with the induced pathological changes (hypertension, atherosclerosis, diabetes mellitus) – 2, 3, 4, 5, 6, 7, 8, 9, 10, 11. As the peripheral arteries were of minimal interest to the researchers, we decided to determine the activities of acid phosphatase, B-galactosidase, N-acethyl-D-glucosaminidase, lipase and sulphatase in the internal and middle layers of iliac arteries in rabbits.

MATERIAL AND METHODS

The studies were performed in 135 rabbits from the Department of Genetics and Animal Improvement Methods of Agricultural Academy in Cracow. The material used is presented in Table 1. The animals aged 70 and 140 days included: New Zealand, white breed (NZ), black, bay breed (BB) and cross-breed-NZ female and BB male (NZX) and BB female and NZ male (BBX).

Table 1. Least square means for activities of lysosomal enzymes of internal and middle coat of the wall of the common and external iliac arteries and aorta of rabbits (in nanomoles/1mg of protein/1 h of incubation)

Enzyme	x	SE	Artery			Breed				Sex		Age	
			Common iliac	External iliac	Aorta	NZ	NZX	Czp	Czpx	♂	♀	70 days	140 days
Acid phospatase	1.4507	0.1413	1.5424	1.3722	1.4376	A, B 2.2776	A 1.0031	a 1.6492	Ba 0.8730	A 0.9600	A 1.9415	B 0.6030	B 2.2980
b-galacto-sidase	0.2847	0.0268	0.3258	0.2539	0.2746	A 0.1583	B 0.5454	A,B,C 0.1694	C 0.2659	a 0.2244	a 0.3451	b 0.2216	b 0.3479
NAGL	0.3759	0.0185	A 0.3272	B 0.3188	A,B 0.4817	0.3246	0.3723	0.3781	0.4288	0.3550	0.3968	A 0.1287	A 0.6231
Lipase	0.6436	0.0310	0.6306	0.6088	0.6913	A 0.7556	B 0.2938	A,B,C 0.8165	c 0.7084	A 0.6115	A 0.6757	B 0.1147	B 0.1725
Sulphatase	0.1634	0.0151	0.1640	0.1800	0.1464	Aa 0.1221	A,B,C 0.2456	B 0.0746	Ca 0.2115	A 0.1015	A 0.2254	B 0.0522	B 0.2447

The number with the same letters are significantly different (p#0.05)
– small letters, or higly significantly different (p#0.01) – capital letters.

Table 2. Phenotypic correlations between activities of lysosomal enzymes of internal and middle coat of common iliac artery of rabbit

Enzyme	Acid phosphatase	β-galactosi-dase	Nagl	Lipase	Sulphatase
Acid phosphatase	—	0.0830	0.2833xx	0.3343xx	0.1023
β-galactosi-dase		—	0.0936	0.2760xx	0.6073xx
Nagl			—	0.3506xx	0.0050
Lipase				—	0.1739
Sulphatase					—

xx – highly significantly differences (p#0.01).

Table 3. Phenotypic correlations between activities of lysosomal enzymes of internal and middle coat of external iliac artery of rabbit

Enzyme	Acid phosphatase	β-Galactosi-dase	Nagl	Lipase	Sulphatase
Acid phosphatase	—	0.0830	0.2833xx	0.3343xx	0.1023
β-galactosi-dase		—	0.0936	0.2760xx	0.6073xx
Nagl			—	0.3506xx	0.0050
Lipase				—	0.1739
Sulphatase					—

xx – highly significantly differences (p#0.01).

Table 4. Phenotypic correlations between activities of lysosomal enzymes of internal and middle coat of aorta of rabbit

Enzyme	Acid phosphatase	b-galactosi-dase	Nagl	Lipase	Sulphatase
Acid phosphatase	—	0.2097xx	0.0948	0.4655xx	0.1438
b-galactosi-dase		—	0.0182	0.0123	0.0152
Nagl			—	0.3601xx	0.0520
Lipase				—	0.0762
Sulphatase					—

xx – highly significantly differences (p#0.01).

The animals were routinely sacrificed and exsanguinated. The iliac arteries-common and external and the end-part of aorta were washed with cooled physiological saline and frozen to –20°C. The vessels were homogenized in 2 ml of 0.1 M phosphatic buffer (pH 6.0) containing 0.1% Triton X-100 as a lysosomal membrane tearing factor in the teflon homogenizer at ice-melting temperature. The homogenate was centrifuged for 20 minutes at 12 r.p.m. and 4şC (K-24 Janetzka centrifuge). The supernatant obtained was used for further studies. The lysosomal enzyme activity was determined on the basis of the decomposition of suitable substrates and the release of 4-methyl-umbelipherol.

The statistical analysis was performed in the Department of Genetics and Animal Improvement Methods of Agricultural Academy in Cracow using Mixed model least squares and maximum likelihood computer program PC-1 (W. Harwey, 1987, USA). On the basis of variance analysis, the significance of differences between the vessels, genetic, sex and age groups was estimated. The difference significance was determined using the repeated F. Dunkan test.

RESULTS

The least square averages of the lysosomal enzyme activities of the iliac arterial and aortic walls with regard to breed, sex and age of the rabbits studied are presented in Table 1.

The acid phosphatase activity in internal and middle layers does not show the statistically significant changes in various vessels. The highly significant enzyme activity differences are observed comparing NZ breed and cross-breed groups while significant differences are found comparing BB and BBX groups. The highly significant differences are related to sex and age of rabbits.

Likewise, the B-galactosidase activity showed no statistically significant differences concerning the vessel type. The highly significant differences were observed comparing NZ and NZX with BB, and BB with BBX groups. The activity of this enzyme was significantly higher in females than in males and in 140-day-old rabbits than in younger ones.

The N-acethyl-B-D-glucosaminidase activity decreased with the vessel division and the differences were highly statistically significant, but they were not significant with regard to breed and sex. However, the enzyme activity increased with the animal age – the highly significant differences.

The lysosomal lipase activities were similar in the arteries examined. The highly significant differences were observed comparing NZ, NZX groups with BB group and BB with BX group, males with females and 70-day-old with 140 day-old animals.

The sulphatase activity was similar in the vessels studied. However, the differences were highly statistically significant comparing NZ and NZX groups, NZX and BB, BBX groups as well as NZ and BBX groups. The sulphatase activity was highly significantly increased in females compared with males and in sexually mature animals compared with younger ones.

Table 2 presents the phenotype correlations between the enzyme activities in the internal and middle layers of the common iliac arterial wall after eliminating the variations due to experimental factors. The highly significant correlations are found between acid phosphatase and N-acethyl-B-D-glucosaminidase and lipase. The identical results were obtained for the external iliac artery, which is presented in Table 3.

The enzymes of aortic wall show highly significant correlations between acid phosphatase activity and B-galactosidase and lipase activities as well as between N-acethyl-B-D-glucosaminidase and lipase activities (Table 4).

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Otrz.: 1998.12.06

STRESZCZENIE

Badania przeprowadzono na 135 królikach obejmujących zwierzęta rasy nowozelandzkiej białej (NZ), czarnej podpalanej (CzP) oraz krzyżówek obukierunkowych: samice NZ i samce Czp (NZX), samców Czp i samic NZ (CzpX), w wieku 70 i 140 dni. Zwierzęta zabijano w sposób tradycyjny i skrwawiano. Do dalszych badań pobierano tętnice biodrowe wspólne i zewnętrzne oraz końcowy odcinek aorty brzusznej. Oznaczono aktywność enzymów lizosomalnych ścian tętnic biodrowych i aorty brzusznej. Wyniki badań opracowane statystycznie przedstawiono w tabelach.