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Vagotomy and accompanying pyloroplasty down-regulates ghrelin mRNA but does not affect ghrelin secretion

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ABSTRACT

In this study, we have examined how the lack of vagus activity affects the long-term secretion of total and active ghrelin. We subjected mice to sham-operation, pyloroplasty or vagotomy and pyloroplasty. The study lasted for 2 weeks, during which body weight development and daily food intake was monitored. At the end of the study, the mice were sacrificed, and serum and fundus were collected. Measurements of total and active serum ghrelin revealed no difference between the surgical groups and sham-operated mice, despite the fact that fundic ghrelin mRNA was down-regulated. The results presented here suggest that the vagus activity is not required for the long-term secretion of neither total nor active ghrelin in mice. They also suggest that fundic ghrelin mRNA expression is affected by pyloroplasty and vagotomy but that this effect does not translate into changes in ghrelin levels in the circulation.

1. Introduction

Ghrelin is a 28 amino acid peptide produced in the X/A-like cells of the fundic mucosa in the stomach [1,2]. Ghrelin was originally discovered as a growth hormone (GH) secretagogue binding to the GH-secretagogue receptor 1a (GHS-R1a) [2]. Serum and mRNA levels of ghrelin increase in response to fasting and decrease postprandially [1,3–5]. Consequently, injections of ghrelin have been reported to initiate feeding [6,7]. Also, ghrelin has been reported to increase gastric emptying [8], promote adipogenesis [9] and affect thermogenesis [10].

The vagus nerve is involved in the regulation of food intake by mediating the satiating effects of certain hormones such as cholecystokinin (CCK) and leptin. Furthermore, the nodose ganglion of the vagus nerve has been reported to express GHS-R1a mRNA [11], CCK-1 receptor mRNA [12] and the active form of the leptin receptor (Ob-Rb) mRNA [13]. The meal-initiating effect of ghrelin has been suggested to be vagus-dependent [14]. Also, the role of the vagus nerve in regulation of ghrelin's effect on gastric acid secretion has previously been reported [15]. In this study, we investigated how the lack of vagus nerve activity to the stomach would affect ghrelin mRNA expression and the secretion of both total and active ghrelin.

2. Materials and methods

2.1. Animals

Eighteen ($n=18$) female Naval Marine Research Institute (NMRI)-mice were obtained from B&K (Sollentuna, Sweden). The mice were housed three per cage in Macrolon cages under standard housing conditions (21 ± 1 °C, 12 h light/dark cycle). All groups had free access to tap water and standard rat chow (R36, Lactamin, Kimsta, Sweden). The study lasted for 3 weeks and the mice were fasted overnight before sacrifice by decapitation. The experiments described here were approved by the Local Animal Welfare Committee, Lund, Sweden.

2.2. Vagotomy

The mice were allowed a one-week period of habituation, then divided into weight-matched groups and subjected to either vagotomy with an additional pyloroplasty (VtPP; $n=6$), pyloroplasty alone (PP; $n=6$) or sham-operation ($n=6$). They were anaesthetized with an intraperitoneal injection of a mixture of fluanisone/fentanyl/midazolam (15/0.5/7.5 mg kg^{-1}). Vagotomy was performed by dividing and cutting both vagal trunks immediately below the diaphragm and accompanied by a pyloroplasty in order to prevent food retention and fatal gastric dilatation. Sham-operation consisted of a mid-line abdominal incision and gentle manipulation of the stomach. At the start of the study, all mice weighed approximately 31 g (31.5 ± 0.2 g). Clean but not sterile instruments were used to perform the surgery. A successful vagotomy was indicated by elevated levels of serum gastrin [16].

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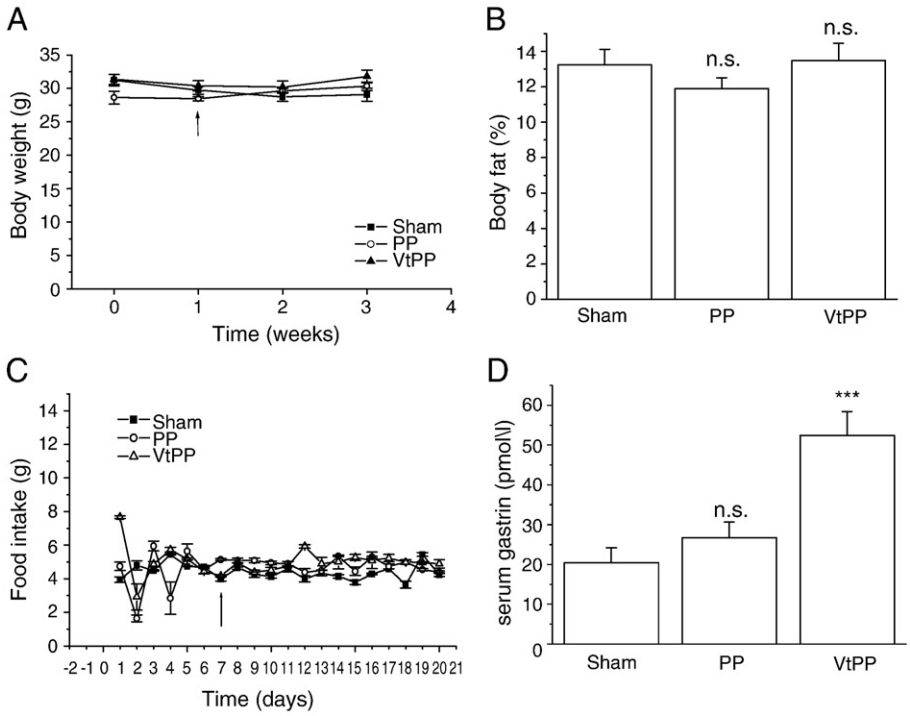

Fig. 1. Body weight was measured weekly for the duration of the study and food intake was measured daily. Neither pyloroplasty nor vagotomy affected body weight development compared to sham-operated mice (A). Furthermore, body fat (B) and daily food intake (C) were unaffected by pyloroplasty and vagotomy compared to sham-operation. A successful vagotomy was indicated by elevated serum gastrin levels. Arrows in Fig. 1 A and C indicate the time of surgery. PP, pyloroplasty; VtPP, vagotomy. ***, $p<0.005$.

2.3. RNA extraction

Total RNA was extracted from snap-frozen fundus using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions and stored at −80 °C until analysis.

2.4. Northern blotting

The quality and concentration of the RNA was determined by measuring the absorbance at 260 and 280 nm; $A_{260}/A_{280}>1.7$ was thought to indicate sufficient purity. 20 μg of total RNA was separated on 1.0% agarose-formaldehyde gel and transferred to a nylon membrane (Zeta-Probe, Bio-Rad, Hercules, CA, USA). The ghrelin probe was labelled as previously described [4]. The plate was analyzed using an FLA 3000 Phosphoimager (Fuji, Tokyo, Japan) and the software Image Reader (Fuji). The signals were quantified using the software Image Gauge (Fuji). As reference for the amount of RNA in each lane, the membranes were rehybridized with an 18S rRNA probe labelled as previously described [4] at 37 °C overnight. The ghrelin mRNA levels were compared with that of the 18S rRNA control and expressed as percent of the sham-operated group.

2.5. Serum analysis

Total serum ghrelin was determined using a commercially available radioimmunoassay (RIA) kit from Phoenix Pharmaceuticals (Burlingame, CA, USA). Active serum ghrelin was determined using a commercially available RIA kit from Linco Research (Millipore, Billerica, MA, USA) according to the protocol provided by the manufacturer. Serum gastrin concentration was determined by RIA as previously described [17], using antiserum no. 2604 (a kind gift from Dr. J. F. Rehfeld, Copenhagen, Denmark) and expressed as picomole equivalents of rat gastrin-17 per litre. For the detection of serum free fatty acids, a colorimetric kit (NEFA C; Wako Chemicals, Richmond, VA, USA) was used. Serum triglycerides were analyzed by GPO Trinder (Sigma, St Louis, MO, USA) and serum leptin was determined using a enzyme-linked immunosorbent assay (ELISA) from Crystalchem (Downers Grove, IL, USA).

2.6. Dual-energy X-ray analysis (DEXA) of body composition

At the time of sacrifice, percentage of body fat in whole mouse was assessed by dual energy X-ray absorptiometry (DEXA; PIXImus, Lunar Corporation, Madison, MI, USA).

2.7. Statistical analysis

Data are presented as mean±SEM. Differences were analyzed statistically by analysis of variance (ANOVA) with Bonferoni's post-hoc test. A p-value<0.05 was considered statistically significant.

3. Results

3.1. Weight development and daily food intake

Body weight development and daily food intake was measured starting 1 week prior to surgery. At the start of the study, there was no difference in body weight between the groups, and the surgery (PP or VtPP) did not affect body weight development (Fig. 1A) or body fat

Table 1
Effect of pyloroplasty and vagotomy on serum levels of free fatty acids, triglycerides and leptin

	Sham	PP	VtPP
FFA (mmol/l)	1.0±0.1	0.94±0.2	1.27±0.2
TG (mmol/l)	10.5±1.2	8.7±0.8	11.1±1.2
Leptin (pmol/l)	22.4±4.0	20.6±3.2	22.7±3.3

Neither pyloroplasty nor vagotomy affected FFA, TG or leptin compared to sham-operated mice. Data are presented as mean±SEM. FFA, free fatty acids; TG, triglycerides; PP, pyloroplasty; VtPP, vagotomy.