ORIGINAL ARTICLE

Effect of ghrelin on activities of some lysosomal hydrolases in rabbits

Bożena Witek[1], Ewa Ochwanowska[1], Jan Rafay[2], Adam Kołątaj[3], Peter Chrenek[2], Karin Suvegova[2], Rastislav Jurcik[2], Alexander Sirotkin[2] & Krzysztof Darlak[4]

[1] Department of Genetics, Institute of Biology, Świętokrzyska Academy, Kielce, Poland.
[2] Research Institute of Animal Production, Nitra, Slovak Republic.
[3] Institute of Genetics and Animal Breeding, Polish Academy of Sciences in Jastrzębiec, Wólka Kosowska near Warsaw, Poland.
[4] International Department of Sciences Inc. Peptides, Louisville, 40299 Kentucky, USA.

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: Observing the changes of activity of some lysosomal enzymes in blood serum of female rabbits subjected to injection of 10 μg of ghrelin/kg of body weight.
METHODS: In the blood serum the activity of cathepsins D and L, alanine aminopeptidase, acid phosphatase, lysosomal lipase and lysosomal esterase was determined.
RESULTS: As a result of ghrelin injection the activity of all the enzymes examined in blood serum increased markedly.
CONCLUSION: Changes of lysosomal enzymes activities in the blood serum caused by the effects of ghrelin should be regarded as the response of the lysosomal system.

Introduction

Ghrelin, an endogenous GH secretagouge, discovered in rat and human stomach, is a 28-amino-acid peptide with a unique *n*-octanoyl modification on serine residue 3 that is necessary for its biological activity [1]. This modification plays a decisive role for the biological activity of ghrelin. Ghrelin may have other functions in some tissues other than pituitary, because the GHS receptor is expressed in heart, lung, pancreas, intestine, and adipose tissue [2]. Apart from potential paracrine effects, ghrelin may also link endocrine between stomach, hypothalamus and pituitary, suggesting an involvement in regulation of energy balance [3].

The problem of stress reactivity in animals is still the focus of interest of biologist and breeders. In response to the stressor, which is a strong informatic and energetic excitation, the organism reacts with the increased energy expenses [4–8]. The lysosomal enzymes react quickly by starting the defensive mechanisms in the states of threat

to the organism's homeostasis. Lysosomal enzymes were discussed in different aspects [9–11].

However, we have not found in the literature reports concerning the effect of ghrelin – which is involved in the process of food intake – on the activity of lysosomal hydrolases in blood plasma of New Zealand rabbit.

Material and methods

The experiment was carried out on 36 females of New Zealand rabbit at the 180 days, weighed 3 800 g ± 150 g, coming from a farm of the Research Institute of Animal Production in Nitra [Slovak Republic]. All rabbits were maintained in identical conditions of nutrition and nursing. The animals were divided into 2 groups [n = 18 individuals]. The animals of the experimental group injected intramuscularly [right femoral muscle] with ghrelin [Peptides International Incl., Louisville, Kentucky, USA] per 10 μg/kg of body weight for seven days, daily at 8:00–9:00 a.m. The control rabbits received 1000 μl 0.9% sodium chloride solution, analogously. In 5 day of administration of ghrelin, the females were stimulated by application of PMSG [pregnant mare serum gonadotrophin, SERGON, Bioveta, Czech Republic] in dose 25 IU/female. After end of administration of ghrelin females were subjected artificial insemination. In aim of stimulation of the insemination females received supergestran Lecirelinum [Ferring-Leciva, Czech Republic], applied as superanalogue LH-RH [releasing factor luteinizing hormone] in volume 0,1ml/individual. Blood to analyses' was taken before application of ghrelin and in 7 day his administration. After that time, blood was taken from the ear vein and placed in heparinized test tubes.

In the blood serum the activity of the acid phosphatase [AcP, EC 3.1.3.2] according to [12], cathepsin D and L [Cath. D, EC 3.4.23.5; Cath. L, EC 3.4.22.15] according to [13], using 2% azocasein in 6M urea as substrate, alanine aminopeptidase [AlaAP, EC 3.4.11.2] according to [14], lysosomal lipase [LL, EC 3.1.1.13] and lysosomal esterase [EL, EC 3.1.1.2] according to [12] was determined. Protein was also determined in the blood serum [15]. The enzyme activity has been expressed in μmol/l/h.

All substrates were from Serva Feinbiochemica GmbH & Co., Heidelberg, Germany. The results obtained were analyzed statistically according to the Student's *t-test*. The experiment has been confirmed by the University Ethics Commission for Animal Research of the Świętokrzyska Academy in Kielce.

Results

The Table 1 and Figure 1 indicate that, versus the control, in the blood serum ghrelin administration increased statistically the activity of Cath. D and L [to 141,23], AcP [to 83,57], LL [to 90,91], and EL [to 58,26].

Discussion

The ghrelin is at present in area of the endocrinologists', nutritionists', internists' and pediatrician's interests. This hormone is predominantly produced in the stomach, but its presence has been also detected among others in the bowel, pancreas, kidney, pituitary and hypothalamus [16]. The actions of ghrelin are mediated by specific receptors. Two types of receptors, referred to as GHS-R types 1a and 1b. Both receptors are widely distributed in central [brain, pituitary] and peripheral organs [17–20]. GHS-R 1a is predominantly expressed in the pituitary. It is also distributed in other central and peripheral tissues, but at much lower levels [21]. Ghrelin and its receptors were found also in the epsilon cells of pancreatic islands recently. The same explorers show that the epsilon cells develop from the same precursor cells, as the beta cells, which take part in insulin production.

Structure-function analysis of ghrelin has demonstrated that the first five amino acids of this peptide, including the *n*-octanoyl modification at position 3, are capable of binding the GHS receptor to stimulate the release of intracellular calcium [17].

Ghrelin secretion rises before food intake [22–23], and it has been shown to stimulate food consumption [24–25] and adipogenesis [26]. Ghrelin stimulates the secretion of insulin and gastric acid [27–28], and ghrelin possibly is leptyn's antagonist, which acts on hypothalamic centres to regulate food intake and energy expenditure [29–30].

Research shows that in starved animals' blood higher level of ghrelin was affirmed than in control animal [25]. In addition, it has been found that fasting plasma level of ghrelin is negatively correlated with body mass

Table 1. Activity [$\bar{X}$ ± SD] of studied enzymes [in μmol/l/h] of New Zealand rabbits after ghrelin injection

Enzyme	Control	Ghrelin %	
Cath. D and L	120, 7 ± 32,1	141,23 ± 33,4*	117
AlaAP	101,3 ± 25,6	115,5 ± 48,2	114
AcP	65,87 ± 17,8	83,57 ± 19,3**	127
LL	69,93 ± 9,14	90,91 ± 25,5***	130
EL	48,15 ± 22,04	58,26 ± 19,21*	121

* P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001 – statistically confirmed differences

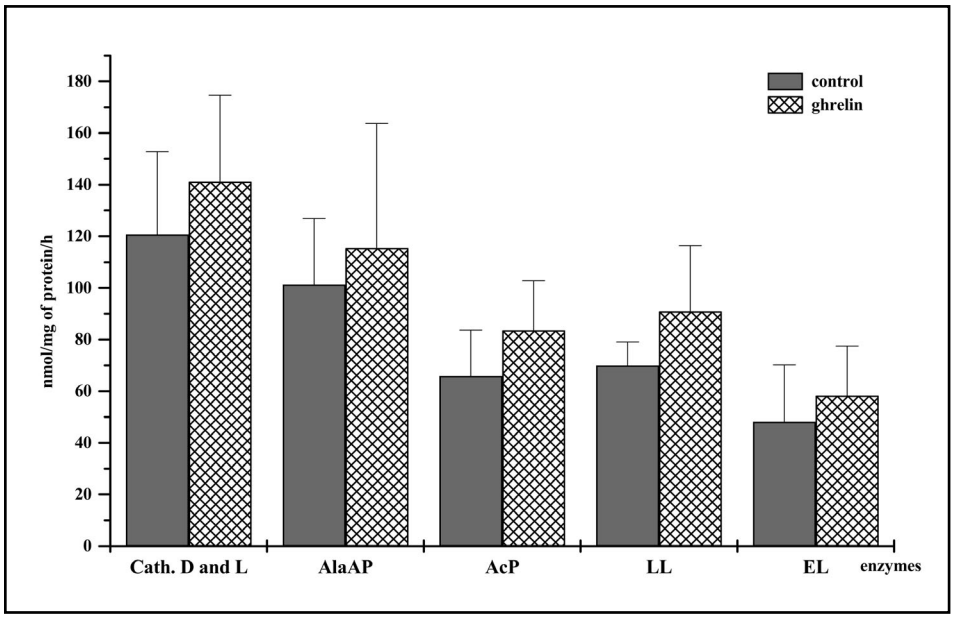

Figure 1: Activity of studied enzymes [in µmol/l/h] of New Zealand rabbits after ghrelin injection

index [31]. Mice which ghrelin was administered to had enlarged hunger and, as a result, raised body mass [32]. Whereas the decrease of body mass was observed in mice which leptyn's blood was injected to. It also turned out, that the mutations of genes which code leptyn's are the cause of the obesity consequential from excessive gorging in mice. Relationship between ghrelin and insulin effect and whether ghrelin takes part in metabolism of glucose remain unknown [28]. Probably further research over ghrelin will have essential influence on the treatment of nourishing disorders, such as obesity or devastation [26].

The physiological stability of organisms depends largely, amongst other factors, on the reactivity of enzymatic cell complexes and endocrine status. Numerous studies indicate that lysosomal system is designed not only for intracellular digestion, but – due to the high reactivity of hydrolases contained in it – it also has an important role in the adaptation processes. The activities of investigated lysosomal enzymes increased after the injection of ghrelin. We suggest that changes in the reactivity of the examined lysosomal enzymes caused by the action of ghrelin can be regarded as manifestations of the organism's adjustment to biochemical stressors disturbing their normal cell homeostasis.

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