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Somatostatin infusion lowers plasma ghrelin without reducing appetite in adults with Prader-Willi syndrome

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ABSTRACT Prader-Willi syndrome (PWS) is characterized by life-threatening childhood-onset hyperphagia, obesity and, uniquely, high plasma levels of ghrelin, the orexigenic gastric hormone. Somatostatin suppresses ghrelin secretion in normal subjects. We therefore examined the effect of somatostatin on plasma ghrelin and appetite in four male PWS adults fasted overnight in a double-blind, placebo-controlled, randomized cross-over study. Subjects received an intravenous infusion of somatostatin (250 μg/hr) or saline for 300min, and had blood samples taken every 30min for measurement of plasma ghrelin and PYY3-36 (anorexigenic intestinal hormone) by radio-immunoassay, and glucose. Appetite was measured by counting sandwiches eaten over a 60min free food access period from +120min. Despite somatostatin lowering fasting plasma ghrelin by 60 ± 2% (P=0.04) to levels seen in non-PWS men, there was no associated reduction in food intake (105 ± 9% of food intake during saline infusion, P=0.6). Somatostatin also lowered plasma PYY levels by 45 ± 16% (P=0.04), and produced post-prandial hyperglycemia (P=0.04). We conclude that either hyperghrelinemia may not contribute to hyperphagia in PWS adults, or perhaps concomitant reductions in anorexigenic gastrointestinal hormones by somatostatin counteracted any anorexigenic effect of lowering orexigenic ghrelin. Somatostatin analogues may therefore not be an effective therapy for obesity in PWS. Larger chronic studies with long-acting somatostatin analogues will be needed to determine their benefits and risks in treating PWS obesity.

Introduction

Prader-Willi syndrome (PWS) is the most common genetic syndromal cause of human obesity, characterized by life-threatening hyperphagia from childhood, for which effective treatments are lacking (1). PWS results from absent expression of imprinted genes on chromosome 15q11-q13, but the pathophysiological cause of their hyperphagia is unclear, though it is felt to be hypothalamic in origin (1). The control of appetite includes interactions between circulating hormones, particularly those secreted by the gastrointestinal tract, and hypothalamic feeding neuropeptides (2).

Anorexigenic hormones include peptide YY $_{3\text{-}36}$ (PYY), whose secretion from the intestine increases post-prandially in proportion to calories consumed (3). Ghrelin is an orexigenic gastric hormone, which stimulates feeding through the GH-secretagogue receptor (GHS-R), at least in part through activation of orexigenic neuropeptide Y (NPY) and agouti-related related peptide (AGRP) hypothalamic neurons (4). NPY and AGRP neurons appear normal on post-mortem examination of PWS hypothalami (5). Chronic peripheral or central administration of ghrelin to rodents causes obesity (4,6), and ghrelin acutely stimulates appetite when infused in humans (7). Plasma ghrelin levels are high before meals and fall after food (8).

Plasma ghrelin is usually low in obesity, but is uniquely elevated around 2 to 3-fold in PWS subjects, compared to obese controls (9-13). The cause and consequences of hyperghrelinemia in PWS remains unclear (1,12,13), but it has been hypothesized to contribute to their hyperphagia (9,10).

Somatostatin is able to suppress circulating ghrelin in normal subjects (14,15). We therefore hypothesized that somatostatin would lower plasma ghrelin and hence appetite in patients with PWS.

Materials and Methods

Subjects. We studied four male adults with PWS (mean ± SEM: age 25.6 ± 0.4 years, body mass index (BMI) 31.4 ± 2.9 kg/m^2, % body fat 28.4 ± 6.6% (bio-impedance analysis, Bodystat 1500, Isle of Man, UK), body weight 70.2 ± 7.1 kg), all of whom suffered from childhood-onset hyperphagia. Local ethical approval was obtained, and informed consent obtained from subjects and carers.

Somatostatin infusions. Subjects were studied in a double-blind, placebo-controlled, randomized cross-over manner. Subjects were given a fixed 450kCal supper (98g carbohydrate, 8g protein, 4g fat) at 19.00h the evening before each of two separate study days, and then fasted overnight. On the next day they received an intravenous infusion of somatostatin (Stilamin: Serono, Geneva, Switzerland, 250 μg/hr) for 300min starting at 09.30h (0min), or a control infusion of 0.9% saline. From +120 to +180min, they were given free access to cottage cheese sandwich quarters (61.5kCal, 8g carbohydrate, 3g protein, 2g fat per quarter), along with 200ml of orange juice, to assess appetite (16). The number of sandwiches presented was kept constant to provide a constant food stimulus. The number of sandwiches consumed was measured every 5min during this 60min period. Blood was taken at 30min intervals for assay of plasma glucose (oxidase method, Roche Modular Analytics, Lewes, UK), and immediately spun, plasma separated and stored at -20°C for radio-immunoassay (RIA) of ghrelin (EDTA) and PYY (lithium heparin containing aprotinin, Bayer, Newbury, UK, 2.7% v/v). After termination of the infusion at +300min, sampling was continued until +420min.

Overnight fasting and post-prandial blood samples were also assayed from five non-PWS obese male adults (age 38.2 ± 3.9 years, BMI 34.5 ± 3.0 kg/m^2, body fat 31.1 ± 3.3%). Samples were collected from these non-PWS subjects before and after (every 30min for 180min) a fixed breakfast (522kCal, 77g carbohydrate, 15g protein, 19g fat) for determination of fasting, trough and peak post-prandial levels.

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Radioimmunoassays. Samples for RIA were measured in duplicate in the same assay. Ghrelin-like immunoreactivity was measured with a specific and sensitive RIA that measures both octanoyl and des-octanoyl ghrelin, and does not cross-react with any known gastrointestinal or pancreatic peptide hormones. The antisera (SC-10368, Santa Cruz Biotechnology, CA, USA) was used at a final dilution of 1:50,000. ^{125}I ghrelin was prepared by Bolton & Hunter reagent (Amersham International, UK) and purified by reverse phase-HPLC. The specific activity of ghrelin label was 48 Bq/fmol. The assay was performed in total volume of 0.7ml of 0.06M phosphate buffer pH7.2, containing 0.3% BSA and incubated for 3 days at 4°C before charcoal-absorption separation. The assay detected changes of 25pmol/L of plasma ghrelin with 95% confidence limit, with an intra-assay coefficient of variation (CV) of 5.5%. PYY was assayed using an established RIA (3).

Statistical analysis. Comparisons between treatment and subject groups and different time points were made using the paired or unpaired Student's t-test. Statistical significance was taken as $P<0.05$.

Results

Somatostatin lowered ghrelin levels compared to saline, both before and during the meal (Fig.1A), reaching 60.4 ± 2.3% (range 54.8 - 65.8%) suppression at +120min (pre-meal saline: 742 ± 205 vs. somatostatin: 287 ± 78 pmol/L, P=0.038). By comparison the fasting ghrelin levels in five similarly obese non-PWS adult males were 442 ± 49 pmol/L, falling to a post-prandial trough of 368 ± 48 after a 522 kCal breakfast (Fig.1A, P=0.12 and 0.38 vs. pre-meal ghrelin levels in PWS subjects receiving somatostatin). Post-prandially, plasma ghrelin tended to be lower in PWS subjects receiving somatostatin compared to saline (P=0.07-0.21) throughout the rest of the study (Fig.1A). Plasma ghrelin fell post-prandially by a maximum of 54.0 ± 4.9% (P=0.002 vs. pre-meal) while receiving saline, but by only 27.9 ± 3.5% (P=0.004 vs. pre-meal) while receiving somatostatin (% fall P=0.03 saline vs. somatostatin).

Despite this marked lowering of plasma ghrelin in PWS subjects before and during the period of free access to food, somatostatin had no significant effect on the cumulative or total amount of food eaten during this period (P=0.20-1.00, Fig.2). The total food intake while receiving somatostatin was 105.2 ± 9.1% (range 91.5 - 131.9%, P=0.61) of that when receiving saline. Two of the four subjects were still eating food at the end of the 60min period during both infusions.

Somatostatin lowered PYY levels compared to saline, both before and at the end of the meal (Fig.1B), reaching 44.6 ± 15.7% suppression at +120min (saline: 18.6 ± 3.1 vs. somatostatin: 11.6 ± 4.6 pmol/L, P=0.044). Plasma PYY increased to similar levels in the two groups after the meal had been completed (P=0.28-0.93, Fig.1B). Before access to food, somatostatin lowered fasting plasma glucose levels slightly at +60 and +90min (Fig.1C), but no subject had symptomatic or biochemical hypoglycemia, with glucose levels always > 2.8 mmol/L (50 mg/dL). Somatostatin had no significant effect on plasma glucose at the start or during the food access period (+120 to +180min, Fig. 1C). By +210min somatostatin had caused significant hyperglycemia compared to saline (saline: 6.5 ± 0.9 vs. somatostatin 12.7 ± 1.8 mmol/L (117 ± 16 vs. 229 ± 32 mg/dL), P=0.037, Fig.1C).

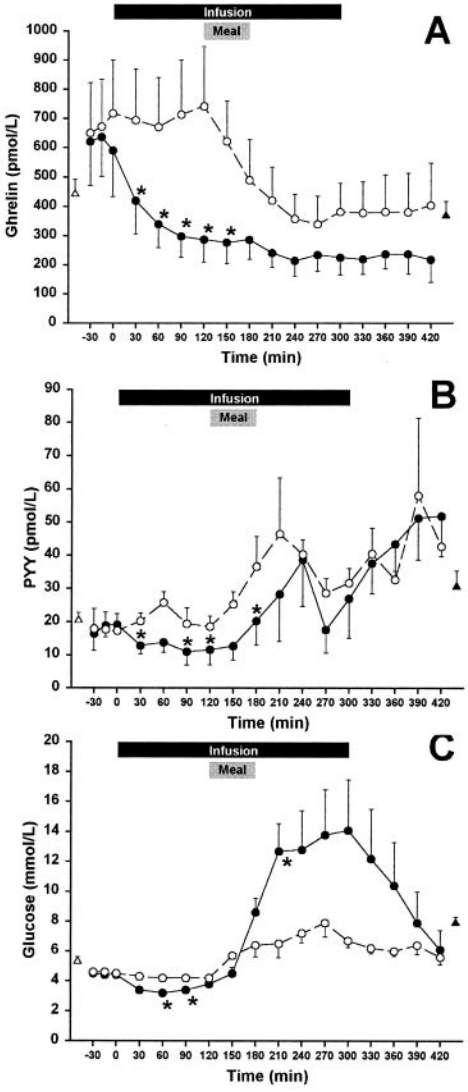

FIGURE 1. *Effect of somatostatin on plasma ghrelin, PYY and glucose levels in PWS.*
Effect of a somatostatin (250μg/hr: •, solid line) or saline (○, dashed line) infusion on plasma (A) ghrelin, (B) PYY or (C) glucose in four PWS male adults. Duration of infusion (black bar) and access to food (grey bar) are indicated. Δ represents fasted levels, and ▲ post-prandial trough (A) or peak (B,C) levels after a 522kCal breakfast in five non-PWS obese male adults. Data represents mean ± SEM. * $P<0.05$ somatostatin vs. saline at same time point. Note that somatostatin lowers both plasma ghrelin and PYY, and causes post-prandial hyperglycemia in PWS subjects. To convert ghrelin units from pmol/L to pg/mL multiply by 3.4, PYY units from pmol/L to pg/mL multiply by 4.3, and glucose units from mmol/L to mg/dL multiply by 18.0.

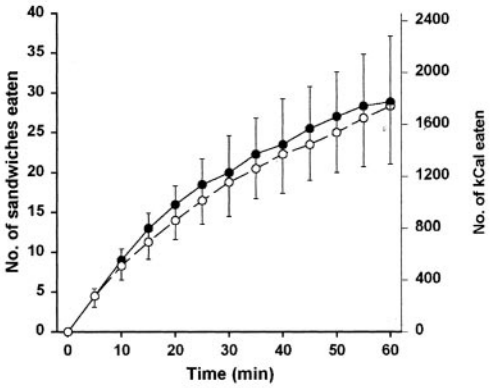

FIGURE 2. *Effect of somatostatin on food intake in PWS.* Effect of a somatostatin (●, solid line) or saline (○, dashed line) infusion on cumulative consumption of food (no. of sandwiches or kCal eaten) during a 60min period of free access to food in four PWS male adults. Data represents mean ± SEM. Note that somatostatin has no significant effect on food intake in PWS subjects.

Discussion

We found that an intravenous somatostatin infusion acutely suppressed fasting plasma ghrelin levels in PWS male adults by around 60%, similar to that seen in non-PWS subjects (14,15). A similar lowering of plasma ghrelin was recently reported after 5-7 days of subcutaneous treatment with the somatostatin analogue, octreotide, in PWS children, but that study did not examine the effect on food intake (17). Using the same ghrelin RIA as in the current study, we have found that fasting ghrelin levels are elevated up to 2.2-fold in PWS adults compared to non-PWS adults adjusting for their % body fat, and elevated up to 1.8-fold for their insulin sensitivity (13). The 60% suppression of fasting plasma ghrelin achieved with somatostatin in our individual PWS patients would therefore have fully corrected their hyperghrelinemia, and as a result their fasting ghrelin levels were no higher than those seen in similarly obese non-PWS men. Despite somatostatin normalizing fasting plasma ghrelin we were unable to observe any reduction in food intake when the PWS subjects were given free access to food during the infusion.

Somatostatin also lowered plasma PYY levels, which is consistent with the ability of somatostatin or its analogues to reduce secretion of many gastrointestinal hormones, including cholecystokinin, GLP-1 and pancreatic polypeptide (18,19). Indeed these and most other gastrointestinal hormones so far identified to affect appetite are anorexigenic (2,20-23), with only ghrelin known to stimulate feeding (7). The post-prandial hyperglycemia induced by somatostatin would be consistent with the recognized suppression of insulin secretion (18).

The lack of any reduction in food intake despite correcting hyperghrelinemia in PWS subjects suggests either that (i) hyperghrelinemia does not contribute to hyperphagia in PWS, or (ii) concomitant reductions in anorexigenic hormones, such as PYY, by somatostatin counteract any anorexigenic effect achieved through lowering orexigenic ghrelin (2,3,20). A lack of a contribution to hyperphagia by hyperghrelinemia in PWS may result from (i) an overriding and persisting orexigenic effect of other abnormalities, such as deficient or defective anorexigenic neurons in the hypothalamic paraventricular nucleus, which impairs satiety (ii) hypothalamic abnormalities in PWS that hinder a normal response to changes in ghrelin, or (iii) the chronic hyperghrelinemia down-regulating or densensitizing GHS-R (1,16,24-26).

This suggests that somatostatin analogues alone may not be an effective therapy for hyperphagia in PWS. Furthermore although it has been postulated that somatostatin analogues may help reduce hypothalamic obesity from suprasellar tumors through suppression of hyperinsulinemia-stimulated adipogenesis (27), the relative hypoinsulinemia in PWS subjects (seemingly related to reduced visceral adiposity and insulin resistance) may limit the benefit of this effect in PWS obesity (28,29), while still predisposing to hyperglycemia.

Although ghrelin infusions are able to stimulate feeding after only a few hours (7), and hour-to-hour changes in ghrelin are thought to play a role in meal initiation (8), it does remain possible that more prolonged or greater suppression of hyperghrelinemia, perhaps in combination with other therapies, will be needed to reduce hyperphagia and hence obesity in PWS. Chronic studies with long-acting somatostatin analogues in larger numbers of both children and adults with PWS and the development of ghrelin antagonists will be needed to answer these questions.

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