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Long-term infusion of nutrients (total parenteral nutrition) suppresses circulating ghrelin in food-deprived rats

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Abstract

Background: Ghrelin derives from endocrine cells (A-like cells) in the stomach (mainly the oxyntic mucosa). Its concentration in the circulation increases during fasting and decreases upon re-feeding. This has fostered the notion that the absence of food in the upper gastrointestinal (GI) tract stimulates the secretion of ghrelin. The purpose of the present study was to determine the concentration of ghrelin in serum and oxyntic mucosa after replacing food with intravenous (iv) infusion of nutrients for 8 days using the technique known as total parenteral nutrition (TPN).
Materials and methods: Male Sprague–Dawley rats (200–250 g) were given nutrients (lipids, glucose, amino acids, minerals and vitamins) by iv infusion for 8 days during which time they were deprived of food and water; another group was deprived of food for 24–48 h (fasted controls), while fed controls had free access to food and water. Serum ghrelin, gastrin and pancreastatin concentrations were measured together with the ghrelin content of the oxyntic mucosa. Plasma insulin and glucose as well as serum lipid concentrations were also determined.
Results: Fasted rats had higher serum ghrelin than TPN rats and fed controls. The oxyntic mucosal ghrelin concentration (and content) was lower in TPN rats than in fasted rats or fed controls. The serum gastrin and pancreastatin concentrations were lower in TPN rats and fasted rats than in fed controls. The plasma insulin concentration was 87 pmol/l ± 8 (SEM) in TPN rats compared to 101 ± 16 pmol/l in fed controls; it was 26 ± 14 pmol/l in fasted rats. The basal plasma glucose level was 11 ± 0.6 mmol/l in TPN rats and 12 ± 0.8 mmol/l in fed controls; it was 7 ± 0.3 mmol/l in fasted rats. In TPN rats, the serum concentrations of free fatty acids, triglycerides and cholesterol were increased by 100%, 50% and 25%, respectively, compared to fed controls. Fasted rats had higher circulating concentrations of free fatty acids (20%) and lower concentrations of triglycerides (−40%) than fed controls; fasted rats did not differ from fed controls with respect to serum cholesterol.
Conclusion: The circulating ghrelin concentration is high in situations of nutritional deficiency (starvation) and low in situations of nutritional plenty (free access to food or TPN). The actual presence or absence of food in the GI tract seems irrelevant. Circulating insulin and glucose concentrations did not differ much between TPN rats and fed controls; serum lipids, however, were elevated in the TPN rats. We suggest that elevated blood lipid levels contribute to the suppression of circulating ghrelin in rats subjected to TPN for 8 days.

Keywords: Ghrelin; A-like cells; Gastrin; Pancreastatin; TPN

1. Introduction

Ghrelin is a 28 amino acid peptide hormone discovered in human and rat stomach. It acts as a natural ligand for the growth hormone (GH) secretagogue receptor (GHS-R) and stimulates GH release [1,2]. GHS-Rs are expressed in the hypothalamus and pituitary [1,3–5] as well as in the stomach [1,4], intestine [1] and pancreas [1].

Ghrelin is said to have orexigenic and adipogenic properties [6–9], which is in line with the observation that the concentration of circulating ghrelin increases during fasting and decreases upon re-feeding [10,11].

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Total parenteral nutrition (TPN) means that nutrients (lipids, glucose and amino acids) are made available via the systemic circulation and not via the gastrointestinal (GI) tract. In TPN rats, the absence of food in the GI tract will create a situation analogous to fasting. Although the nourishment provided through TPN is sufficient for maintaining normal growth of the animals during the experiment [12], the gastrointestinal tract and pancreas will undergo atrophy [13,14].

Since circulating ghrelin increases during fasting and decreases postprandially [10,11], we anticipated that long-term TPN would raise the serum ghrelin concentration. The purpose of the study was to investigate the effect of 8 days of TPN on the concentration of gherlin in serum and in the oxyntic mucosa. For comparison, we studied the effect of TPN for 8 days on the serum concentration of gastrin and pancreastatin, markers of gastrin-cell and ECL-cell activity, respectively. In addition, we measured the circulating concentrations of glucose, insulin, free fatty acids, triglycerides and cholesterol.

2. Materials and methods

2.1. Animals

The study, which was approved by the local animal welfare committee, Lund, Sweden, comprised 45 male Sprague–Dawley rats (B and K, Sollentuna, Sweden) (180–250 g). Before the experiment, the animals were given a standard pellet diet (B and K, Sollentuna, Sweden) and tap water ad libitum. They were housed for 5 days prior to use in cages with constant temperature (22 °C) and a 12 h light/dark cycle. The 15 rats intended for TPN were anaesthesised by an intraperitoneal injection of 5% chloral hydrate (1 ml/100 g body weight) before the operation. The neck of the rat was shaved and the operative field washed with iodine solution. The operation was performed under sterile conditions. A silicon rubber catheter was inserted into the right external jugular vein. The catheter was delivered to the skull subcutaneously and connected to a swivel via a protective coil attached to the skin of the skull. After surgery the rats were housed individually in metabolic cages and infused continuously with 5% glucose at a rate of 1 ml/h over night. Thereafter, infusion of the TPN solution started and continued for 8 days. During this period the rats were not allowed any oral intake of either food or water. Thirty control rats were subjected to identical surgery, including insertion of a catheter, but no TPN solution was infused and they resumed free intake of food upon recovery from anaesthesia. The catheters in both groups were flushed with 100 U/kg /day of low molecular weight heparin (Fragmin®; Pharmacia, Uppsala, Sweden) every second day. A detailed description of the method has been published previously [15,16]. The composition of the TPN solution was as follows (in g/l): Total nitrogen 2.60, amino acids 16.60, glucose 16.80 glycerol 4.30 and soybean oil 39.20. This gives a total of 1066 kcal (nitrogen energy not included). The drugs used were Vamin®, Glucose 40%, Intralipid® 20%, Soluvit®, Vitalipid Adult®, and Tracel®. The solution also contained the following electrolytes; Na^+ 3678.4 mg, K^+ 1563.9 mg, Ca^{2+} 27 mg, Mg^{2+} 121.5 mg, Cr^{2+} 7.84 μg, Cu^{2+} 1.02 mg, Fe^{2+} 0.86 mg, Mn^{2+} 0.21 mg, Zn^{2+} 5.10 mg, I^- 0.10 mg, Mo^{4+}14.90 μg and Se^{2-} 25.10 μg. The TPN solution was given in an amount corresponding to 270 kcal /kg /day. The TPN rats were kept in metabolic cages for 8 days. The control rats were left to recover for a week and then deprived of food (free access to water) for 24–48 h before sacrifice or allowed continued free access to food and water. All rats were killed in the morning (8–10 a.m.). There was no difference between TPN rats and control rats with respect to body weight either at the start or at the end of the experiments (TPN: 235±5 g at the start and 257±6 g at the end of the study. Control rats (freely fed): 220±4 g at the start and 253±4 g at the end of the study). At sacrifice (exsanguination under chloral hydrate anaesthesia, 300 mg/kg intraperitoneally), blood was drawn from the abdominal aorta for determination of plasma insulin and glucose and serum ghrelin, gastrin, pancreastatin, free fatty acids, triglycerides and cholesterol. Each stomach was taken out and opened along the major curvature and rinsed in saline. Thereafter the acid producing (oxyntic) mucosa was scraped off the wall of the stomach. Serum and plasma were frozen and stored at −20 °C until analysis. The mucosa was weighed, frozen and extracted in boiling 0.5 M acetic acid for 10 min (100 mg tissue per ml). After centrifugation at 5000×*g* for 20 min, the supernatants were lyophilised and reconstituted in the assay buffer (0.04 M Na_2 HPO_4 $2H_2O$, 0.01 M NaH_2PO_4. H_2O, 4 mM NaN_3, 7 mM EDTA, 5% Trasylol, 0.25% BSA), giving a concentration of 1 mg tissue per ml buffer.

2.2. Drugs and chemicals

The radioimmunoassay (RIA) kit for determination of insulin was obtained from Diagnostica (Falkenberg, Sweden). Kits for the spectrophotometric measurement of free fatty acids, triglycerides and cholesterol were from Wako Chemicals (Neuss, Germany) and Boehriger Mannhein (Indianapolis, IN, USA), respectively. The ingredients used to prepare the TPN solution were from Fresenius-Kabi (Uppsala, Sweden). All other drugs and chemicals were from British Drug Houses (Poole, UK) or Merck (Darmstadt, Germany).

2.3. Determination of insulin

Plasma insulin was determined by RIA [17].