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GHRELIN IS MULTIFACETED HORMONE

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Ghrelin is one of the most studied hormones with regards to its effects on growth hormone release and appetite regulation. Although many additional functions of ghrelin have been described this review focused on the data regarding regulation of ghrelin and its receptors, some information regarding the effect of ghrelin on the pain, growth and appetite regulation.

Key words: pain, appetite, growth, ghrelin receptors

INTRODUCTION

Ghrelin is a 28 amino-acid peptide with a fatty acid chain modification on the N-terminal third amino acid – serine. Ghrelin, identified as an endogenous ligand for the growth hormone secretagogue receptor (GHS-R) was isolated from rat stomach (KOJIMA et al., 1999). The gastric endocrine X/A-like cells in rats and P/D_1 cells in human are the major source of circulating desacyl, acyl and n-decanoyl ghrelin (HIEJIMA et al., 2009). It must be also noticed out that about 80-90% of circulating ghrelin is not acylated (des-acyl-ghrelin), and it still remains unclear whether or not des-acyl-ghrelin represents a precursor or a degradation product of the acylated peptide. Moreover, des-acyl-ghrelin does not replace radio labeled ghrelin at pituitary and hypothalamic binding sites, nor it seems capable of inducing growth hormone (GH) release. Several *in vitro* studies have demonstrated that radio labeled ghrelin and des-acyl-ghrelin bind to the membranes of PC-3 prostate tumor cells, H9C2 cardiomyocytes and isolated adipocytes, none of which expressed the GHS-R (MUCCIOLI et al., 2004, FERRINI et al., 2009).

Ghrelin is present in the circulation in the active and inactive forms and is easily hydrolyzed by proteases. In order to measure the plasma level of ghrelin in the plasma or tissues, the samples should be taken to EDTA tubes with aprotinin. It must be also noticed that most of circulating ghrelin is in total form including acyl- and des-acyl ghrelin. Enzymatic hydrolysis *in vitro* releases free form of active ghrelin and the plasma level of its peptide is increased from 150-200pg/ml to 2500-3000pg/ml in mice (YANG et al., 2008). Pla-

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sma ghrelin concentration is increased in fasting conditions and decreased after feeding, suggesting that ghrelin may be as an signal for food intake.

The main source for circulating ghrelin is stomach, particularly fundus with four types of endocrine cells –ECL, D, enterochromaffin and X/A-like cells. Ghrelin immunoreactive cells were found in the duodenum, jejunum, ileum, colon.

Ghrelin is expressed also in many brain structures, pituitary, immune cells, lung, placenta, ovary, testis, kidney and pancreas as well as in different tumors (in review of Korbonits et al., 2004). The pancreas is a ghrelin-producing organ during fetal development and during postnatal life. Ghrelin has been found in the hypothalamic arcuate nucleus, ghrelin-containing neurons send fibers to NPY neurons, AgRP and may stimulate the release of orexigenic peptides.

The regulation of ghrelin secretion is under many factors: upregulating- fasting (low BMI), leptin, GHRH, thyroid hormones, testosterone and downregulating- food intake, glucose, insulin, somatostatin, GH, opioids (Korbonits et al., 2004, Pierzchala-Koziec et al., 2006).

Ghrelin displays strong growth hormone (GH) releasing actions mediated by the activation of the GHS-R type 1a. Apart from stimulating GH secretion, ghrelin exhibits hypothalamic activity that results in stimulation of prolactin, ACTH secretion, also influences the pituitary–gonadal axis, pituitary-adrenal axis, stimulates appetite and positive energy balance, influences behavior, controls gastric motility and acid secretion as well as glucose metabolism (reviewed in Korbonits et al.,2004, Van Der Lely et al., 2004, Pierzchala-Koziec et al., 2006, reviewed in Stengel and Tache, 2012).

Ghrelin bounds to the typical GPCR with seven transmembrane domains (GHS-R) expressed in the brain structures and peripheral organs in animals and humans (Howard et al., 1996, Chung et al.,2008). Two distinct ghrelin receptor cDNAs have been isolated. The first – GHS-R1a serves a role as ghrelin's receptor, the other GHS-R type 1b is produced by an alternative splicing mechanism (Howard et al., 1996, Henderson and Mcknight, 1997). The ghrelin receptor superfamily contains receptors of ghrelin, motilin, neuromedin U and neurotensin. All of these peptides were found in the gastrointestinal system. The ghrelin receptor is well conserved across all vertebrate species-mammals, chicken, amphibian, fish. Ghrelin receptor mRNA is expressed in many brain areas – arcuate nuclei, hippocampus, pituitary (Guan et al., 1997). Method of RT-PCR showed ghrelin receptor mRNA expression in peripheral organs: heart, liver, lung, kidney, pancreas, stomach, intestine, adipose tissue and immune cells. The existence of ghrelin and its receptor in many organs indicate that ghrelin has multiple functions in these tissues (Masayasu and Kangawa, 2005).

Although many additional functions of ghrelin have been described this review focused on the data regarding regulation of ghrelin and its receptors, some information regarding the effect of ghrelin on the pain, growth and appetite regulation.

Ghrelin and pain

The impact of ghrelin on the pain has been the subject of very recent investigations, and currently available evidence indicates that ghrelin acts as an antinociceptive signal at both peripheral and central sites (Ferrini et al., 2009).

The first convincing evidences that ghrelin may has antinociceptive effects was provided by Sibilla et al., (2006). These authors showed that ICV injections of the hormone dose-dependently reduce mechanical hyperalgesia and paw edema in rats model of acute inflammatory pain. Because the action of ghrelin was reversed by ICV injections of the opioid antagonist naloxone, it was suggested that ghrelin centrally interacts with opioid neurons (Bodnar, 2011; Dykstra et al., 2011). They postulated that peripherally administered ghrelin increased AgRP synthesis and relase, what in turn, enhances release of β-endorphins from POMC neurons. Also, it is possible that increase of nitric oxide (NO) synthase activity after ghrelin administration may stimulate the antinociceptive effects of endogenous opioid peptides (Heinzen et al., 2005).

It was also shown that ghrelin significantly enhanced inhibitory (GABAergic/glycinergic) neurotransmission in a subpopulation of deep dorsal horn neurons in mice. The effect was due to interaction of ghrelin with GHS-R type 1a, and GHS-R type 1a antagonist prevented the ghrelin effect (Vergnamo et al., 2008, Ferrini et al., 2009).

Considerable work is needed to proof the role of ghrelin in pain central mechanisms. However, the data reported suggest that interaction with opioid/NPY expressing neurons should be further

investigated to better understand the role of ghrelin as a pain modulator.

Ghrelin and growth hormone

The era of ghrelin started with the observation that opioid like peptides and their chemical analogs stimulate GH secretion through orphan receptor called GHS-R (Bowers et al., 1977, Bowers, 1993, Korbonits and Grossman, 1995). Isolation and identification of endogenous ligand for this receptor by Kojima et al. (1999) led to named it for its GH-releasing activity – ghrelin.

The target cells of ghrelin are somatotrophs in the pituitary gland. In an *in vivo* assay, ghrelin stimulated primary pituitary cells and increased their intracellular Ca^{2+} concentration, indicating that the GHS-R is expressed in pituitary cells. Also, ghrelin has been found in the pituitary gland itself where it may influence the release of GH in an autocrine or paracrine manner. The expression level of ghrelin in the pituitary is high after birth and declines with puberty. Pituitary tumors, such as adenomas, corticotroph, and gonadotroph contain ghrelin peptides.

It was found that GH-releasing activity of ghrelin is similar to that of GHRH i.v. injected to rats. However, the maximal stimulation effected by ghrelin is two to three times greater than that of GHRH. Ghrelin stimulates GH release both *in vitro* and *in vivo* in a dose-dependent manner. Intravenous injection of ghrelin induces potent GH release both in rats and in humans (Arvat et al., 2000).

The involvement of the hypothalamus in ghrelin-mediated stimulation of GH release has been also strongly suggested (Kojima et al., 1999). Exogenous treatment with GH, somatostatin or its analogs suppress plasma ghrelin level (Korbonits et al., 2004).

Ghrelin has also been shown to induce GH release in chicken (Kaiya et al., 2002), fish (Kaiya et al., 2003), and frog (Kaiya et al., 2001). In addition, high doses of ghrelin in humans increase ACTH, prolactin, and cortisol levels (Takaya et al., 2000).

Ghrelin and food intake

Ghrelin secretion is regulated by many different factors, but the strongest stimulator is feeding, mainly lower level of glucose. On the other hand, high-fat meal decrease plasma level of ghrelin. Plasma ghrelin is increased during night sleep but only in lean persons, and in patients with anorexia nervosa and bulimia nervosa.

Ghrelin is the only known hormone served as a hunger signal from peripheral tissues. Intravenous and subcutaneous injections of ghrelin increase food intake; peripherally injected ghrelin stimulates hypothalamic neurons and food intake (Nakazato et al., 2001).

Ghrelin has been found in the hypothalamic arcuate nucleus, an important region for controlling appetite (Kojima et al., 1999). Recent study has reported the presence of ghrelin in hypothalamic neurons adjacent to the third ventricle between the dorsal, ventral, paraventricular, and arcuate hypothalamic nuclei (Date et al., 2001, Sato et al., 2012). These ghrelin-containing neurons send efferent fibers to neurons that contain neuropeptide Y (NPY) and agouti-related protein (AgRP) and may stimulate the release of these orexigenic peptides. The localization of ghrelin confirm a role in controlling food intake. It was also found that injection of ghrelin into the cerebral ventricles of rats potently stimulates food intake.

Central ghrelin action is of physiological relevance in the control of adipocyte metabolism and suggests that ghrelin could trigger meal preparation processes in the central nervous system (CNS). It seems probable that ghrelin activates metabolic pathways that would lead to a more efficient storage of calories. Intracerebroventricular injection of ghrelin increases cumulative food intake and decreases energy expenditure what results in body weight gain (Shintani et al., 2001). This orexigenic effect of hypothalamic ghrelin is regulated through a neuronal network involving food intake. On the other hand, to suppress the release of the anorexigenic peptide, ghrelin-containing neurons send efferent fibers onto proopiomelanocortin (POMC) neurons (Cowley et al., 2003). The ARC is also a target of leptin, an appetite-suppressing hormone produced in adipose tissues (Flier, 2004). Leptin inhibits appetite-stimulating effects of NPY and AgRP, on the other hand hypothalamic ghrelin augments NPY gene expression and blocked leptin-induced feeding reduction. It may be suggested that ghrelin and leptin have a competitive interaction in feeding regulation.

Fetissov et al. (2010) reviewed recent papers about ghrelin involvement into regulation of appetite and found that although ghrelin stimulates feeding, it inhibits drinking and activates neurons of the hypothalamic subfornical organ usually associated with dehydration. They suggest that circulating ghrelin may signal to the brain via the circumventricular organs not necessarily associated with the median eminence. Ghrelin interacts with gastrointestinal hormones signaling satiety such as cholecystokinin, bombesin, peptide YY, glucagon-like peptide, pancreatic polypeptide, and amylin which may inhibit ghrelin secretion or antagonize its action in the appetite regulatory neurons in the brain. Since plasma levels of ghrelin normally fall after a meal, it is possible to measure it as a marker to evaluate the satietogenic properties of different macronutrients for the development of various nutritional antiobesity approaches.

SUMMARY

The structure of ghrelin, and the tissue in which it is produced, is unique in endocrinology.

Ghrelin exists in mammalian and in nonmammals species maintaining GH release, energy homeostasis, controlling pain, immune system and stress reactions. Further research will answer questions about the undiscovered roles of this unique hormone and elucidate its biochemical and physiological characteristics.

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REFERENCES

Arvat, E., L. Di Vito, F. Broglio, M. Papotti, G. Muccioli, F. Dieguezc, F. Casanueva, R. Deghenghi, F. Camanni, and E. Ghigo. 2000. Preliminary evidence that Ghrelin, the natural GH secretagogue (GHS)-receptor ligand, strongly stimulates GH secretion in humans. *J Endocrinol. Invest.* 23: 493–495.

Bodnar, R.J. 2011. Endogenous opiates and behawior: 2010. *Peptides* 32: 2522-2552.

Bowers, C.Y. 1993. GH-releasing peptides – structure and kinetics. *J. Pediatr. Endocrinol.* 6: 21-31.

Bowers, C.Y., J. Chang, F.A. Momany, and K. Folkers. 1977. Effect of the enkephalins and enkephalin analogues on release of pituitary hormones *in vitro*. *Mol. Endocrinol.* 287-292.

Chung, S., T. Funakoshi, and O. Civelli. 2008. Orphan GPCR research. *Br. J. Pharmacol.* 153: S339-S345.

Cowley, M.A., R.G. Smith, S. Diano, M. Tschop, N. Pronchuk, K.L. Grove, C.J. Strasburger, M. Bidlingmaier, M. Esterman, M.L. Heiman, L.M. Garcia-Segura, E.A Nillni, P. Mendez,, M.J. Low, P. Sotonyi, J.M. Friedman, H. Liu, S. Pinto, W.F. Colmers, R.D. ConE, and T.L. Horvath. 2003. The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron* 37: 649-66187.

Date, Y., M. Nakazato, S. Hashiguchi, K. Dezaki, M.S. Mondal, H. Hosoda, M. Kojima, K. Kangawa, T. Arima, H. Matsuo, T. Yada, and S. Matsukura. 2002. Ghrelin is present in pancreatic alpha-cells of humans and rats and stimulates insulin secretion. *Diabetes* 51: 124-129.

Dykstra, L.A., B.D. Fischer, R.E. Balter, F.E. Henry, K.T. Schmidt, and L.L. Miller. 2011. Opioid antinociception, tolerance and dependence: interactions with the N-methyl-D-aspartate system in mice. *Behav. Pharmacol.* 22: 540-547.

Ferrini, F., C. Salio, L. Lossi, and A. Merighi. 2009. Ghrelin in central neurons. *Curr. Neuropharmacol.* 7: 37-49.

Fetissov, I., A. Laviano, S. Kalra, and A. Inui. 2010. Update on ghrelin. Int. J. Pept. 2010, 1-4.

Flier, J.S. 2004 Obesity wars: molecular progress confronts an expanding epidemic. *Cell* 116: 337-350.

Guan XM, H. Yu, O.C. Palyha, K.K. McKee, S.D. Feighner, D.J. Sirinathsinghji, R.G. Smith, L.H. Van der Ploeg, and A.D. Howard. 1997. Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Brain Res.* 48: 23–29.

Heinzen, E.L., R.G. Booth, and G.M. Pollack. 2005. Neuronal nitric oxide modulates morphine antinociceptive tolerance by enhancing constitutive activity of the mu-opioid receptor. *Biochem. Pharmacol.* 69: 679-688.

Henderson, G., and A.T. Mcknight. 1997. The orphan opioid receptor and its endogenous ligand-nociceptin/orphanin FQ. *Trends Pharmacol. Sci.* 18,: 293-300.

Hiejima, H., Y. Nishi, H. Hosoda, J. Yoh, H. Mifune, M. Satou, H. Sugimoto, S. Chiba, Y. Kawahara, E. Tanaka, H. Yoshimatsu, N. Uchimura, K. Kangawa, and M. Kojima. 2009. Regional distribution and the dynamics of n-decanoyl ghrelin, another acyl-form of ghrelin, upon fasting in rodents. *Reg. Pept.* 156: 47-56.

Howard, A.D., S.D. Feighner, D.F. Cully, P.A. Liberator, J.P. Arena, C.I. Rosenblum, M.J. Hamelin, D.L. Hreniuk, O.C. Palyha, J. Anderson, P.S. Paress, C. Diaz, M. Chou, K. Liu, K. Kuljumckee, S.-S. Pong, L.Y. Chaung, A. Elbreicht, M. Dashkevicz, R. Heavens, M. Rigby, D.J.S. Sirinathsinghji, D.C. Dean, D.G. Melillo, A,A. Patchett, R. Nargund, P.R. Griffin, J.A. Demartino, S.K. Gupta, J.M. Schaeffer, R.G.

Smith, and L.H.T. Van Der Ploegh. 1996. A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science*, 273: 974-977.

Kaiya H, M. Kojima, H. Hosoda, S. Moriyama, A. Takahashi, H. Kawauchi and K. Kangawa. 2003. Peptide purification, complementary deoxyribonucleic acid (DNA) and genomic DNA cloning, and functional characterization of ghrelin in rainbow trout. *Endocrinology* 144: 5215–5226.

Kaiya H., S. Van Der Geyten, M. Kojima, H. Hosoda, Y. Kitajima, M. Matsumoto, S. Geelissen, V.M. Darras, and K. Kangawa. 2002. Chicken ghrelin: purification, cDNA cloning, and biological activity. *Endocrinology* 143: 3454–3463.

Kaiya, H., M. Kojima, H. Hosoda, A. Koda, K. Yamamoto, Y. Kitajima, M. Matsumoto, Y. Minamitake, S. Kikuyama, and K. Kangawa. 2001. Bullfrog ghrelin is modified by n-octanoic acid at its third threonine residue. *J. Biol. Chem.* 276: 40441–40448.

Kojima, M. H., Y. Hosoda, M. Date, H. Nakazato, H. Matsuo and K. Kangawa. 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 402: 656–660.

Korbonits, M. and A.B. Grossman. 1995. Growth hormone- releasing peptide and its analogues; novel stimuli to growth hormone release. *Trends Endocrinol. Metab.* 6: 43-49.

Korbonits, M., A.P. Goldstone, M. Gueorguiev and A.B. Grossman. 2004. Ghrelin-a hormone with multiple functions. *Front. Neuroendocrinol.* 25: 27-68.

Masayasu K., and K. Kangawa. 2005. Ghrelin: Structure and Function. *Physiol Rev.* 85: 495-522.

Muccioli, G., N. Pons, C. Ghe, F. Catapano, R. Granata, and E. Ghigo. 2004. Ghrelin and des-acyl ghrelin both inhibit isoproterenol induced lipolysis in rat adipocytes via a non-type 1a growth hormone secretagogue receptor. *Eur. J. Pharmacol.* 498: 27-35.

Nakazato, M., N. Murakami, Y. Date, M. Kojima, H. Matsuo, K. Kangawa, and S. Matsukura. 2001. A role for ghrelin in the central regulation of feeding. *Nature* 409: 194-198.

Pierzchala-Koziec, K., J. Zubel, and E. Kanitz. 2006. The effect of ghrelin on the opioid responses to mannan oligosaccharides treatment in growing lambs. *Acta Biol. Cracoviensia ser. Zool.* 48: 25-29.

Sato, J., Y. Nakamura, Y. Shiimura, H. Ohgusu, K. Kangawa, and M. Kojima. 2012. Structure, regulation and function of ghrelin. *Biochem.*151(2):119–128.

Shintani, M., Y. Ogawa, K. Ebihara, M. Aizawa-Abe, F. Miyanaga, K. Takaya, T. Hayashi, G. Inoue, K. Hosoda, M. Kojima, K. Kangawa, and K. Nakao. 2001. Ghrelin, an endogenous growth hormonese cretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. *Diabetes* 50:227-232

Sibilla, V., N. Lattuada, D. Rapetti, F. Pagani, D. Vincenza, I. Bulgarelli, V. Locatelli, F. Guidobono, and C. Netti. 2006. Ghrelin inhibits inflammatory pain in rats: Involvement of the opioid system. *Neuropharmacology* 51: 497-505.

Stengel, A., and Y. Tache. 2012. Ghrelin – a pleiotropic hormone secreted from endocrine X/A-like cells of the stomach. *Front. Neurosci.* 6: 24..

Takaya K, H. Ariyasu, N. Kanamoto H. Iwakura, A. Yoshimoto, M. Harada, K. Mori, Y. Komatsu, T. Usui, A. Shimatsu, Y. Ogawa, K. Hosoda, T. Akamizu, M. Kojima, K. Kangawa, and K. Nakao. 2000. Ghrelin strongly stimulates growth hormone release in humans. *J. Clin. Endocrinol. Metab.* 85: 4908–4911.

Van Der Lely, A.J., M. Tschop, M.L. Heiman, and E. Ghigo. 2004. Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. *Endocrine Rev.* 25: 426-457.

Vergnano, A.M., F. Ferrini, C. Salio, A. Baratta, and A. Merighi. 2008. The gastrointestinal hormone ghrelin modulates inhibitory neurotransmission in deep laminae of mouse spinal cord dorsal horn. *Endocrinology* 149: 2306-2312.

Yang, J., M.S. Brown, G. Liang, N.V. Grishin, and J.L. Goldstein. 2008. Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. *Cell* 132: 387-396.