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The effect of exogenous glycerol on the activity of lysosomal enzymes in the blood plasma of young bulls (short communication)

Summary

The study was performed on 3 young bulls of the Lowland Black-and-White breed. The animals received daily, during three days about 400 g through the gastric fistula of the glycerol. In the period 2, 4 and 8 hours after the glycerol infusion the blood was taken and in the plasma obtained the activities the enzymes BGRD, BGAL, BGLU, NAGL, AP, AAP, LAP, LL and EL were determined. Administration of glycerol had a significant influence on the activity of BGRD, BGAL, NAGL, AP, LAP and EL. The great variability of activities of enzymes has been observed.

Key words: glycerol, lysosomal enzymes, biochemical stress, bulls

Zusammenfassung

Titel der Arbeit: **Der Einfluß von Exoglyzerin auf die Aktivität der lysosomalen Enzyme im Blutplasma junger Bullen** (Kurzmitteilung)
Die Aktivitäten der lysosomalen Enzyme wurden im Blutplasma von 3 Jungbullen bestimmt. Die Tiere erhielten mittels Infusion an drei Tagen 2 g/kg Körpergewicht Exoglyzerin. 2, 4 und 8 Stunden nach Glyzerininfusion wurden die Aktivitäten von BGRD, BGAL, BGLU, NAGL, AP, AAP, LAP, LL und EL untersucht. Exoglyzerin hatte wesentlichen Einfluß auf die Aktivitätsveränderungen von BGRD, BGAL, NAGL, AP, LAP und EL. Es konnte eine große Enzymaktivität beobachtet werden.

Schlüsselwörter: Glyzerin, Lysosomale Enzyme, Bullen, Streß

Introduction

The problem of biochemical stress in animals is still the focus of interest of biologists and breeders (SPENCER, 1995; KOŁĄTAJ, 1993; DALLMAN, 1991; RABIN et al., 1990). Glycerol is in the ruminants, above all in the cattle the substrate into glucose exchanged. The oscillation of access of this substrate can be directly or indirectly accountable for changes in the glucose metabolism and its concentration in blood can be the expression of homeostase (MONTMINY and GALIGOIS, 1994; LAYCHOCK, 1990; KOŁĄTAJ et al., 1996a; WAPNIR et al., 1996; DE SANCTIS et al., 1996; MÜLLER-WIELAND et al., 1993). In our opinion the artificial introduction of an excess of the glycerol to the blood of the cow or bull is the biochemical stressor which disturbs an actual homeostase of animal.
In literature we have not found the reports concerning the reactivity of lysosomal enzymes in the cattle, therefore we have taken into account a glycerol infusion as a

model of the biochemical stressor and the activity of these enzymes as the model markers of that kind of stress.

Material and Methods

The study was carried out on 3 young bulls, of the Lowland Black-and-White breed of the body weight 180 - 250 kg, at the age of 6 months, which came from the breeding farm of the Research Institute of Animal Production in Nitra, Slovakia. The animals were provided with equal conditions of standard food, nursing and they had a professional veterinary care. The bulls received a food in daily ratio of about 7.5 kg which consisted of 1.5 kg of good quality hay, 4 kg of corn grain, 2 kg of barley, and water in unlimited amounts.

The experiment was performed from 17 - 19 April with animals which had been previously starved for 24 hour. The animals received the glycerol in the amount 2 g/kg of body weight (about 400 g/bull) daily, during three days, at 6.30 a.m. through gastric fistula. Before the administration of glycerol the control blood samples were taken from the neck vein of all individuals. In the period 2, 4 and 8 hours after the glycerol administration the blood samples were taken again and in plasma the activities of the beta-glucuronidase (BGRD - EC 3.2.1.31); beta-galactosidase (BGAL - EC 3.2.1.23); beta-glucosidase (BGLU - EC 3.2.1.21); N-acetyl-beta-glucosaminidase (NAGL - EC 3.2.1.30); acid phosphatase (AP - EC 3.1.3.2); alanyl aminopeptidase (AAP - EC 3.4.11.1); leucyl aminopeptidase (LAP - EC 3.4.11.1); lysosomal esterase (EL - EC 3.1.1.2) and lysosomal lipase (LL - EC 3.1.1.3) were determined.

The activities BGRD, BGAL, BGLU, NAGL and AP were determined according to the BARRETT'S method (1972); AAP according to PFLEIDERER et al. (1964); LAP by method of PFLEIDERER and CELLIERS (1963); EL and LL according to the modified method of MAIN (1960).

Enzyme activity was expressed in nmol/mg of protein/hour.

The results obtained were statistically analysed according to the three way analysis of variance.

Results

From Table 1 we can see that for example during first day after 2, 4 and 8 hours after glycerol administration BGLU activity in blood plasma of first bull decreased significantly to 73%, 76% and 56% suitable. AAP activity for example during first day decreased after 4 hours to 74%. During second day after 4 hours from glycerol administration BGLU activity was lower significantly to 78% of control value. The remained enzymes did not change statistically their activities.

From Table 2 we see that for second bull the differences statistically confirmed after glycerol administration showed during first day BGAL past 2 h (78%) only and AAP during third day past 8 h (78%).

Table 3 informs, that during first day in third bull revealed the statistically confirmed

Table 1
The activities (in nmol/mg of protein/hour and per cent) of the examined enzymes in the blood plasma of I bull after the glycerol infusion. Control = 100 % (Aktivität der untersuchten Enzyme im Blutplasma von einem Bullen nach Glyzerininfusion)

		Time after glycerol infusion					
		1st day					
Enzyme	Control	2 h	%	4 h	%	8 h	%
BGRD	0.205	0.232	113	0.236	115	0.210	102
BGAL	0.367	0.314	85	0.356	97	0.356	97
BGLU	0.290	0.213 *	73	0.222 *	76	0.162 *	56
NAGL	0.892	0.782	88	0.804	90	0.846	95
AP	0.786	0.726	92	0.854	109	0.871	111
AAP	1.320	1.080	82	0.980 *	74	1.306	99
LAP	0.840	0.808	96	0.800	95	0.830	99
LL	1.100	1.084	98	1.040	94	1.068	97
EL	0.420	0.432	103	0.442	105	0.430	102
				2nd day			
BGRD	0.190	0.198	104	0.204	107	0.195	103
BGAL	0.324	0.315	97	0.319	98	0.328	101
BGLU	0.182	0.160	88	0.142 *	78	0.200	110
NAGL	0.941	0.900	96	0.860	91	0.942	100
AP	0.848	0.684	81	0.704	83	0.756	89
AAP	1.040	0.880	85	0.980	94	1.010	97
LAP	0.800	0.756	94	0.780	97	0.805	101
LL	1.050	1.040	99	1.030	98	1.036	99
EL	0.440	0.436	99	0.432	98	0.452	103
				3th day			
BGRD	0.178	0.180	101	0.198	111	0.190	107
BGAL	0.342	0.296	86	0.315	92	0.330	96
BGLU	0.128	0.137	107	0.111	87	0.111	87
NAGL	0.920	0.831	90	0.864	94	0.864	94
AP	0.903	0.754	83	0.752	83	0.768	85
AAP	0.980	1.006	103	0.940	96	0.990	101
LAP	0.780	0.760	97	0.776	99	0.788	101
LL	1.120	1.090	97	1.110	99	1.100	98
EL	0.480	0.462	96	0.478	99	0.470	98

changes after glycerol administration for NAGL past 2 h (75%) only.
The data of analysis of variance show in the Table 4 the statistically confirmed differentiation between the experimental animals for all enzymes obtained except BGLU, AAP and LL. The values for differences between days were confirmed for BGRD [F = 4.367], BGAL [F = 11.170], BGLU [F = 5.704], NAGL [F = 11.034], AAP [F = 4.697] and EL [F = 17.276], the values for differentiation of the samples for BGRD [F = 4.866], BGAL [F = 6.796], NAGL [F = 7.586], AP [F = 6.246], LAP [F = 4.544] and EL [F = 3.318].
The statistically significant values too have been observed for interaction animals x days for BGAL [F = 8.984], NAGL [F = 19.547], LAP [F = 6.925], LL [F = 10.478], EL [F = 14.495] and for interaction days x samples for BGAL [F = 3.559] only.

Table 2
The activities (in nmol/mg of protein/hour and per cent) of the examined enzymes in the blood plasma of II bull after the glycerol infusion. Control = 100 % (Aktivität der untersuchten Enzyme im Blutplasma von einem zweiten Bullen nach Glyzerininfusion)

		Time after glycerol infusion					
		1st day					
Enzyme	Control	2 h	%	4 h	%	8 h	%
BGRD	0.168	0.178	106	0.202	120	0.190	113
BGAL	0.410	0.320 *	78	0.342	83	0.392	95
BGLU	0.141	0.115	81	0.165	117	0.135	96
NAGL	0.964	0.965	100	0.903	94	0.942	98
AP	0.846	0.794	94	0.800	94	0.815	96
AAP	1.040	0.880	85	1.080	104	0.980	94
LAP	0.800	0.810	101	0.816	102	0.816	102
LL	1.050	1.006	96	1.040	99	1.063	101
EL	0.510	0.492	96	0.482	94	0.496	97
				2 nd day			
BGRD	0.171	0.170	99	0.200	117	0.190	111
BGAL	0.380	0.421	111	0.360	95	0.369	97
BGLU	0.132	0.142	107	0.134	101	0.150	114
NAGL	0.904	0.806	89	0.860	95	0.874	97
AP	0.860	0.805	94	0.816	95	0.855	99
AAP	1.040	0.940	90	0.982	94	0.994	95
LAP	0.780	0.764	98	0.758	97	0.770	99
LL	1.040	1.030	99	1.050	101	1.050	101
EL	0.524	0.514	98	0.518	99	0.532	101
				3 th day			
BGRD	0.170	0.182	107	0.205	120	0.190	112
BGAL	0.405	0.320	79	0.358	88	0.382	94
BGLU	0.151	0.139	92	0.121	80	0.142	94
NAGL	0.880	0.902	102	0.864	98	0.852	97
AP	0.840	0.810	96	0.792	94	0.820	98
AAP	1.020	0.900	88	0.930	91	0.800 *	78
LAP	0.760	0.750	99	0.764	100	0.770	101
LL	1.050	1.100	105	1.060	101	1.080	103
EL	0.490	0.464	95	0.492	100	0.502	102

Discussion

Although glycerol is product of fat metabolism it can be not use by the tissues immediately. It diffuses to the blood plasma and from there it comes to the liver and kidneys. The existent there enzyme, glycerol kinase, catalyzes its conversion to glucose (MAYES and LAKER, 1981; MC GARRY and FOSTER, 1980). The glucose has been transported from these organs to fat tissue and glycerol comes back from it to the liver and kidney again so that it may be able to glucose resynthesis (WATFORD, 1988; NOEL et al., 1997; PILKIS et al., 1988).

The changes of activity of lysosome enzymes under the influence of glycerol in the young bulls appear interesting. It was possible to suppose that the excess of glycerol introduced to the blood can be the biochemical stressor for animal and effects a disturb its normal metabolic homeostasis. Activities of examined enzymes changed statistically significant. The changes of the BGRD, BGAL, NAGL, AP, LAP and EL

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Table 3
The activities (in nmol/mg of protein/hour and per cent) of the examined enzymes in the blood plasma of III bull after the glycerol infusion. Control = 100 % (Aktivität der untersuchten Enzyme im Blutplasma von einem dritten Bullen nach Glyzerininfusion)

	Time after glycerol infusion						
		1st day					
Enzyme	Control	2 h	%	4 h	%	8 h	%
BGRD	0.188	0.239	127	0.384	204	0.205	109
BGAL	0.360	0.296	82	0.320	89	0.344	95
BGLU	0.170	0.152	89	0.140	82	0.162	95
NAGL	0.683	0.512 *	75	0.615	90	0.655	96
AP	0.916	0.816	89	0.848	92	0.869	95
AAP	1.100	1.006	91	1.036	94	1.050	95
LAP	0.860	0.818	95	0.824	96	0.840	98
LL	1.120	1.084	97	1.080	96	1.102	98
EL	0.420	0.400	95	0.416	99	0.408	97
				2nd day			
BGRD	0.231	0.242	105	0.255	110	0.228	99
BGAL	0.362	0.391	108	0.352	97	0.354	98
BGLU	0.171	0.107	62	0.167	98	0.165	96
NAGL	0.822	0.792	96	0.796	97	0.816	99
AP	0.816	0.825	101	0.804	98	0.830	102
AAP	0.940	0.936	99	0.902	96	0.936	99
LAP	0.904	0.772	85	0.890	98	0.890	98
LL	1.090	1.056	97	1.068	98	1.088	100
EL	0.450	0.444	99	0.426	95	0.438	97
				3th day			
BGRD	0.171	0.188	110	0.204	119	0.198	116
BGAL	0.354	0.302	177	0.346	98	0.362	102
BGLU	0.171	0.117	68	0.137	80	0.154	90
NAGL	0.816	0.726	89	0.748	92	0.796	97
AP	0.870	0.817	94	0.848	97	0.854	98
AAP	0.982	1.002	102	0.940	96	1.005	102
LAP	0.880	0.880	100	0.894	101	0.888	101
LL	0.990	0.982	99	1.060	107	1.040	105
EL	0.440	0.420	95	0.416	94	0.432	98

Table 4
Analysis of variance for lysosomal enzyme activities in the blood plasma of studied bulls; n_1 (animals) = 3; n_2 (days) = 3; n_3 (samples of blood plasma) = 4 (Varianzanalyse der lysosomalen Enzymaktivität im Blutplasma der untersuchten Bullen)

Source of variation		Sum of squares	Mean squares	F	F
	BGRD				
Animals (bulls)		0.0113	0.0057	8.033	0.0032**
Days		0.0062	0.0031	4.367	0.0284*
Samples		0.0103	0.0034	4.866	0.0119*
Interaction	A x D	0.0052	0.0013	1.856	0.1621
Interaction	D x S	0.0050	0.0008	1.184	0.3582
Remainder		0.0127	0.0007		
	BGAL				
Animals		0.0323	0.0162	26.596	0.0000***
Days		0.0136	0.0068	11.170	0.0007***
Samples		0.0124	0.0041	6.796	0.0029**
	A x D	0.0218	0.0055	8.984	0.0004***
	D x S	0.0130	0.0022	3.559	0.0168*
Remainder		0.0110	0.0006		

Table 4 (continued)

Source of variation		Sum of squares	Mean squares	F	F
BGLU					
Animals		0.0076	0.0038	3.417	0.0552
Days		0.0127	0.0063	5.704	0.0121*
Samples		0.0071	0.0023	2.113	0.1343
	A x D	0.0105	0.0026	2.356	0.0925
	D x S	0.0035	0.0006	0.525	0.7818
Remainder		0.0200	0.0011		
NAGL					
Animals		0.1838	0.0919	83.458	0.0000***
Days		0.0243	0.0121	11.034	0.0007***
Samples		0.0251	0.0084	7.586	0.0017**
	A x D	0.0861	0.0215	19.547	0.0000***
	D x S	0.0025	0.0004	0.381	0.8818
Remainder		0.0198	0.0011		
AP					
Animals		0.0213	0.0107	7.520	0.0042**
Days		0.0049	0.0025	1.741	0.2036
Samples		0.0265	0.0088	6.246	0.0043**
	A x D	0.0081	0.0020	1.435	0.2631
	D x S	0.0055	0.0009	0.647	0.6923
Remainder		0.0255	0.0014		
AAP					
Animals		0.1053	0.0526	2.295	0.1295
Days		0.2155	0.1077	4.697	0.0228*
Samples		0.0958	0.0319	1.393	0.2773
	A x D	0.0734	0.0184	0.800	0.5406
	D x S	0.2271	0.0378	1.650	0.1910
Remainder		0.4129	0.0229		
LAP					
Animals		0.0497	0.0249	49.766	0.0000***
Days		0.0015	0.0007	1.513	0.2470
Samples		0.0068	0.0023	4.544	0.0154*
	A x D	0.0138	0.0035	6.925	0.0015**
	D x S	0.0042	0.0007	1.417	0.2619
Remainder		0.0090	0.0005		
LL					
Animals		0.0033	0.0017	2.421	0.1172
Days		0.0040	0.0020	2.886	0.0818
Samples		0.0040	0.0013	1.921	0.1623
	A x D	0.0289	0.0072	10.478	0.0001*
	D x S	0.0046	0.0008	1.106	0.3971
Remainder		0.0124	0.0007		
EL					
Animals		0.0362	0.0181	205.77	0.0000***
Days		0.0030	0.0015	17.276	0.0001***
Samples		0.0009	0.0003	3.318	0.0434*
	A x D	0.0051	0.0013	14.495	0.0000***
	D x S	0.0005	0.0008	0.956	0.4815
Remainder		0.0016	0.0008		

D.F. for all enzymes = for animals: 2; for days: 2; for samples: 3; for interaction days x samples: 6; for interaction animals x days: 4; for remainder: 8;

activities we have observed earlier during the different aspects of our studies

(KOŁĄTAJ et al., 1998, 1996; WITEK et al., 1996, 1995, 1994). In consideration synthesis and degradation of the protein in the cells they seem to be a good model of the stress reactivity after the biochemical excitation. We supposed that according to our earlier results, the excess of exogenous glycerol introduced to blood of the animal initiates the chain of adaptative reactions in lysosomal space of cells probably mainly in the liver and kidneys. Our results presented in this paper are connected with the studies of the activities of these enzymes in the blood serum. On the basis of analysis of variance we have found the differences of these activities in six enzymes in the presence of nine examined after glycerol infusion.There is the statistically confirmed variability between the animals and days of experiment. The studies of this phenomen ought be continued.

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