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Peptides associated with hyperphagia in adults with Prader–Willi syndrome before and during GH treatment

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Abstract

Prader–Willi syndrome (PWS) is a complex genetic disorder characterised by mild mental retardation and distinct physical, behavioural, and psychiatric features. One of the cardinal symptoms is excessive eating, which – if left untreated – leads to extreme obesity. In the present study we have examined circulating levels of peptides with documented association to hyperphagia in young adults with PWS. Since growth hormone (GH) is often used nowadays to correct GH insufficiency during childhood PWS, we also studied the impact of GH administration on the peptides. Seventeen adults, 9 men and 8 women, 17–32 years of age with a mean BMI of $35 \pm 3.2\,kg/m^2$ participated. All had clinical PWS (Holm's criteria). Genetic testing was performed in all patients and in 11 the diagnosis was confirmed. They were randomized to treatment with either placebo or GH (Genotropin, Pharmacia Corporation) for 6 months. Subsequently all received open label treatment to provide all subjects with 12 months active GH treatment. Doses were individually titrated. Weight, BMI, oxytocin (baseline only), leptin, Neuropeptide Y (NPY), and ghrelin were evaluated at baseline and after 6 and 12 months. At baseline plasma mean oxytocin was within and serum ghrelin just above the normal range (14.7 ± 1.2 pmol/L and 0.87 ± 0.12 μg/L, respectively). Serum leptin levels were high above and plasma NPY levels within the lower normal range (47.8 ± 29.1 μg/L and 13 ± 1pmol/L, respectively). Results were independent of genotype. No changes in mean BMI, ghrelin, leptin or NPY were seen following GH treatment. *Conclusion*: Leptin levels were in general high reflecting obesity and as a consequence NPY levels were low. In simple obesity oxytocin levels are high, while ghrelin levels are suppressed. In view of the adiposity oxytocin circulated in abnormally low and ghrelin in abnormally high concentrations in our patients. GH treatment of PWS patients did not change ghrelin, leptin or NPY. We suggest that both oxytocin and ghrelin are involved in the pathogenesis of hyperphagia seen in PWS.
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Keywords: Prader–Willi syndrome; Adults; Obesity; Peptides; Hyperphagia; GH treatment

1. Introduction

Prader–Willi syndrome (PWS) is a complex genetic disorder characterised by mild to moderate mental retardation and distinctive physical, behavioural, and psychiatric features [1]. After the age of two years, one of the cardinal symptoms is excessive eating, and this necessitates a lifelong strict diet in order to avoid extreme obesity. It is not known whether the hyperphagia in PWS is caused by an increased hunger or a decreased satiation [2–4].

The signs and symptoms of PWS indicate hypothalamic–pituitary aetiology, but a structural lesion has not yet been fully identified. A decreased number of neuropeptide Y (NPY) and oxytocin secreting neurons has however, been found in the hypothalamus at autopsy [5]. Furthermore, hypogonadism and low growth hormone (GH) secretion are common findings, and both of these conditions may be treated by replacement therapy

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– at least during childhood and adolescence. Body composition in PWS is abnormal with more body fat than lean body mass [1]. We have recently shown that GH treatment in adult PWS patients has beneficial effects on body composition [6].

It is well known that GH is an important regulator of glucose, lipid, and amino acid metabolism [7], and GH treatment could theoretically through an increase in lean body mass increase the metabolic rate. Both NPY and oxytocin are important hormones regulating hunger and satiation [8], NPY being orexigenic and oxytocin anorexigenic. Other peptides involved in appetite regulation are leptin and ghrelin. Leptin is synthesised and secreted exclusively by adipose tissue [9], while ghrelin is mainly synthesised and secreted from the upper gastrointestinal tract [10]. Leptin is associated with anorexigenic abilities [8], and ghrelin is a strong orexigen [11,12]. We have previously shown that leptin levels are high in obese PWS adults and correlate positively with % body fat [13]. Ghrelin has recently been found to circulate in abnormally high concentrations in PWS patients [14,15]. It is not known in detail if GH treatment changes the levels of the various peptides.

In the present study we have analysed peptides of importance for hyperphagia in young adults with PWS. We have measured peripheral levels of oxytocin, ghrelin, leptin and NPY, and subsequently studied the impact of GH treatment on circulating ghrelin, leptin, and NPY.

2. Patients

The study group of 17 adults, 9 men and 8 women, 17–32 years, mean 25 years, with clinical PWS) (Table 1), has been described previously [6,13]. All, except two, were obese. They were recruited consecutively among adults with PWS referred to us from other clinics in Sweden or via the Swedish Prader–Willi patient organisation. Informed consent to participate was obtained from the patients and their caretakers. The study was approved by the committee for medical ethics at the Karolinska Institute and the Swedish Medical Product Agency.

The cohort was for practical and ethical reasons established according to the diagnostic criteria for PWS [16], which all fulfilled. Genotyping was performed using the methylation test in all patients [17], and in 11 patients the genotype was confirmed as methylation positive. All patients had been urged to follow a strict diet of about 1000 kcal/day since many years. All were reported to have behavioural problems. The GH responses following insulin-hypoglycemic test and arginine stimulation showed a wide variation, but 50% had severe GH deficiency defined as a peak GH response <3 μg/L [13].

Table 1
Data (numbers or mean ± SEM) in 17 patients with clinical Prader–Willi syndrome

Number of subjects	17
Males/females	9/8
Age (yr)	25 ± 1
BMI (kg/m^2)	35.0 ± 2.3
IGF-I SDS	-2.0 ± 0.4
Hypogonadism	12/17

3. Methods

3.1. Study protocol

Insulin growth factor-I (IGF-I), insulin, oxytocin, NPY, ghrelin, and leptin were measured in blood samples drawn at baseline. The patients were then randomized to either treatment with placebo or GH (Genotropin, Pharmacia) 0.8 IU (0.26 mg) daily for one month, and then 1.6 IU (0.53 mg) daily for 5 months given subcutaneously at bedtime. Subsequently all received open label treatment to provide each patient with 12 months active treatment. GH doses were individually titrated during this period to keep serum IGF-I concentrations within the normal range for age. Samples for measurement of IGF-I, insulin, ghrelin, leptin, and NPY were drawn every 6 months during the study. Oxytocin was only measured at baseline due to insufficient sample material. The patients were asked not to change their dietary habits, and to confirm this a four day diet recall was performed at baseline and thereafter every 6 months.

3.2. Assays

All blood samples were taken in the fasting state in the morning between 08.00 and 09.00 h, and plasma and serum were stored at −20 °C until the assays were performed.

Oxytocin was determined in EDTA plasma by radioimmunoassay (RIA) after SEP-PAK$_{18}$-extraction (Waters, MA, USA) as described previously [18]. The detection limit was 3.2 pmol/L. In a previous study the normal mean ± SE in non-obese individuals were 15 ± 5 pmol/L [19].

Serum ghrelin was determined by a novel in house RIA, using recombinant human octanoylated ghrelin and ^{125}I-labelled ghrelin. The assay was calibrated against a commercially available RIA kit from Phoenix Pharmaceuticals (Belmont, CA, USA), and compatibility was complete. The lower detection limit was estimated to ~0.125 μg/L, and the within assay coefficient of variation averaged less than 5%. All samples were measured in one assay in duplicate. Normal values according to the Phoenix kit were 0.20–0.80 μg/L.

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Serum leptin was measured with Human Leptin RIA kit (LINCO research, St. Charles, MO, USA). Mean reference leptin values ± SD in individuals with BMI 18–25 kg/m^2 were for men: 3.8 ± 1.8 μg/L and for women: 7.4 ± 3.7 μg/L.

NPY was determined in plasma by RIA after SEP-PAK$_{18}$-extraction. The lower detection limit was 1.90 pmol/L and normal plasma levels were below 50 pmol/L.

IGF-I was determined by RIA [20]. Normal range of IGF-I in adults was established in healthy subjects [21].

GH and insulin were measured by fluoroimmunoassays (Delfia hGH and autodelfia insulin respectively Wallac Oy, Turku, Finland).

4. Statistics

Results are presented as mean ± SEM or median and range. When values were normally distributed comparisons within and between groups were carried out with paired and unpaired Student's *t* test. Otherwise the Mann–Whitney rank sum test was used. Linear regression analysis was performed by Pearson's correlation when values were normally distributed; otherwise with Spearman's correlation. Changes over time were analysed by one-way repeated measures analysis of variance (ANOVA).

Since ghrelin, leptin and NPY did not differ between the initial six months GH and placebo treatment groups, values are shown for the entire patient group at baseline, 6 and 12 months of active GH treatment.

5. Results

5.1. Baseline evaluation (Tables 2 and 3)

Plasma oxytocin levels measured at baseline were within the normal range (14.7 ± 1.2 pmol/L). There were no significant correlations between oxytocin and body weight, BMI, body fat, insulin or IGF-I (data not shown). Furthermore, there were no significant correlations between oxytocin and ghrelin or NPY, while the correlation between oxytocin and leptin was significant ($r = -0.574$, $p = 0.02$).

At baseline mean serum ghrelin was 0.87 ± 0.12 μg/L, but the levels showed wide variations. Ghrelin correlated neither with body weight, BMI, body fat, insulin, IGF-I, oxytocin, leptin, or NPY.

Serum leptin levels were high (47.8 ± 29.1 μg/L), and showed significant positive correlations with body weight ($r = 0.629$, $p < 0.05$), BMI ($r = 0.643$, $p < 0.05$) and body fat ($r = 0.801$, $p < 0.05$). Leptin did not correlate with insulin, IGF-I, ghrelin, or NPY. The correlation with oxytocin was negative (see above).

Plasma NPY levels were within the lower range (13 ± 1 pmol/L) and NPY did not correlate significantly with body weight, BMI, body fat, insulin, IGF-I, oxytocin, ghrelin, or leptin.

We did not see any differences in baseline plasma oxytocin, serum ghrelin, serum leptin, or plasma NPY between patients, who were methylation positive and patients, who were methylation negative (Table 3). Similar results were also obtained, when we calculated the above mentioned relations for patients with the PWS genotype, with the exception of a positive correlation

Table 2
Plasma oxytocin (baseline only), plasma ghrelin, serum leptin and plasma NPY (mean ± SEM) in 17 adult Prader–Willi patients before and during 12 months GH treatment

	Baseline	6 Months	12 Months	*P* value[a]
Oxytocin (pmol/L)	14.7 ± 1.2			
Ghrelin (μg/L)	0.87 ± 0.12	0.85 ± 0.10	1.02 ± 0.13	0.735
Leptin (μg/L)	45.2 ± 7.6	43.1 ± 7.2	42.5 ± 7.5	0.965
NPY (pmol/L)	13 ± 1	8 ± 3	10 ± 5	0.151
IGF-I (μg/L)	164 ± 18	269 ± 29	268 ± 25	$P < 0.001$

[a] ANOVA baseline to 12 months GH treatment.

Table 3
Comparison of baseline plasma oxytocin, plasma ghrelin, serum leptin and plasma NPY (mean ± SEM) between methylation positive and methylation negative adult Prader–Willi patients

	Methylation positive patients[a]	Methylation negative patients[a]	*P* value[a]
Oxytocin (pmol/L)	14.9 ± 1.6	14.4 ± 2.1	0.840
Ghrelin (μg/L)	0.89 ± 0.16	0.78 ± 0.15	0.625
Leptin (μg/L)	48.8 ± 8.3	45.7 ± 12.3	0.832
NPY (pmol/L)	14 ± 4	8 ± 2	0.832

[a] Routine genetic testing.

between oxytocin and ghrelin in the methylation positive group ($r = 0.692$, $p = 0.03$).

In the following, results are therefore referring to the entire cohort of 17 patients with clinical PWS.

5.2. Effects of GH treatment (Table 2)

None of the patients changed their caloric intake during the study, as judged from the four day diet recalls, and this was in accordance with the impression of the caretakers and relatives. IGF-I increased significantly during GH treatment (Table 2). Body weight and BMI were unchanged, while body fat decreased significantly – on average 2.2% – during GH treatment [6].

Ghrelin (Fig. 1), leptin, and NPY concentrations in the circulation did not change during twelve months of GH treatment (Table 2). Similar results were obtained for the methylation positive and the methylation negative subgroup.

6. Discussion

In adult obese PWS patients we found normal oxytocin levels, and slightly elevated ghrelin concentrations, while leptin was high and NPY within the lower normal range. GH treatment did not significantly change the levels of ghrelin, leptin, or NPY.

We did not in this study establish control groups relevant to the PWS patients, but rather decided to compare values from our patients to values given as "normal range" in the various assays. The assays we used have all been tested in patients with simple obesity with consistent results as mentioned below. Establishment of a number of "control" populations relevant to PWS is most likely needed, e.g., matched for body weight, body fat, visceral fat or muscle mass in addition to gender and age. Therefore, the present results have to be considered as pilot data in our endeavour to design a more conclusive trial.

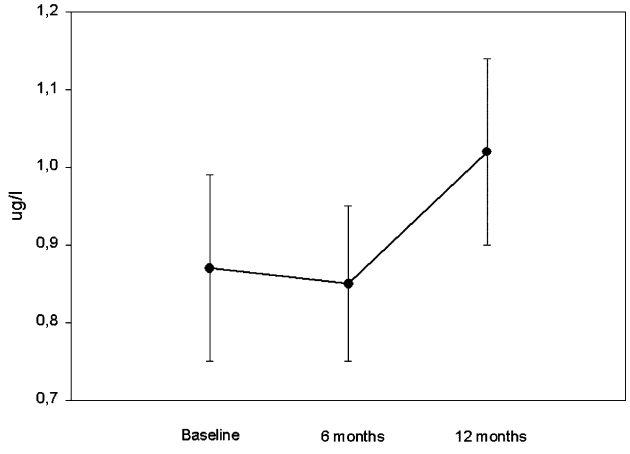


Fig. 1. Serum ghrelin values in 17 adults with Prader–Willi syndrome at baseline and after 6 and 12 months GH treatment (mean ± SEM).

Animal experiments have shown that the parvocellular oxytocin neurones in the paraventricular nuclei (PVN) are crucial for the regulation of food intake, i.e., lesions in rat PVN cause overeating and obesity [22] and central administration of oxytocin to rats has anorectic effects [23]. A previous study has shown, that the oxytocin producing neurons in the PVN are reduced in PWS [5] potentially linking reduced oxytocin to hyperphagia. In another study, however, it was found that oxytocin in cerebrospinal fluid from PWS patients were higher than in obese patients without PWS [24]. On the other hand, plasma oxytocin levels have been reported to be higher in simple obesity than in normal weighted controls (53 ± 6 versus 15 ± 5 pmol/L) [19], and similar results have also been found recently (K Uvnäs-Moberg et al., personal communication). In contrast, our obese PWS patients had normal values (mean 14 pmol/L). Although the physiological significance of normal oxytocin levels in PWS patients remains to be clarified, low oxytocin values in relation to obesity in PWS are in concordance with oxytocin being an anorexigenic peptide.

Two recent studies of patients with PWS have shown elevated levels of plasma ghrelin [14,15]. Ghrelin is known to be a very strong orexigen involved in mealtime hunger [11,12,25], and also associated with weight regulation in rodents and man [10,26]. It has been shown previously that total circulating ghrelin levels are low in patients with adiposity [27,28] and high in patients with anorexia nervosa as compared to normal weight adults [27]. In normal subjects ghrelin levels tended to be low under conditions of positive energy balance and high during negative energy balance, suggesting a dynamic relationship between ghrelin and energy balance [26]. Furthermore, it has been shown that feeding decreases plasma levels of ghrelin in humans [12]. Obviously the ghrelin values reported in our patients are not as high as those reported by Cummings et al. [14] and DelParigi et al. [15], but similar to the levels reported by Haqq et al. [29] in PWS children. The reason for these discordant results is not clear at present. Problems comparing values from different assays of this relatively new hormone have to be considered. Our samples were obtained during standardized conditions at fixed hours in order to reduce variability. However, a number of known (and unknown factors) contribute to the short-term oscillations of ghrelin-including caloric restrictions and interaction with other peptides like leptin. Since ghrelin was not completely suppressed, which theoretically might be expected in these grossly obese individuals, our results are consistent with the suggestion that ghrelin may be one of the significant contributors to hyperphagia in PWS-in agreement with the findings by Haqq et al. [29]. The negative correlation between ghrelin and insulin, BMI, fat mass and leptin shown in other studies [15,30]

did not reach statistical significance in our patients. On the other hand we found a positive correlation between ghrelin and oxytocin in the patients with the PWS genotype, indicating a co-variation in secretion of these peptides. We did not see any change in ghrelin levels during GH treatment. This is in accordance with experience from GH treatment of other GH deficient patients [30].

Obese animals and humans with normal leptin gene are characterised by high plasma leptin levels and/or high adipose tissue ob (leptin) expression, and there is a strong correlation between leptin levels and percent body fat [31]. Elevated NPY levels stimulate eating [32], and leptin inhibits the gene expression of NPY in the arcuate nucleus. This relationship between NPY and leptin might lead to a decreased secretion of NPY at the CNS-level. Thus, although we do not have any measurements of NPY at the hypothalamic level, we suggest that the levels of NPY centrally are low and that NPY is not one of the peptides leading to hyperphagia in PWS.

Increased amount of body fat results in higher leptin levels, which physiologically should inhibit eating [8]. Despite this, obese persons do not decrease their food intake [8], indicating the existence of central leptin resistance. Most of our PWS patients were obese, and we have previously shown that their leptin levels were significantly related to percentage body fat [13]. The same results have been found in children with PWS [33]. Thus, leptin cannot explain hyperphagia in our cohort. Leptin levels decreased insignificantly in our patients during GH treatment maybe because the reduction in percent body fat was too small to significantly affect leptin secretion.

It should be recognised that our group of adults with clinically diagnosed PWS is heterogeneous in the sense that not all patients (6 out of 17) were subsequently confirmed to have the diagnosis also on genetic testing. However, in this small cohort we could not discover any differences between the two groups of PWS patients in the parameters tested [6,13].

In summary this group of adult PWS patients had low plasma oxytocin and elevated serum ghrelin in relation to their obesity. These peptides might therefore be among the explanations of hyperphagia. Leptin levels were in general high reflecting obesity and as a consequence NPY levels were low. Ghrelin, leptin, and NPY levels did not change during GH treatment. Our observations support peptides being involved in the pathogenesis of hyperphagia in PWS.

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