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The effect of exogenous glucose on the activity of lysosomal enzymes in some organs of rabbits (short communication)

Summary

The 6-month-old male rabbits, New Zealand White, were given twice daily for 7 days by infusion 40% solution of exogenous glucose in the amount of 1 ml/kg of body weight. Administration of glucose decreased significantly the activity of BGLU and AP in the liver; BGRD, BGAL, NAGL, AP and LL in the kidney; NAGL and LL in the muscle.

Key Words: glucose, lysosomal enzymes, rabbits

Zusammenfassung

Titel der Arbeit: **Der Einfluß von Exoglukose auf die Aktivität der lysosomalen Enzyme in einigen Organen bei Kaninchen** (Kurzmitteilung)

Die Aktivitäten der lysosomalen Enzyme wurden in der Leber, den Nieren und den Muskeln bei Kaninchen bestimmt. Die Tiere erhielten zweimal pro Tag während 7 Tagen je 1 ml/kg Körpergewicht 40% Exoglukoselösung. Nach der Glukoseinjektion wurden die Senkung der BGLU- und AP-Aktivität in der Leber; BGRD-, BGAL-, NAGL-, AP- und LL-Aktivität in der Niere; NAGL- und LL-Aktivität im Muskel beobachtet.

Schlüsselwörter: Exoglukose, Lysosomale Enzyme, Kaninchen

Introduction

If we introduce to the blood of animal an excess of exogenous glucose, this sugar becomes itself a metabolic stressor because its level can be the expression of homeostase (LAYCHOCK, 1990; KOŁĄTAJ et al., 1998; RANDLE et al., 1988).
We did not find the reports connected with the reactivity of lysosomal enzymes during metabolic stimulation (DESJARDINS, 1995; STORRIE and DESJARDINS, 1996). In our experiment we studied the changes of that reactivity in some organs of rabbits on glucose model of the biochemical stress.

Material and Methods

The experiment was carried out on twenty 6-month-old male rabbits of the New Zealand White breed, coming from a farm of the Institute of Animals Production in Nitra (Slovakia). The animals weighed 2.0 - 2.2 kg and were maintained in identical conditions of nutrition and nursing. They received an industrial feed mixture, 19% of crude protein (Altromin Standard Diets 1320 Totally Pathogene Free TPF; GmbH International; Germany). All animals had a continuous access to water. The rabbits have been divided into control (n = 10) and experimental group (n = 10). The animals of the experimental group received 40% solution of glucose in the amount of 1 ml/kg

of body weight twice daily at 10^{00} - 11^{00} a.m. and 6^{00} - 7^{00} p.m. during the 7 days to the ear vein. The control rabbits received analogously the 0.9% NaCl solution.
After 7 days the rabbits were killed by interrupting of spinal cord and slices of the liver, kidney and muscle (*musculus longissimus dorsi*) at the height of the last rib immediately were taken to analysis. The slices of the liver have been subjected to perfusion by solution of 0.9% NaCl cooled to + 5^0 C and similarly to slices of the kidney and muscle were suspended in 0.1 M phosphate buffer pH 7.0 at the temperature + 5^0 C at ratios 1 g of tissue/6 ml buffer. The whole was homogenized in glass Potter homogenizer at 200 rotations/min. Differential fractioning of the liver homogenates was carried out according to the method of MARZELLA and GLAUMANN (1980), kidney and muscle homogenates according to the method of BEAUFAY (1972).

The activity of ß-glucuronidase (BGRD - EC 3.2.1.31); ß-galactosidase (BGAL - EC 3.2.1.23); ß-glucosidase (BGLU - EC 3.2.1.21) and N-acetyl-β-glucosaminidase (NAGL - EC 3.2.1.30) was determined on the basis of p-nitrophenyl substrate by use the micro-spectrophotometric BARRETT'S method (1972); acid phosphatase (AP - EC 3.1.3.2) according to HOLLANDER (1970); alanyl aminopeptidase (AAP - EC 3.4.11.2) according to PFLEIDERER et al., (1964); leucyl aminopeptidase (LAP - EC 3.4.11.1) according to PFLEIDERER and CELLIERS (1963); lysosomal esterase (EL - EC 3.1.1.2) and lysosomal lipase (LL - EC 3.1.1.3) according to the modified MAIN'S method (1960) and activity of cathepsin D (Cath. D - EC 3.4.23.5) was estimated according to the method of LANGNER et al., (1973) using 2% azocasein in 6M urea as substrate. All reagents have been made by Serva (Feinbiochemica GmbH & Co., Heidelberg, Germany). The enzyme activity has been expressed in nmol/mg of protein/hour.

In the lysosomal fraction obtained the protein was also determined by a modified Lowry's method (KIRSCHKE and WIEDERANDERS, 1984).
The results were statistically analysed according to analysis of variance and Student's tests.

Results

Tables 1 – 3 show that the activities of all estimated enzymes of the liver, kidney and muscle except AAP, LAP and Cath. D decreased in comparison with the values of control group.
The data of analysis of variance are presented in Table 4. Statistically confirmed differences in the liver were revealed for BGLU [F = 5.01] and AP [F = 7.90]; in the kidney for BGRD [F = 8.27], BGAL [F = 14.42], NAGL [F = 8.56], AP [F = 13.07] and LL [F = 17.40]; in the muscle for NAGL [F = 5.39] and LL [F = 9.94].

Discussion

It is known that glucose is a main energetic source on the pathways of metabolic processes in the human and in the majority of the mammalians. Its level in the blood serum has already been discussed (LEWIS et al., 1996; RUBIO et al., 1997; KOŁATAJ et al., 1998).

Table 1
The activity of lysosomal enzymes ($\bar{x} \pm S_d$) in the liver of the rabbits (in nmol/mg of protein/hour) after the 40% solution of glucose administration; control = 100% (Aktivität der lysosomalen Enzyme in der Leber von Kaninchen, Kontrollgruppe = 100%)

Enzyme	Liver		
	Control	Experiment	%
BGRD	0.194 ± 0.007	0.183 ± 0.007	94
BGAL	0.127 ± 0.004	0.113 ± 0.002	89
BGLU	0.032 ± 0.001	0.023 ± 0.008 *	72
NAGL	0.144 ± 0.003	0.138 ± 0.003	96
AP	0.574 ± 0.001	0.376 ± 0.002 **	65
AAP	0.135 ± 0.006	0.159 ± 0.05	118
LAP	0.032 ± 0.001	0.036 ± 0.001	112
EL	0.027 ± 0.0009	0.023 ± 0.0008	85
LL	0.073 ± 0.002	0.068 ± 0.002	93
Cath.D	0.317 ± 0.007	0.323 ± 0.003	102

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ - the differences statistically confirmed

Table 2
The activity of lysosomal enzymes ($\bar{x} \pm S_d$) in the kidney of the rabbits (in nmol/mg of protein/hour) after the 40% solution of glucose administration; control = 100% (Aktivität der lysosomalen Enzyme in der Niere von Kaninchen, Kontrollgruppe = 100%)

Enzyme	Kidney		
	Control	Experiment	%
BGRD	0.062 ± 0.003	0.033 ± 0.001 **	53
BGAL	0.065 ± 0.003	0.038 ± 0.001 ***	58
BGLU	0.037 ± 0.002	0.025 ± 0.0005 **	67
NAGL	0.556 ± 0.018	0.347 ± 0.001 **	62
AP	0.915 ± 0.027	0.542 ± 0.017 ***	59
AAP	0.438 ± 0.005	0.469 ± 0.013	107
LAP	0.126 ± 0.009	0.149 ± 0.008	118
EL	0.026 ± 0.001	0.020 ± 0.0009	77
LL	0.066 ± 0.002	0.036 ± 0.001 ***	54
Cath.D	0.367 ± 0.020	0.468 ± 0.025	127

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ - the differences statistically confirmed

The activity of lysosomal enzymes in the course of the programmed glucose stress in the rabbits is unknown. On the basis of the results obtained it is possible to suggest that the introduce of exogenous glucose had a significant influence on changes in the activity of BGLU and AP in the liver; BGRD, BGAL, NAGL, AP, LL in the kidney; NAGL and LL in the muscle. The range of those changes dependend on the kind of enzyme and the tissue.

The activities of all estimated enzymes of all examined organs except AAP, LAP and Cath.D decreased in comparison with control group.

The high glucose concentration in the blood serum may be an indicator of diabetes (ANDERSON, 1997; HENRY, 1996) or an indicator of the oxidative stress in the tissues of animal.The reactive oxygen forms may disturb the normal carbohydrate metabolism (RANDLE et al., 1988).

On this way the glucose happens a metabolic stressor (HALL and BROWN, 1979; ANDERSON, 1997; NONOGAKI and UGUCHI, 1997).

Table 3
The activity of lysosomal enzymes ($\bar{x} \pm S_d$) in the muscle of the rabbits (in nmol/mg of protein/hour) after the 40% solution of glucose administration; control = 100% (Aktivität der lysosomalen Enzyme im Muskel von Kaninchen, Kontrollgruppe = 100%)

Enzyme	Muscle		
	Control	Experiment	%
BGRD	0.254 ± 0.013	0.226 ± 0.015	89
BGAL	0.322 ± 0.011	0.258 ± 0.010	80
BGLU	0.089 ± 0.005	0.072 ± 0.002	81
NAGL	1.090 ± 0.032	0.794 ± 0.025 **	73
AP	0.497 ± 0.020	0.417 ± 0.015	84
AAP	0.387 ± 0.019	0.378 ± 0.019	98
LAP	0.275 ± 0.011	0.288 ± 0.007	105
EL	0.037 ± 0.002	0.035 ± 0.007	94
LL	0.245 ± 0.008	0.147 ± 0.005 ***	60
Cath.D	0.454 ± 0.025	0.462 ± 0.025	102

* p < 0.05; ** p < 0.01; *** p < 0.001 - the differences statistically confirmed

Table 4
Analysis of variance for the activities of lysosomal enzymes in the liver, kidney and in the muscle of experimental rabbits; A – between groups; B – within groups; D.F. – in all cases for A – 1; for B – 18 (Varianzanalyse für die Aktivität der lysosomalen Enzyme in der Leber, der Niere und im Muskel von Kaninchen, Experimentalgruppe)

Enzyme and source of variance	Liver		Kidney		Muscle	
	Mean square	F	Mean square	F	Mean square	F
BGRD				**		
A	0.0006	0.1383	0.0044	8.2695	0.0039	0.1881
B	0.0045		0.0005		0.0205	
BGAL				***		
A	0.0011	1.2001	0.0061	14.4197	0.0206	1.8117
B	0.0009		0.0004		0.0114	
BGLU		**				
A	0.0005	5.0099	0.0007	0.1344	0.0014	0.8368
B	0.0001		0.0003		0.0017	
NAGL				**		**
A	0.0001	0.1526	0.2176	8.5654	0.4422	5.3937
B	0.0010		0.0254		0.0820	
AP		**		***		
A	0.1954	7.9034	0.6945	13.0723	0.0316	0.9566
B	0.2470		0.0531		0.0330	
AAP						
A	0.0029	1.0026	0.0050	0.4732	0.0004	0.0105
B	0.0029		0.0105		0.0362	
LAP						
A	0.0001	0.5101	0.0026	0.3696	0.0007	0.0834
B	0.0002		0.0071		0.0089	
EL						
A	0.0001	0.7096	0.0002	1.3528	0.0000	0.0770
B	0.0015		0.0001		0.0003	
LL				***		***
A	0.0001	0.3868	0.0047	17.3994	0.0483	9.9435
B	0.0061		0.0003		0.0049	
Cath. D						
A	0.0030	0.0876	0.0506	0.9991	0.0003	0.0048
B	0.6090		0.0506		0.0631	

* p < 0.05; ** p < 0.01; *** p < 0.001 - the differences statistically confirmed

The programmed glucose stress in the young bulls has been presented in our earlier paper (KOŁĄTAJ et al., 1998). We did not meet another communications connected with the reactivity of lysosomal enzymes under the influence of glucose in animal

tissues.

We suppose that the revealed changes in the activity of these enzymes are one of the elements of adaptation reaction to biochemical stress caused by the excess of exogenous glucose.

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