

Insulin-induced changes of proteolytic activity of the lysosomal enzymes

Bozena Witek¹, Ewa Ochwanowska¹, Adam Kolataj², Teodora Król¹,
Danuta Baranowska¹ & Jan Rafay³

¹ Department of Genetics, Institute of Biology, Swietokrzyska Academy, POLAND

² Institute of Genetics and Animal Breeding, Polish Academy of Sciences in Jastrzebiec near Warsaw, POLAND

³ Research Institute of Animal Production in Nitra, SLOVAK REPUBLIC

Correspondence to: Dr. Bozena Witek
Department of Genetics,
Institute of Biology, Swietokrzyska Academy,
Swietokrzyska 15, 25-406 Kielce, POLAND
EMAIL: b.witek@pu.kielce.pl
FAX: +4841 368 66 42

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Abstract

OBJECTIVES: Changes in the activity of alanine aminopeptidase, leucine aminopeptidase and cathepsins D and L in the liver and kidney of male and female of mice, injected with 0,4 IU/kg b.w. insulin for 4 and 8 days.

METHODS: The homogenates of the liver and kidney were taken for examination. The activity of alanine aminopeptidase, leucine aminopeptidase and cathepsins D and L has been determined according to [1] method.

RESULTS: The activity of alanine aminopeptidase, leucine aminopeptidase, cathepsins D and L in the liver and kidney of male and female of mice decreased in effect of insulin injections for 4 and 8 days.

CONCLUSION: The changes of enzyme activities showed a stimulating effect of the insulin injection on the labilization of lysosomal membranes. The range of the reaction remained in a relationship with the kind of the organ, the type of enzyme, time over which insulin introduced operates in the organism, and with the sex.

Introduction

The mechanisms regulating the activity of the lysosomal system, and the secretion of its enzymes evoke considerable interest in numerous biochemical laboratories as indicated by the increasing number of papers on this topic found in literature [2-11]. The results of these investigations showed that the changes the activity of the degradative system of the lysosomal compartment are a significant factor maintaining the cell in a state of dynamic homeostasis as well as an important indicator of reactivity to stress.

In our experiment we studied the changes of the activity of the proteolytic enzymes in the liver and kidney of mice exposed to the action of the pharmacological doses of the exogenous insulin.

Material and methods

The study was performed out on 56-day-old 45 male and 45 female of mice chosen at random, weighed about 22.0 ± 1.10 g, from the Institute of Genetics and Animal Breeding of the Polish Academy of Sciences in Jastrzebiec [Poland]. The animals were kept in standard cages in a

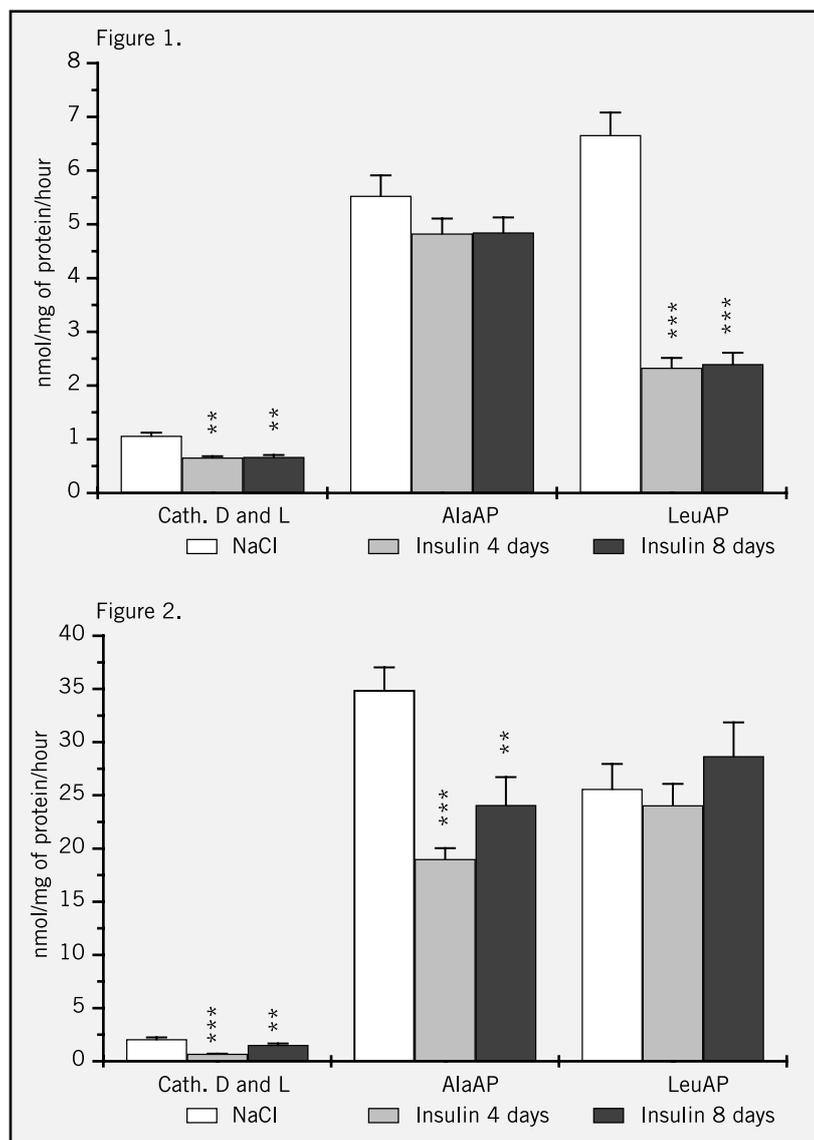


Figure 1. The activity [$\bar{x} \pm SD$] of the cathepsins D and L, alanine aminopeptidase, and leucine aminopeptidase (in nmol/mg of protein/hour) in the liver of males of mice after 4 and 8 days of insulin administration.

Figure 2. The activity [$\bar{x} \pm SD$] of the cathepsins D and L, alanine aminopeptidase, and leucine aminopeptidase (in nmol/mg of protein/hour) in the kidney of males of mice after 4 and 8 days of insulin administration.

After a suitable time, mice were killed by cervical dislocation, and slices of the liver, and kidney were taken immediately. The slices of the liver were subjected to perfusion 0,9% NaCl solution cooled to +5°C and similarly with the slices of the kidney were suspended in 0,1 M phosphate buffer, pH 7,0 at the temperature +5°C, at 500 mg tissue/per 4 ml solution ratio. The material was homogenized in a teflon homogenizer at 200 rotations/min. Differential centrifugation of the liver and kidney homogenates was carried out according to [12] method.

In the lysosomes of the liver and kidney the activity [in nanomol/mg of protein/hour] of leucine aminopeptidase [LeuAP, EC 3.4.11.1], alanine aminopeptidase [AlaAP, EC 3.4.11.2] was estimated according to [1] method, and total proteolytic activity of cathepsins D and L without inhibitors [Cath. D, EC 3.4.23.5; Cath. L, EC 3.4.22.15] was estimated according to [13] method. Protein was also determined in the lysosomes of the liver and kidney according to [14] method, and glucose level in the blood plasma according to the enzymatic method using “Bio-Lachema-Test” [Brno, Slovak Republic]. All substrates were from Serva Feinbiochemica GmbH & Co. [Heidelberg, Germany]. The results obtained were analyzed statistically according to the Student-Fisher *t*-test.

The experiment was approved by the University Ethics Commission for Animal Research of the Swietokrzyska Academy in Kielce.

ventilated room in natural photoperiod at 21°C. They were fed with standard food mixture [16% of protein – Murigran, product of Poland from “Animal Food Company” from Motycz near Lublin], with constant access to water. All animals received good veterinary care. Mice have been divided into four experimental groups [male I–II; female III–IV], and control groups [male–V; female–VI; n = 15 individuals in each group]. Mice of the experimental groups [I and III] were injected subcutaneously with 0,4 IU/ per kg of body weight of exogenous insulin [Insulin semilente ChO-S, Polfa Tarchomin S.A., Poland] in the amount of 250 µl twice daily at 8:00–9:00 a.m. and 6:00–7:00 p.m. for four days. The mice of the experimental groups [II and IV] were injected with insulin analogously for eight days. Mice of the control groups [V and VI] received subcutaneously 250 µl of 0,9% NaCl for four days.

Table 1. Glucose level [$\bar{x} \pm SD$] in blood plasma of the males and females after 4 and 8 days of insulin administration;

Traits	Control [before insulin injection]	after 4 days of insulin administration	after 8 days of insulin administration
Glucose [mmol/L] males	5.71 ± 1.90	4.82 ± 1.24*	4.73 ± 1.03*
females	5.80 ± 1.74	5.01 ± 1.19	4.79 ± 1.20*

* P < 0,05 – statistically confirmed differences;

Figure 3. The activity [$\bar{x} \pm SD$] of the cathepsins D and L, alanine aminopeptidase, and leucine aminopeptidase (in nmol/mg of protein/hour) in the liver of females of mice after 4 and 8 days of insulin administration.

Figure 4. The activity [$\bar{x} \pm SD$] of the cathepsins D and L, alanine aminopeptidase, and leucine aminopeptidase (in nmol/mg of protein/hour) in the kidney of females of mice after 4 and 8 days of insulin administration.

Results

Table 1 shows that the glucose level in the blood serum of the males after 4 and 8 days insulin injection decreased significantly to 4.82 ± 1.24 mmol/L and to 4.73 ± 1.03 mmol/L, respectively. Glucose level in the blood serum of females decreased markedly to 4.79 ± 1.20 mmol/L, after 8 days of insulin injection, only.

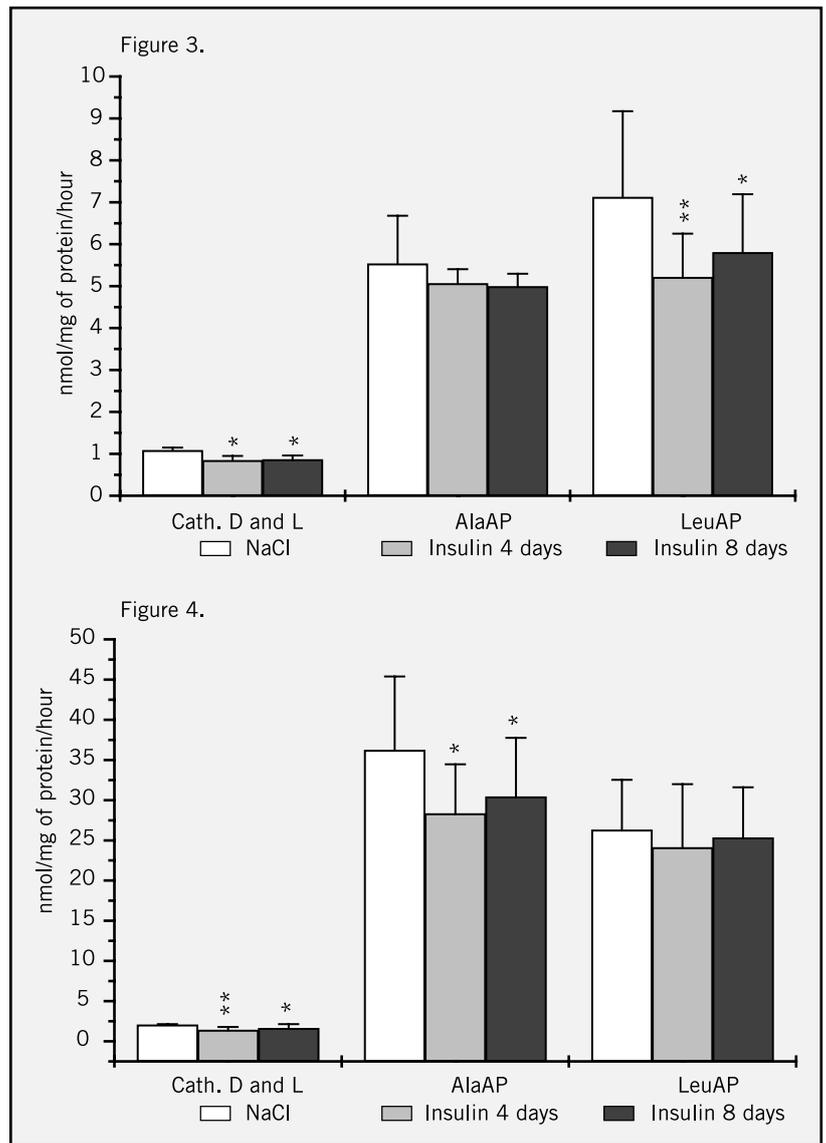
Figures 1–4 show that the activities of all estimated proteolytic enzymes of the liver and kidney of males [Figures 1 and 2] and females [Figures 3 and 4] decreased in comparison with the values of the control group.

As can be seen from the Figure 1, total proteolytic activity of cathepsins D and L in the liver of males decreased significantly after 4 and 8 days of insulin administration [to 0.660 ± 0.025 and to 0.670 ± 0.037 , respectively]. The leucine aminopeptidase activity decreased markedly after 4 and 8 days [to 2.33 ± 0.184 and to 2.40 ± 0.210], suitable.

Figure 2 informs that cathepsins D and L activity in the kidney decreased significantly after 4 and 8 days of insulin administration [to 0.704 ± 0.014 , and to 1.534 ± 0.137 , respectively]. The alanine aminopeptidase activity decreased statistically confirmed to 19.02 ± 1.01 and to 24.09 ± 2.62 after 4 and 8 days of insulin injection, respectively.

According to Figure 3, activity of cathepsins D and L in the liver of females decreased markedly after 4 and 8 days of insulin injection [to 0.843 ± 0.105 and to 0.860 ± 0.098 , suitably]. Leucine aminopeptidase activity decreased to 5.21 ± 1.04 after 4 days, and to 5.80 ± 1.39 after 8 days of insulin administration.

As can be seen in Figure 4, the activity of cathepsins D and L in the kidney of females decreased to 1.38 ± 0.401 and to 1.61 ± 0.529 , and activity of alanine



aminopeptidase decreased markedly to 28.31 ± 6.15 and to 30.42 ± 7.33 after 4 and 8 days of insulin administration, respectively.

Discussion

The environmental unfavourable effects on animals cause among others, stress responses. In the opinion of many authors stress in an adaptation response which involves almost all the structure of body of lively organism, beginning from the molecular level [15–19]. One of the cell arrangements which takes part in those responses is the lysosomal complex.

The lysosomal compartment, participating in the processes of transport and liquidation of exogenous substances as well as already used cell organella, reacts as one of the first cytoplasmic systems by activating the resistance mechanisms in situations constituting a threat to the maintenance of the existing homeostasis [20]. Proteolytic lysosomal enzymes e.g. cathepsin D are widely accepted tissue markers of tumorous and the important diagnostic factors [21–22]. Catabolism of cell protein plays a decisive role during the embryogenesis [23–24].

The results of our investigations on the effect of injecting mice with exogenous insulin indicated significantly decreased of the activity of cathepsins D and L, alanine aminopeptidase and leucine aminopeptidase. The insulin is an anabolic hormone, which modify

carbohydrates, lipids and proteins metabolism [25–26]. It regulates the glucose metabolism by increasing production of glycogen, fatty acids, triglycerides, glycerol and dioxide [27–29]. The insulin intensify intracellular protein synthesis and has significant influence on production of ATP – using in amino acids activation [30–31]. As the research showed [32] the insulin injection increased its participating in protein biosynthesis and decreased the range of proteolytic process, causing simultaneously decrease of amino acids level in the blood [33].

On the basis of our results obtained, one can see a different influence of the insulin on the activities of investigated proteolytic lysosomal enzymes. The insulin injected for 4 and 8 days in dose 0,4 IU/per kg of body weight decreased the activities of the examined hydrolases in the liver and kidney of males and females. The significant decrease of the activity of these enzymes depend principally on the type of enzyme, time over which insulin introduced operates in the organism, on the sex, and on the organ. Similar observations were conducted [34]. These investigations indicate, that insulin had a stabilizing effect on the lysosomal membranes when exposure to the insulin was prolonged.

Our results suggest that the insulin can act as metabolic stressor eliciting adaptative responses in hepatocytes and kidney cells. Presumably these changes are connected with the function of the insulin as a compound which is able to intensify processes of degradation in the lysosomal compartment of the cell. We suggest that changes in the reactivity of the examined proteolytic lysosomal enzymes caused by the action of exogenous insulin can be regarded as manifestations of the organisms adjustment to biochemical stressors disturbing their normal cell homeostasis.

REFERENCES

- Mc Donald JK, Barrett AJ. Exopeptidases. In: Mammalian Proteases: A Glossary and Bibliography Acad Press, London, 1986; 111–144.
- Kolataj A, Bulla J, Poltarsky J, Witek B, Król T. Activities of some leucocyte lysosomal hydrolases of pigs under the effects of diverse stress models. *J Anim Physiol Anim Nutr* 1996; 191–198.
- Król T, Schmidt M, Kolataj A, Witek B. Vinblastine-induced autophagy in mouse liver. *Comp Biochem Physiol* 1994; 1:165–169.
- Raben N, Plotz P, Byrne BJ. Acid alpha-glucosidase deficiency [glycogenesis type II, Pompe disease]. *Curr Mol Med* 2002; 2:145–166.
- Rohrer J, Kornfeldt R. Lysosomal hydrolase mannose-6-phosphate uncovering enzyme resides in the trans-Golgi network. *Mol Biol Cell* 2001; 12:1623–1631.
- Sommer A, Witek B, Kolataj A. The effect of exogenous glycerol on the activity of lysosomal enzymes in the blood plasma of young bulls. *Arch Tierz* 1999; 5:451–458.
- Tanaka Y, Tanaka R, Kawabata T, Noguchi Y, Himeno M. Lysosomal cysteine protease, cathepsin B, is targeted to lysosomes by the mannose-6-phosphate-independent pathway in rat hepatocytes: site-specific phosphorylation in oligosaccharides of the proregion. *J Biochem* 2000; 128:39–48.
- Witek B, Kolataj A. Effect of ethanol administration on activities of some lysosomal hydrolases in the mouse. *Gen Pharmacol* 1999; 32: 163–168.
- Witek B, Legath J, Kolataj A, Kalinska O, Banasik A. The effect of small doses of mercury on the level of selected lysosomal enzymes in the plasma and lymphocytes of sheep. *Gen Pharmacol* 1996; 27: 901–903.
- Witek B, Król T, Kolataj A, Ochwanowska E, Stanislawski I, Slewa A. The insulin, glucose and cholesterol level and activity of lysosomal enzymes in the course of the model alloxan diabetes. *Neuroendocrinology* 2001; 22:240–244.
- Witek B, Ochwanowska E, Slewa A, Kolataj A. Effect of hydrocortisone on the activity of lysosomal enzymes in mice. *Neuroendocrinology* 2002; 23:105–108.
- Beaufay H. Methods for isolation of lysosomes. W: Lysosomes [Dingle T. Ed.]. A Laboratory Handbook, North-Holland Publ. Co., [Amsterdam] 1972; 1–30.
- Langner J, Wakil A, Zimmermann M, Ansorge S, Bohley P, Kirschke H, Wiederanders B. Aktivitätsbestimmung proteolytischer Enzyme mit Azokasein als Substrat. *Acta Biol Med. Germ* 1973; 31:1–18.
- Kirschke H, Wiederanders B. Methoden zur Aktivitätsbestimmung von Proteinasen. Martin-Luther-Universität, Halle-Wittenberg Wissenschaftl Beitr Halle/Salle 1984; 11–17.
- Dallaire A. Stress and behaviour in domestic animals. *Ann New York Acad Science* 1993; 697:269–274.
- Janssens CJG, Helmond FA, Wiegant VM. The effect of chronic stress on plasma cortisol concentrations in cyclic female pigs depends on the time of day. *Domest Anim Endocrinol* 1995; 12:167–177.
- Kolataj A. Phenomenon of the stress. Wydawnictwo Naukowe Wyższej Szkoły Pedagogicznej, Kielce Poland 1993; 1–205.
- Mc Carty R, Gold PE. Catecholamines, stress, and disease: A psychobiological perspective. *Psychosom Med*. 1996; 58:590–597.
- Olsson IAS, De Jonge FH, Schurman T, Helmond FA. Poor rearing conditions and social stress in pigs: repeated social challenge and the effect on behavioural and physiological responses to stressors. *Behav Process* 1999; 46:201–217.
- Karageorgos LE, Isaac EL, Brooks DA, Ravenscroft EM, Davey R, Hopwood JJ, Meikle PJ. Lysosomal biogenesis in lysosomal storage disorders. *Exp Cell Res* 1997; 234:85–98.
- Duffy MJ. Proteases as prognostic markers in cancer. *Clin Cancer Res* 1996; 2:613–618.
- Kageshita T, Yoshi A, Kimura T, Maruo K, Himeno M, Nishimura Y. Biochemical and immunohistochemical analysis of cathepsins B, H, L, and D in human melanocytic tumors. *Arch Dermatol Res* 1995; 287:266–272.
- Agarwal S, Sohal RS. Age and proteolysis of oxidised proteins. *Arch Biochem Biophys* 1994; 3089:24–28.
- Banay-Schwartz M, De Guzman T, Kenessey A, Palkovits M, Lajtha A. The distribution of cathepsin D activity in adult and ageing human brain regions. *J Neurochem* 1992; 50:2207–2211.
- Doherty JJ, Kay DG, Lai WH, Posner BI, Bergeron JJM. Selective degradation of insulin within rat liver endosomes. *J Cell Biol* 1990; 108:2093–2099.
- Seabright PJ, Smith GD. The characterization of endosomal insulin degradation intermediates and their sequence of production. *Biochem J* 1996; 320:947–956.
- Lewis GF, Zinman B, Steiner G, Vranic M, Giacca A. Peripheral effects of insulin on hepatic glucose production (HGP) in humans. An important role for glucagon. *Diabetes* 1995; 44:196A–199A.
- Satake S, Moore MC, Igawa K, Converse M, Farmer B, Neal DW, Cherrington AD. Direct and indirect effects of insulin on glucose uptake and storage by the liver. *Diabetes* 2002; 51:1663–1671.
- Sindelar DK, Balcom JH, Chu C A, Neal DW, Cherrington AD. A comparison of the effects of selective increases in peripheral or portal insulin on hepatic glucose production in the conscious dog. *Diabetes* 1996; 45:1594–1604.
- Komatsu M, Sato Y, Yamada S, Yamauchi K, Hashizume K, Aizawa T. Triggering of insulin release by a combination of cAMP signal and nutrients: an ATP – sensitive K⁺ channel – independent phenomenon. *Diabetes* 2002; 51:29–32.
- Waldegger S, Busch GL, Kaba NK, Zempel G, Ling H, Heidland A, Haussinger D, Lang F. Effect of cellular hydration on protein metabolism. *Miner Electrolyte Metab* 1997; 23:201–205.
- Fawcett J, Hamel FG, Duckworth WC. Characterization of the inhibition of protein degradation by insulin in L cells. *Arch Biochem Biophys* 2001; 385:357–363.
- Sidney M, Morris PJr. Regulation of enzymes of the urea cycle and arginine metabolism. *Annu Rev Nutr* 2002; 22:87–105.
- Stvolinskaya N, Poljakova E, Nikulina P, Korovkin B. Effect of insulin on permeability of lysosome membrane in primary monolayer hepatocyte culture of newborn rats under anoxia conditions. *Skand J Clin Lab Invest* 1992; 52:791–796.