Ghrelin tissue distribution: Comparison between gene and protein expression

S. Ghelardoni, V. Carnicelli, S. Frascarelli, S. Ronca-Testoni, and R. Zucchi

Dipartimento di Scienze dell'Uomo e dell'Ambiente, University of Pisa, Pisa, Italy

ABSTRACT. Ghrelin, the natural ligand of the GH secretagogue (GHS) receptor, was originally isolated from the stomach and detected in several tissues, but a systematic study of its tissue distribution has not been performed. In the present investigation, we evaluated ghrelin gene expression (by RT-PCR technique) and ghrelin protein concentration (by enzyme immunoassay technique) in tissues obtained from control rats as well as in rats subjected to 48-h fasting. The ghrelin gene was expressed in stomach, small intestine, brain, cerebellum, pituitary, heart, pancreas, salivary gland, adrenal, ovary and testis, with maximum expression occurring in the stomach, while no significant expression was detected by standard RT-PCR in liver, lung, kidney and skeletal muscle. Ghrelin protein was detected in stomach, small intestine, brain, cerebellum, pituitary, lung, skeletal muscle pancreas, salivary gland, adrenal, ovary and testis, at concentrations ranging from 0.05 to 1.43 ng/mg of homogenate protein (the highest concentration occurred in the lung, followed by the brain). Ghrelin was not detectable in the heart, liver and kidney. Therefore, gene and protein expression were dissociated. Fasting did not produce significant changes in ghrelin gene expression, while the distribution of ghrelin between different tissues was significantly modified: protein concentration increased in the brain, cerebellum, lung and salivary gland, while it decreased in the stomach.

(J. Endocrinol. Invest. 29: 115-121, 2006)

INTRODUCTION

Ghrelin is the endogenous ligand for the GH secretagogue (GHS) receptor, first identified from rat stomach by Kojima et al. (1) as a 28-amino acid endocrine peptide, n-octanoyled in serine 3 residue.

Ghrelin is secreted into the bloodstream mostly by stomach fundus (2) and in small amounts by placenta, kidney, pituitary and hypothalamus (3-5).

To date, ghrelin has been said to stimulate GH secretion, feeding and adiposity (6), and to regulate energy homeostasis (7). Non-endocrine actions have also been reported (8, 9). Several findings indicate that ghrelin is an important stomach hormone, which is sensitive to nutritional intake and may connect enteric nutrition with secretion of GH, insulin and gastrin (10).

In humans, ghrelin plasma levels increase before and decrease after every meal, indicating that ghrelin could play a physiological role in meal initiation and hunger (11, 12).

In rodents, ghrelin plasma concentration increases with prolonged fasting, and its blood levels are reduced by re-feeding or infusion of nutrients (13), while intracerebroventricular ghrelin administration increases food intake and body weight (14).

In anorexia nervosa, hyperghrelinemia has been detected, indicating that ghrelin is related to food intake in disease-associated malnutrition (15). Ghrelin has recently also been found to play a role in determining the type of metabolic substrate (16, 17), and circulating ghrelin levels are influenced by the ratio of fat, protein and carbohydrate content in the ingested diet (10, 18).

Highly sensitive techniques such as real-time PCR suggest that in humans the ghrelin gene is expressed in most tissues (19) but the functional relevance of such findings is unclear, and the correlation between gene and protein expression has not been extensively studied. In the present investigation, we decided to carry out a comparison between ghrelin gene expression and ghrelin protein concentration in several rat tissues, under control conditions and after 48-h fasting.

Key-words: Ghrelin, fasting, gene expression, enzyme immunoassay, growth hormone.

Correspondence: R. Zucchi, MD, Dipartimento di Scienze dell'Uomo e dell'Ambiente, Sezione di Biochimica, Università di Pisa, Via Roma 55, 56126 Pisa, Italy.

E-mail: r.zucchi@med.unipi.it

Accepted September 15, 2005.

MATERIALS AND METHODS

Experimental protocol

Wistar rats of both sexes (275-300 g body weight) were used (no.=16). Control rats (no.=8) were fed *ad libitum* with standard chow and free access to water. Some rats (no.=8) were subjected to fasting, obtained by food deprivation for 48 h with free access to water. All rats were anesthetized with a mixture of ether and air and sacrificed by decapitation. Tissue samples were quickly excised, washed with ice-cold saline, immediately frozen in liquid nitrogen and stored at –80 C until use.

Plasma and tissue concentration

Blood samples were collected and allowed to clot at 4 C, then centrifuged at 1000xg for 10 min to collect plasma. Tissue samples were obtained from brain, cerebellum, pituitary, heart, hind leg muscle, lung, stomach, small intestine (jejunum), liver, pancreas, salivary gland (submaxillary gland), kidney, adrenal, ovary and testis. The samples were homogenized in five volumes of buffer containing 0.9% NaCl, 50 mM Tris-HCl, 12 µM leupeptin, 100 µM PMSF, 10 µM peptin, using a Potter-Elvejheim homogenizer set at 800 rpm and cooled in ice. The homogenate was filtered on cheesecloth and stored at –80 C until use. Plasma and tissue ghrelin levels were measured using a ghrelin enzyme immunoassay (EIA) kit (Phoenix Pharmaceuticals Inc., Belmont, CA, USA). This assay evaluates total (ie acylated and desacyl) ghrelin. The minimum detectable concentration was 0.1 ng/ml. Results were normalized for protein concentration, which was assessed by the Lowry method.

Gene expression

Ghrelin gene expression was determined by RT-PCR. Total RNA was extracted from rat brain, cerebellum, pituitary, heart, hind leg muscle, lung, stomach, small intestine (jejunum), liver, pancreas, salivary gland (submaxillary gland), kidney, adrenal, ovary, and testis using TRIzol Reagent (Invitrogen Life Technologies, Paisley, UK) and quantified spectrophotometrically. DNAseI (Sigma-Aldrich, St.Louis, MO, USA)-treated RNA was used as template in a one-step RT-PCR (SuperScript One-Step RT-PCR with Platinum Taq kit, Invitrogen Life Technologies, Paisley, UK). Primers based on rat ghrelin cDNA sequences were used to detect respective mRNAs.

Rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA was used as an internal standard. The forward primers for ghrelin and GAPDH were 5′-TAAACTGCAGCCACGAGCTCT-3′, and 5′-TGGCTTATGGCGACCCCTCTTT-3′, respectively. The reverse primers were 5′-TTGAACCTGATTTCCAGCTCCT-3′, and 5′-CCGCAATGGCCAAGAACACAT-3′, respectively. The lengths of amplified fragments were 93, and 135 bp, respectively. Each reverse primer was labeled at 5′ terminal with 5-carboxyfluorescein (FAM) fluorochrome so that RT-PCR products could be detected on ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and measured by Gene Scan fragment analysis software. The identity of the amplified fragments was confirmed by sequencing, and they corresponded to the coding region of each gene. Ghrelin signals were normalized on the basis of the GAPDH signal.

Ghrelin and GAPDH RT-PCR were performed according to the kit protocol. Primer concentrations were 0.2 µM for ghrelin and 0.4 µM for GADPH; 50 µg of total RNA was used as template. One-step RT-PCR was carried out with the following cycle profile: 30 min at 50 C (cDNA synthesis), 2 min at 94 C (cDNA denaturation), 26 cycles of denaturation at 94 C for 15 sec and annealing-extension at 68 C for 1 min (amplification), followed by 5 min at 68 C of final extension. These conditions were chosen on the basis of the results of preliminary experiments, in which increasing amounts of

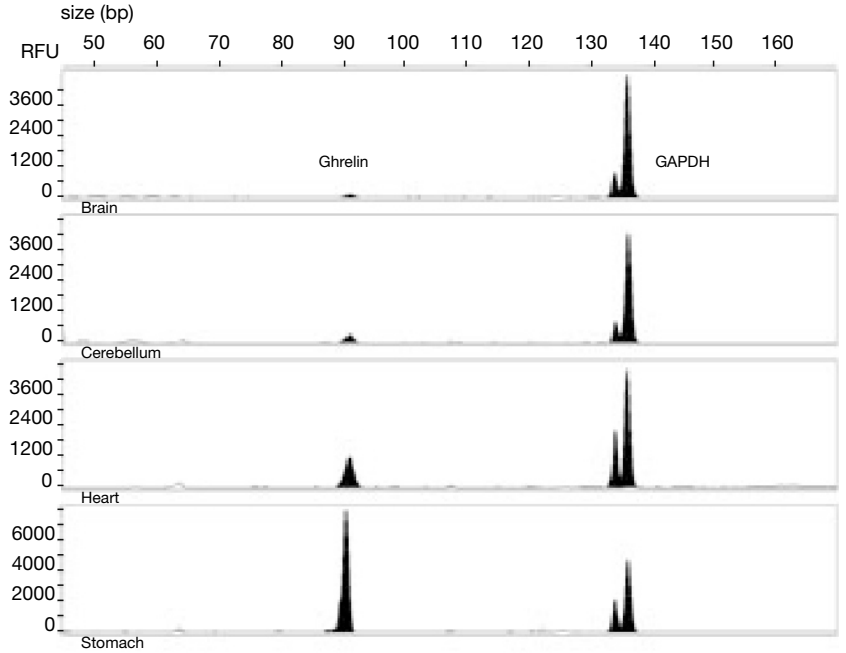

Fig. 1 - *Representative results of RT-PCR assays. Ghrelin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNAs were amplified by RT-PCR. The amplified fragments were separated by capillary electrophoresis and detected fluorimetrically in an ABI 310 GeneticAnalyzer. The area underlying each peak was calculated through Gene Scan fragment analysis software. Representative fluorometric tracings obtained from brain, cerebellum, heart and stomach tissues are shown. RFU: relative fluorescence units.*

RNA were subjected to different numbers of cycles (22 to 30) to establish the linear range of amplification.

Statistical analysis

Results are expressed as means±SEM. Comparison between groups was performed by one-way or two-way analysis of variance (ANOVA), as appropriate. The threshold of statistical significance was taken as $p<0.05$. GraphPad Prism version 4.01 for Windows (GraphPad Sofware, San Diego, CA) was used for data processing and statistical analysis.

RESULTS

Ghrelin mRNA tissue distribution

Representative fluorometric tracings showing ghrelin mRNA amplification by RT-PCR are reported in Figure 1. Significant gene expression was observed in most tissues, although ghrelin mRNA was undetectable in skeletal muscle, lung, liver and kidney. Pilot experiments showed that a high number of amplification cycles (in excess of 30) allowed occasional detection of the ghrelin gene also in these tissues, which were however excluded from the following statistical analysis.
The level of gene expression, as evaluated by comparison with GAPDH gene expression, showed remarkable inter-tissue variability ($p=0.0015$ by one-way ANOVA). Maximum expression occurred in the stomach, followed by the intestine, while in the other tissues gene expression was about one order of magnitude higher than in the stomach. Interestingly, ghrelin mRNA levels were slightly higher in the heart than in the brain or cerebellum (Fig. 2).

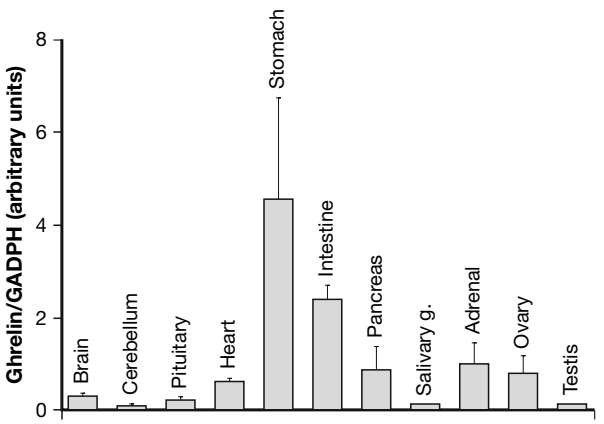

Fig. 2 - *Ghrelin gene expression. Ghrelin mRNA was measured by RT-PCR and normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA. Only tissues in which significant gene expression was detected are shown here. Bars represent mean±SEM of 4 tissue samples in each group, except for ovary and testis, in which no.=2 (SEMs which are very low may not be clearly visible). Analysis of variance (ANOVA) yielded* p*=0.0015 for differences among groups.*

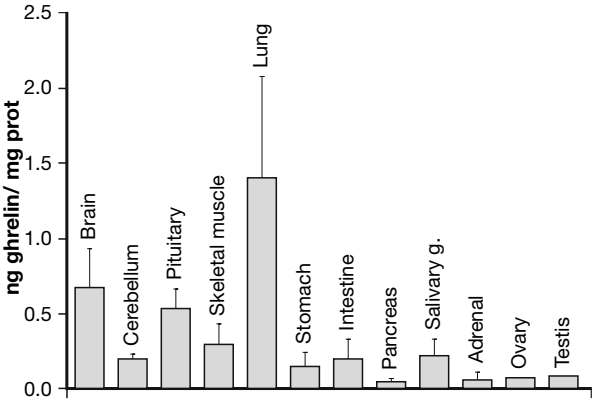

Fig. 3 - *Ghrelin protein concentration. Ghrelin concentration was measured in tissue homogenate by enzyme immunoassay (EIA) and expressed as ng/mg of total homogenate protein. Only tissues in which ghrelin concentration exceeded the sensitivity limit of the assay are shown here. Bars represent mean±SEM of 4 tissue samples in each group, except for ovary and testis, in which no.=2 (SEMs which are very low may not be clearly visible). Analysis of variance (ANOVA) yielded* p*=0.0006 for differences among groups.*

Ghrelin protein tissue distribution

The assays performed by EIA technique showed that ghrelin was detectable in many tissues, although its concentration was below the sensitivity of the assay in the heart, liver and kidney. If ghrelin concentration was normalized to tissue protein, significant inter-tissue variability was observed (Fig. 3), and the highest levels were measured in the lung, followed by the brain ($p=0.0006$ by one-way ANOVA).
The average concentration of ghrelin in the above-mentioned tissues ranged from 1.43 ng/mg in the lung to 0.05 ng/mg in the pancreas. Stomach concentration averaged 0.14 ng/mg. Assuming tissue protein concentration in the range of 100-200 mg/g of wet weight (20), our findings imply tissue concentration in the range of 5-280 ng/g of wet weight, corresponding to 1.5-85 pmol/g of wet weight. By comparison, in control animal plasma ghrelin concentration averaged 1.2±0.2 pmol/ml.

Effect of fasting

Fasting is known to be associated with increased plasma ghrelin concentration (10, 11) and it is believed to be the major physiological stimulus to ghrelin synthesis and secretion (15, 21, 22). Therefore, it seemed interesting to evaluate the effects of fasting on tissue ghrelin distribution.
Results of RT-PCR and EIA assays performed in rats subjected to 48 h-fasting are shown in Figures 4 and 5. While no major change was observed with regard to ghrelin mRNA expression, ghrelin

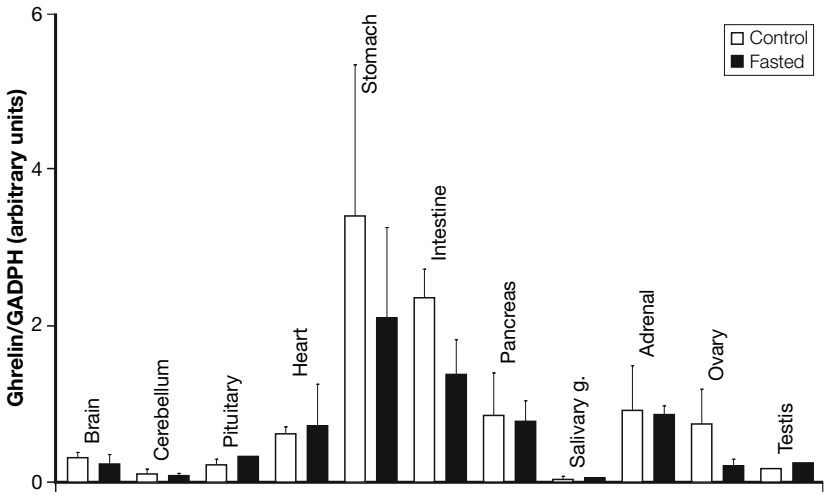

Fig. 4 - *Effect of fasting on ghrelin gene expression. Ghrelin mRNA was measured by RT-PCR and normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA in control rats and in rats subjected to 48-h fasting. Bars represent mean±SEM of 4 tissue samples in each group, except for ovary and testis, in which no.=2 (SEMs which are very low may not be clearly visible). Two-way analysis of variance (ANOVA) yielded* p*=0.0006 for the effect of tissue and* p*=ns for the effect of fasting.*

protein concentration was significantly modified in fasted rats (two-way ANOVA yielded p=0.04 for the effect of fasting). In general, protein concentration was increased in most tissues (particularly lung, brain, cerebellum, and salivary gland), while it was decreased in the stomach. As expected, plasma ghrelin concentration was also increased after fasting (4.9±1.1 vs 1.2±0.2 pmol/ml)

DISCUSSION

Ghrelin is an n-octanoylated 28-amino acid peptide, which has been isolated from gastric tissue and identified as the endogenous ligand for the GHS receptor (1). Its chief effects are stimulation of both GH secretion and feeding (23). In humans, ghrelin administration consistently produces hunger and increases food intake (24). So ghrelin is regarded as a gastric hormone which acts predominantly on the pituitary and central nervous system (particularly on the hypothalamus), although additional effects have been observed in peripheral tissues, such as the heart and blood vessels, which have been attributed to the presence of peripheral GHS receptors (25, 26)
Extra-gastric ghrelin production has been observed, particularly in the placenta, kidney, pituitary and hypothalamus (3-5). Therefore, we decided to investigate and compare the expression of ghrelin gene and the presence of ghrelin protein in different tissues. Since fasting is the major physiological stimulus to ghrelin secretion, experiments were performed both in control rats and in rats subjected to 48-h fasting.

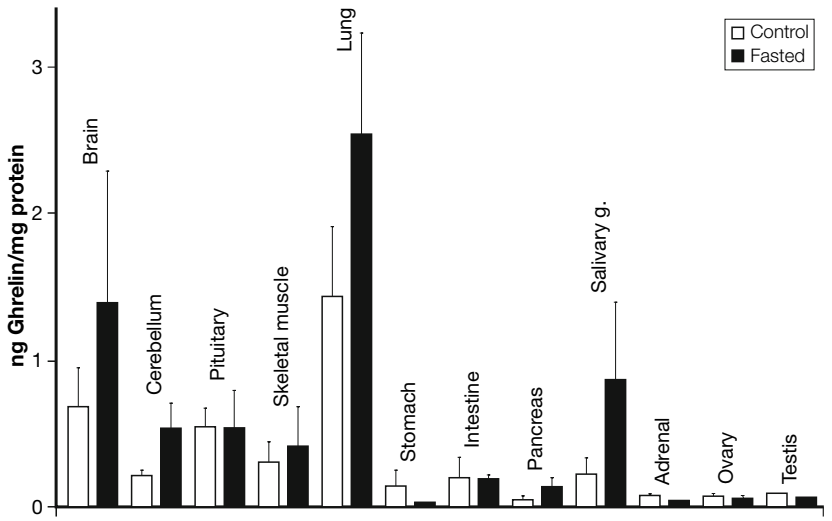

Fig. 5 - *Effect of fasting on ghrelin protein concentration. Ghrelin concentration was measured by enzyme immunoassay (EIA) in tissue homogenate obtained from control rats and rats subjected to 48-h fasting and it is expressed as ng/mg of total homogenate protein. Bars represent mean±SEM of 4 tissue samples in each group, except for ovary and testis, in which no.=2 (SEMs which are very low may not be clearly visible). Two-way analysis of variance (ANOVA) yielded* p*=0.0001 for the effect of tissue and* p*=0.04 for the effect of fasting.*

We observed that ghrelin mRNA was detectable by standard RT-PCR in most of the tissues which we examined, with the exception of skeletal muscle, lung, liver and kidney. However, by increasing the number of amplification cycles, some signal could also be obtained in these tissues. Therefore, our results are consistent with a widespread expression of the ghrelin gene, as already suggested by previous reports (5, 7, 9, 19, 27-30). The results obtained in the cerebellum, heart and salivary gland are particularly interesting, since ghrelin mRNA had not been detected in these tissues.

EIA assays also showed that ghrelin was present in most tissues. Negative results were obtained only in the heart, liver and kidney, although we cannot exclude that ghrelin may be present at very low concentrations which lie below the sensitivity limit of our assay, but might still produce some biological effects.

Some of our findings are consistent with previous reports. Ghrelin peptide has been detected in several extra-gastric tissues, including the small intestine, pituitary, hypothalamus, pancreas, ovary, testis (27-30), as well as in fetal human lung (31), and in rhesus monkey muscle (32). On the other hand, the presence of ghrelin in the cerebellum, adrenal and salivary gland represents to our knowledge a novel finding.

Other results are at variance with previous reports. In particular, we could not detect ghrelin in rat kidney homogenate, while ghrelin has been identified by radioimmunoassay (RIA) in mouse kidney by Mori et al. (4), although at low concentration. In addition, a much higher ratio of gastric to cerebral ghrelin concentration has been reported in mouse (33). Differences in species (eg mouse vs rat), sample processing, homogenization technique and assay procedure might account for these discrepancies. The lack of consensus about the "physiological" range of plasma ghrelin is a well-known issue that emphasizes the difficulty in identifying a "gold standard" for ghrelin assay.

In any case, a remarkable and unexpected finding is that protein expression appeared to be dissociated from gene expression. Ghrelin could be detected by EIA in tissues where ghrelin mRNA was undetectable by standard RT-PCR, such as the lung and skeletal muscle. On the other hand, ghrelin concentration was below the sensitivity of our assay in the heart, in spite of significant gene expression. Notably, in the stomach gene expression was maximum, while protein concentration was remarkably lower than in the lung.

These observations suggest that different tissues may show significant differences in the rate of ghrelin mRNA translation, or in post-translational events. The latter may be represented by ghrelin hydrolysis, secretion and storage. In particular, it might be speculated that tissue ghrelin concentration is largely dependent on the presence of specific binding sites, which ghrelin may reach from the bloodstream even if it is not produced in situ.

GHS receptor represents an obvious binding site for ghrelin, but GHS receptor density appears to be too low to account for tissue ghrelin concentration. For instance, in hypothalamic membranes, GHS receptor concentration has been reported to be in the order of 50 fmol/mg of protein (27), while according to our results in unfractionated brain homogenate ghrelin concentration is in the order of 0.7 ng/mg of protein, corresponding to about 200 fmol/mg of protein. The existence of different binding or storage sites is also suggested by the absence of detectable ghrelin in the heart, where GHS receptor density is higher than observed in most other peripheral tissues (34, 35).

Assuming tissue protein concentration in the order of 100-200 mg/g of wet weight, tissue ghrelin concentration was estimated in the range of 1.5-85 pmol/g of wet weight. This value appears to be substantially higher than plasma ghrelin concentration, which averaged about 1 pmol/ml, in good agreement with previous reports (33, 36). The existence of a tissue-plasma gradient further suggests that ghrelin-containing tissues can either produce or accumulate ghrelin.

A limit of our investigation is that we could not identify the specific cellular or subcellular localization of ghrelin, since whole tissue homogenate was used. Immunohistochemistry might provide the means for direct evaluation of this issue.

Fasting is the best known stimulus able to increase plasma ghrelin concentration (11, 37), as confirmed in our study. We observed that 48 h of fasting were associated with decreased ghrelin concentration in the stomach and increased ghrelin concentration in the lung, brain, cerebellum and salivary gland, while gene expression was not significantly modified. Decreased ghrelin levels have already been observed in the stomach of fasting animals, which has been interpreted as evidence that fasting mobilizes gastric ghrelin (37). Our findings are in agreement with this hypothesis. We also suggest that fasting favors ghrelin binding to or storage in extra-gastric tissues.

The functional implications of our findings remain to be determined. Ghrelin accumulation in brain and salivary glands might play a role in hunger and in other behavioral responses to fasting. The high ghrelin concentration found in the lung, and its increase after fasting, are more difficult to interpret. Since pulmonary capillaries drain all blood coming from the splanchnic circulation, it might be hypothesized that this is a temporary storage site, or a sort of buffer in ghrelin redistribution.

The lack of ghrelin gene overexpression after fasting is surprising, and it is at variance with the findings reported by Toshinai et al. (37). It is possible that in our experimental model, 48 h of fasting may not be sufficient to allow detection of changes in gene expression. Investigations involving longer fasting times are needed to clarify this issue.
In conclusion, ghrelin is a peptide hormone synthesized and secreted mainly by the stomach but also expressed in other tissues. Significant dissociation between gene expression and protein expression has been observed in control and fasting conditions, suggesting that ghrelin metabolism and distribution are a complex and still unresolved issue.

REFERENCES

1. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 1999, 402: 656-60.
2. Date Y, Kojima M, Hosoda H, et al. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. Endocrinology 2000, 141: 4255-61.
3. Gualillo O, Caminos J, Blanco M, et al. Ghrelin, a novel placental-derived hormone. Endocrinology 2001, 142: 788-94.
4. Mori K, Yoshimoto A, Takaya K, et al. Kidney produces a novel acylated peptide, ghrelin. FEBS Lett 2000, 486: 213-6.
5. Korbonits M, Bustin SA, Kojima M, et al. The expression of the growth hormone secretagogue receptor ligand ghrelin in normal and abnormal human pituitary and other neuroendocrine tumors. J Clin Endocrinol Metab 2001, 86: 881-7.
6. Shuto Y, Shibasaki T, Otagiri A, et al. Hypothalamic growth hormone secretagogue receptor regulates growth hormone secretion, feeding, and adiposity. J Clin Invest 2002, 109: 1429-36.
7. Cowley MA, Smith RG, Diano S, et al. The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. Neuron 2003, 37: 649-61.
8. Frascarelli S, Ghelardoni S, Ronca-Testoni S, Zucchi R. Effect of ghrelin and synthetic growth hormone secretagogues in normal and ischemic rat heart. Basic Res Cardiol. 2004, 43: 294-9.
9. Ghigo E, Broglio F, Arvat E, Maccario M, Papotti M, Muccioli G. Ghrelin: more than a natural GH secretagogue and/or an orexigenic factor. Clin Endocrinol (Oxf) 2005, 62: 1-17.
10. Lee HM, Wang G, Englander EW, Kojima M, Greeley GH Jr. Ghrelin, a new gastrointestinal endocrine peptide that stimulates insulin secretion: enteric distribution, ontogeny, influence of endocrine, and dietary manipulations. Endocrinology 2002, 143: 185-90.
11. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 2001, 50: 1714-9.
12. Tschop M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML. Circulating ghrelin levels are decreased in human obesity. Diabetes 2001, 50: 707-9.
13. Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature 2000, 407: 908-13.
14. Nakazato M, Murakami N, Date Y, et al. A role for ghrelin in the central regulation of feeding. Nature 2001, 409: 194-8.
15. Marchesini G, Bianchi G, Lucidi P, Villanova N, Zoli M, De Feo P. Plasma ghrelin concentrations, food intake, and anorexia in liver failure. J Clin Endocrinol Metab 2004, 89: 2136-41.
16. Sanchez J, Oliver P, Palou A, Pico C. The inhibition of gastric ghrelin production by food intake in rats is dependent on the type of macronutrient. Endocrinology 2004, 145: 5049-55.
17. Wortley KE, Anderson KD, Garcia K, et al. Genetic deletion of ghrelin does not decrease food intake but influences metabolic fuel preference. Proc Natl Acad Sci USA 2004, 101: 8227-32.
18. Beck B, Musse N, Stricker-Krongrad A. Ghrelin, macronutrient intake and dietary preferences in long-evans rats. Biochem Biophys Res Commun 2002, 292: 1031-5.
19. Gnanapavan S, Kola B, Bustin SA, et al. The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. J Clin Endocrinol Metab 2002, 87: 2988.
20. Long C. Biochemists Handbook. London: E&FN Spon LTd. 1961.
21. Ariyasu H, Takaya K, Tagami T, et al. Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. J Clin Endocrinol Metab 2001, 86: 4753-8.
22. Torsello A, Scibona B, Leo G, et al. Ontogeny and tissue-specific regulation of ghrelin mRNA expression suggest that ghrelin is primarily involved in the control of extraendocrine functions in the rat. Neuroendocrinology 2003, 77: 91-9.
23. Wren AM, Small CJ, Ward HL, et al. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology 2000, 141: 4325-8.
24. Akamizu T, Takaya K, Irako T, et al. Pharmacokinetics, safety, and endocrine and appetite effects of ghrelin administration in young healthy subjects. Eur J Endocrinol 2004, 150: 447-55.
25. Nagaya N, Kojima M, Uematsu M, et al. Hemodynamic and hormonal effects of human ghrelin in healthy volunteers. Am J Physiol Regul Integr Comp Physiol 2001, 280: R1483-7.
26. Tigno XT, Selaru IK, Angeloni SV, Hansen BC. Is microvascular flow rate related to ghrelin, leptin and adiponectin levels? Clin Hemorheol Microcirc 2003, 29: 409-16.
27. Muccioli G, Papotti M, Locatelli V, Ghigo E, Deghenghi R. Binding of 125I-labeled ghrelin to membranes from human hypothalamus and pituitary gland. J Endocrinol Invest 2001, 24: RC7-9.
28. Talebizadeh Z, Kibiryeva N, Bittel DC, Butler MG. Ghrelin, peptide YY and their receptors: Gene expression in brain from subjects with and without Prader-Willi syndrome. Int J Mol Med 2005, 15: 707-11.

29. Rindi A, Torsello A, Locatelli V, Solcia E. Ghrelin expression and actions: a novel peptide for an old cell type on the diffuse endocrine system. Exp Biol Med 2004, 229: 1007-16.
30. Korbonits M, Goldstone AP, Gueorguiev M, Grossman AB. Ghrelin - a hormone with multiple functions. Front Neuroendocrinol 2004, 25: 27-68.
31. Volante M, Fulcheri E, Allia E, Cerrato M, Pucci A, Papotti M. Ghrelin expression in fetal, infant, and adult human lung. J Histochem Cytochem. 2002, 50: 1013-21.
32. Angeloni SV, Glynn N, Ambrosini G, et al. Characterization of the rhesus monkey ghrelin gene and factors influencing ghrelin gene expression and fasting plasma levels. Endocrinology. 2004, 145: 2197-205.
33. Ariyasu H, Takaya K, Iwakura H. Transgenic mice overexpressing des-acyl ghrelin show small phenotype. Endocrinology 2004, 146: 355-64.
34. Halmos G, Schally AV, Comaru-Schally AM, Nagy A, Irimpen A. Absence of binding of targeted analogs of somatostatin carrying cytotoxic radicals or radionuclides to growth hormone secretagogue receptors on human myocardium. Life Sci 2003, 72: 2669-74.
35. Papotti M, Ghe C, Cassoni P, et al. Growth hormone secretagogue binding sites in peripheral human tissues. J Clin Endocrinol Metab 2000, 85: 3803-7.
36. Patterson M, Murphy KG, le Roux CW, Ghatei MA, Bloom SR. Characterisation of Ghrelin-like immunoreactivity in human plasma. J Clin Endocrinol Metab 2005, 90: 2205-11.
37. Toshinai K, Mondal MS, Nakazato M, et al. Upregulation of ghrelin expression in the stomach upon fasting, insulin-induced hypoglycemia, and leptin administration. Biochem Biophys Res Commun 2001, 281: 1220-5.