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NEUROMODULATORY ACTION OF OBESTATIN ON THE SECRETORY ACTIVITY OF THE HYPOTHALAMIC-PITUITARY AXIS

Abstract

In 2005, Zhang and co-workers presented information about the new peptide – obestatin. It is a small peptide which is the product of the GHRL gene also responsible for encoding another protein – ghrelin. Obestatin and ghrelin, are produced by post-translational processing of a common precursor preproghrelin and what intriguing characterized by different physiological activity. Obestatin originally has been isolated from cells located in the mucosa of the rat gastric fundus, but successive studies have shown the obestatin presence in other tissues of the body. It was also found that this peptide affinity for the orphan G protein-coupled receptor 39 (GPR39) and receptor for glucagon-like peptide-1 (GLP-1R).

As mentioned, obestatin and ghrelin are mainly synthesized by the cells lining the bottom of the stomach. Both peptides are transferred into the blood circulation, where they exhibit an endocrine activity. So far, the information about the obestatin action at the central nervous system (CNS) level are fragmentary and often contradictory. This is due to both, an ambiguous receptor determination and the lack of knowledge about the peptide agonists and antagonists. For this reason, in their deliberations we will focus on obestatin actions in the hypothalamic-pituitary axis.

Keywords: obestatin, central nervous system, hypothalamus

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Introduction

In 2005, Zhang and co-workers presented information about the new peptide – obestatin (*lat. obedere* – devour, and *statin* – inhibit). It is a 23 amino acid peptide (mass of about 2.5 kDa), which is the product of the *GHRL* gene also responsible for encoding the ghrelin protein. Both of these peptides arise as a result of post-translational processing of a common precursor preproghrelin, and what is intriguing they show different physiological activity: ghrelin is a orexigenic peptide, which stimulate appetite while obestatin exhibits a anorexigenic effect, affecting on the food intake inhibition. Obestatin originally has been isolated from cells located in the mucosa of the rat stomach fundus, but subsequent studies also demonstrated its presence in other tissues such as the duodenum, jejunum, colon, pancreas, liver, mammary glands, testies (Leydig cells), the lungs, saliva and blood plasma (Dun et al. 2006; Zhang et al. 2008; Zhang et al. 2005). Results of research conducted by Huda et al. (2008) showed that obestatin levels in the blood does not change significantly after the procedure of gastrectomy, what confirms that the stomach is not the only place in the body where obestatin is synthesized. Both obestatin and ghrelin are then transferred into the blood circulation, where they exhibit a endocrine activity.

As mentioned earlier obestatin and ghrelin are encoded by a single gene, which in human, is located on chromosome 3p26-p25, whereas in the rat is on chromosome 4q4. This gene consists of four exons, three introns and additional small (20 bp) exon 0, which are responsible for encoding of 117 amino acid protein – preproghrelin (Kanamoto et al. 2004; Nakai et al. 2004). Preproghrelin contains a signal peptid and a 94-amino acid segment called proghrelin, which undergoes a proteolytic processing that leads to the generation of the mature native ghrelin peptide, and also, to an additional C-terminal peptide which is named C-ghrelin. C-ghrelin can subsequently undergo further proteolytic processes generating an alternative, functional peptide – obestatin (Sato et al. 2012).

In the last few years an extra exon (–1) in the *GHRL* gene has been discovered. Analysis of the *GHRL* gene expression allowed to establish that functional peptide – obestatin with amino acid formula: FNAPFDVGIKLSGAQYQQHGRAL-NH2 is formed from exons – 1, 3, 4 as a result of alternative splicing (Figure 1) (Seim et al. 2010; Seim et al., 2007; Soares and Leite-Moreira 2008).

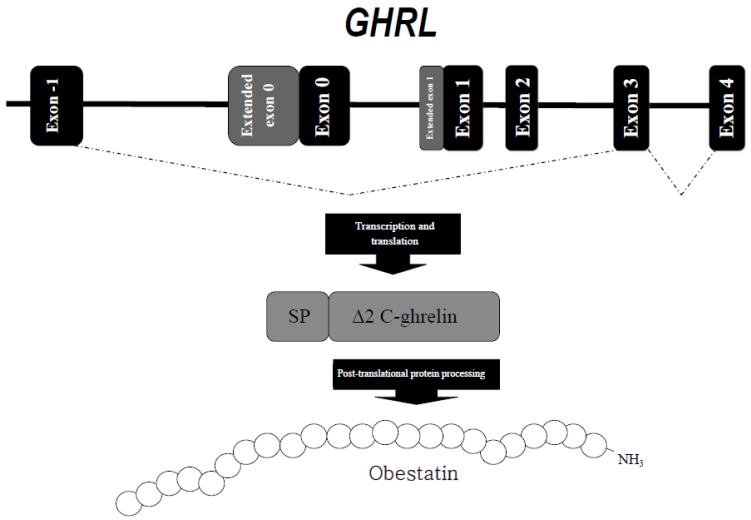

Figure 1. The structure of *GHRL* gene and obestatin synthesis processing pathway

Receptors

In the course of research Zhang et al. (2005, 2008) found that obestatin shows affinity for the orphan G protein-coupled receptor 39 (GPR39). This receptor belongs to the family of rhodopsin-like receptors, and has been identified previously as a highly specific receptor for growth hormone (GH) (McKee et al. 1997). Analysis of expression of GPR39 receptor gene revealed the existence of two isoforms of it. GPR39-1a isoform is composed of 423 amino acids (aa), while second isoform – GPR39-1b consists of 284 aa. High expression of GPR39-1a was observed in organs such as liver, pancreas, kidneys or in adipose tissue. Interestingly, this receptor is not expressed in the CNS, while isoform GPR39-1b has a high expression in central nervous system (Egerod et al. 2007). Surprisingly, further studies with knockout GPR39$^{-/-}$ mice did not confirm the obestatin affinity to GPR39 receptor (Tremblay et al. 2007; Moechars et al. 2006). However, the data from nuclear magnetic resonance analysis of human obestatin structure showed that obestatin have the necessary domain required for full activation of the GPR39 receptor (Alén et al. 2012).

The research conducted by Italian researchers on human pancreas suggest the possibility of obestatin binding to the receptor for glucagon-like peptide-1

(GLP-1R) (Granata and Ghigo 2013; Granata et al. 2008). Its expression has been found in various tissues of the body, also in the CNS, where it can act as a factor for appetite regulation. Also in this case we can find in literature articles which say that there is no interaction between the GLP-1R and obestatin (Unniappan et al. 2008).

Endocrine effects of obestatin

It is assumed that, both obestatin as ghrelin have the ability to penetrate the blood-brain barrier. In studies using intravenously given radiolabelled obestatin, rapid influx of this peptide into the brain structures and the simultaneous rapid degradation has been shown (Pan et al. 2006). This effect can be explained by the short half-life of obestatin in the blood plasma. Many researchers stress the importance of obestatin in the regulation of the body metabolism at the CNS level. It was further proven that obestatin can modulate by auto- and/or paracrine way the release of other hormones such as for example growth hormone (GH) (Grönberg et al. 2008). However information about the acting of obestatin in the CNS are fragmentary and often conflicting against each other. This results from the ambiguous of determination of receptor assay and the lack of knowledge about the peptide agonists and antagonists.

Obestatin administered directly into the arcuate nucleus of the hypothalamus caused a significant reduction in daily calorie intake and weight gain of the animals. However, the results of studies where obestatin was administered directly into the hypothalamus did not show its influence on the mRNA level for both, anorexigenic factors (cocaine and amphetamine regulated transcript – CART, corticotropin-releasing hormone – CRH, pro-opiomelanocortin – POMC) and orexigenic factors (agouti-related protein – AgRP, neuropeptide Y – NPY, orexin-A) (Nogueiras et al. 2007). No change in the level of dopamine, norepinephrine and serotonin was also found in animals treated with obestatin. However it was observed, that the obestatin administration induces dose-dependent inhibition of dopamine release associated with the depolarization of nerve cells. Moreover, it was demonstrated, that obestatin blocked the effect of ghrelin-dependent inhibition of serotonin secretion in hypothalamic cells (Brunetti et al. 2010). The research on the anorexigenic action of obestatin in brain showed, that both: the intracerebroventricular and peripheral obestatin

administration caused a significant reduction in the level of vasopressin secretion (Samson et al. 2008; Samson et al. 2007).

In the course of experiments it was found that ghrelin stimulates the pituitary hormones secretion such as prolactin (PRL), adrenocorticotropic hormone (ACTH) and thyroid stimulating hormone (TSH), therefore researchers set out to test the effect of obestatin on release of these hormones (Figure 2) (Arvat et al. 2001; Hataya et al. 2001; Kojima et al. 1999). Interestingly, intravenous and intraventricular obestatin administration did not affect the change in hormone levels in the blood plasma (Yamamoto et al. 2007). So far, in the literature there is no information on the obestatin effects on the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) by pituitary cells.

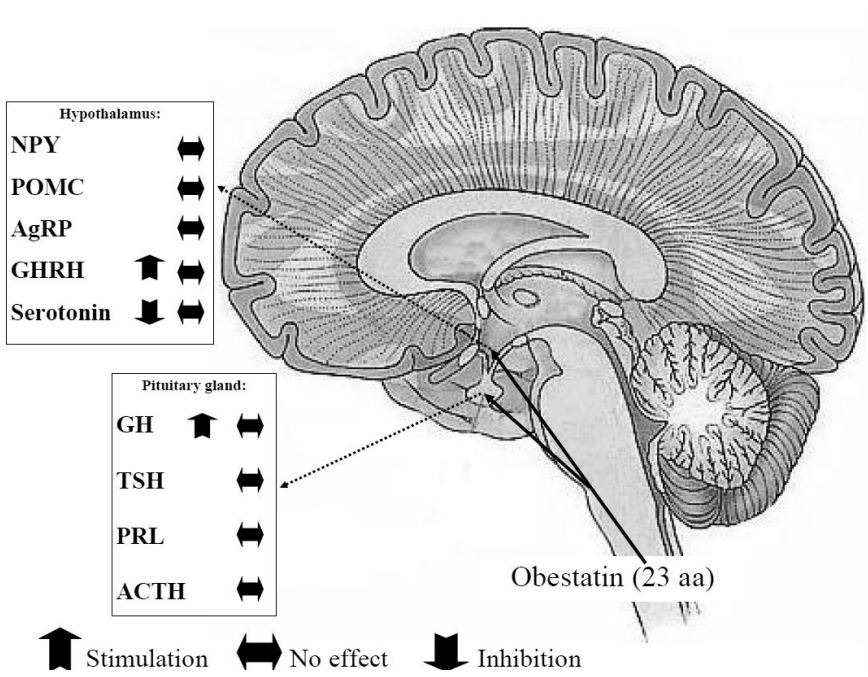

Figure 2. Action of obestatin in the chosen brain structures

The effect of obestatin on the somatotropic axis

Interesting observations were made by Feng et al. (2011), who checked the effect of ghrelin and obestatin on GH releasing hormone (GHRH). In their studies, they demonstrated that obestatin administered *in vitro* does not affect the release of GHRH from hypothalamic neurons. Simultaneously, obestatin causes the abolition of stimulating effects of ghrelin on GHRH release. Also Hassouna et al. (2012) in their work showed that obestatin may block ghrelin-dependent release of GH, acting through the NPY and GHRH neurons. Majority of the research on obestatin focuses on understanding its impact of the release of GH from pituitary cells. However the results of the effect of obestatin on GH secretory activity are ambiguous and often contradictory. *In vitro* studies have shown no increase of GH release from the cultured rat pituitary cells treated with exogenous obestatin (Zhang et al. 2005). *Ex vivo* experiments where pituitaries from rats was incubated with obestatin (which was added to the medium during 15-min periods) did not change basal GH levels but markedly inhibited ghrelin-induced GH secretion (Zizzari et al. 2007). Very interesting observations were made in studies where cell lines (rat tumor somatotroph cells also named growth cells – GC) were treated with different doses of obestatin administered to the culture medium. It turned out that obestatin at dose of 200 nM resulted in increased secretion of GH by the somatotroph cancer cell line (GC cells line), but only in the first 15 minutes after its administration. Longer incubation of tissue caused the disappearance of this effect. This phenomenon can be explained by the short half-life of obestatin (Pazos et al. 2009). Also unpublished results of our study allow us to assume that intracerebroventricular administration of obestatin stimulates the secretion of GH affecting both, its concentration in blood plasma and GH pulsatile parameters.

Conclusions

Since the characterization of obestatin in 2005 it has been demonstrated that it fulfills many important functions in the regulation of physiological processes of the body. Especially significant for the maintenance of homeostasis is its endocrine actions on the periphery of the body and at the CNS level. However, the current state of knowledge on the participation of obestatin in the mechanisms regulating the function of the hypothalamic-pituitary axis is still not fully understood, and the data obtained so far are fragmentary and often ambiguous.

It is necessary to make further investigate of the physiological action of obestatin at the CNS level and throughout the body.

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NEUROMODULACYJNE DZIAŁANIE OBESTATYNY NA AKTYWNOŚĆ SEKRECYJNĄ OSI PODWZGÓRZE-PRZYSADKA

Streszczenie

W 2005 roku Zhang i współpracownicy przedstawili informacje dotyczące nowego peptydu – obestatyny. Jest to niewielki peptyd będący produktem genu GHRL, odpowiedzialnego również za kodowanie innego białka – greliny. Oba te peptydy – obestatyna i grelina, powstają w wyniku potranslacyjnej obróbki wspólnego prekursora preprogreliny i, co intrygujące, cechują się odmienną aktywnością fizjologiczną. Obestatyna pierwotnie została wyizolowana z komórek zlokalizowanych w błonie śluzowej dna żołądka szczura, jednak kolejne badania wykazały jej obecność również w innych tkankach organizmu. Ustalono również, że wykazuje ona powinowactwo do sierocego receptora 39 sprzężonego z białkiem G (GPR39) oraz do receptora dla glukagono – podobnego peptydu typu 1 (GLP-1R).

Jak wspomniano, zarówno obestatyna, jak i grelina syntetyzowane są głównie przez komórki dna błony śluzowej żołądka. Oba peptydy trafiają następnie do krwiobiegu, gdzie wykazują działanie o charakterze endokrynnym. Dotychczasowe informacje dotyczące działania obestatyny w ośrodkowym układzie nerwowym są fragmentaryczne a często nawet sprzeczne ze sobą. Jest to spowodowane zarówno niejednoznacznym określeniem receptora, jak i brakiem wiedzy na temat agonistów i antagonistów peptydu. Z tego też powodu w swoich rozważaniach skupimy się na przybliżeniu działania obestatyny w obszarze osi podwzgórzowo-przysadkowej.

Słowa kluczowe: obestatyna, centralny układ nerwowy, podwzgórze

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