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Influence of protein starvation on some lysosomal enzyme activities in blood serum of sheep

Summary

Reactivity of none lysosomal enzymes in blood plasma of sheep has been estimated. After 48 h protein deprivation the activity of BGLU, BGAL, AAP and LAP increased significantly, NAGL and LL activity decreased, EL and KF activity remained unchanged.

Key Words: sheep, lysosomal enzymes, protein starvation, blood serum

Zusammenfassung

Titel der Arbeit: **Der Einfluss von Eiweißmangel auf die Aktivität von lysosomalen Enzymen im Blutspiegel beim Schaf**

Die Aktivitäten der lysomalen Enzyme wurden in Blutplasma der Schafe nach 48 stündiger Eiweißhungerperiode bestimmt. Die Aktivitäten von BGLU, BGAL, AAP und LAP erhöhten sich, die von NAGL und LL verminderten sich signifikant. Keine Veränderungen zeigten die Aktivitäten von EL und KF.

Schlüsselwörter: Schaf, lysosomale Enzyme, Eiweißmangel, Blutplasma

Introduction

The role of the cell lysosomal compartment in the processes of protein degradation is connected with the adaptation ability of an organism (BAIRATI et al., 1997; MIYAWAKI, 1988; PFEFFER, 1991; SEGLEN and BOHLEY, 1992; STORRIE, 1995; STORRIE and DESJARDINS, 1996). Lysosomal enzymes may constitute a good model for studies of these adaptation processes on the cell level (BRUNK et al., 1996; CALDER, 1989; DESJARDINS, 1995; HICKS, 1995; KOŁĄTAJ et al., 1996; LOMBARDO et al., 1996; WITEK et al., 1994; 1995; 1996).

Also the problem of stress in animal breeding is becoming an important research problem, connected with its negative effects on productivity (HERSHOCK and VOGEL, 1989; KOŁĄTAJ et al., 1998; 1996; 1995;).

In the literature available no information was found on lysosomal enzyme activities in the tissues of sheep, which would present the animals in the physiological conditions and in response to stress. WITEK et al. (1996) observed the activity of lysosome enzymes in leucocytes and blood plasma of sheep as result of mercury poisoning only.

The present studies are connected with the activity of model lysosomal enzymes in the blood plasma of sheep maintained in farm conditions, fed normally and next restrictived for a short period. Fasting or restriction of food is some specific type of stress (FIDANZA, 1980; KOŁĄTAJ, 1993). In domestic animals it still occurs quite often (KLUSEK et al., 1997).

Material and Methods

The studies were performed on 21 Booroola rams 1-4 year old (Table 1) which came from the breeding farm belonging to the Polish Academy of Sciences, Institute of Genetics and Animal Breeding in Jastrzębiec. The animals received unlimited hay, silage and concentrate according to norm for farm. They had a constant access to water. All the animals were in good conditions of breeding, nursing and professional veterinary care.

Table 1
Least squares means (LSM) and (± se) for activity of studied lysosomal enzymes in blood serum of sheep (nMol/mg protein/hour)

	Treatment		**Group of animals (years)***			
	before starvation (1)	**after starvation (2)**	***1***	***2***	***3***	***4***
N	21	21	14	16	6	6
BGRD	0,208 ±0,034	0,280^{ns} ±0,034	0,256 ±0,037	0,238 ±0,035	0,268 ±0,057	0,215^{ns} ±0,057
BGLU	0,160 ±0,011	0,216*** ±0,011	0,204 ±0,012	0,184 ±0,012	0,162 ±0,019	0,202^{ns} ±0,019
NAGL	4,377 ±0,187	2,409*** ±0,187	4,360^{AaB} ±0,207	2,844^{A} ±1,194	2,840^{B} ±0,316	3,528^{a} ±0,316
BGAL	0,506 ±0,031	0,597* ±0,031	0,560 ±0,034	0,524 ±0,032	0,635 ±0,052	0,488^{ns} ±0,052
LL	0,797 ±0,092	0,462* ±0,092	0,432^{A} ±0,101	0,487^{B} ±0,095	0,987^{AB} ±0,155	0,613 ±0,155
EL	2,707 ±0,169	2,483^{ns} ±0,169	2,181 ±0,187	2,693 ±0,175	2,743 ±0,285	2,762^{ns} ±0,285
KF	2,242 ±0,513	2,233^{ns} ±0,513	3,455 ±0,568	1,855 ±0,531	1,778 ±0,867	1,862^{ns} ±0,867
AAP	9,520 ±0,420	12,210*** ±0,420	11,100 ±0,470	10,930 ±0,440	9,910 ±0,710	10,520^{ns} ±0,710
LAP	79,960 ±3,060	109,380*** ±3,060	89,800 ±3,380	93,970 ±3,170	94,270 ±5,170	100,630^{ns} ±5,170

ns - non significant; * P ≤ 0.05, ** P ≤ 0.001
$^{AB..}$ Within each rows, for group, means bearing the different superscript differ significantly at : a-a -P ≤ 0.05; A-A, B-B, P ≤ 0.01;
^{x}\ significance of differences between means was estimated according to the contrast method;

The animals maintained in these conditions were treated as blood donors of the control group. Next, the animals were deprived for 48 hours the silage and concentrate, therefore the protein components but they had a constant access to straw only. We did not apply the full starvation in order to not evoke the emotional stress connected with a total lack of food. The essence of our intention was a deprivation of food protein during this period. After 48 hours of that protein starvation the blood was drawn again. Both times, before 48 h period of food restriction (control) and after this time, 10 ml of blood were drawn between 8^{30} - 10^{30} a.m. to test tubes containing heparin and centrifuged for 15 minutes at 20.000 g in K-24 centrifuge. The blood samples were

taken from the neck vein of all individuals. In the blood serum obtained the following lysosomal enzymes were determined: ß-glucoronidase (BGRD) - EC 3.2.1.31; ß - glucosidase (BGLU) - EC 3.2.1.21; N-acetyl -ß-glucosaminidase (NAGL) - EC 3.2.1.30; ß-galactosidase (BGAL) - EC 3.2.1.23; lysosome lipase (LL) - EC 3.1.1.2; alanine aminopeptidase (AAP) - EC 3.4.11.2; leucine aminopeptidase (LAP) - EC 3.4.11.1; acid phosphatase (KF) - EC 3.1.3.2; lysosome esterase (EL) - EC 3.1.1.2.
The activity of BGRD, BGLU, NAGL, BGAL and KF was determined according to the method of BARRETT (1972), LAP - by method of PFEIDERER and CELLIERS (1963), AAP by method of PFEIDERER et al. (1964), EL and LL - by MAIN'S method (1960). The enzymatic activity was expressed in nMol/mg of protein/hour. Plasma protein was estimated according to the method of KIRSCHKE and WIEDERANDERS (1984).
The reagents used were produced by Sigma (Sigma - Aldrich Corp.) firm. The results obtained were analysed statistically using the two-way analysis of variance.

Results

The results obtained are presented in Tables 1 - 3 and Figure 1. After 48 hours of protein fasting increased significantly the activity of BGLU (F=12.385), BGAL (F=4.280), AAP (F=20.516), LAP (F=46.216). The activity of NAGL (F= 55.356) and LL (F=6.675) decreased significantly while BGRD, EL and KF remained unchanged.

Table 2
The example of the interaction (in one case only) for least squares means (LSM) and (± se) for activity of studied lysosomal enzyme KF [x\]

		KF	
Factor	N	LSM	se
Treatment x group			
1 x 1 years	7	1.859[a]	0.803
1 x 2 years	8	2.465	0.751
1 x 3 years	3	2.123	1.227
1 x 4 years	3	2.520	1.227
2 x 1 years	7	5.051[a]	0.803
2 x 2 years	8	1.245	0.751
2 x 3 years	3	1.433	1.227
2 x 4 years	3	1.203	1.227

Means bearing the different superscript differ significantly at: a, a: P≤ 0.05
[x] \ significance of differences between means was estimated by Duncan's test;

The analysis of variance (Table 3) indicated, that as regards NAGL and LL the age of the animals played a significant role in determining the enzyme activity (F=10.984, P ≤ 0.001 and F=3.302, P ≤ 0.03, respectively). The age x treatment interaction revealed the significant value (F=3.244,P< 0.034) only in relation to acid phoshatase - KF (Fig.).

Discussion

Stress has already been defined many times (FRIEND, 1980; MOBERG, 1985; SPENCER, 1995; YOUSEF, 1985). There are the suggestions that stress should be understood as the sudden informatic and energetic excitation of the cell or the organism as a whole (KOŁĄTAJ, 1993). It is known that numerous regulatory

possibilities of homeostasis reveal under the pressure of environment (KLASSING, 1985). An analysis of our results obtained indicates that 48 h protein fasting is an important stress factor in relation to lysosomal enzymes and it can cause the changes of their activities. The activity of four enzymes investigated increased and the activity of two enzymes decreased.

Table 3
The analysis of variance for the lysosomal enzyme activities in blood serum of studied sheep

Enzyme	Factor	DF	Sum of squares	Mean squares	F	P (probability)
BGRD	Treatment (T)	1	0.4446	0.4446	2.291	0.1394
	Age (A)	3	0.0110	0.0037	0.189	0.9034
	T x A	3	0.0231	0.0077	0.396	0.7567
	Error	34	0.6627	0.1950	-	-
BGLU	Treatment (T)	1	0.0263	0.0263	12.385	0.0013
	Age (A)	3	0.0087	0.0029	1.367	0.2694
	T x A	3	0.0075	0.0025	1.181	0.3316
	Error	34	0.0723	0.0021	-	-
NAGL	Treatment (T)	1	33.1673	33.1673	55.356	0.0000
	Age (A)	3	19.7430	6.5810	10.984	0.0000
	T x A	3	2.7651	0.9217	1.538	0.2223
	Error	34	20.3717	0.5992	-	-
BGAL	Treatment (T)	1	0.7026	0.7026	4.280	0.0462
	Age (A)	3	0.7813	0.2604	1.586	0.2107
	T x A	3	0.1142	0.3807	2.319	0.0929
	Error	34	0.5581	0.1642	-	-
LL	Treatment (T)	1	0.9593	0.9593	6.675	0.0142
	Age (A)	3	1.4236	0.4745	3.302	0.0318
	T x A	3	0.3046	0.1015	0.706	0.5548
	Error	34	4.8860	0.1437	-	-
EL	Treatment (T)	1	0.4289	0.4289	0.880	0.3549
	Age (A)	3	2.7252	0.9084	1.863	0.1545
	T x A	3	1.1563	0.3854	0.790	0.5077
	Error	34	16.5796	0.4876	-	-
KF	Treatment (T)	1	0.0006	0.0006	0.000	0.9908
	Age (A)	3	24.3727	8.1242	1.800	0.1658
	T x A	3	43.9206	14.6402	3.244	0.0339
	Error	34	153.4656	4.5137	-	-
AAP	Treatment (T)	1	62.4357	62.4357	20.516	0.0001
	Age (A)	3	8.7480	2.9160	0.958	0.4236
	T x A	3	10.4747	3.4916	1.147	0.3441
	Error	34	103.4729	3.0433	-	-
LAP	Treatment (T)	1	7407.3790	7407.3790	46.216	0.0000
	Age (A)	3	503.7801	167.9267	1.048	0.3840
	T x A	3	738.2594	246.0865	1.535	0.2231
	Error	34	5449.3956	160.2763	-	-

** $P < 0.01$, *** $P < 0.001$

The decrease of NAGL and LL activity in the blood plasma during fasting was observed earlier on rabbits by WITEK et al. (1995).Their activity may be connected with the changed rate of synthesis or the degradation of fats and carbohydrates in the cell.

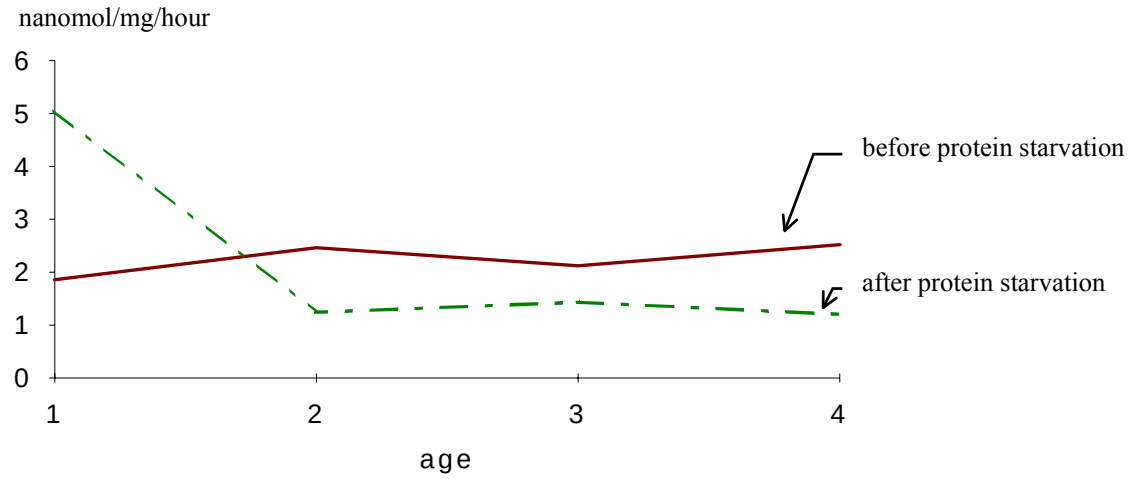

Figure: The example of interaction treatment x age for acid phosphatase (KF) activity

The activity of BGLU, BGAL, AAP and LAP enzymes increased. It may be connected probably with the degradation intensity of protein and protein - carbohydrate compounds in the cell. Such selective changes may suggest the different of breaking stages of the lysosome arrangement membranes. This phenomenon may be treated as an example of the adaptation of the cell lysosome arrangement to conditions of 48 h lasted protein fasting. In this period the glycoprotein and lipoprotein metabolism rate increases, as well as the rate of passage of aminoacids from the degraded proteins to mitochondria in order to maintain a correct level of oxidative phosphorylation.It is known that different types of proteins have a different period of half-life. It proves to their differently controlled degradation because metabolism must take place also during protein fasting.

The problems of human and animal adaptation to the starvation and to the restricted feeding remain in the centre of interest of many researches (CHEREL et al., 1992; FIDANZA, 1980; KOŁĄTAJ, 1993; WITEK et al., 1995).

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