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Kierownik: prof. dr hab. Zbigniew Wójtowicz

Zakład Genetyki i Metod Udoskonalania Zwierząt
Akademii Rolniczej w Krakowie
Kierownik: prof. dr hab. Andrzej Żarnowiecki

ZBIGNIEW WÓJTOWICZ, GRZEGORZ PIETRAS,
RYSZARD MACIEJEWSKI, JÓZEF BIENIEK,
ELŻBIETA ANNA JĘDRZEJEWSKA

Lysosomal enzyme activity of internal and middle layers of renal arterial wall in rabbits

Aktywność enzymów lizosomalnych wewnętrznej i środkowej warstwy ściany tętnicy nerkowej u królików

The lysosomes are the cell organelles containing approximately 100 various types of enzymes, mainly acid hydrolases involved in the metabolism of proteins, carbohydrates and lipids (1,2). Moreover, the lysosomal enzymes take part in the degradation of the connective tissue elements including glycosaminoglycans composing the vascular wall (3-7). The activity of these enzymes was determined by histochemical and quantitative methods using the specific substrates. The studies concerned mostly 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) – 4-7.

The peripheral arteries were of minimal interest. Thus, the studies concerning the effects of experimental diabetes on the morphological and biochemical structure of peripheral arterial walls were aimed at the determination of acid phosphatase, β-galactosidase, N-acethyl -β-D-glucosaminidase, lipase and sulphatase activities in the internal and middle layers of the renal arterial wall in rabbits.

MATERIAL AND METHODS

The studies were performed in 77 rabbits from the Department of Genetics and Animal Improvement Methods, Agricultural Academy in Cracow. 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) or BB female and NZ male (BBX) of both sexes. The rabbits were routinely sacrificed and exsanguinated. The renal arteries were washed with physiological saline and frozen to – 20°C. The biopsy specimens of abdominal aorta from the renal artery origin were collected for comparative studies. 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 agent in the homogenizer with teflon piston at ice-
-melting temperature. The homogenate was centrifuged for 20 minutes at r.p.m. and 4°C (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 the suitable substrates and the release of 4-methylumbelipherol (1). The detailed description of this method was presented in the previous paper.

The statistical analysis was carried out in the Department of Genetics and Animal Improvement Methods, Agricultural Academy in Cracow using mixed model least-squares and maximum likelihood computer program PC-1 (W. Harwey, 1987, USA). On the basis of the variance analysis the significance of the enzyme activity differences in various vessels, genetic groups, males, females and age groups were estimated. The difference significance was determined using the repeated F. Dunkan test.

RESULTS AND DISCUSSION

The least square averages of the lysosomal enzyme activities in the internal and middle layers of the renal artery and abdominal aorta with regard to breed, age and sex of the rabbits studied are presented in Table 1.

The highest activity in the vessels was observed for acid phosphatase, the markedly lower for lipase, β-galactosidase and N-acethyl-β-D-glucosaminidase and minimal for sulphatase. The enzyme activity differences between renal artery and aorta were not statistically significant.

In various breeds only the differences in acid phosphatase activities were statistically significant, between NZ and BBX-highly significant while between NZ and NZX, BB-significant. The activities of acid phosphatase, N-acethyl-β-D-glucosaminidase, lipase and sulphatase increased comparing 70 and 140-day-old groups, i. e. were higher in the most intensive developmental period till the sexual maturity (the highly significant differences). No statistically significant differences in the lysosomal enzyme activities were observed with regard to sex.

Table 1. Least square means for activities of lysosomal enzymes of internal and middle coat of renal artery and aorta of rabbits

Enzyme	X	S.E.	Artery		Breed				Age		Sex	
			Renal artery	Aorta	Nz	Nzx	Czp	Czpx	70-days	140-days	♂	♀
Acid phosphatase	1.2064	0.1698	1.0534	1.3594	Aab 2.0103	a 1.0023	b 1.1763	A 0.6367	A 0.5944	A 1.8184	0.9460	1.4669
β-galactosidase	0.5640	0.4138	0.2037	0.9242	1.4023	0.0842	0.6118	0.1575	0.8238	0.3041	0.1433	0.9846
NAGL	0.4603	0.0278	0.4331	0.4875	0.3533	0.4780	0.4669	0.5431	A 0.1351	A 0.7855	0.1279	0.1611
Lipase	0.7648	0.0505	0.925	0.012	0.8435	0.466	0.5414	0.8559	A 0.1475	A 1.3462	0.618	0.319
Sulphatase	0.1445	0.0164	0.1466	0.1425	0.1351	0.0833	0.1605	0.1993	A 0.0421	A 0.2470	0.129	0.1611

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.

Table 2. Phenotypic correlation between activities of lysosomal enzymes of intima and media renal artery in rabbits

Enzyme	Acid phosphatase	β-galacto-sidase	N-acety-β-glucosamini-dase	Lipase	Sulphatase
Acid phos-phatase	–	0.1645	-0.0108	-0.3410**	-0.2200
β-galacto-sidase		–	0.0026	-0.1628	0.0157
N-ace-tyl-b-glucosa-minidase			–	0.5186**	0.2398*
Lipase				–	0.4135
Sulphatase					–

** $p \leq 0.01$.

* $p \leq 0.05$.

Table 3. Phenotypic correlation between activities of lysosomal enzymes of intima and media abdominal aorta in rabbits

Enzyme	Acid phosphatase	β-galacto-sidase	N-acety-β-glucosamini-dase	Lipase	Sulphatase
Acid phos-phatase	–	0.2097**	0.0948	0.4655**	0.1438
β-galacto-sidase		–	0.0182	-0.1628	-0.0152
N-acety-β-glucosamini-dase			–	0.3601**	0.0520
Lipase				–	0.0762
Sulphatase					–

** $p \leq 0.01$.

Table 2 presents the phenotype correlations between the lysosomal enzyme activities of the renal arterial wall after eliminating the variations due to experimental factors (sex, age, breed). The negative correlation is found between acid phosphatase and lipase ($p \leq 0.01$) and between N-acethyl-β-D-glucosaminidase and lipase ($p. \leq 0.01$) and sulphatase ($p. \leq 0.05$).

The lysosomal enzymes of the abdominal aorta show the positive highly significant phenotype correlations between acid phosphatase and β-galactosidase and lipase and between N-acethyl-β-D-glucosaminidase and lipase (Table 3).

The enzyme activity in another muscular-type artery – the basilar artery–differs from the above mentioned. The highest activity in that vessel is N-acethyl-β-D-glucosaminidase and lipase, the lowest – galactosidase (8, 9). The lysosomal enzyme activities observed in the elastic type artery pulmonary trunk (10) were similar to the above – the most active was lipase. This may prove a slightly different course of metabolic processes in the renal artery wall in comparison with other vessels.

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STRESZCZENIE

Badania przeprowadzono na 77 królikach rasy nowozelandzkiej białej (NZ), czarnej podpalanej (CzP) oraz krzyżówek obukierunkowych samice NZ i samce CzP (NZX) lub samica CzP i samiec NZ (CzPX) w wieku 70 i 140 dni. Króliki zabijano w sposób tradycyjny i skrwawiano. Do

badań pobierano tętnice nerkowe oraz wycinek aorty brzusznej z miejsca odejścia tętnic nerkowych. Oznaczono aktywność enzymów lizosomalnych ściany tętnicy nerkowej oraz aorty brzusznej. Wyniki badań opracowano statystycznie i przedstawiono w tabelach.