EXPERIMENTAL STUDY

Influence of thyroid status and growth hormone deficiency on ghrelin

J E Caminos, L M Seoane, S A Tovar, F F Casanueva and C Dieguez
Department of Physiology and 1Department of Medicine, University of Santiago, Santiago de Compostela E-15700, Spain
(Correspondence should be addressed to C Dieguez, Faculty of Medicine, University of Santiago, Santiago de Compostela E-15700, Spain; Email: fscadigo@usc.es)

Abstract

Objective: To assess whether some of the alterations in energy homeostasis present in thyroid function disorders and GH deficiency could be mediated by ghrelin.

Design: To assess the influence of thyroid status on ghrelin, adult male Sprague-Dawley rats were treated with vehicle (euthyroid), amino-triazole (hypothyroid) or l-thyroxine (hyperthyroid). The influence of GH on ghrelin was assessed in wild-type (control) and GH-deficient (dwarf) Lewis rats. Evaluation of gastric ghrelin mRNA expression in the stomach was carried out by Northern blot. Circulating levels of ghrelin were measured by radioimmunoassay.

Results: Hypothyroidism resulted in an increase in gastric ghrelin mRNA levels (euthyroid: 100 ± 3.2% vs hypothyroid: 127 ± 6.5%; P < 0.01), being decreased in hyperthyroid rats (70 ± 5.4%; P < 0.01). In keeping with these results, circulating plasma ghrelin levels were increased in hypothyroid (euthyroid: 124 ± 11 pg/ml vs hypothyroid: 262 ± 39 pg/ml; P < 0.01) and decreased in hyperthyroid rats (75 ± 6 pg/ml; P < 0.01). Using an experimental model of GH deficiency, namely the dwarf rat, we found a decrease in gastric ghrelin mRNA levels (controls: 100 ± 6% vs dwarf: 66 ± 5.5%; P < 0.01) and circulating plasma ghrelin levels (controls: 124 ± 12 pg/ml vs dwarf: 81 ± 7 pg/ml; P < 0.01).

Conclusion: This study provides the first evidence that ghrelin gene expression is influenced by thyroid hormones and GH status and provides further evidence that ghrelin may play an important role in the alteration of energy homeostasis and body weight present in these pathophysiological states.
between plasma ghrelin levels and body mass index has been reported (9).

Alterations in thyroid function are associated with a large variety of symptoms, including changes in body weight and sleep. Thus, weight loss is present in 85% of patients with thyrotoxicosis, while hypothyroidism is associated with weight gain in 59% of patients (10). Similarly, there is also an important inter-relationship between GH and body weight homeostasis. Thus, it is well established that GH reduces fat mass in normal and obese subjects while patients with GH deficiency exhibit increased fat mass. Furthermore, data obtained in rats have shown that GH stimulates food intake (11).

The aim of this paper was to assess whether some of the alterations in energy homeostasis present in thyroid function disorders and GH deficiency could be mediated by ghrelin. Thus, we assessed ghrelin mRNA levels in the stomach and serum ghrelin concentrations in normal, hypothyroid, hyperthyroid and GH-deficient rats.

Materials and methods

Animals

Male rats were maintained in plastic cages at 23°C under a 12 h light:12 h darkness cycle (lights on 0700–1900 h), with ad libitum access to food and water. All animal experiments were conducted in accordance with the standards approved by the Faculty Animal Committee.

To assess the influence of thyroid status on ghrelin, adult male Sprague–Dawley rats were used. Hypothyroidism was induced by the addition of 0.1% amino-triazole (3-amino-1,2,4-triazole; Sigma, St Louis, MO, USA) to the drinking water and hyperthyroidism was induced by chronic subcutaneous administration of L-thyroxine (100 μg/day L-thyroxine sodium salt pentahydrate; Sigma) (12). The treatment regimes were maintained for 2 weeks and at the end of the experiment the blood was collected by decapitation and tissue dissected. The efficiency of the treatments was confirmed as previously described (12). Briefly, amino-triazole treatment significantly increased plasma thyrotrophin (TSH) levels (control rats: 3.17 ± 0.38 ng/ml; amino-triazole-treated rats: 27.17 ± 1.71 ng/ml; *P* < 0.05). On the other hand, administration of L-thyroxine significantly decreased plasma TSH levels (control rats: 3.17 ± 0.38 ng/ml; L-thyroxine-treated rats: 0.42 ± 0.09 ng/ml; *P* < 0.05). As expected, weight gain was impaired in hypothyroid (163 ± 3 g/3 weeks) and hyperthyroid rats (9.78 ± 3.27 g/3 weeks) in comparison with euthyroid control rats (66.3 ± 2.8 g/3 weeks; *P* < 0.001). Finally, the effect of GH deficiency on ghrelin was assessed in two groups of 7-week-old male Lewis rats, one wild-type and one dwarf.

RNA isolation and Northern blot analysis

Total RNA of the gastric fundus was extracted by the acid guanidinium thiocyanate–phenol–chloroform method (13). Twenty micrograms of total RNA was denatured with formaldehyde, electrophoresed in 1.5% agarose gel and blotted onto a Hybond N* membrane (Amersham Pharmacia Biotech, Buckinghamshire, UK). The membranes were hybridized with a 32P-labelled cDNA probe for ghrelin mRNA in the stomach as previously described (14). The total integrated densities of hybridization signal were normalized using an image analyzer (Gel 2000; Bio-Rad, Hercules, CA, USA) and normalized for the 18S RNA signal intensity and expressed as a percentage of euthyroid control rats or as a percentage of control Lewis rats.

Radioimmunoassay for rat ghrelin

Plasma levels of ghrelin were assayed using reagent kits and methods provided by Phoenix Pharmaceuticals. Truncal vein plasma was obtained by decapitation from the three groups of rats. It was collected in tubes containing EDTA.2Na (1 mg/ml blood) and aprotonin (500 units/ml blood) (Sigma), centrifuged immediately and the plasma ghrelin determined by means of a double-antibody radioimmunoassay using materials and protocols supplied by the provider (Phoenix Peptide Inc., Belmont, CA, USA). All samples were assayed in duplicate within one assay and results expressed in terms of the ghrelin standard. The limit of the assay sensitivity was 2 pg/ml, the intra- and interassay levels were respectively 5% and 13%.

Statistical analysis

All values are expressed as means ± S.E.M. (*n* = 8 rat per group). Analysis by non-parametric Mann–Whitney test was used to assess the differences among groups. *P* < 0.05 was considered statistically significant.

Results

As shown in Fig. 1, hypothyroidism resulted in a marked increase in gastric ghrelin mRNA levels (euthyroid: 100 ± 3.2% vs hypothyroid: 127 ± 5.6%; *P* < 0.01), being decreased in hyperthyroid rats (70 ± 5.4%; *P* < 0.01). In keeping with this (Fig. 1), circulating plasma ghrelin levels were increased in hypothyroid (euthyroid: 124 ± 11 pg/ml vs 262 ± 39 pg/ml; *P* < 0.01) and decreased in hyperthyroid rats (75 ± 6 pg/ml; *P* < 0.01).

Using an experimental model of GH deficiency, namely the dwarf rats, we found a decrease in gastric ghrelin mRNA levels (controls: 100 ± 6% vs dwarf: 66 ± 5.5%; *P* < 0.01) and circulating plasma ghrelin levels (controls: 124 ± 12 pg/ml vs dwarf: 81 ± 7 pg/ml; *P* < 0.01) (Fig. 2).
Discussion

The isolation of ghrelin is one of the most important breakthroughs in our understanding of the neuroregulation of GH secretion as well as in the regulatory mechanisms involved in the regulation of food intake and body weight (15). Regarding the latter, it has been shown that ghrelin administration, either centrally or peripherally, stimulates short-term food intake as potently as any known peptide (7). The orexigenic effect of ghrelin in rodents and humans can be observed at doses that are near the physiological range (15). Furthermore, animal experiments have implicated ghrelin in the long-term regulation of body weight. Thus, rats treated with ghrelin for a period of several weeks showed increased food intake, decreased fat utilization, increased adiposity and a positive energy balance (8, 16). Although the mechanisms by which ghrelin exerts its effects on feeding and metabolism are still unclear, it is likely that these effects are brought about by the interaction of ghrelin with hypothalamic circuits controlling body weight. Finally, measurements of circulating ghrelin levels have linked this hormone with meal initiation and long-term maintenance of body weight. Thus, ghrelin levels are decreased in obesity (9) and increased in states of negative energy balance such as caloric restriction, cancer anorexia, chronic exercise and anorexia nervosa (17, 18).

Energy intake and energy expenditure are tightly regulated, and it has been shown that there is an important inter-relationship between thyroid status and this energy homeostasis. It is well known that starvation is associated with a depression of the thyroid axis. Moreover, patients with alterations in thyroid function, either hypo- or hyperthyroidism, exhibit alterations in food intake, energy expenditure and body weight (10, 19). We therefore hypothesized that the ghrelin system could be a potential mediator of

Figure 1 (A) Representative Northern blots, (B) densitometric analysis of gastric ghrelin mRNA levels and (C) rat plasma ghrelin levels in euthyroid (control), hypothyroid and hyperthyroid rats. Values \( (n = 9/\text{group}) \) are expressed as means \( \pm \) S.E.M. *\( P < 0.01 \).

Figure 2 (A) Plasma ghrelin levels and (B) densitometric analysis of gastric ghrelin mRNA levels in controls and GH-deficient (dwarf) rats. Values \( (n = 7 \text{ animals/group}) \) are expressed as means \( \pm \) S.E.M. **\( P < 0.01 \).
the effects of thyroid hormones on energy homeostasis. Our data showing a decrease in ghrelin levels in hyperthyroid rats were somewhat surprising since in states of negative energy balance ghrelin levels are usually increased. Furthermore, we found increased ghrelin levels in hypothyroid rats. Taking into account that hypothyroidism is usually associated with decreased food intake, our data suggest that hypothyroidism leads to an state of resistance in relation to the orexigenic effects of ghrelin, the nature of which is unclear at present.

Finally, it is well established that GH has a potential to reduce fat mass in obese subjects. These effects are more likely to be mediated by an increase in metabolic rate and energy expenditure. Thus, patients with GH deficiency exhibit increased fat mass and body weight, while exogenous GH administration leads to breakdown of adipose tissue and increased body protein accretion (20). Furthermore, GH has been shown recently to inhibit voluntary food intake in experimental animals. Our data showing that ghrelin gene expression is decreased in GH-deficient rats suggest that this hormone is unlikely to be the mechanism responsible for either the increase adiposity observed in states of GH deficiency or in the inhibitory effect exerted by exogenous GH administration on food intake. In a recent study carried out in humans it has been shown that circulating ghrelin levels were unaffected following 1 year of replacement therapy with human GH. In the same study, the authors also failed to find significant differences between control and GH-deficient patients although the mean values were much lower in the latter (21). Whether these discrepancies are due to interspecies differences or to the fact that we used an experimental model of genetic GH deficiency is unclear. Regarding the latter, it is noteworthy that the GH receptor is present in the stomach. It being generally accepted that ghrelin gene expression is influenced by thyroid deficiency .

In summary, this study provides the first evidence that ghrelin gene expression is influenced by thyroid hormones and GH status and provides further evidence that ghrelin may play an important role in the alterations of energy homeostasis and body weight present in these pathophysiological states.

Acknowledgements

This study was supported by grants from the DGICYT, Fondo de Investigación Sanitaria, Spanish Ministry of Health and the Xunta de Galicia.

www.eje.org

References

12. Lopez M, Seoane L, Senaris RM & Dieguez C. Prepro-orexin mRNA levels in the rat hypothalamus, and orexin receptors mRNA levels in the rat hypothalamus and adrenal gland are not influenced by the thyroid status. Neuroscience Letters 2001 300 171–175.


Received 22 February 2002
Accepted 10 April 2002