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The effect of intraperitoneally administered glucagon on the lysosomal enzyme activity in the liver of the mice

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The experiment was carried out on 20 Swiss male mice divided into experimental (E, n=10) and control (C, n=10) group. E mice were intraperitoneally injected with glucagon (15 µg/kg b.w.) twice daily for eight days, while mice C with 250 µl 0.9% NaCl/mouse (to exclude the injection effect only). In the lysosomal fraction of the liver the activities of acid phosphatase, β-N-acetyl-hexosaminidase, β-glucuronidase, β-glucosidase, lysosomal arylesterase, lysosomal lipase, leucine aminopeptidase and alanine aminopeptidase were determined. Glucagon caused an increase of activity of all studied enzymes except β-glucuronidase and β-glucosidase, where reduced activities were observed.

KEY WORDS: adaptation / glucagon / glucose / lysosomal enzymes / mice

Endocrine pancreatic functions in the course of diabetes were a subject of numerous studies [Cryer 2008, Henkel *et al*. 2005].

The lysosomal enzymes are important in the cell degradation processes [Beaujouin and Liaudet-Coopman 2008, Minazaki *et al*. 2008, Witek *et al.* 2007, 2008].

The participation of lysosomal enzymes in glucagon-induced autophagocytosis was first suggested by Ashford and Porter [1962]. It has been found that the formation of autophagic vacuoles after glucagon treatment *in vivo* was accompanied by an increase in fragility and osmotic sensitivity of lysosome membranes [De Duve and Wattiaux 1966, Deter and De Duve 1967].

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In this paper the effect of exogenous glucagon on the activity of some eight important lysosomal enzymes in mouse hepatocytes is discussed. Its influence on the activity of these enzymes is yet unexplained. Results could be useful to better interpretation of diabetes course and glucose metabolism.

Material and methods

The study was carried out on twenty Swiss male mice with18.5-20.3 g b.w., eight weeks old, and chosen randomly from the population maintained at the Institute of Genetics and Animals Breeding, Polish Academy of Sciences, Jastrzębiec. The animals were kept in standard cages at 21°C under 12 h of light *vs.* 12 h of darkness, were fed standard granulated feed (16% of protein) produced by Animal Food Company, Łomna near Warsaw and had free access to water. They received good veterinary care.

Animals were divided into two groups – experimental (E) and control (C), ten mice in each. E mice were injected intraperitoneally with glucagon - 15 μg/kg body weight (*Glucagoni hydrochloridum*, Novo Nordisk A/S; Bagsvaerd, Denmark) at a volume of 250 μl. C mice were injected with 250 μl of 0.9% NaCl. Both injections were continued over a period of eight days, twice daily, at 8.00 a.m. and 6.00 p.m.

Ten hours after last injection, mice of both groups were killed by cervical breaking. The liver was taken immediately and subjected to perfusion in 0.9% NaCl at +5°C, placed in 0.1 M phosphate buffer at +5°C, pH 7.0, at ratio 500 mg tissue/5 ml buffer. Next it was homogenized in the Potter-Elvehjem homogenizer with a Teflon piston at 200 rpm. Liver homogenates were centrifuged in Sorvall centrifuge for 8 min at 5500 rpm, and again for 20 min at 14000 rpm according to Beaufay [1972]. The lysosome precipitate was suspended in a 0.1 M phosphate buffer, pH 6.0 with Triton X100 and finally frozen at -20°C. In the supernatant the following activities were determined: acid phosphatase (AcP, EC 3.1.3.2), β-N-acetyl-hexosaminidase (Hex, EC 3.2.1.52), β-glucuronidase (β-GlcUr, EC 3.2.1.31), β-glucosidase (β-Glu, EC 3.2.1.21), lysosomal arylesterase (EL, EC 3.1.1.2), lysosomal lipase (LL, EC 3.1.1.13), leucine aminopeptidase (LeuAP, EC 3.4.11.1) and alanine aminopeptidase (AlaAP, EC 3.4.11.2).

The activities of AcP, Hex, β-GlcUr, β-Glu EL and LL were determined spectrophotometrically as 4-nitrophenyl derivatives at 420 nm according to Barrett's and Heath's [1977] micro-method. The activity of LeuAP and AlaAP was determined as Fast Blue BB salt (4-benzoyloamino-2.5-diethoxybenzene-diazonium chloride) derivatives at 540 nm according to the method of Mc Donald and Barrett [1986]. All substrates were produced by SERVA GmbH & Co (Heidelberg, Germany). The protein was determined with Kirschke and Wiederanders method [1984]. The activity of lysosomal enzymes was expressed in nmol/mg of protein/hour. Glucose content of blood plasma was determined with the enzymatic BIO-LACHEMA test method (Brno, Slovak Republic). The results obtained were evaluated according to the Student-Fisher

t-test. The experiment was approved by the Local Commission for Ethics in Animal Experimentation in the Institute of Genetics and Animal Breeding Polish Academy of Sciences, Jastrzębiec, Certificate S.6.9/99.

Results and discussion

Table 1 shows, that after eight days of injection, the mean blood plasma glucose concentration in the C group was 8.6 mmo/l (SD=0.3, n=10), while in group E – 12.9 mmo/l (SD=1.1, n=10, P≤0.01).

Table 1. Means and standard deviations (SD) for glucose concentration and lysosomal enzyme activity (nmol/mg protein/h) in the liver of mice after 8 days intraperitoneally glucagon injection (group E)

Glucose/enzyme		Control group (C)	Experimental group (E)	E as per cent of C
Glucose (mmol/l)	mean	8.6^{A}	12.9^{A}	150
	SD	0.3	1.1	
AcP	mean	9.06^{a}	11.20^{a}	124
	SD	0.760	0.635	
Hex	mean	5.53^{A}	9.07^{A}	164
	SD	1.08	1.00	
β-GlcUr	mean	0.711^{A}	0.401^{A}	56
	SD	0.034	0.034	
β-Glu	mean	0.586^{A}	0.175^{A}	30
	SD	0.077	0.024	
EL	mean	2.24^{a}	2.88^{a}	129
	SD	0.237	0.445	
LL	mean	2.72	3.09	114
	SD	0.226	0.275	
AlaAP	mean	5.53	6.19	112
	SD	0.381	0.306	
LeuAP	mean	6.66^{A}	9.26^{A}	139
	SD	0.422	0.810	

$^{aA\ldots}$Within lines means bearing the same superscripts differ significantly at: small letters – P≤0.05; capitals – P≤0.01.

The glucagon injection increased activity of AcP, Hex, EL and LeuAP (to 124, 164, 129 and 139%) in relation to the C group, respectively. Simultaneously, the significantly reduced activities of β-GlcUr and β-Glu (to 56 and 30% of the control). The activities of LL and AlaAP were not affected significantly by glucagon injections.

We have not found any reports concerning the influence of glucagon on the lysosomal hydrolases activity.

It is known that AcP affects the metabolic rate of phosphates. So, we can suppose, that glucagon stimulated AcP activity and in this way increased the glucose phosphorylation turnover in the liver.

The increase of Hex activity was probably connected with acceleration of the hydrolysis of glycopeptides and glycoproteins in tissues.

The increased EL activity can be related to the mobilization of lipids at higher level of glucagon in blood and more intensive their degradation under these conditions.

LeuAP hydrolyses the end-amino acids of protein chains, simultaneously showing the transferase and esterase activity. Exogenous glucagon increasing the LeuAP activity speeds up these processes. A considerable increase in LeuAP activity in liver can also be a result of increased synthesis of this enzyme [Yin *et al.* 2008].

Reduction of β-GlcUr and β-Glu activity was observed. According to Schmizu *et al*. [2000] glucagon injection may decrease the activity of these enzymes. This phenomenon can be related to the decrease of metabolic rate of glycosylation by this enzyme in liver, as more glucose molecules are utilized for energetic reactions.

LL and AlaAP did not change significantly under glucagon injection. It is possible, that this hormone did not alter the rate of lipid metabolism and degradation of amino acids of protein chains from their alanine side.

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Ewa Ochwanowska, Bożena Witek, Adam Kołątaj

Wpływ iniekcji glukagonu na aktywność enzymów lizosomowych w wątrobie myszy

S t r e s z c z e n i e

Eksperyment przeprowadzono na 20 samcach myszy Swiss, podzielonych na dwie grupy – doświadczalną (E) i kontrolną (C). Myszom grupy E podawano w iniekcji dootrzewnowej glukagon w dawce 15 µg/kg masy ciała, w objętości 250 µl 0,9% NaCl, dwa razy w ciągu dnia, w ciągu 8 dni. Myszom grupy C podawano w taki sam sposób 250 µl 0.9% NaCl. W lizosomowej frakcji wątroby oznaczano aktywność fosfatazy kwaśnej, β-N-acetylo-hexozaminidazy, β-glukuronidazy, β-glukozydazy, aryloesterazy lizosomowej, lipazy lizosomowej, aminopeptydazy leucynowej i aminopeptydazy alaninowej. Glukagon spowodował wzrost aktywności fosfatazy kwaśnej, β-N-acetyl-hexozaminidazy, lizosomowej aryloesterazy i aminopeptydazy leucynowej, a zmniejszył aktywność β-glukuronidazy i β-glukozydazy.