Administration of ghrelin to young uraemic rats increases food intake transiently, stimulates growth hormone secretion and does not improve longitudinal growth

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Abstract

Background. Ghrelin administration stimulates appetite and growth hormone (GH) secretion. Whether these effects are preserved in young individuals with chronic renal failure (CRF) and their potential benefit on growth is questioned.

Methods. Three experiments were performed in subtotally nephrectomized young rats (Nx). (i) Food intake was monitored in CRF rats receiving saline intraperitoneally or a ghrelin dose (30 nmol) shown to increase food intake over 2 and 24 h in rats with normal renal function. (ii) Plasma levels of GH were measured after a dose of intravenous ghrelin (3 nmol) was given to three groups of five rats each: Nx, sham-operated fed ad libitum (SAL) and sham-operated pair-fed with Nx (SPF). (iii) Growth of Nx rats treated with intraperitoneal ghrelin (3 nmol) for 7 days (Nx-Ghr) was compared with that of SAL and Nx groups receiving saline (n = 8–10 per group).

Results. In CRF rats, the dose of 30 nmol of ghrelin increased food consumption for 2 h (1.3 ± 0.2 g vs 0.5 ± 0.2 g, P < 0.05) but not 24-h food intake (12.5 ± 0.6 g vs 12.2 ± 0.5 g). Ghrelin (3 nmol) increased plasma levels of GH, which were maximal 10 min after injection, no differences being observed among groups (SAL: 666.2 ± 104.6 ng/ml; Nx: 691.6 ± 90.7 ng/ml; SPF: 577.8 ± 125.4 ng/ml). Return to basal GH levels was delayed in Nx. Ghrelin did not improve body length and weight gains, longitudinal bone growth rate or food intake in the Nx-Ghr group.

Conclusions. In young uraemic rats, ghrelin increases appetite but not 24-h food intake, stimulates GH secretion and does not improve growth.

Keywords: chronic renal failure; food intake; ghrelin; growth; growth hormone; rat

Introduction

The biological, physiological and pharmacological aspects of the 28 amino acid acylated peptide described as ghrelin at the end of the 1990s have been thoroughly reviewed recently [1]. The main source of circulating ghrelin is the stomach, but it is also expressed in various tissues, including the kidney. Ghrelin acts as an endogenous ligand of the growth hormone secretagogue receptor (GHS-R) to stimulate secretion of growth hormone (GH) in several species, including humans. Ghrelin probably plays an important role in the regulation of energy balance, an effect independent of its GH secretion stimulatory activity. In rodents [2], as well as in healthy humans [3], ghrelin has been found to stimulate food intake.

Few studies have examined the metabolism of ghrelin in chronic renal failure (CRF) [4–7]. Yoshimoto et al. [4] found increased plasma ghrelin levels in bilaterally nephrectomized mice and markedly increased levels of plasmatic desacyl ghrelin, an inactive form of ghrelin, in patients with CRF. High plasmatic ghrelin concentrations have also been reported in a short number of adults with end-stage renal disease [5] and in children with CRF [6]. The elevation of circulating ghrelin in CRF is likely the result of decreased clearance or degradation by the kidney. Wynne et al. [7] reported transient enhancement of acute food intake following ghrelin injection in malnourished adult patients on maintenance peritoneal dialysis.

Anorexia, low caloric intake and malnutrition are common manifestations of CRF and play an important pathogenic role in growth retardation of children...
with CRF. In these patients, growth impairment occurs in the presence of elevated circulating levels of GH and may be reversed, at least partly, with administration of high doses of exogenous GH [8], indicating a state of resistance to GH in CRF.

This study was designed to explore the effect of ghrelin administration on appetite and GH secretion in young uremic rats, as well as the effects of ghrelin administration on longitudinal growth on the basis that a positive response to ghrelin in terms of nutrition and GH secretion might result in a beneficial effect on growth rate.

**Animals and methods**

Male Sprague–Dawley rats aged $21 \pm 1$ days and weighing $45 \pm 5$ g were housed in individual cages under controlled conditions of light (12-h light/dark cycle) and temperature ($21–23^\circ$C). After 3 days of acclimation, rats underwent subtotal nephrectomy or sham operation. All animals had free access to tap water and received standard rat chow with a protein content of $17.2\%$ (A04, Panlab, Barcelona, Spain).

Nephrectomy by excision of, approximately, 5/6 of the renal mass, or renal decapsulation without loss of renal mass (sham operation), were performed under anaesthesia in two stages, on days 0 and 4 of the protocol. The degree of renal failure was assessed by measuring plasma concentrations of urea nitrogen and creatinine with a Kodak Ektachem analyser (Eastman Kodak Company, Rochester, NY, USA).

Ghrelin was purchased from NeoMPS (NeoMPS Inc., San Diego, CA, USA), resuspended in saline at a concentration of $1 \text{ mg/ml}$ and stored at $-20^\circ$C until use.

**Experiment 1: Orexigenic activity of ghrelin**

Firstly, to find out the lower intraperitoneal ghrelin dose able to stimulate feeding in young rats, seven groups of normal renal function sham-operated rats ($n=10$ per group) received intraperitoneal injections of saline or ghrelin ($0.1, 0.3, 1, 3, 10$ and $30 \text{ nmol}$) on day 11 of the protocol. Food was weighed 2 and 24 h after ghrelin or saline injection using an electronic balance (Ohaus GT 2100, Florham Park, NJ, USA). The dose able to increase feeding over 2 and 24 h or a similar volume of saline was injected intra-peritoneally into two groups ($n=6$ per group) of nephrectomized rats (Nx-Ghr and Nx, respectively).

**Experiment 2: Effect of ghrelin on GH secretion**

To examine the effect of ghrelin on GH secretion, three groups of five rats each were included: nephrectomized, sham-operated fed ad libitum (SAL) and sham-operated pair-fed with the Nx group (SPF). On day 23 of the protocol, rats were catheterized through the right internal jugular vein under anaesthesia. A blood sample of 0.3 ml was obtained to determine basal GH concentrations. Then, a single dose of intravenous ghrelin (3 nmol) was given to each animal. Successive blood samples were collected 10, 15, 20, 40 and 60 min after ghrelin administration. Each blood sample was immediately centrifuged and $250 \mu$l of plasma was separated and frozen at $-20^\circ$C until radioimmunoassay. Red cells were resuspended in $250 \mu$l of sterile $0.9\%$ saline and infused into the rat to avoid blood volume contraction. Plasma GH concentration was determined using a commercially available double antibody radioimmunoassay (Rat Growth Hormone RIA kit, Linco Research, St Charles, MO, USA). The intra and interassay coefficients of variation were $4.4$ and $4.5\%$, respectively.

**Experiment 3: Effect of repeated ghrelin administration on body growth**

To examine the effect of ghrelin treatment on longitudinal growth, three groups of animals ($n=8–10$ per group) were used: SAL, nephrectomized (Nx) and nephrectomized treated with ghrelin (Nx-Ghr). From day 11 on, rats received two intraperitoneal injections of saline (SAL and Nx) or $3 \text{ nmol}$ of ghrelin (Nx-Ghr) at 10 and 17 h, approximately.

Body growth of the animals was assessed by measuring body weight and nose to tail-tip length gain. Food intake and body weight were measured daily at 10 h using an electronic balance (Ohaus GT 2100, Florham Park, NJ, USA). Nose to tail-tip length gain was measured under anaesthesia on days 0, 4 and 18.

Three days before sacrifice, which was carried out on day 18, each animal received $20 \text{ mg/kg}$ of calcein (Sigma, St Louis, MO, USA) by intraperitoneal route. At sacrifice, tibiae were removed and the proximal ends embedded in methyl metacrilate. Longitudinal growth rate was measured in 10-μm thick frontal sections of the proximal end of tibiae obtained using a rotary microtome (HM355S, Microm, Barcelona, Spain) fitted with tungsten carbide blades. Sections were examined under an Olympus incident light fluorescence microscope (Olympus BX41, Olympus Optical España, Barcelona, Spain) coupled to a digital camera (Olympus DP11, Olympus Optical España, Barcelona, Spain) to detect calcein label. Images were captured and the distance between the chondro–osseous junction and the calcein label was measured using an image analysis system (Scion Image, Scion Corporation, Frederick, MD, USA).

The average value of these measurements divided by three was considered as the daily longitudinal bone growth during ghrelin or vehicle administration.

**Statistical analysis**

Values for each group are expressed as mean$\pm$SEM. Comparisons among groups were carried out by analysis of variance at a level of significance of 95%. Student’s $t$-test was performed for comparisons between two groups. A $P$-value $<0.05$ was considered significant. All datasets were analysed using SPSS 11.0 software package (SPSS Inc., Chicago, IL, USA).

**Results**

**Experiment 1: Orexigenic activity of ghrelin**

The dose of ghrelin that significantly stimulated feeding in young rats with normal renal function (plasma urea nitrogen $16 \pm 2 \text{ mg/dl}$, plasma creatinine $0.4 \pm 0.0 \text{ mg/dl}$) was $30 \text{ nmol}$ (Figure 1). In comparison to the group of animals receiving saline, this dose...
increased food intake over 2 h (1.8 ± 0.2 g vs 0.8 ± 0.2 g, P < 0.05) and 24 h after injection (20.0 ± 0.4 g vs 18.2 ± 0.7 g, P < 0.05). In rats with renal failure (plasma urea nitrogen 75 ± 4 mg/dl, plasma creatinine 1.0 ± 0.1 mg/dl), the dose of 30 nmol of ghrelin increased food consumption in the 2-h period following injection (1.3 ± 0.2 g vs 0.5 ± 0.2 g, P < 0.05) but did not modify 24-h food intake (12.5 ± 0.6 g vs 12.2 ± 0.5 g).

**Experiment 2. Effect of ghrelin on GH secretion**

Significantly increased plasma concentrations of creatinine (1.3 ± 0.2 mg/dl) and urea nitrogen (60 ± 5 mg/dl) were observed in Nx animals when compared with SAL (0.5 ± 0.0 mg/dl; 13 ± 0.0 mg/dl) and SPF (0.4 ± 0.0 mg/dl; 9 ± 1 mg/dl) groups.

As shown in Figure 2, basal plasma GH concentrations were similar in all groups (SAL: 32.1 ± 8.8 ng/ml; Nx: 39.8 ± 11.7 ng/ml; SPF: 37.3 ± 7.4 ng/ml). Intravenous administration of ghrelin gave rise to marked elevation of plasma levels of GH which were maximal 10 min after ghrelin injection in all animals, no statistical differences being observed among groups (SAL: 666.2 ± 104.6 ng/ml; Nx: 691.6 ± 90.7 ng/ml; SPF: 577.8 ± 125.4 ng/ml). Basal GH levels were again reached at 40 min in SAL and SPF animals, whereas Nx animals had not reached basal GH levels even 60 min after ghrelin injection.

**Experiment 3: Effect of repeated ghrelin administration on body growth**

Table 1 shows data on renal function, food consumption and growth in the three groups of animals. Plasma concentrations of creatinine and urea nitrogen were significantly increased, about 2- and 4-fold higher than those of SAL animals, respectively, in Nx and Nx-Ghr rats. In comparison with SAL, body length and weight gain, longitudinal bone growth rate and food intake were significantly and similarly reduced, in Nx and Nx-Ghr groups when compared with the SAL group. Representative images of tibial growth plate sections showing the differences in longitudinal bone growth rate are shown in Figure 3.

**Discussion**

Firstly, it is of note that the dose of ghrelin able to increase food intake 2 and 24 h after injection in normal rats was extremely high in comparison with that previously reported in adult rats. Intraperitoneal administration of 10 nmol of ghrelin has been shown to stimulate food consumption in male Wistar rats [2]. In our laboratory, doses as low as 1 nmol enhanced food ingestion in adult male rats with normal renal function.

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**Table 1. Renal function, food intake and growth of the three groups of rats (n=8–10 animals/group)**

<table>
<thead>
<tr>
<th></th>
<th>SAL</th>
<th>Nx</th>
<th>Nx-Ghr</th>
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<tbody>
<tr>
<td>Plasma urea nitrogen</td>
<td>18 ± 1</td>
<td>77 ± 4*</td>
<td>76 ± 3*</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td></td>
<td></td>
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<tr>
<td>Plasma creatinine</td>
<td>0.4 ± 0.1</td>
<td>1.0 ± 0.1*</td>
<td>0.9 ± 0.1*</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulative length gain</td>
<td>6.9 ± 0.1</td>
<td>2.6 ± 0.2*</td>
<td>2.7 ± 0.7*</td>
</tr>
<tr>
<td>(cm)</td>
<td></td>
<td></td>
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<tr>
<td>Longitudinal bone</td>
<td>196 ± 6</td>
<td>60 ± 12*</td>
<td>51 ± 12*</td>
</tr>
<tr>
<td>growth rate (μm/d)</td>
<td></td>
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<tr>
<td>Cumulative weight</td>
<td>39.1 ± 1.9</td>
<td>18.9 ± 4.2*</td>
<td>19.9 ± 4.6*</td>
</tr>
<tr>
<td>gain (g)</td>
<td>137.0 ± 3.3</td>
<td>81.1 ± 4.5*</td>
<td>81.8 ± 5.0*</td>
</tr>
<tr>
<td>Cumulative food</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>intake (g)</td>
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</tbody>
</table>

Data are given as mean ± SEM. SAL: sham-operated rats fed *ad libitum*. Nx: nephrectomized rats. Nx-Ghr: nephrectomized rats treated with ghrelin.

*Significantly different (P < 0.05) from SAL.
(data not shown). Therefore, our study indicates that, in terms of appetite, sensitivity to ghrelin is influenced by age. The reason why the dose of exogenous ghrelin needed to stimulate food intake is much higher in young than in adult rats is unknown. It could simply be argued that physiological voraciousness of fast growing young rats makes it difficult to further stimulate food intake. Alternatively, it is likely that the role of ghrelin in the metabolic control of appetite and energy balance is less relevant in young rodents. In fact, circulating ghrelin levels are very low in newborn rats and increase with age up to 90 days [9]. Studies in rodents have also shown that the hypothalamic metabolic circuits involved in the modulation of food intake and influenced by the action of ghrelin are not completely developed in the first weeks of postnatal life [10], so explaining the partial resistance to ghrelin observed in the rats of our study. In a similar way, ghrelin administration failed to stimulate food intake in suckling and weaned rats, whereas it resulted in enhanced food ingestion in 7-week-old rats [11], also suggesting the immaturity of neural circuits responsible for mediating some of the ghrelin actions in the first weeks of life. However, stimulated GH release in response to ghrelin has been found in rats of 1–3 weeks of age [12] and, therefore, the development of the hypothalamic and pituitary pathways involved in this secretory response occurs at a much earlier age.

Under our experimental conditions, young rats with normal renal function injected with a single 30 nmol dose of ghrelin ingested more food and energy over the following 24 h than rats receiving saline. However, the same dose of ghrelin failed to increase the overall 24-h food intake in young CRF rats. In these animals, a 30 nmol dose of ghrelin transiently increased food intake during the first 2 h. The acute increment in food consumption induced by ghrelin was, in terms of percentage, similar to that observed in animals with normal renal function but the effect was counterbalanced by a subsequent reduction in feeding, so that food intake within the 24-h period was equal to that of CRF animals untreated with ghrelin. Our study does not provide any explanation on the mechanism of this compensating alimentary behaviour, although elevation of circulating leptin, an appetite-inhibiting peptide, found in advanced CRF [13] might play a role. In addition, recent experimental data suggest that desacyl ghrelin, a metabolite usually considered inactive [1] which is elevated in CRF [4], might neutralize the orexigenic action of ghrelin [14], although this inhibitory effect of desacyl ghrelin on appetite has not been confirmed in other studies [15]. Regardless of the underlying mechanism, our results indicate that the transient effect of ghrelin on appetite does not result in a sustained increase of energy intake in uraemic individuals, at least when given once a day. Whether a different dosage of ghrelin, for instance more injections at short intervals, might be useful to increase food intake remains to be determined. However, the need for repeated administration obviously would limit to a great extent its clinical application. In adult humans on chronic peritoneal dialysis, administration of a single dose of subcutaneous ghrelin at 3.6 nmol/kg increased energy intake immediately after but not over the following 24, 48 or 72 h periods in comparison with the same patients receiving placebo [7]. In this clinical study, ghrelin-treated patients tended to ingest more calories within the first 24 h but, as observed in the young uraemic rats of our study, the difference with the administration of placebo was not statistically significant.

GH secretory response to ghrelin was not different between uraemic and normal renal function rats, showing that the ability to secrete GH in response to ghrelin remains unaltered in this model of experimental CRF. Former studies from our group demonstrated that the in vitro ability of dispersed pituitary cells to release GH when stimulated with different doses of GHRH is...
preserved in moderate renal failure [16] but adversely impaired in severe uraemia [17]. Therefore, it might be hypothesized that a more advanced degree of renal failure might interfere with the GH secretory response to ghrelin. However, Wynne et al. [7] recently demonstrated that intravenous ghrelin administration caused high amplitude peaks of circulating GH in adult patients with end-stage renal disease on peritoneal dialysis. In our study, return of GH concentrations to basal values was delayed in uraemic rats compared to animals with normal renal function. This finding is consistent with decreased metabolic clearance and prolonged half-life of GH in CRF.

The positive GH secretory response to ghrelin led us to test if the same dose of ghrelin able to stimulate GH secretion was useful to accelerate the growth rate of nephrectomized rats on the basis that CRF induces partial resistance to GH action [18] and that growth retardation of CRF individuals can be ameliorated with high GH doses [8]. Ghrelin was administered twice a day because this is the way GH has usually been injected into uraemic rats [19]. Higher doses of ghrelin potentially able to stimulate appetite were not used because experiment 1 showed that, in CRF animals, the effect on food consumption was transient and was not accompanied by a sustained increase in daily food intake. On the other hand, the high cost of ghrelin precluded its utilization in repeated doses every day. With the protocol used in our study, ghrelin did not modify weight or length gains, cumulative food intake or growth velocity in CRF rats in comparison with untreated CRF animals. Thus, in spite of its ability to stimulate GH release, daily treatment with ghrelin failed to improve longitudinal growth. This lack of effect on length gain might be related to the resistance to GH found in CRF. In any case, ghrelin produced a marked stimulation of GH release but the peaks of circulating GH obtained in GH-treated uraemic rats are likely much higher because the very high doses, up to 5–10 IU/kg/day [20], usually employed in those experimental models. The effects on growth of longer periods of ghrelin treatment remain to be examined.

In conclusion, the present study shows that high doses of ghrelin transiently stimulate appetite in young rats with CRF. Unlike animals with preserved renal function, this effect does not increase daily food intake. Although a much lower dose of ghrelin is able to enhance pituitary GH release, repeated administration of this dose does not produce any beneficial effect on longitudinal growth of stunted uraemic rats.

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Conflict of interest statement. None declared.

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