Physiol. Res. 54: 403-408, 2005

Plasma Ghrelin Levels in Patients with End-Stage Renal Disease

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Received October 10, 2003
Accepted September 15, 2004
On-line available December 9, 2004

Summary

Ghrelin is an acylated peptide stimulating secretion of the growth hormone (GH). It was originally isolated from the rat stomach as an endogenous ligand for the growth hormone secretagogue receptor. Although being predominantly produced by endocrine cells of the gastric fundus, its secretion has been found in various tissues including the kidney. To study the influence of renal failure on plasma ghrelin levels we examined 16 patients with end-stage renal disease (ESRD) receiving hemodialysis (8 men and 8 women) and 19 controls (10 men and 9 women). Both groups were comparable in age and BMI. In all subjects we assessed plasma levels of ghrelin, leptin, soluble leptin receptor, insulin, IGF-I, IGFBP-1, IGFBP-3 and IGFBP-6. Ghrelin levels were significantly higher in the group of dialyzed patients (4.49±0.74 vs. 1.79±0.15 ng/ml; $p<0.001$). These patients had significantly higher levels of GH, IGFBP-1, IGFBP-6, leptin and percentage of body fat ($p<0.05$). In the group of patients with ESRD plasma ghrelin levels positively correlated with IGFBP-1 ($p<0.01$). In the control group, ghrelin positively correlated with GH concentrations ($p<0.01$) and negatively correlated with the levels of insulin and creatinine ($p<0.05$). In conclusion, patients with ESRD have higher ghrelin concentrations, which might be caused by a decreased excretion/metabolism of ghrelin in the kidney during renal failure.

Key words

Ghrelin • Leptin • Soluble leptin receptor • End-stage renal disease

Introduction

Ghrelin, an endogenous ligand for the growth hormone secretagogue receptor (GHS-R), is a novel peptide hormone predominantly produced by the stomach (Kojima *et al.* 1999, Date *et al.* 2000, Rosická *et al.* 2002). It is a 28-amino acid peptide with a unique structure, where serine at position 3 is octanoylated (Bednarek *et al.* 2000). Des-acyl ghrelin, which lacks a hydrophobic chain substitution, is another form of ghrelin devoid of its endocrine functions. It predominates in the systemic circulation (Hosoda *et al.* 2000).

Ghrelin displays a strong growth hormone (GH) releasing activity, which is even stronger than that of the growth hormone-releasing hormone (GHRH) (Kojima *et al.* 1999, Seoane *et al.* 2000). Via NPY/AGRP neurons in the hypothalamus ghrelin stimulates food intake and causes body weight gain due to a significant increase in

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fat tissue (Wren *et al*. 2000, Nakazato *et al*. 2001). Plasma ghrelin levels are decreased in obesity, elevated in cachexia and show a diurnal rhythm (Tschöp *et al.* 2001, Shiiya *et al.* 2002, Rosická *et al*. 2003).

Leptin is a protein hormone produced predominantly by adipocytes, which suppresses appetite and decreases food intake by inhibition of the NPY/AGRP system in hypothalamus (Ahima *et al*. 1996). Serum leptin levels are markedly elevated in patients with end-stage renal disease (ESRD), which suggests a possible role for leptin in the development of anorexia and protein-energy malnutrition in these patients (Haluzík *et al.* 2000).

Meanwhile, ghrelin production has been identified in many tissues and organs including the hypothalamus, pituitary, pancreas, bowel, thyroid or kidney (Date *et al.* 2000, Mori *et al*. 2000, Kršek *et al.* 2002). However, not much is known about ghrelin clearance or metabolism, and what factors other than nutrition affect the circulating ghrelin concentrations. To address this issue we determined plasma ghrelin concentrations in patients with ESRD and their relations to other studied parameters, such as leptin, the soluble leptin receptor, GH, IGF-I, IGF-I binding proteins and insulin.

Methods

We examined 16 patients with ESRD receiving hemodialysis (8 men and 8 women) and 19 control healthy subjects (10 men and 9 women). Both groups were comparable in age (patients 66.25±2.46 years, controls 64.16±2.18 years) and BMI (patients 25.14±1.0 kg/m^2, controls 25.21±1.03 kg/m^2). In all subjects we assessed levels of ghrelin, leptin, soluble leptin receptor, insulin, GH, IGF-I, IGFBP-1, IGFBP-3 and IGFBP-6. The present study was conducted under written informed consent and approved by the Ethical Committee of the First Faculty of Medicine of the Charles University.

In the control group blood samples were collected at 08:00 h after an overnight fast. In patients with ESRD receiving hemodialysis, blood was collected similarly at 08:00 h before hemodialysis after an overnight fasting. All subjects were weighed and measured on the same day. The hemodialysis procedures were conducted with low flux polysulphone dialyser (1.5 m^2) and bicarbonate dialysis solution, Kt/V index (OCM module) was above 1.2 in all patients.

The human plasma ghrelin levels were determined using a commercial RIA kit measuring total plasma ghrelin, the sum of octanoylated ghrelin and des-acyl ghrelin (Linco Research, USA).

The human serum leptin levels and soluble leptin receptor levels were detected using the commercial ELISA kits (Bio Vendor, Czech Republic). GH and IGF-I serum levels were determined using the commercial IRMA kits (Immunotech, Czech Republic), IGFBP-1, IGFBP-3 and IGFBP-6 were measured by commercial IRMA kits (DSL, USA). Serum insulin levels were determined using commercial RIA kits (CIS Bio International, France). Body fat was determined using Best´s calliper.

Using SigmaStat statistical analysis software (Jandel Scientific, San Rafael, CA, USA) the statistical analysis of the differences between both groups was performed by Student´s t-test for unpaired data distribution or by the Mann-Whitney non-parametric test. Interdependence between variables within the separate groups were evaluated using Pearson´s or Spearman´s correlation. All data are presented as means ± S.E.M. P<0.05 values were considered significant.

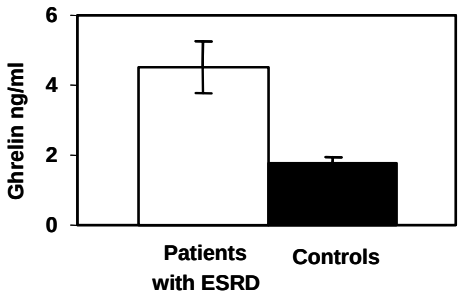

Fig. 1. Serum ghrelin levels in patients with end-stage renal disease (ESRD) compared with healthy subjects.

Results

Both studied groups were comparable in age and BMI, but significantly differed in serum creatinine level (patients 726±51 μmol/l, controls 94±3 μmol/l, p<0.0001). Ghrelin plasma levels were significantly higher in the group of patients with ESRD (4.49±0.74 vs. 1.79 ±0.15 ng/ml, p<0.001, Fig. 1). The serum levels of GH, IGFBP-1, IGFBP-6, leptin and percentage of body fat were also significantly higher in this group (p<0.05). Comparison of laboratory parameters of the two groups is given in Table 1.

The relationships between laboratory variables in the group of patients with ESRD are given in Table 2.

In the group of patients with ESRD plasma ghrelin levels positively correlated with IGFBP-1 ($r = 0.53$, $p<0.05$), in the control group plasma ghrelin positively correlated with GH ($r = 0.59$, $p<0.02$) and leptin serum levels ($r = 0.48$, $p<0.05$) and negatively correlated with serum levels of insulin ($r = –0.51$, $p<0.05$) and creatinine ($r = –0.57$, $p<0.02$).

Table 1. Comparison between laboratory parameters of patients with end-stage renal disease (ESRD) and healthy controls.

	Patients with ESRD	**Controls**
Ghrelin (ng/ml)	4.49±0.74	1.79±0.15**
Leptin (ng/ml)	26.53±4.64	15.58±2.77*
Leptin receptor (ng/ml)	25.52±3.69	21.88±2.36
Insulin (µIU/ml)	23.64±3.14	21.11±2.60
Growth hormone (mIU/l)	13.54±3.57	5.05±2.32*
IGF-I (µg/l)	139.94±15.21	155.33±16.93
IGFBP-1 (µg/l)	175.76±36.23	37.72±4.41*
IGFBP-3 (mg/l)	4.27±0.36	3.81±0.17
IGFBP-6 (mg/l)	2.26±0.56	0.39±0.03*
Creatinine (µmol/l)	725.94±51.16	93.79±3.24***

IGF-I – insulin-like growth factor-I; IGFBP-1 – insulin-like growth factor binding protein-1; IGFBP-3 – insulin-like growth factor binding protein-3; IGFBP-6 – insulin-like growth factor binding protein-6; *p<0.05, **p<0.001, ***p<0.0001

Table 2. Interdependence between Laboratory Variables in the Group of Patients with ESRD

	BMI	**Ghrelin**	**GH**	**IGF-I**	**IGFBP-1**	**Leptin**	**Leptin-R**	**Creatinin**
BMI		r=-0.447	r=0.198	r=0.087	r=-0.206	**r=0.519**	r=-0.364	r=-0.411
		p=0.083	p=0.463	p=0.749	p=0.443	**p=0.039**	p=0.165	p=0.114
Ghrelin	r=-0.447		r=0.222	r=0.209	**r=0.525**	r=-0.192	r=0.318	r=0.399
	p=0.083		p=0.408	p=0.436	**p=0.037**	p=0.476	p=0.230	p=0.126
GH	r=0.198	r=0.222		r=0.104	**r=0.586**	r=0.186	r=0.264	r=-0.287
	p=0.463	p=0.403		p=0.701	**p=0.017**	p=0.491	p=0.324	p=0.282
IGF-I	r=0.087	r=0.209	r=0.104		r=0.028	r=0.114	r=0.147	r=0.218
	p=0.749	p=0.436	p=0.701		p=0.919	p=0.674	p=0.588	p=0.418
IGFBP-1	r=-0.206	**r=0.525**	**r=0.586**	r=0.028		r=-0.069	r=0.485	r=-0.005
	p=0.443	**p=0.037**	**p=0.017**	p=0.919		p=0.799	p=0.57	p=0.987
Leptin	**r=0.519**	r=-0.192	r=0.186	r=0.114	r=-0.069		r=-0.455	r=0.050
	p=0.039	p=0.476	p=0.491	p=0.674	p=0.799		p=0.077	p=0.853
Leptin-R	r=-0.366	r=0.318	r=0.264	r=0.147	r=0.485	r=-0.455		r=-0.019
	p=0.165	p=0.230	p=0.324	p=0.588	p=0.057	p=0.077		p=0.946
Creatinine	r=-0.411	r=0.399	r=-0.287	r=0.218	r=-0.005	r=0.050	r=-0.019	
	p=0.114	p=0.126	p=0.282	p=0.418	p=0.987	p=0.853	p=0.946	

BMI – body mass index; GH – growth hormone; IGF-I – insulin-like growth factor-I; IGFBP-1 – insulin-like growth factor binding protein-1; Leptin-R – soluble leptin receptor; r – correlation coefficient; p – p value

Discussion

Ghrelin is a novel peptide hormone which was originally isolated as an endogenous ligand of the growth hormone secretagogue receptor (Kojima *et al.* 1999). Stomach is the main source of circulating ghrelin

(Toshinai *et al.* 2001), but not much is known about its clearance and metabolism.

Previous observations reported a negative correlation between ghrelin and BMI, body fat mass and plasma leptin levels (Tschöp *et al.* 2001). Low plasma ghrelin levels were found in obese individuals (English *et al.* 2002), whereas malnutrition was associated with high ghrelin levels (Otto *et al.* 2001).

Our group of patients with ESRD receiving hemodialysis was characterized by a significantly higher percentage of body fat. When compared with a group of controls with a comparable BMI, a suppression of plasma ghrelin levels could be expected, but we found higher ghrelin levels in this group. This could be caused by a decreased renal excretion.

A positive correlation between total plasma ghrelin and creatinine in renal failure has been previously reported. No correlation was found between acylated ghrelin and creatinine (Yoshimoto *et al.* 2002). In our study we measured total plasma ghrelin, which did not correlate with creatinine. The reason for this is not clear, we plan further investigations in patients with mild-to-severe renal failure.

Ghrelin has an orexigenic effect in humans, where its administration leads to hunger sensations (Cummings *et al.* 2002). Anorexia is often present in patients with renal failure so that low ghrelin levels could be expected in this condition. Our results, however, show significantly increased ghrelin levels. Higher ghrelin levels associated with anorexia were already found in malnutrition (Ariyasu *et al.* 2001) and anorexia nervosa (Becker *et al.* 1999). One possible explanation might be a resistance of hypothalamic centers to ghrelin in the presence of its high concentrations. Ghrelin is not the only compound affecting appetite; markedly elevated serum leptin levels in renal failure, which we also confirmed, at least contribute to anorexia. Because both leptin and ghrelin affect antagonistically appetite *via* the regulation of NPY/AGRP system in hypothalamus (Nakazato *et al.* 2001), a local interplay between these two compounds could play a role.

Ghrelin is a strong stimulator of GH release (Kojima *et al.* 1999). High GH concentrations in our group of patients with ESRD, which is consistent with previous findings, could thus be at least in part caused by elevated ghrelin levels. In our study we found no correlation between plasma ghrelin levels and GH concentrations, similarly to previous observations (Cappiello *et al.* 2002).

The elevated levels of IGFBP-1 and normal levels of IGF-I in ESRD have been described in numerous studies (Divino *et al.* 1998, Nanba *et al.* 2001). The IGFBP-1 level is thought to be primarily determined by insulin level in portal blood (Suikkari *et al.* 1989). In our study we assessed serum insulin levels, which did not differ between both studied groups. This was also observed by Divino *et al.* (1998). Furthermore, IGFBP-1 concentration as well as its production in liver is markedly increased in malnutrition, a condition which is commonly seen in patients with ESRD (Sanaka 2003).

Patients with chronic renal failure had normal levels of IGF-I. Although there is a reduction of IGF-I gene expression and GH insensitivity in uremia (GH levels are high, but there is downregulation of hepatic GH receptors), the IGF-I level remains in the normal range. This phenomenon could be explained by increased concentrations of IGFBPs in uremia (Tonshoff *et al.* 1997).

Why are plasma ghrelin levels elevated in renal failure? One possibility is a reduction of ghrelin degradation/clearance in end-stage renal disease. However an overproduction of ghrelin in certain tissues might contribute to its higher plasma concentrations. In order to answer this question we plan further investigations.

Acknowledgements

This experiment was supported by the grant of IGA MHCR No. NB/7099-4.

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