Effect of pancreatic polypeptide on gastric accommodation and gastric emptying in conscious rats

Sofie Verschueren,1 Pieter Janssen,1 Lukas Van Oudenhove,1 Leif Hultin,2 and Jan Tack1

1Translational Research Center for Gastrointestinal Disorders, Leuven, Belgium; and 2AstraZeneca R&D, Mölndal, Sweden

Submitted 6 February 2014; accepted in final form 8 April 2014

Verschueren S, Janssen P, Van Oudenhove L, Hultin L, Tack J. Effect of pancreatic polypeptide on gastric accommodation and gastric emptying in conscious rats. Am J Physiol Gastrointest Liver Physiol 307: G122–G128, 2014. First published April 17, 2014; doi:10.1152/ajpgi.00043.2014.—Pancreatic polypeptide (PP) is an anorexigenic hormone released from pancreatic F cells upon food intake. We aimed to determine the effect of PP on gastric accommodation and gastric emptying in conscious Wistar HAN rats to investigate whether effects on motor function could contribute to its anorexigenic effects. Intragastric pressure (IGP) was measured through a chronically implanted gastric fistula during the infusion of a nutrient meal (Nutridrink; 0.5 ml/min). Rats were treated with PP (0, 33 and 100 pmol·kg⁻¹·min⁻¹) in combination with N⁶-nitro-L-arginine methyl ester (L-NAME; 180 mg·kg⁻¹·h⁻¹), atropine (3 mg·kg⁻¹·h⁻¹), or vehicle. Furthermore, the effect of PP was tested after subdiaphragmal vagotomy of the stomach. Gastric emptying of a noncaloric and a caloric meal after treatment with 100 pmol·kg⁻¹·h⁻¹ during nutrient infusion was compared with vehicle treatment. Furthermore, the effect of PP was tested after subdiaphragmal vagotomy of the stomach. Gastric emptying of a noncaloric and a caloric meal after treatment with 100 pmol·kg⁻¹·min⁻¹ PP or vehicle was compared using X-rays. PP significantly increased IGP during nutrient infusion compared with vehicle (P < 0.01). L-NAME and atropine significantly increased IGP during nutrient infusion compared with vehicle treatment (P < 0.005 and 0.01, respectively). The effect of PP on IGP during nutrient infusion was abolished in the presence of L-NAME and in the presence of atropine. In vagotomized rats, PP increased IGP compared with intact controls (P < 0.05). PP significantly delayed gastric emptying of both a noncaloric (P < 0.05) and a caloric (P < 0.005) meal. PP inhibits gastric accommodation and delays gastric emptying, probably through inhibition of nitric oxide release. These results indicate that, besides the well-known centrally mediated effects, PP might decrease food intake through peripheral mechanisms:

- Gastric pressure;
- Nutrient tolerance;
- Nitric oxide;
- Vagus nerve

Both meals, gastric smooth muscle maintains a high resting tone because of the myoelectrical properties of the smooth muscle but also because of a constant cholinergic input from the vagal nerves. The stomach is known to relax upon food intake. This reflex relaxation, also referred to as gastric accommodation (GA), enables the stomach to receive large quantities of food without an increase in intragastric pressure (IGP) (35). Relaxation of the stomach upon food intake is mediated via vago-vagal reflex pathways, eventually leading to activation of mainly nitric nerves in the enteric nervous system (38). Nitric oxide (NO) finally relaxes the smooth muscle cells, and the gastric tone decreases (19).

We recently described a method to assess GA in rats by measuring the IGP during intragastric infusion of a liquid test meal (15). During infusion of a test meal, IGP increased initially until an inflection point was reached, after which the IGP stabilized despite further gastric distension. It was suggested that this inflection point represents the onset of GA, which is proposed by the plateau phase. Pretreatment with N⁶-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase (NOS) inhibitor, resulted in a higher increase of IGP, confirming the role of nitric oxide (NO) as the main mediator of GA.

Gastric function is regulated by a variety of gastrointestinal hormones. Pancreatic polypeptide (PP), a member of the PP family, is a 36-amino acid hormone produced by F cells within the pancreatic islets. It is released upon food intake or in response to other gastrointestinal peptides, and its secretion is mediated through a vagal cholinergic mechanism, since the PP response to a meal or cholecystokinin infusion is reduced after treatment with atropine or after truncal vagotomy (27, 33). Alterations in postprandial PP release have been demonstrated in clinical syndromes associated with abnormal eating behavior, such as Prader-Willi syndrome (decreased PP secretion), anorexia nervosa (increased PP secretion), and obesity (decreased PP secretion) (11, 23, 40). Exogenous PP has been shown to reduce food intake in humans (both healthy volunteers and Prader-Willi subjects), dogs, and mice (5, 6, 24, 34). These observations indicate that PP is involved in the regulation of food intake.

In rats, it has been shown that intravenous infusion of PP caused an increase in antral motility as measured with strain gauges (26). This increase was abolished by pretreatment with atropine and when the animals were vagotomized, suggesting that PP acts through a vagal cholinergic mechanism. The same outcome was seen after microinjection of PP in the dorsal vagal complex, again supporting the theory that PP can affect antral motility through vagal pathways (25).

In humans, gastric emptying (GE) was delayed after PP treatment (32). Also in mice receiving PP and in PP-overexpressing mice a decreased GE rate was observed (3, 20). The gold standard to assess GE in animals makes use of inert markers that in some cases are mixed with a meal and for which the animal has to be killed to determine the GE rate. A disadvantage of this technique is that no paired measurements can be performed.

Because PP is considered to be involved in gastric motility and food intake, we aimed to study the effect of PP on GA during nutrient infusion in conscious rats. To investigate the pathway through which PP exerts its effect, we tested the influence of L-NAME, the muscarinic receptor antagonist atropine, and we performed selective vagotomy of the stomach. In a second series of experiments we aimed to evaluate GE after PP treatment using X-rays to visualize the meal. The results from this study might help us further understand how PP affects food intake and satiety.
METHODS AND MATERIALS

Animals

Twenty three male Wistar HAN IGS rats (Charles River, L’Abresle Cedex, France) were used for this study. Animals were single housed in polypropylene cages in a temperature- and humidity-controlled environment, with a 12:12-h light-dark cycle and free access to water and food pellets. At the start of the experiments, rats weighed 200–250 g. To reduce restraint stress and motion artifacts, rats were regularly accustomed to custom-made Bollmann cages for 30 min a day during the week before the start of the experiments. Experiments were approved by the ethical committee for animal use of the faculty of Medicine of the University of Leuven.

GA Study

Surgery. FISTULA IMPLANTATION. Gastric fistulas were chronically implanted in all animals at least 2 wk before the start of the experiments, as previously described by Janssen et al. (15). In short, rats were anesthetized using inhalation anesthesia (2.5% Isoflurane). A custom-made fistula (made of acryl plastic; outer diameter of 5.5 mm, inner diameter 4.0 mm, and a length of 16.0 mm) was inserted in the stomach through a midline incision of the abdomen. The fistula was attached along the major curvature of the stomach and further exteriorized through the abdominal muscle and the skin. The midline incision was closed, and the skin was sutured. To suppress pain, animals received 0.8 ml/kg of buprenorphin (0.3 mg/ml Temgesic; Reckitt Benckiser Healthcare, Hull, UK) during surgery. Also an injection of an electrolyte solution (Lactretol; Eurovet, Hasundzolder, Belgium) was given to aid recovery after the surgery. From 3 days before surgery until 4 days after the surgery, enrofloxacin (Baytril 10%; Bayer, Diegem, Belgium) was added to the drinking water (2 ml/l). For at least 2 wk, rats were left to recover from the operation while their health status was closely monitored.

VAGOTOMY. In a subset of rats, subdiaphragmatic denervation was performed before fistula implantation during the same surgical procedure. After opening of the abdominal cavity, the esophagus was prepared for the infusion of the test drugs. The experimental protocol was similar to that used in Janssen et al. (15). Animals were fasted overnight before the beginning of each experiment to prevent the presence of food particles in the stomach, and access to water was provided ad libitum. At the start of the experiment, the rats were weighed and then shortly anesthetized to open the fistula and to remove any remaining gastric contents with a syringe. An intravenous line in the tail vein was prepared for the infusion of the test drugs. The opened fistula was connected to a meal infusion system containing a syringe pump to control the infusion speed. The meal, a liquid nutrient (Nutridrink, 1.5 kcal/ml; Nutricia, Bornem, Belgium), was prewarmed to 37°C before infusion into the stomach. A water-filled polyethylene catheter (inner diameter: 0.86 mm, outer diameter: 1.27 mm), connected to an AJP0 (inner diameter: 0.86 mm, outer diameter: 1.27 mm), connected to an

Experimental protocol.

After the start of the infusion (15 min), rats were gavaged with 3 ml of a caloric meal (Nutridrink) or 3 ml of a noncaloric meal (tap water). Hydroxypropylmethylcellulose (0.01 g/ml) was added to the water to match viscosity of Nutridrink (20 mPa-s). BaSO4 (0.5 g/ml) was added to both meals as a radiotracant. Animals were placed in a custom-made Bollmann cage and placed on the platform of a mammographic device (Embrace; Hologic, Villoorde, Belgium). The abdomen was visualized as a dorsal projection using X-ray imaging (29 kV, 40 mAs). X-ray imaging was possible throughout the whole experiment. To estimate the test meal fraction in the stomach, X-rays were taken every 5 or 10 min.

Image analysis. Images were processed using ImageJ software (ImageJ 1.43u; National Institute of Mental Health, Bethesda, MD). After manually outlining the stomach, the software was used to calculate the surface area of the image of the stomach. The surface area directly after gavage of the meal (t = 0) was set at 100%, and GE was expressed as the fraction of the original stomach area over time.

Statistical Analysis

GA. Results are expressed as means ± SE. Maximal IGP increase from baseline and areas under the curve (AUC) were calculated and compared using ANOVA and Tukey’s posttest. Comparisons in experiments with L-NAME, atropine, and vagotomy were made with Mann-Whitney U-test. A P value <0.05 was considered statistically significant.

GE. Mixed-model analysis was performed using proc mixed in SAS 9.3 (SAS Institute, Cary, NC), with fraction of the meal present in the stomach as the dependent variable and “condition” (within subject, 4 levels: water-vehicle, water-PP, nutrient-vehicle, and nutrient-PP) and “time” (within subject, 12 time points after meal) as independent categorical variables. Post hoc t-test with Bonferroni correction for multiple testing was used for further exploring significant main and/or interaction effects.

RESULTS

IGP During Nutrient Infusion and Influence of PP, l-NAME, Atropine, and Vagotomy

Nutrient infusion caused the IGP to increase, after which it stabilized, despite further nutrient infusion (Fig. 1). The maximal pressure increase during control experiments was 6.2 ± 0.4 mmHg. During nutrient infusion, rats receiving PP displayed a higher IGP increase compared with vehicle-treated animals (maximal IGP increase of 8.1 ± 0.5 and 9.1 ± 0.9 mmHg for 33 and 100 pmol·kg<sup>-1</sup>·min<sup>-1</sup> PP, respectively; Fig. 1A). This effect was significant in the high-dose group (P < 0.05) but not in the low-dose group (P > 0.05). Similarly,
AUC was only significantly different between placebo and 100 pmol·kg⁻¹·min⁻¹ PP (P < 0.005).

L-NAME administration significantly increased IGP during nutrient infusion compared with vehicle, with a maximal IGP increase of 11.2 ± 1.2 mmHg (P < 0.005; Fig. 1B). Also, AUC was different (P < 0.005).

Atropine treatment resulted in an IGP increase, reaching 11.2 ± 1.0 mmHg, which was significantly higher compared with vehicle (P < 0.005; Fig. 1C).

In vagotomized animals, the IGP increase during nutrient infusion tended to be less pronounced compared with controls (P = 0.059), but maximal IGP increase did not differ (6.6 ± 0.5 mmHg in controls vs. 6.2 ± 0.5 mmHg in vagotomy; P > 0.5; Fig. 1D).

Possible Mechanisms of Action of PP

In the presence of L-NAME, the effect of PP was abolished, with IGP increasing up to 10.4 ± 1.0 mmHg from baseline (P > 0.5; Fig. 2B). AUCs between L-NAME and L-NAME + PP did not differ (P > 0.05; Fig. 2A).

In the presence of atropine, PP did not induce a significant further increase in IGP (maximal IGP increase of 12.8 ± 1.3 mmHg; P > 0.1; Fig. 2D). Similarly, AUCs between atropine and atropine + PP did not differ significantly (P > 0.05; Fig. 2C).

In vagotomized rats, IGP was increased during PP treatment compared with controls (P < 0.005; Fig. 2E). In addition, the maximal IGP after PP treatment was higher (8.5 ± 0.8 vs. 6.2 ± 0.5 mmHg; P < 0.5; Fig. 2F).

GE and Influence of PP

Initial GE was fast (46.5% emptied in the first 10 min), followed by a slower emptying rate (29.9% emptied during the following 50 min). GE was reproducible since no difference could be observed between two consecutive control experiments (P > 0.1, n = 6).

Independent from caloric content, PP treatment resulted in a delay of GE (P < 0.0001). This effect was observed in both nutrient (65.1 ± 2.3 vs. 53.2 ± 2.1, P < 0.005) and nonnutrient (51.4 ± 2.5 vs. 39.9 ± 2.3, P < 0.05) conditions. Furthermore, a significant effect of nutrient vs. noncaloric was found in both the PP (65.1 ± 2.3 vs. 51.4 ± 2.5, P = 0.001) and vehicle (53.2 ± 2.1 vs. 39.9 ± 2.3, P = 0.007; Fig. 3) conditions.

DISCUSSION

We used IGP measurement during intragastric nutrient infusion as an assessment of GE in conscious rats. We investigated whether cholinergic, nitricergic, and vagal pathways mediated the effect of PP. In addition, we used X-ray to determine the effect of PP on GE.

Nutrient infusion initially caused an increase of IGP. This increase was followed by a plateau phase during which IGP hardly increased despite further nutrient infusion. These findings correspond to results from previous experiments in which the same animal model was used (15). It was suggested that the plateau phase, during which IGP is stable but intragastric nutrient infusion continues, represents a reflex gastric relaxation upon food ingestion and thus GA.

Many studies have confirmed the importance of NO as a mediator in gastric motility and also GA. Indeed, Lefebvre et al. showed that L-NAME reduced IGP decrease during vagal nerve stimulation in anesthetized rats, indicating that NO release is crucial for this response (21). Another group showed that spontaneous antral relaxations were abolished by L-NAME (12). In gastric fundus strips, electrical field...
stimulation-induced relaxation was inhibited by L-NAME (10). In our study, NOS inhibition resulted in a higher IGP increase during nutrient infusion compared with vehicle, and this observation indicates that GA in our model is also mediated by NO.

Also, muscarinic acetylcholine receptors have been shown to be involved in gastrointestinal motility (26). We observed that, during atropine treatment, GA was impaired. This finding is a confirmation of previous findings (15) and suggests that GA in rats is mediated through muscarinic receptor activation and that antagonism of the receptor causes an impaired accommodation response. Outcomes in studies that have investigated the role of cholinergic pathways in gastric motility and accommodation are, however, not consistent. In Sprague-Dawley rats, atropine had no effect on gastric volume response (29). In dogs, gastric motility was decreased upon cholinergic blocking (7). In humans, atropine enhanced GA (22).

Denervation of the stomach was performed by means of subdiaphragmal vagotomy. In vagotomized animals, the pattern of GA during nutrient infusion seemed to be altered compared with outcome in nonvagotomized animals, but the
that gut motility is disturbed, as would be expected (37). However, compensatory mechanisms after vagotomy have been described (1, 13), and such a mechanism may also have developed in our rat model.

We expected an inhibited GA response in vagotomized animals. Although we did not see a clear difference in accommodation, IGP patterns were in fact different, and this might indicate that comparisons of AUC and maximal IGP increase are insufficient to detect more subtle effects. Immediately after the start of nutrient infusion, IGP seems to increase faster in vagotomized rats. From previous experiments, we suggested that the phase during which IGP stabilized reflects accommodation. Apparently, the time period between nutrients starting to enter the stomach and reaching a stabilized IGP might also be relevant. Additional comparison of the AUC during the first 4 min of nutrient infusion (2 ml infused) showed that, compared with controls, IGP increase is significantly faster after vagotomy ($P = 0.0055$; data not shown). This effect might be attributed to the fact that mechanoreceptors fail to efficiently signal to the brain to induce gastric relaxation, indicating that gut motility is disturbed, as would be expected (37).

During L-NAME treatment, PP failed to further increase the IGP response during nutrient infusion. Although a ceiling effect cannot entirely be excluded, this seems very unlikely, since even higher IGPs were observed in the atropine experiments compared with during L-NAME treatment. Our observations thus suggest that the effect of PP involves a nitricergic pathway, i.e., inhibits NO release. To our knowledge, to date, literature investigating whether PP acts on NO release is lacking, and the underlying mechanism deserves further analysis beyond the scope of the current study.

In the presence of atropine, PP failed to cause an enhanced IGP response, indicating that the mechanism of action of PP also involves muscarinic receptor activation. Atropine has been shown to abolish the PP-induced increase in antral motility in anesthetized rats (26).

It is suggested that PP acts via the vagal nerve, since $Y_4$-receptors are located in the dorsal vagal complex, which comprises the vagal lower motor neurons (39). McTigue and Rogers found that bilateral cervical vagotomy diminished the PP-induced increased antral motility (26). We observed that, after vagotomy, PP was still able to increase IGP. A possible explanation for this effect could be the before-mentioned compensatory mechanisms that might have developed (1, 13). It could also be possible that vagal-independent mechanisms contribute to the effect of PP. Banks et al. showed that PP could penetrate the blood-brain barrier, suggesting that PP can exert its effect despite vagal disruption (4).

PP-overexpressing mice and mice receiving PP treatment display a decreased GE rate (3, 20). Schmidt et al. showed that, in healthy subjects, GE of a solid meal was delayed after PP administration, but emptying of water was unaffected (32). We, however, demonstrate that PP was able to slow down GE of both a noncaloric meal and a caloric nutrient meal in rats. A possible difference between our study and the one of Schmidt that might explain the observed difference is the dose of PP that was administered. Even though it is difficult to compare doses between humans and animals, we can state that the dose we used in rats was rather high. The dose used by Schmidt was relatively low (2.25 pmol·kg$^{-1}$·min$^{-1}$), since there are reports from other studies administering higher doses to humans (up to 10 pmol·kg$^{-1}$·min$^{-1}$) (5, 17). This dose might have been too low to induce a delay in GE of water. The use of X-rays over the commonly used phenol red method has some advantages. There is no need to kill animals; hence, far less animals are required to acquire results. In addition, multiple observations per animal are possible, allowing paired comparisons. Disadvantages include the lack of three-dimensional imaging and the radioactive load.

Numerous studies in animals have demonstrated that PP is involved in food intake (2, 3). Also, humans reduce food intake upon PP, accompanied by lower hunger scores (5). In healthy volunteers, pharmacologically induced impaired GA was associated with early satiety and reduced food intake (16). Paired accommodation and early satiety were also correlated in functional dyspepsia patients (36). Our findings fit in this context that PP has a twofold effect. By inhibiting GA by increasing IGP, gastric mechanoreceptors are activated and lead to feelings of fullness (8). In addition, $Y_4$-receptor stimulation in the brain leads to the inhibition of neuropeptide Y release and hence the sensation of satiety, which eventually decreases food intake (3). Interestingly, a number of studies in animals have shown enhanced feeding behavior after intraretroventricular administration of PP (2, 9, 14). Additional experiments investigating the receptor involved in this effect have generated controversial outcomes (18). Some authors have even suggested that a yet unknown receptor with affinity for PP might be involved in the increased food intake evoked by centrally administered PP. Clearly, more research is required to address this hypothesis.

PP has low affinity for the $Y_1$- and the $Y_5$-receptor, but it displays high affinity for $Y_4$-receptors, located in the area postrema in the dorsal vagal complex (30). It has been shown that $Y_4$-receptor activation can alter gastrointestinal functions such as gastric motility, gallbladder contraction, and pancreatic exocrine secretion. $Y_4$-receptors are also expressed on gastric smooth muscle (28). Activation of these $Y_4$-receptors stimulates inositol triphosphate, which in turn increases intracellular Ca$^{2+}$ levels, eventually resulting in contraction, which might result in an increase in pressure. In mice with a knock out of the $Y_4$-receptor, food intake is reduced and body weight is
lowered (31). Up to date, selective and potent Y4-receptor antagonists are lacking, which hampers the study of the exact mechanism of action of PP.

In conclusion, PP impaired GA in conscious rats. The mechanism of action of PP involved the release of NO and the activation of the muscarinic receptor. After vagotomy, PP still affected IGP, indicating a local peripheral mechanism of action. PP also delayed GE of both a noncaloric and a caloric meal. These findings might provide an alternative explanation for the effect of PP on food intake.

GRANTS
This work was supported by a Methusalem grant from the University of Leuven to J. Tack.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
Author contributions: S.V., P.J., and J.F.T. conception and design of research; S.V. performed experiments; S.V. and L.V.O. analyzed data; S.V., P.J., L.V.O., and J.F.T. performed experiments; S.V. and L.V.O. analyzed data; S.V., P.J., L.V.O., and J.F.T. drafted manuscript; P.J., L.H., and J.F.T. edited and revised manuscript; P.J., L.V.O., and J.F.T. interpreted results of experiments; S.V. prepared figures; S.V. drafted manuscript; P.J., L.H., and J.F.T. edited and revised manuscript; P.J., L.V.O., and J.F.T. approved final version of manuscript.

REFERENCES