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# **Assessment of vagal nerve integrity in autonomic neuropathy**

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# **Assessment of vagal nerve integrity in autonomic neuropathy**

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*Aan José  
en aan Merel, Marieke en Berend*

# 1





# **Assessment of vagal nerve integrity in autonomic neuropathy**

General introduction and outline of investigations



## The enteric nervous system

The enteric nervous system is a collection of neurons that constitutes the “brain of the gut” and can function independently of the central nervous system<sup>1</sup>. It is a multifunctional system that controls motility<sup>2,3</sup>, exocrine and endocrine secretions<sup>4</sup>, and microcirculation<sup>5</sup> of the gastrointestinal tract; it is also involved in regulating immune and inflammatory processes<sup>6</sup>.

The enteric nervous system is primarily derived from cells of the vagal segment that arise from the medulla oblongata passing to the oesophagus, heart, bronchi, lungs, stomach and subsequently move caudally to populate the entire gastrointestinal tract<sup>7</sup>. The vagus fibres form a single nerve on each side of the oesophagus. It has been estimated that 80% of the fibers in the vagal trunk are afferent fibers<sup>8</sup>.

Parasympathetic innervation of the pancreas is provided by the vagus nerves. The splanchnic nerves of the pancreas contain the sympathetic fibers and arise from the fifth to the ninth thoracic ganglia. Both the sympathetic and parasympathetic fibers pass the celiac and superior mesenteric plexus before they reach the pancreas. The adrenergic preganglionic fibers end in these plexuses, whereas the parasympathetics pass uninterrupted and end in the intrinsic pancreatic ganglia. From here the adrenergic postganglionic fibers form a perivascular network with the pancreatico-duodenal and splenic vessels<sup>9,10</sup>. The parasympathetic postganglionic fibers are distributed to acini, ducts and smooth muscles<sup>11</sup>.

The innervation of the gallbladder can be separated into an intrinsic and an extrinsic nervous system<sup>12</sup>. The intrinsic nerve system of the gallbladder is suggested to be under cholinergic and peptidergic control. The extrinsic neural control is mainly mediated through parasympathetic and sympathetic nerve fibers<sup>8,13</sup>. Inhibition of the vagal-cholinergic system with atropine or truncal vagotomy results in an increase in fasting gallbladder volume and an impaired gallbladder contraction during the cephalic and gastric phase of digestion<sup>14,15,16,17,18</sup>. The gallbladder smooth muscle contains both alpha and beta-adrenergic receptors<sup>8,19</sup>. Since the gallbladder contains only a small number of alpha-adrenergic receptors, under normal circumstances, adrenergic stimulation results in relaxation of the gallbladder through activation of the more numerous inhibitory beta-receptors<sup>8</sup>. The existence of a non-adrenergic, non-cholinergic, possibly VIP-ergic nerve system has been postulated<sup>8,20</sup>.

The vagal-cholinergic system is of utmost importance during the cephalic and gastric phases of postprandial gallbladder contraction. During the intestinal phase, hormonal factors e.g. cholecystokinin (CCK) or motilin play a major role but the cholinergic system seems to modulate the gallbladder response to these hormones at the end-organ level<sup>21,22,23</sup>.

## Pancreatic polypeptide

The discovery of avian pancreatic polypeptide (PP) was an accidental event associated with studies that involved the isolation of chicken insulin<sup>24</sup>. Chance et al., isolated PP from pancreatic extracts from several mammalian species<sup>25</sup>. Additional studies demonstrated that avian and bovine PP contain 36-amino acid residues with a molecular weight of 4240 and 4226 Dalton, respectively and have identical residues at 16 positions<sup>26</sup>. Human PP differs at two to four positions. The amino acid of Human PP at position 2, 6, 11 and 23 are Proline, Valine, Asparagine and Aspartic acid, respectively<sup>27</sup>. It appears that the activity of PP resides in the C-terminal tyrosine amide portion of the peptide because it was demonstrated that the C-terminal hexapeptide of bovine PP was able to mimic the pharmacologic actions of the whole molecule while bovine PP deprived of the C-terminal did not<sup>28</sup>.

## Distribution

The pancreas appears to be the major site for production and storage of PP because release of PP in the blood could not be demonstrated in patients with previous total pancreatectomy<sup>29,30</sup>. PP producing cells occur almost exclusively in the duodenal part of the pancreas. PP is stored in endocrine F-cells<sup>31,32</sup>. These PP producing cells can be demonstrated with the light microscope by the use of immunocytochemical methods only. With the electron microscope, they are rather small and show round or slightly ovoid granules of small size and of moderate to high electron density<sup>30</sup>. The number PP producing cells increases with age. A more striking degree of hyperplasia is found in all pathological conditions associated with injury to the pancreatic parenchyma: e.g. mucoviscidosis, pancreatitis, exocrine and endocrine tumours, hemochromatosis and diabetes<sup>33</sup>.

## Physiological activities

It has been demonstrated that infusion of PP to plasma concentration as observed as after a meal was able to affect physiological functions<sup>28,34</sup>. In conscious dogs steady gastric acid secretion induced by continuous intravenous infusion of 0,5 µg/kg/hr of pentagastrin was significantly inhibited by bovine PP<sup>28</sup>. Bovine PP (10 µg/kg/hr) also reduced the water and bicarbonate secretion by about 80% in dogs with chronic pancreatic fistula<sup>28,35</sup>. At higher doses, PP has been demonstrated to impair bile flow into the duodenum by relaxing the gallbladder and by increasing choledochal sphincter tone<sup>36,37</sup>. The suppression of pancreatic secretion induced by exogenous or endogenous CCK secretion, the enhancement of gastric emptying and intestinal transit and the initial stimulation followed by inhibition of gut motility are other actions of PP<sup>37-39</sup>. Administration of PP in dogs decreases intraluminal gallbladder pressure with concomitant gallbladder filling following CCK induced gallbladder contraction<sup>40</sup>. Furthermore, exogenous PP promotes gallbladder filling in the basal state<sup>41</sup>. The physiologic importance of these gastrointestinal actions, however, remains to be established.

## Radioimmunoassay of PP and metabolism

In the studies performed for this thesis, plasma PP was measured by a sensitive and specific radioimmunoassay. Radioiodinated PP was prepared by the chloramine-T method as described previously<sup>42</sup>. The specific activity of <sup>125</sup>I-PP used was about 275 µCi/µg. In the radioimmunoassay system, 1 ml of <sup>125</sup>I-PP (1800 cpm corresponding to 0.9 fmol of PP), 0.2 ml of anti-PP serum (final dilution 1:900,000), 0.2 ml of standard or sample, and 0.8 ml of veronal buffer, giving a total volume of 2.2 ml, were incubated for 3 days at 4°C. Human PP was used as standard preparation. All dilutions were made in 0.02 M veronal buffer (pH 8.4) containing 0.02% sodium azide and 0.17% human serum albumin. Separation of free and antibody-bound PP was carried out using albumin-coated charcoal. The ratio of bound to free labelled PP was inhibited by 50% at a concentration of 2.8 pM of incubation mixture. The intraassay variation in the steep part of the standard curve was between 4% and 7%, and the interassay variation was between 6% and 12%. The detection limit of the assay was 0.5 pM of incubation mixture.

At room temperature, PP is stable for at least 24 hours in whole blood and for 10 days in sterile plasma. Plasma PP levels remain stable when stored at -20 °C. The plasma PP level is genuine by freezing and thawing up to 20 times. Pharmacokinetic data of circulating PP have been determined in man after infusion of pure 1-36 PP. The disappearance half-life time of PP from the circulation was calculated to be 6.9 minutes with a clearance rate of 5 ml/kg/min and a distribution volume of 51 ml/kg<sup>43</sup>. In patients with renal failure, basal PP levels are elevated and the plasma half-life time is prolonged demonstrating a significant role of the kidney in the excretion of PP<sup>42,44,45</sup>.

## Regulation of PP secretion

Regulation of pancreatic exocrine secretion is comprised of a complex interplay between hormonal and nervous mechanisms<sup>25</sup>. PP can be released from F-cells in the pancreas by several stimuli. Infusion of exogenous CCK, leading to plasma CCK concentrations found after a meal, caused significant PP release in humans<sup>46</sup>. Cholinergic pathways, however, play a key role, since subsequent administration of atropine abolishes PP release in response to all investigated stimuli<sup>47-50</sup>. This suggests that acetylcholine may be the final common pathway by which PP cells are stimulated.

Ingestion of a protein meal is a potent and rapid stimulus for the release of PP. The amount of the ingested meal corresponds with PP release. A significant increase in plasma PP is seen as early as 3 minutes after the beginning of the ingestion. Intravenously administered fat did not result in release of plasma PP<sup>25,51</sup>, while ingestion of fat resulted in a three times increase of plasma PP. Ingestion of protein, however, revealed the most stimulatory effect on PP<sup>52,53</sup>. Infusion of a mixture of 10 essential amino acids resulted in a significant but much smaller PP release<sup>25</sup>. The PP response pattern of carbohydrates is similar, but the PP increment is lower than that to protein or fat. Distension of the stomach, as produced by the ingestion of celery or water results in a modest stimulatory effect on plasma PP release. Because of the great discrepancy in PP release between ingestion of water/celery and protein/fat/carbohydrates, it is unlikely that distension of the stomach after ingestion of food is the major mechanism for PP release. All these findings and the demonstration that PP is found in the pancreas suggests the existence of an entero-PP axis.

## Erythromycin

Erythromycin (ERY) was originally isolated from an actinomycete in a soil sample from the Philippines<sup>54</sup>. It has become established as a safe and useful macrolide antibiotic with a broad spectrum of action against many commonly acquired bacterial pathogens<sup>55</sup>. Since the introduction of ERY into clinical practice, gastrointestinal side effects, commonly nausea and vomiting, cramping upper abdominal pain, and diarrhoea, have been consistently reported. These side effects may in part be the result of a change in intestinal flora. However, ample studies demonstrated that ERY exerts powerful stimulatory effects on gastro-duodenal motility<sup>56-64</sup>. In addition, ERY also stimulates gallbladder emptying and colonic motility<sup>65-70</sup>. In vitro, the effects of ERY on isolated smooth muscle strips were not inhibited by atropine, hexamethonium, naloxone, diphenhydramine, methysergide, procaine, trypsin, indomethacin, or sodiumnitroprusside but were blocked by nifedipine, indicating a direct calcium dependent effect of ERY, probably by binding to motilin receptors which are abundantly present in the gastric antrum<sup>49,71</sup>. In vivo, however, the effect of ERY on gastrointestinal motility is inhibited by atropine suggesting the importance of cholinergic pathways in this action<sup>67,72-74</sup>. Jebbink et al, demonstrated that intravenously administered ERY also stimulated PP secretion<sup>67</sup>. This effect can be blocked by atropine indicating that cholinergic pathways play an important role in ERY stimulated PP release<sup>67</sup>.

## The role of pancreatic polypeptide in the assessment of vagal nerve integrity

Basal plasma PP correlates with age. Plasma PP increases about 30 pg/ml per decade<sup>75</sup>. Basal plasma PP levels may play a role in clinical practice since these levels can be dependent on different disease states. Abnormally low basal PP values have been reported in patients with obesity<sup>76</sup>, autonomic

neuropathy<sup>77-82</sup>, pancreatic insufficiency and pancreatectomy<sup>83-85</sup>, while high basal PP values have been demonstrated in patients with duodenal ulcer disease<sup>86-89</sup>, diabetes mellitus<sup>90-94</sup>, apudomas<sup>95</sup>, stressful events<sup>96,97</sup> and sleep deprivation<sup>97</sup>.

The vagal-cholinergic system plays an important role in the stimulation of PP release, since atropinisation or total vagotomy decreases PP release in response to several stimuli<sup>56,57</sup>. For this reason, the secretion of PP is a model of choice for studies of vagal control of endocrine systems at the afferent, central, and efferent levels of vago-vagal reflexes. In medical practice, stimulation of PP cells is achieved by central vagal activity induced by sham feeding or acute hypoglycaemia by long vago-vagal or local gastro-pancreatic or enteropancreatic cholinergic reflexes<sup>89,90</sup>. Damage of the vagus nerve may result in a decreased or absent PP response. Sham feeding by the 'chew and spit' technique is a rather specific, but mild stimulation of vagal activity to the gastrointestinal tract resulting in approximately 20% negative results in normal subjects<sup>87,98</sup>, and 'Insulin induced hypoglycaemia' a less specific but a stronger stimulus.

The PP response during a meal is typically biphasic<sup>53</sup>. The initial cephalic phase can be eliminated by vagotomy or atropinisation and can be mimicked by sham-feeding. This phase is followed by a long-lasting, secondary gastrointestinal phase, which is just blunted by vagotomy<sup>53,93</sup>. During modified sham feeding by the 'chew and spit' technique there is stimulation of the cephalic phase of the meal by visual, olfactory, gustatory and masticatory perception. The PP response to modified sham feeding can be further increased by adequate sham feeding, a technique in which patients, equipped with a rubber tube inserted into the distal part of the oesophagus via a gastrostomy to prevent entrance of food into the stomach, are allowed to eat and swallow the meal<sup>49,87</sup>. Distention of the stomach and duodenum in itself, e.g. by a water bolus may also stimulate additional PP release by activation of local or long vago-vagal/gastro-duodeno-pancreatic reflexes<sup>99-101</sup>. Since the intestinal phase of meal stimulated PP release is not completely blocked by atropine, it is suggested that activation of peptidergic messengers like CCK or other gut peptides play a role<sup>102-105</sup>. However, the action of these peptides on PP release is complex and they all seem to act either through a cholinergic mechanism or against a background of permissive cholinergic tone<sup>106</sup>.

PP secretion is not only regulated by food intake but also by blood glucose. Hypoglycaemia stimulates PP release by increasing central vagal activity. Hyperglycaemia inhibits PP release by decreasing central vagal activity. The blood glucose level has no direct effect on the PP cell<sup>107</sup>. Since vagotomy leaves the PP cell unresponsive to changes in blood glucose, cholinergic pathways seem to play a key role<sup>92,98,108-110</sup>. PP release after insulin induced hypoglycaemia can therefore be used in the assessment of vagal nerve integrity.

Electrical stimulation of the vagus nerves causes a rapid and considerable release of PP both in pigs and calves<sup>47,111</sup>. This response is inhibited by atropine and abolished by hexamethonium, suggesting the involvement of nicotinic and muscarinic receptors<sup>102,103</sup>. Electrical stimulation of the vagus nerve is not used in clinical practice.

## Cardiovascular autonomic function tests

The development of simple tests using cardiovascular reflexes has allowed a more precise approach of autonomic nerve function. These tests reflect both, symptoms<sup>112-114</sup> and prognosis<sup>104</sup> and it can be assumed that cardiovascular reflex abnormalities indicate diffuse damage throughout the autonomic nervous system<sup>78,115-117</sup>. Recently an automated programme has been developed, using a Finapres (FINger Arterial PRESsure) device and personal computer, to perform a battery of five cardiovascular reflex tests<sup>118</sup>. With the use of this programme, cardiovascular autonomic dysfunction can be detected in a sim-

ple and quick way. The tests are based on heart rate and blood pressure variability (both under vagal control) during forced breathing, standing up, the Valsalva manoeuvre and sustained handgrip. The cardiovascular reflex tests used in this thesis are also performed by this automated method. With a Finapres device, heart rate (beats/min) and blood pressure (mmHg) are continuously recorded from a finger. The principle of this instrument is based on servoplethysmomanometry, employing the volume clamp technique<sup>119,120</sup>. The digital signal of the Finapres is recorded by a personal computer, by which means a computer programme could be developed to calculate the test results automatically, immediately after the tests have been performed. The Finapres cuff (Finapres model 5 TNO Amsterdam) is wrapped around the middle finger of the non-dominant hand, which is fixed exactly at heart rate level. Before starting the tests the manoeuvres are trained to perform them correctly, according to the timeclock of the computer programme. After 5 minutes of supine rest, the subjects perform 6 consecutive maximal respirations in 1 minute. Five minutes later the subjects stand up and remain in upright position for 2 minutes. After 2 minutes rest in the sitting position the Valsalva manoeuvre is performed three times, each after 1 minute rest. Finally the patients are asked to exert 30% of their previously determined maximum voluntary contraction for 3 minutes on a handgrip dynameter, 2 minutes after the last Valsalva manoeuvre.

During deep breathing at 6 breaths/min, the mean difference between the highest heart rate during inspiration and the lowest during expiration for six consecutive breathing cycles is calculated<sup>118,121</sup>. During the standing up test the programme calculates the difference between the maximum heart rate after the manoeuvre and the control heart rate before and also the quotient of maximum heart rate and minimum heart rate after standing up<sup>113</sup>. Furthermore the difference in averaged diastolic blood pressure between 50 and 80 seconds in the standing position and during the control period is calculated<sup>122</sup>. The highest Valsalva ratio of the 3 manoeuvres performed, defined by the maximum heart rate divided by the lowest heart rate after the manoeuvre, is used to evaluate the heart rate variability during this test<sup>123</sup>. During sustained handgrip the highest increase in average diastolic blood pressure over 5 seconds is considered. The test results are defined as abnormal if the testparameter is below the 5<sup>th</sup> percentiles of normal<sup>123</sup>. Autonomic dysfunction is defined as an abnormal score of 2 or more of the 5 tests<sup>118</sup>.

## Aims and outline of investigations

The purpose of the studies presented in this thesis is to contribute to the knowledge of the physiology and pathophysiology of PP release or gallbladder contraction after stimulation of the vagal-cholinergic system by several stimuli in healthy subjects and patients with disorders that may involve the autonomic nerve system.

In chapter 2 and 3 we investigated to what part chemoreceptors are involved in the induction of vagal activity during MSF. We therefore studied the relevance of the composition of the meal in MSF induced gallbladder contraction and PP release.

To determine whether long vagal-cholinergic pathways or the gastric antrum are involved in ERY induced PP release, we studied in chapter 4 the effect of an intravenously administered bolus of ERY in vagotomized patients with or without antrectomy.

Chapter 5 through 7 deal with ERY or insulin hypoglycaemia stimulated PP release in different patient groups. In chapter 5 and 6, ERY was administered in different dosages to determine if ERY stimulates PP release in a dose dependent way. Insulin hypoglycaemia is the most potent stimulus for vagal-cholinergic PP secretion. As a test, it can be used to determine vagal nerve integrity. Since this stimulation test is time-consuming and accompanied by transient side-effects, we studied whether ERY stimulated PP release could replace the insulin hypoglycaemia test. This is especially important



for patients with diabetes mellitus because it is difficult, time-consuming and hazardous to induce an acute standardized hypoglycaemia in these patients. Furthermore, we investigated the possible clinical practical value of the different tests. The results of ERY and insulin hypoglycaemia stimulated PP release are compared with the cardiovascular reflex test results with respect to gastrointestinal symptoms.

Finally, in chapter 8 the results of the various studies reported in this thesis are discussed and summarized in the light of the questions posed in this chapter.

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# Gallbladder responses to modified Sham feeding: Effects of the composition of a meal

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## Summary

Changes in gallbladder contraction and plasma cholecystokinin release were studied following modified sham feeding of three different isocaloric meals rich in either fat, protein or carbohydrates in healthy volunteers, and results were compared with those following real feeding of comparable meals. In contrast to carbohydrate rich meals ( $8 \pm 19 \text{ ml} \cdot 120 \text{ min}$ ), fat- ( $-412 \pm 46 \text{ ml} \cdot 120 \text{ min}$ ) and protein rich meals ( $-352 \pm 42 \text{ ml} \cdot 120 \text{ min}$ ) reduced integrated gallbladder volume ( $p < 0.05$ ) in response to modified sham feeding. Plasma cholecystokinin levels were not significantly influenced by modified sham feeding of fat, protein or carbohydrates. Real feeding of a carbohydrate rich meal also failed to significantly reduce gallbladder volume and to stimulate cholecystokinin release ( $-45 \pm 40 \text{ ml} \cdot 120 \text{ min}$  and  $51 \pm 48 \text{ pM} \cdot 120 \text{ min}$ , respectively), while real feeding of both fat- and protein rich meals distinctly reduced gallbladder volume ( $-679 \pm 76$  and  $-564 \pm 53 \text{ ml} \cdot 120 \text{ min}$ , respectively;  $p < 0.05$ ) and increased cholecystokinin release ( $651 \pm 72$  and  $504 \pm 44 \text{ pM} \cdot 120 \text{ min}$ , respectively;  $p < 0.05$ ). This study demonstrates that gallbladder contraction during the cephalic phase of meal stimulation is dependent on the fat-, protein and carbohydrate percentages of a meal, and is activated by different mechanisms than the intestinal phase of a meal.

## Introduction

Sham feeding has been shown to stimulate gastric acid production, gallbladder contraction and pancreatic exocrine secretion in several species, including man<sup>1-6</sup>. It is well established, that the responses to modified and real sham feeding are abolished by atropine and vagotomy<sup>5-10</sup>. As a result sham feeding is thought to stimulate vagal cholinergic activity. The mechanisms by which vagal cholinergic actions are triggered by sham feeding are not completely understood. Although gustatory, olfactory, acoustic and visual signals are known to be involved in the cephalic phase of meal stimulation<sup>3,11</sup>, no data are available on the stimulating effects of the different components of a meal when sham fed. Thus, this study investigated the contribution of fat, protein and carbohydrates in the stimulation of vagal cholinergic activity on gallbladder contraction following modified sham feeding in healthy volunteers. In addition plasma CCK levels were measured during modified sham feeding and the results were compared with those from real feeding of comparable meals in the same subjects.

## Methods

Six healthy volunteers (2M, 4F; mean age  $58 \pm 10$  years) participated in the study. None was taking medication or had a history of gastrointestinal disease or surgery. The studies were approved by the ethical committee. All subjects gave informed consent. Volunteers were studied on three different days with at least one week in between. After an overnight fast, basal gallbladder volumes were measured and blood was collected to determine CCK. Meals were subsequently presented to subjects in random order. Isocaloric (1050 kJ, 250 kcal), isothermic, homogenized meals rich in either fat (walnuts; 66 g fat, 15 g protein, 7 g carbohydrate per 100 g), protein (codfish; 1 g fat, 23 g protein per 100 g) or carbohydrates (bananas; 1 g protein, 22 g carbohydrate per 100 g) were sham fed for thirty minutes by the 'chew and spit' technique<sup>12</sup>. Gallbladder volumes were determined at fifteen minute intervals twice before and for two hours after the onset of modified sham feeding. Blood samples to determine CCK were taken at the same intervals, except for the first 30 minutes following the onset of modified sham feeding, when blood was drawn at 10-minute intervals.

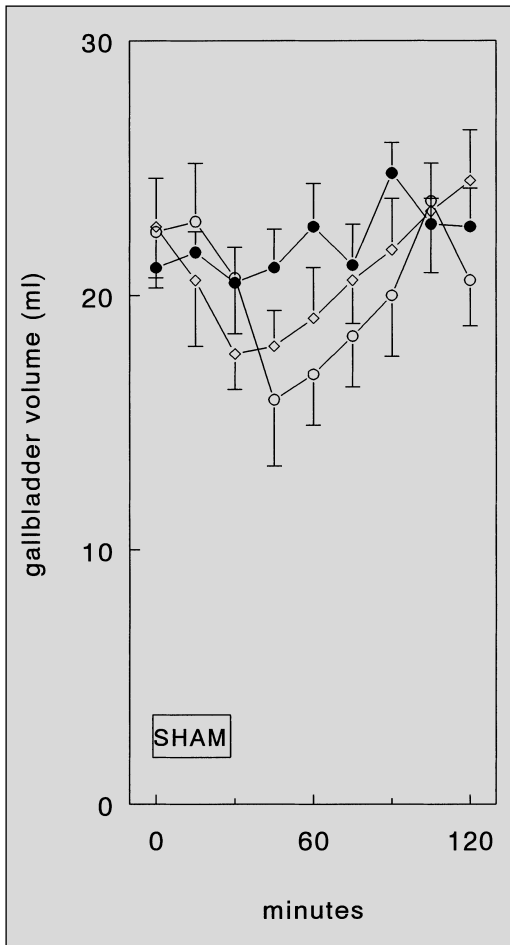


Figure 1a. Gallbladder volume-time curves for the three different meals during and after modified sham feeding in six healthy volunteers. Solid dots indicate 'carbohydrate meals', open dots indicate 'fat meals' and open diamonds indicate 'protein meals'. Rectangle, 0-30 min, indicates the sham-fed meal period. Results are expressed as mean  $\pm$  SEM.

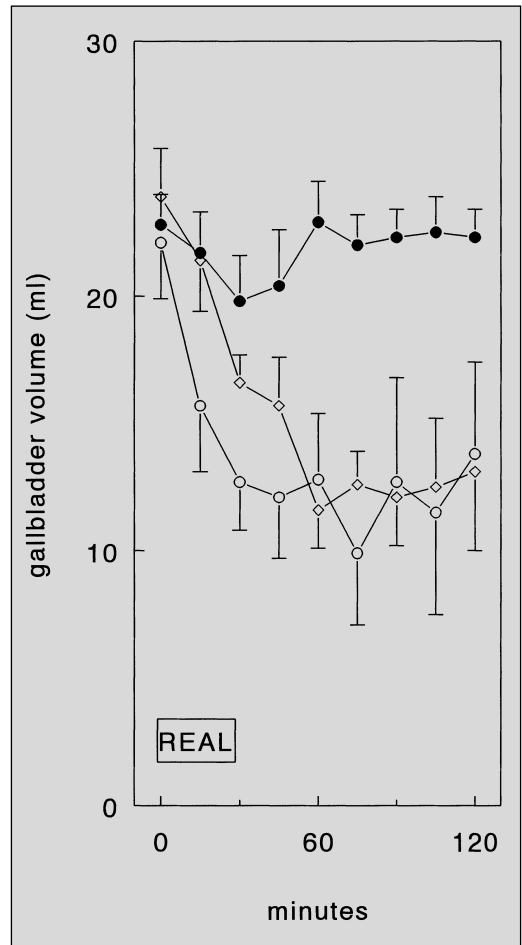


Figure 1b. Gallbladder volume-time curves for the three different meals during and after real feeding in six healthy volunteers. Solid dots indicate 'carbohydrate meals', open dots indicate 'fat meals' and open diamonds indicate 'protein meals'. Rectangle, 0-30 min, indicates the real meal period. Results are expressed as mean  $\pm$  SEM.

Two hours later, comparable meals were ingested and gallbladder volumes and plasma CCK concentrations were determined for another 2 h according to the same protocol as the modified sham feeding period. At each time interval, longitudinal and transverse echograms of the gallbladder were obtained by real-time ultrasonography as previously described<sup>13</sup>. Gallbladder volumes were calculated by computer, based on the sum of cylinders method described by Everson et al.<sup>14</sup>. Ultrasound shots were blindly interpreted by two experts who were unbiased to the type of meal or its manner of presentation. Plasma CCK was measured by a sensitive and specific radioimmunoassay<sup>15,16</sup>. Antibody T<sub>204</sub> binds to all carboxyl-terminal-specific CCK peptides. The antibody shows insignificant (<2%) binding to sulphated gastrins and does not bind to unsulphated gastrins and structurally unrelated peptides.

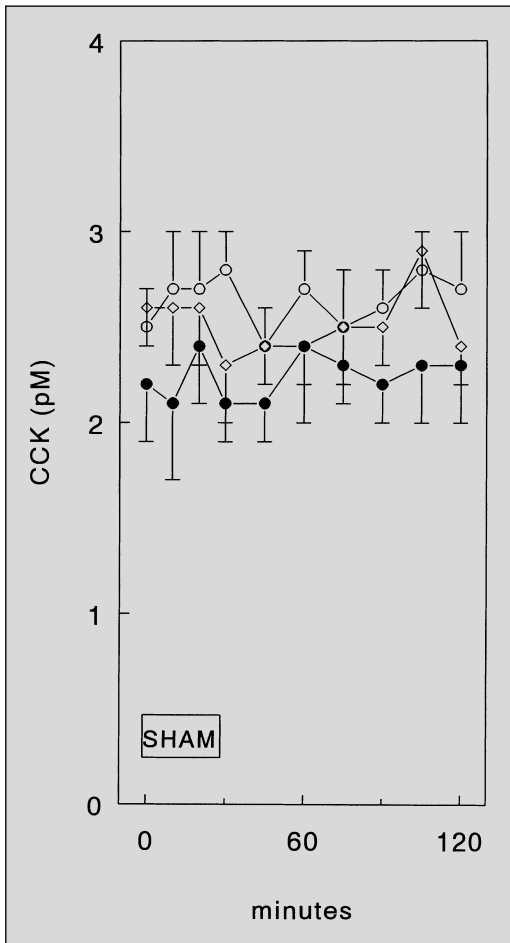


Figure 2a. Plasma cholecystikinin concentration-time curves for the three different meals during and after modified sham feeding in six healthy volunteers. Solid dots indicate 'carbohydrate meals', open dots indicate 'fat meals' and open diamonds indicate 'protein meals'. Rectangle, 0-30 min, indicates the sham-fed meal period. Results are expressed as mean  $\pm$  SEM.

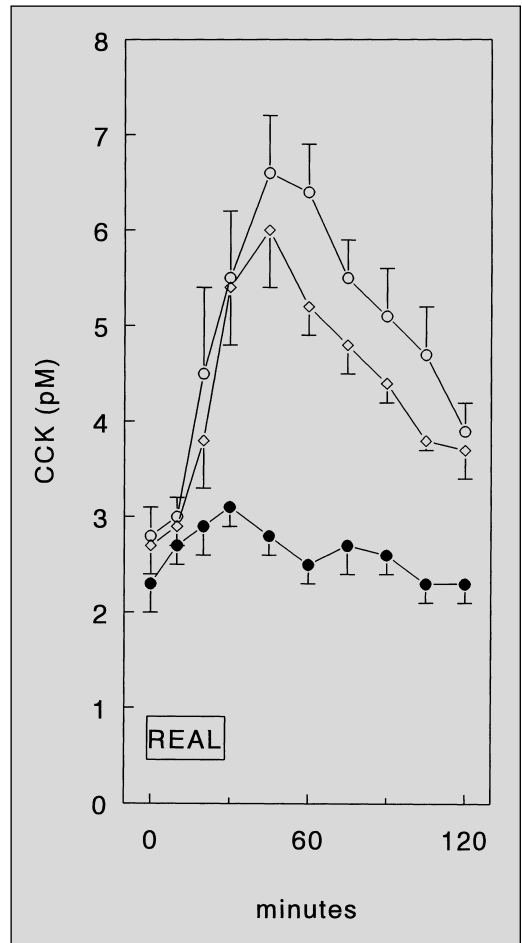


Figure 2b. Plasma cholecystikinin concentration-time curves for the three different meals during and after real feeding in six healthy volunteers. Solid dots indicate 'carbohydrate meals', open dots indicate 'fat meals' and open diamonds indicate 'protein meals'. Rectangle, 0-30 min, indicates the real meal period. Results are expressed as mean  $\pm$  SEM.

CCK<sub>33</sub> coupled to <sup>125</sup>I-hydroxy-phenylpropionic acid succinimide ester (Bolton-Hunter reagent) was used as label. Synthetic CCK<sub>33</sub> was used as standard. Separation between free and antibody-bound hormone was performed by a double-antibody technique. The detection limit of the assay was 0.5 pM. The 50%-inhibition dose was 2.1 pM. CCK was extracted from plasma by ethanol with a recovery of 85  $\pm$  2% when 2 - 10 pM of CCK<sub>33</sub> was added to hormone-free plasma and 90  $\pm$  2% when 2 - 10 pM of CCK<sub>8</sub> was added. The intra-assay variation ranged from 4.6 to 11.5% in the working range of the assay. All samples were measured in the same run.

Results are expressed as mean  $\pm$  standard error of the mean (SEM). Integrated gallbladder- and CCK-responses were determined by calculating the area under the gallbladder volume-time curve

and the plasma CCK concentration–time curve, respectively, employing the trapezoidal rule after subtraction of basal values<sup>17</sup>.

Statistical analysis was performed using ANOVA. Differences were considered significant when a two-tailed p-value was less than 0.05.

## Results

Gallbladder volume–time curves for the different meals before and after modified sham feeding or real feeding are depicted in figure 1a and 1b, respectively. Modified sham feeding of walnuts (fat) and codfish (protein) markedly reduced gallbladder volume. Integrated gallbladder volume decreased by  $-412 \pm 46 \text{ ml} \cdot 120 \text{ min}$  and  $-352 \pm 42 \text{ ml} \cdot 120 \text{ min}$ , respectively ( $p < 0.05$ ). However, modified sham feeding of bananas (carbohydrate) failed to significantly reduce gallbladder volume ( $8 \pm 19 \text{ ml} \cdot 120 \text{ min}$ ). Plasma CCK concentrations were not significantly influenced by modified sham feeding of the different meals (figure 2a). As in the modified sham feeding experiments, marked gallbladder responses were only observed after real feeding of walnuts ( $-679 \pm 76 \text{ ml} \cdot 120 \text{ min}$ ) and codfish, ( $-564 \pm 53 \text{ ml} \cdot 120 \text{ min}$ ;  $p < 0.01$ ), but not after real feeding of bananas ( $-45 \pm 40 \text{ ml} \cdot 120 \text{ min}$ ). The gallbladder response to real feeding of walnuts and codfish was more pronounced than to modified sham feeding ( $p < 0.05$ ). In contrast to modified sham feeding, real feeding of both walnuts and codfish, induced significant increases ( $P < 0.05$ ) in plasma concentrations of CCK of  $651 \pm 72 \text{ pM} \cdot 120 \text{ min}$ , and  $504 \pm 44 \text{ pM} \cdot 120 \text{ min}$ , respectively (figure 2b). Real feeding of bananas only induced a slight increase in plasma CCK concentrations ( $51 \pm 48 \text{ pM} \cdot 120 \text{ min}$ ).

## Discussion

The present study shows that gallbladder contraction in response to modified sham feeding is dependent on the composition of the meal. Fat and protein, but not carbohydrates are potent stimuli for gallbladder contraction in response to modified sham feeding. The gallbladder response to modified sham feeding shows striking qualitative similarities to the gallbladder response to real feeding of fat, protein or carbohydrate enriched meals<sup>18</sup>, suggesting activation of a comparable perceptive mechanism during cephalic and intestinal stimulation. However, the subsequent events that are responsible for the reduction in gallbladder volume after perception of the stimulus of modified sham feeding and real feeding are different. In contrast to modified sham feeding, real feeding of fat or protein significantly stimulates plasma CCK concentrations. CCK has been shown to be an important stimulus for gallbladder contraction under physiological conditions<sup>19–23</sup>. Infusion of CCK to values comparable to those obtained after ingestion of walnuts and codfish has been shown to induce similar gallbladder responses<sup>23</sup>. The reduction in gallbladder volume in response to real feeding of walnuts and codfish can therefore largely be explained by the release of CCK.

The absence of CCK responses to modified sham feeding also indicated that the volunteers were adequately sham fed and that meals were not swallowed during the modified sham feeding tests.

While the gallbladder response to modified sham feeding can be abolished by atropine and truncal vagotomy<sup>1–10</sup>, this substance and process do not fully suppress gallbladder responses or CCK release to real feeding<sup>24–26</sup>. Furthermore, a complete suppression of gallbladder contraction in response to real feeding, but not to modified sham feeding, has been observed after administration of specific CCK receptor antagonists<sup>19,27</sup>.

All these data indicate that gallbladder contraction in response to modified sham feeding is under

vagal cholinergic control, while gallbladder contraction during real feeding is largely mediated by CCK release.

How modified sham feeding of fat or protein stimulates efferent routes to the brainstem, remains to be elucidated. Mechanical, gustatory, visual, acoustic and olfactory factors may all be involved. Conditioned acoustic and visual signals were probably not involved in gallbladder emptying after sham feeding of protein or fat in the present study, since no gallbladder contraction was observed before the homogenized meals were tasted, since acoustic stimuli were comparable due to randomization and since the three meals were hardly distinguishable visually. A delay in gallbladder emptying in response to acoustic and visual stimuli overlapping the response tasting meals is also unlikely, since no gallbladder emptying was observed after sham-feeding of carbohydrates, while acoustic and visual stimuli that preceded meal tasting were comparable. Activation of mechanoreceptors by mastication<sup>11,28</sup> probably cannot explain the difference in gallbladder response between fat and protein meals on one hand and the carbohydrate meal on the other either, since all meals were homogenized. Activation of osmoreceptors or thermoreceptors is also unlikely, since all homogenized meals were isocaloric and served at room temperature. However, activation of olfactory factors by the different meals could not be excluded, since the odor of the meals could not be neutralized.

Since gallbladder responses to modified sham feeding and real feeding show similarities depending on the chemical composition of a meal, it would seem that although chemoreceptors sensitive to protein and fat but not to carbohydrates, are the major perceptive receptors in digestive tract mucosa, these receptors subsequently activate other mechanisms to obtain gallbladder responses, depending on where the stimulus is received. In the present study, olfactory and gustatory chemoreceptors activated by protein and fat but not by carbohydrates stimulated CCK-independent vagal cholinergic mechanisms via the brain stem during sham-feeding, while intestinal chemoreceptors activated by protein and fat, but not by carbohydrates stimulated the release of CCK and induced gallbladder emptying during real feeding.

In conclusion, gallbladder contraction in response to modified sham feeding appears to be dependent on the composition of the meal. The same nutrients that stimulate gallbladder contraction during the intestinal phase also stimulate gallbladder contraction during the cephalic phase of a meal, although via different mechanisms, since real feeding but not modified sham feeding stimulates the release of CCK.

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# Nutrient specific effects of modified sham feeding on pancreatic polypeptide release

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## Abstract

**Objective:** To study the effect of meal composition on pancreatic polypeptide release during modified sham feeding.

**Design:** In random order and on separate occasions, iso-caloric, iso-thermic, iso-osmotic, homogenized meals (250 kcal) either rich in fat (walnuts; 66 g fat, 7 g carbohydrate, 15 g protein per 100 g), protein (codfish; 1 g fat, 23 g protein per 100 g) or carbohydrate (bananas; 22 g carbohydrate, 1 g protein per 100 g) were sham fed for 30 minutes by tasting and spitting out the meal. The plasma pancreatic polypeptide response was monitored by radioimmunoassay at 10 minute intervals from 20 minutes before to 120 minutes after modified sham feeding.

**Setting:** Department of Gastroenterology and Hepatology of a University Hospital.

**Subjects:** Seven healthy volunteers: 3 male, 4 female; age 45 (range 30-77 yrs) were studied.

**Results:** Integrated plasma pancreatic polypeptide responses to modified sham feeding of codfish ( $1088 \pm 395$  pM\*120min;  $p < 0.05$ ) and walnuts ( $1200 \pm 542$  pM\*120min) were distinctly higher ( $p < 0.05$ ) than to modified sham feeding of bananas ( $-390 \pm 291$  pM\*120min).

**Conclusions:** These results demonstrate that the pancreatic polypeptide response to modified sham feeding is dependent on the composition of the meal.

## Introduction

Sham feeding of a meal has been shown to stimulate gastric acid production, gallbladder contraction and pancreatic exocrine secretion in different species including man<sup>1-6</sup>. Several studies have demonstrated that these responses to sham feeding are under vagal cholinergic control<sup>5-11</sup>. Although the mechanism by which sham feeding triggers vagal cholinergic activity is not completely understood, it is believed that gustatory, olfactory, acoustic, mechanic and visual signals are involved<sup>3,12-14</sup>. To what part the composition of a meal is of importance is presently not known. We therefore have studied the effect of bananas (carbohydrate), codfish (protein) and walnuts (fat) on the pancreatic polypeptide (PP) response to modified sham feeding (MSF) in healthy volunteers.

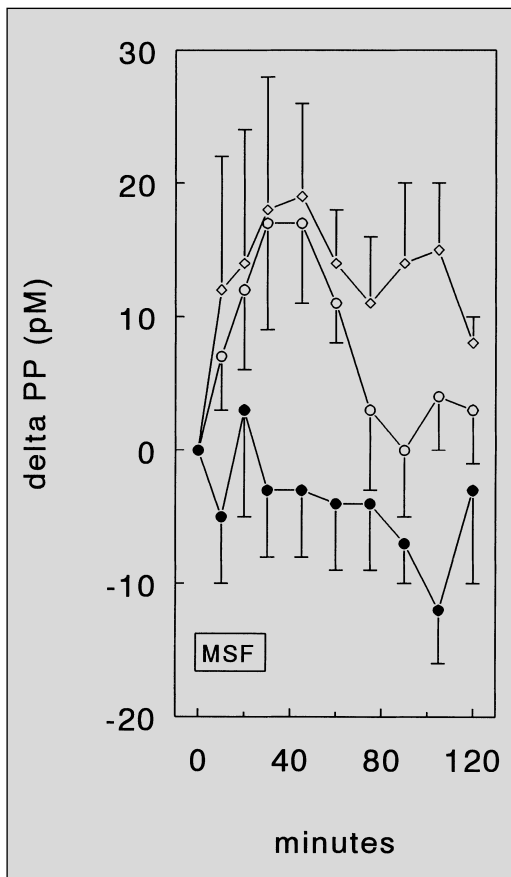
## Methods

Ten healthy volunteers (4M, 6F) with a median age of 48 years (33 to 77 years), participated. None of the volunteers used any medication, had a history of pancreatic disease or had undergone abdominal surgery that could possibly interfere with the PP response to MSF. The subjects were studied on three different days separated by at least one week. In random order, after an overnight fast iso-caloric (1050 kJ; 250 kcal), iso-thermic, iso-osmotic, homogenized meals either rich in fat (walnuts; 66 g fat, 15 g protein, 7 g carbohydrate per 100 g), protein (codfish; 1 g fat, 23 g protein, 0 g carbohydrate per 100 g) or carbohydrate (bananas; 0 g fat, 1 g protein, 22 g carbohydrate per 100 g) were sham-fed for 30 minutes by tasting and spitting out the whole meal<sup>7</sup>. The subjects were strongly encouraged not to swallow any food. At the end of each study they were asked to score appetizing and savory aspects of the different meals on a visual analogue scale (0-10; 5 being neutral). Blood samples for PP and cholecystokinin (CCK) were taken at 10 minute intervals, two times before and for two hours after the start of MSF of the different meals. In order to demonstrate that the subjects who participated in this study were able to release PP, 30 g of fat was consumed several weeks before the MSF tests, and PP responses were determined at 15 min intervals for 120 minutes. Only those subjects who showed

an increase in plasma PP levels of at least 50 pM in response to this fat meal were selected for the MSF tests.

The basal PP level was taken as the mean of two unstimulated samples. Plasma PP and CCK were measured by a sensitive and specific radioimmunoassay. Radioiodinated PP was prepared by the chloramine-T method<sup>14,15</sup>. The specific activity of <sup>125</sup>I-PP used in this study was approximately 275  $\mu$ Ci/ $\mu$ g. In the radioimmunoassay system, 1 ml of <sup>125</sup>I-PP (corresponding to 0.9 fmol of PP), 0.2 ml of anti-PP serum (final dilution 1:2000,000), 0.2 ml of standard or sample, and 0.8 ml of assay buffer, giving a total volume of 2.2 ml, were incubated for 4 days at 4°C. Human PP was used as standard preparation. All dilutions were made in 0.02 M sodium barbital buffer pH 8.4, containing 0.02% sodium azide and 0.17% human serum albumin. Separation of free and antibody-bound PP was carried out using albumin-coated charcoal. The ratio of bound to free labelled PP was inhibited by 50% at a concentration of 4.5 pM of incubation mixture. The intra-assay variation in the steep part of the standard curve ranged between 4% and 7%, while the inter-assay variation ranged between 6% and 12%. The detection limit of the assay was 0.5 pM of incubation mixture. All samples were measured in duplicate in the same run.

Integrated PP responses were determined by calculating the area under the plasma PP concentration-time curve by the trapezoidal rule after subtraction of basal values. Results are expressed as mean  $\pm$  standard error of the mean (SEM). Differences were tested for statistical significance by the analysis of variance (ANOVA) and subsequently by the least significant difference method (LSD)<sup>16</sup>. Differences were considered significant when the probability value was equal or lower than 0.05. The study protocol was approved by the local ethical committee and all subjects gave their informed consent.



## Results

Two female volunteers (both age 48) were excluded from the study because their increase in PP-release after ingestion of a 30 g fat meal never exceeded 50 pM, suggesting that PP-cells might not be intact in these subjects. After each MSF the subjects were asked if they accidentally had swallowed a part of the meal. One female volunteer (age 67) was excluded from the study because she accidentally had ingested parts of the carbohydrate and protein rich meals during sham feeding. The possibility of meal ingestion was fur-

Figure 1: Delta PP response-time curves for walnut, codfish and banana meals after modified sham feeding in seven healthy volunteers. closed dots indicate 'banana meal', open dots indicate 'walnut meal' and open diamonds indicate 'codfish meal'. Results are expressed as mean  $\pm$  SEM.

ther eliminated in the MSF experiments by failure to demonstrate any CCK release (data not shown).

Plasma PP concentration–time curves for each of the different meals in response to MSF in the remaining 7 healthy subjects are depicted in figure 1. Unstimulated plasma PP-levels at -10 and at 0 min were  $45\pm 12$  pM and  $44\pm 13$  pM for bananas,  $37\pm 12$  pM and  $33\pm 9$  pM for codfish and  $29\pm 5$  pM and  $33\pm 9$  pM for walnuts, respectively (not significant). Integrated PP-responses markedly increased after MSF of codfish (protein) and walnuts (fat) to  $1080\pm 395$  pM\*120min ( $p < 0.05$ ) and  $1200\pm 542$  pM\*120min ( $p = 0.05$ ) respectively, while MSF of bananas (carbohydrates) failed to significantly increase integrated plasma PP values ( $-390\pm 291$  pM\*120min). The PP responses to codfish and walnuts were significantly ( $p < 0.05$ ) higher than the PP responses to bananas. Most subjects enjoyed the presentation and sight of the meals. Scores were identical for the different meals (all  $> 7$ ). One subject found MSF of bananas offensive, and 2 other subjects disliked walnuts (scores  $< 5$ ). The PP responses in these series, however, were not different from the results obtained from the other volunteers.

## Discussion

The present study demonstrates that the PP response to MSF is dependent on the composition of the meal. In contrast to bananas; codfish and walnuts proved to be potent stimuli for the release of PP by MSF. Numerous studies have demonstrated that during and after consumption of a meal, PP is released in a biphasic manner: the initial phase of PP release is predominantly under cholinergic control, whereas the secondary prolonged PP response is the result of a synergistic action of hormonal stimulation and vagal activity. Of the hormones, cholecystokinin (CCK) is a major regulator of PP release<sup>1,2,17-22</sup>. Previously, we have demonstrated that consumption of fat and protein, but not carbohydrates, potently stimulates CCK release<sup>23,24</sup>. Therefore, it is likely that fat and protein activate the release of peptidergic messengers like CCK, that in turn mediate release of PP during the intestinal phase of a meal. However, CCK is not responsible for the effects of fat, protein or carbohydrates during the cephalic phase of a meal, since CCK is not released during this phase<sup>24,25</sup>. The absence of CCK release in the present study also demonstrates that meals were not swallowed accidentally, since 2 grams of consumed fat already stimulate the release of CCK<sup>26</sup>. Furthermore, CCK has been demonstrated to be entirely responsible for the release of PP during the intestinal phase of a meal<sup>27</sup>, while during the gastric phase of a meal PP is only released by gastric distention to an extent that normally cannot be achieved by ingestion of a small part of the sham fed meals<sup>11</sup>. Therefore, PP release in our experiments can only be explained by cephalic stimulation in response to sham feeding.

How MSF of protein and fat stimulates central vagal mechanisms and vagal efferent routes to the PP-cell, is presently not known. It is believed that activation of mechano-receptors by distention may play an important role in the stimulation of PP release. This is illustrated by MSF of water which does not release PP, while real sham feeding (MSF and swallowing while the meal is diverted from the oesophagus before it reaches the stomach) of a meal results in a more intense release of PP<sup>7,28</sup>. Since all meals were homogenized and different responses were obtained from carbohydrates and fat or protein, activation of mechano-receptors by mastication does not explain the differences in PP response to the different meals in the present study. Activation of osmo-receptors or thermo-receptors is also unlikely, since all meals were iso-osmotic and served at room temperature. Due to randomization of the different experiments, activation by conditioned acoustic signals that could result in anticipated PP release could not be an important factor. The visual aspects of the different meals also played a minor role because all subjects enjoyed the appetizing appearance of each of the meals equally. These results correspond with those of Feldman and Richardson<sup>3</sup> who demonstrated that the sight of a meal was of minor importance in cephalic stimulation of gastric acid secretion. Therefore, activation of gustatory

and olfactory mechanisms are the only remaining factors that play an important role in PP release during the cephalic phase of these meals. Aside from a difference in smell and taste of the meals, that may after all largely be determined by the kind of protein, fat or carbohydrates in the meals, our data suggest that PP-release is mainly the result of the amount of protein or fat in the meals. The fact is that a similar pattern of PP release is seen in response to the intestinal phase of protein, fat or carbohydrate meals<sup>29</sup>, which suggests that the amounts of fat, protein and carbohydrates in a meal play a more important role than its smell or taste. We therefore suggest that the same type of (chemo)receptors are triggered by nutrients throughout the intestine, but that these (chemo)receptors subsequently activate endocrine, paracrine or neurocrine mechanisms depending on their location in the intestinal tract.

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# **Effect of erythromycin on pancreatic polypeptide release: Role of vagal nerve**

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## Abstract

To determine whether long vagal cholinergic pathways are involved in erythromycin induced pancreatic polypeptide release, erythromycin was administered as an intravenous bolus injection to 9 healthy volunteers (group A) and to 13 patients (group B) with impaired vagal function as a result of truncal vagotomy or accidental vagotomy after anti-reflux surgery. In 7 of these patients (group B<sub>1</sub>) an antrectomy was also performed, while in the other 6 patients (group B<sub>2</sub>) the antrum was not removed. Pancreatic polypeptide was measured by radioimmunoassay at 5 minute intervals twice before and at 2, 5, 10, 15, 30, 45 and 60 minutes after a 3.5 mg/kg injection of erythromycin. On another day, a standard meal was administered and plasma pancreatic polypeptide was measured at 10 minute intervals for 1 h. Erythromycin injection resulted in a lower integrated pancreatic polypeptide response in the patients of group B<sub>1</sub> ( $247 \pm 89$  pM\*15min;  $p=0.005$ ) and group B<sub>2</sub> ( $497 \pm 111$  pM\*15min;  $p=0.05$ ) when compared to the healthy subjects of group A ( $1136 \pm 227$  pM\*15min). The pancreatic polypeptide response to erythromycin in group B<sub>1</sub> was reduced when compared to group B<sub>2</sub> but the difference just failed to reach statistical significance ( $0.05 < p < 0.1$ ). In the first 30 minutes after ingestion of a meal (cephalic phase) pancreatic polypeptide release was also markedly lower in group B<sub>1</sub> ( $1461 \pm 304$  pM\*30min;  $p < 0.005$ ) and group B<sub>2</sub> ( $1452 \pm 215$  pM\*30min;  $p < 0.005$ ) when compared to group A ( $3541 \pm 452$  pM\*30min). However, in the second 30 minutes after ingestion of a meal (intestinal phase), no significant difference in pancreatic polypeptide response was observed between group A, B<sub>1</sub> and group B<sub>2</sub> ( $3689 \pm 690$  pM\*30min,  $3319 \pm 938$  pM\*30min and  $3119 \pm 761$  pM\*30min, respectively). These last results demonstrate that pancreatic polypeptide cells were in fact functional in all patients and controls. We conclude that the effect of erythromycin on pancreatic polypeptide release is at least in part dependent on intact long vagal cholinergic pathways. Furthermore, preservation of the antrum seems to be of importance for erythromycin induced pancreatic polypeptide release.

## Introduction

Erythromycin (ERY) has been demonstrated to exert stimulatory effects on gastroduodenal, colonic and gallbladder motility<sup>1-10</sup>. In vitro, the effects of ERY on isolated smooth muscle strips were not inhibited by atropine, hexamethonium, naloxone, diphenhydramine, methysergide, procaine, trypsin, indomethacin or sodium nitroprusside but were blocked by nifedipine, indicating a direct calcium-dependent effect of ERY. This is probably the result of binding of ERY to motilin receptors, which are abundantly present in the gastric antrum<sup>2,11</sup>. In vivo, however, the effect of ERY on gastrointestinal motility is blocked by atropine suggesting that cholinergic pathways play an important role in this action<sup>12-14</sup>. The release of pancreatic polypeptide (PP) is largely controlled by the cholinergic system and cholecystokinin (CCK)<sup>15</sup>. Recently, it has been shown that intravenously administered ERY also stimulates the secretion of PP<sup>6</sup>. The effects of ERY on PP-release can be blocked by atropine but not by the specific CCK receptor antagonist loxiglumide indicating that cholinergic pathways play a major role<sup>6</sup>. It is not known whether the stimulation of PP-cells by ERY is mediated either centrally via the vagal nerve or locally by gastro/entero-pancreatic cholinergic reflexes. The present study was performed to determine the role of long vagal and local gastropancreatic reflexes in ERY induced PP-release.

## Subjects and methods

Nine healthy subjects (group A; 3 F, 6 M; age  $49 \pm 12$  years) and 13 patients (group B; 1F, 12 M; age  $53 \pm 8$  years) with impaired vagal nerve function as a result of intentional ( $n=6$ ) or accidental vagotomy after anti-reflux surgery as demonstrated by a very low PP release (PP peak increment  $< 50$  pM) in response to insulin hypoglycaemia<sup>15</sup> participated in the study. The patients and healthy subjects were without any medication for 7 days prior to the tests. No pancreatic disease, renal failure, diabetes mellitus or liver dysfunction was demonstrable in the patients. In 7 patients the antrum had also been removed (group B<sub>1</sub>; 1 F, 6 M;  $55 \pm 8$  years). In two of these patients a Billroth I anastomosis was constructed while in the remaining 5 a Billroth II reconstruction was performed. All patients of group B<sub>2</sub> (6 M,  $51 \pm 7$  years) had an intact antrum. In 2 patients of group B<sub>2</sub> a highly selective vagotomy was performed, and in 2 other patients a pyloroplasty. Patients were studied at least 6 months after surgery.

After an overnight fast, blood samples for PP were taken at 5 minute intervals twice before and at 2, 5, 10, 15, 30, 45 and 60 minutes after an intravenous bolus injection of 3.5 mg/kg ERY (erythrocin<sup>®</sup> I.V., Abbott). On another day a standard meal consisting of 2 slices of buttered bread with 50 g of cheese, a boiled egg and 200 cc of tea with 10 g of sugar, was consumed over a 20 minute period and plasma PP values were monitored for one hour at 10 minute intervals. Plasma PP was measured by a sensitive and specific radioimmunoassay<sup>16</sup>. The specific activity of <sup>125</sup>I-PP used in the assay was 275  $\mu$ Ci/ $\mu$ g. In the radioimmunoassay system, 1 ml of <sup>125</sup>I-PP (1800 cpm corresponding to 0.9 fmol of PP), 0.2 ml of anti-PP serum (final dilution 1:2000,000), 0.2 ml of standard or sample, and 0.8 ml of barbital (veronal<sup>®</sup>) buffer containing 0.02% sodium azide and 0.17% human serum albumin, pH 8.4, were incubated for 4 days at 4°C. Human pancreatic polypeptide was used as standard preparation. Separation of free and antibody-bound PP was carried out using albumin-coated charcoal. The ratio of bound to free labelled PP was inhibited by 50% at a concentration of 4.5 pM of incubation mixture. The intraassay variation in the steep part of the standard curve ranged between 4% and 7%, and the interassay variation ranged between 6% and 12%. The detection limit of the assay was 0.5 pM of incubation mixture.

The mean of 2 unstimulated samples was taken as the basal PP level. The integrated incremental PP values were determined by calculating the area under the individual plasma concentration-time curves after subtraction of the mean basal value. Statistical analysis was performed a two sample t-test. Values are expressed as mean  $\pm$  SEM. The study protocol was approved by the local ethical committee and all subjects gave their informed consent.

## Results

PP-release in response to insulin hypoglycaemia in healthy subjects (group A) and in patients with truncal or accidental vagotomy is shown in figure 1. Delta PP-concentration-time curves for groups A, B<sub>1</sub> and B<sub>2</sub> after stimulation with ERY are depicted in figure 2, while figure 3 illustrates the results after a meal. Stimulation with ERY resulted in a distinctly lower ( $p < 0.001$ ) integrated PP response in the patients of group B ( $362 \pm 76$  pM\*15min) when compared to the healthy subjects of group A ( $1136 \pm 227$  pM\*15min). Integrated PP values after injection of ERY just failed to reach a statistically significant difference between vagotomized patients with (group B<sub>1</sub>:  $247 \pm 89$  pM\*15min) or without (group B<sub>2</sub>:  $497 \pm 111$  pM\*15min) antrectomy ( $0.05 < p < 0.1$ ). Integrated PP-values in group B<sub>1</sub> and group B<sub>2</sub> were significantly lower than those in group A ( $p=0.005$  and  $p=0.05$ , respectively). During the cephalic phase of the standard meal (0-30 min), integrated PP release was also markedly lower

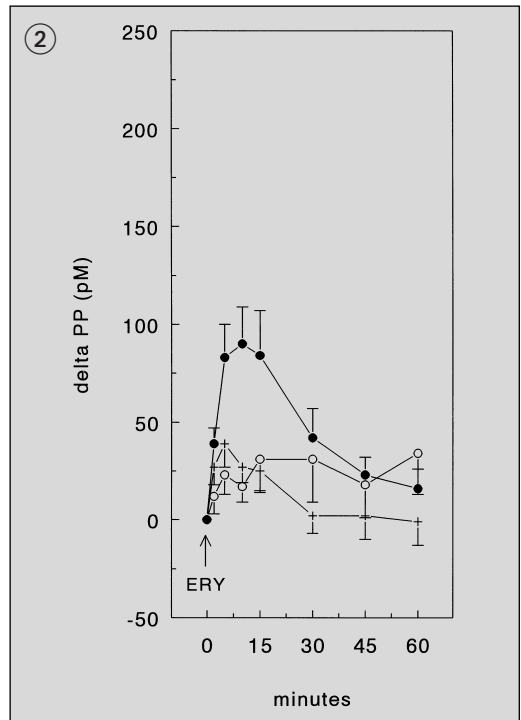
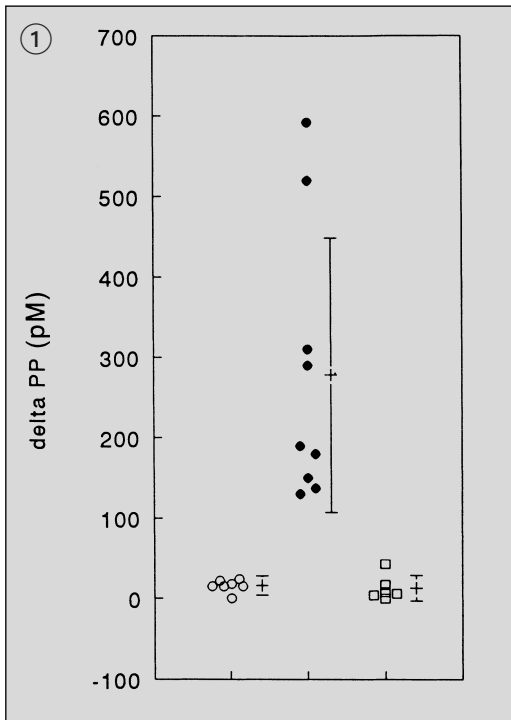


Figure 1: PP responses to insulin (0.1 E/kg of Actrapid) induced hypoglycaemia in the 7 patients with accidental vagotomy due to antireflux surgery (open circles;  $16 \pm 8$  pM), in 6 patients with truncal vagotomy (open squares;  $13 \pm 16$  pM) and in 9 healthy subjects (closed dots;  $278 \pm 171$  pM). Results are expressed as mean  $\pm$  SD.

Figure 2: Delta PP-time curves after stimulation with an intravenously administered bolus injection of ERY (3.5mg/kg) in 9 healthy volunteers (group A; closed dots), 7 vagotomized patients with (group B<sub>1</sub>; open dots) and 6 vagotomized patients without (group B<sub>2</sub>; crosses) antrectomy. Results are expressed as mean  $\pm$  SEM.

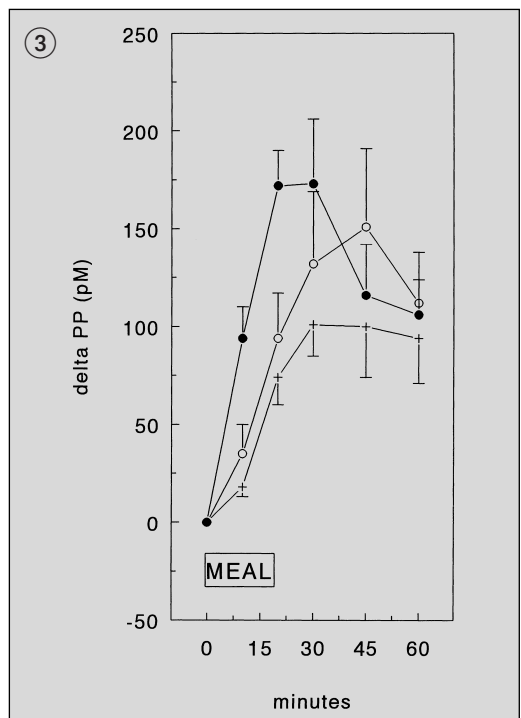


Figure 3: Delta PP-time curves after ingestion of a meal in 9 healthy volunteers (group A; closed dots), 7 vagotomized patients with (group B<sub>1</sub>; open dots) and 6 vagotomized patients without (group B<sub>2</sub>; crosses) antrectomy. Results are expressed as mean  $\pm$  SEM



( $p < 0.001$ ) in group B ( $1457 \pm 184 \text{ pM} \cdot 30\text{min}$ ) when compared to group A ( $3541 \pm 452 \text{ pM} \cdot 30\text{min}$ ). During the intestinal phase of the meal (30–60 min) no statistically significant differences in integrated PP responses were observed between groups A and B ( $3689 \pm 690 \text{ pM} \cdot 30\text{min}$  and  $3227 \pm 591 \text{ pM} \cdot 30\text{min}$ , respectively). Integrated meal-stimulated PP responses in groups B<sub>1</sub> and B<sub>2</sub> did not significantly differ from each other in the cephalic phase ( $1461 \pm 304 \text{ pM} \cdot 30\text{min}$  vs  $1452 \pm 215 \text{ pM} \cdot 30\text{min}$ ), and in the intestinal phase ( $3319 \pm 938 \text{ pM} \cdot 30\text{min}$  vs  $3119 \pm 761 \text{ pM} \cdot 30\text{min}$ ).

## Discussion

The present study demonstrates that ERY stimulates the release of PP into the systemic circulation, and that this release is strongly dependent on long vagal nerves innervating an intact antrum. In addition the present study confirms that PP-release during the cephalic phase of a meal is impaired in patients with vagal nerve damage<sup>15</sup>. The finding that PP release during the intestinal phase of a meal did not significantly differ between patients and healthy subjects indicates that the decreased PP response to the cephalic phase of a meal and to ERY administration in patients with truncal or accidental vagotomy was not the result of incapability of PP-cells to produce PP.

The mechanism by which ERY stimulates PP-release remains speculative. Theoretically ERY may stimulate PP-release by direct effects on PP-producing cells in the pancreas or by indirect effects mediated by endocrine, paracrine or neurocrine mechanisms. A cholinergic dependent neural mechanism seems quite probable, since PP release in response to ERY can be blocked by atropine<sup>6</sup>. Thus, activation of local gastroduodenopancreatic cholinergic mechanisms, peripheral activation of long vago-vagal mechanisms, or direct stimulation of vagal cholinergic mechanisms in the central nervous system may be responsible for PP release after injection of ERY.

It has previously been demonstrated that ERY binds to motilin receptors, which are abundantly present in the gastric antrum<sup>2,11,21</sup>. Binding of Leu<sup>13</sup>-motilin to motilin receptors on smooth muscle cells has also been demonstrated to stimulate the release of acetylcholine from enteric nerves<sup>17</sup>. Therefore, binding of ERY to motilin receptors in the antrum and subsequent activation of local gastrop pancreatic cholinergic pathways may at least in part account for the small amounts of PP released by ERY injection in patients with intentional or accidental vagotomy and an intact stomach. In antrectomized patients, the number of motilin receptors is consequently diminished, resulting in a further reduction of PP release to ERY injection, as shown in the present study. Activation of long vagal pathways, however, remains the most important mechanism to explain PP release by ERY infusion, since in comparison with control subjects, the majority of PP release in response to ERY is suppressed in vagotomized patients. However, it is not clear whether ERY activates central or peripheral mechanisms that are dependent on intact long vagal pathways. By stimulating peripherally located vagal sensory endings or motilin receptors in the upper gastrointestinal tract, ERY may induce PP release through vago-vagal nerves switching in the brain stem. This is supported by recent findings in rats and ferrets, where it has been demonstrated that such digestive vagal sensory endings exhibit chemosensitivity<sup>18-20</sup>.

Motilin receptors are not only present peripherally in the stomach, but also in the central nervous system, where motilin immunoreactivity has also been demonstrated<sup>22</sup>. Therefore, ERY may bind to motilin receptors in the central nervous system to stimulate PP release through efferent vagal cholinergic pathways. However, ERY does not pass readily into the spinal fluid. Even at high serum levels (40 ug/ml) after intravenously administration of comparable doses of ERY (300 mg) as used in the present study, spinal fluid levels remained low (0.04 ug/ml)<sup>23</sup>. Since we have not measured serum or liquor concentrations of ERY and since we do not know the threshold concentration of ERY that ac-

tivates PP-release, we are not able to answer the question whether ERY activates PP-release either by central or peripheral mediated vagal cholinergic mechanisms.

Because differences in the PP response to 3.5 mg/kg of ERY were highly significant between vagotomized patients and controls, we have not tested lower doses of ERY to investigate whether the small release of PP in vagotomized patients disappears by administration of lower doses of ERY. In addition, we cannot exclude with certainty that the small amounts of PP released by ERY, or during the cephalic phase of the meal, in vagotomised patients are explained by other mechanisms, for instance, by enhancement of gastric emptying, which is delayed after vagotomy. By accelerating gastric emptying in vagotomized patients, ERY infusion or cephalic stimulation may result in delivery of gastric juice or meal residues to the duodenum and subsequently in the release of PP by intestinal distention by the juice or by the intestinal phase of a meal<sup>15</sup>, despite the fact that patients restrained from eating for 15 h prior to the study.

Whatever the mechanism of ERY induced PP release may be, long vagal cholinergic pathways seem to be involved. ERY-induced PP release may therefore be helpful in detecting long vagal cholinergic damage, for instance, after anti-reflux surgery or in diabetics with autonomic neuropathy. Since the insulin hypoglycaemia test and the sham-feeding test that are currently used to demonstrate vagal nerve damage<sup>15</sup>, are not without risks and often difficult to achieve especially in diabetics, ERY infusion may replace these tests to demonstrate long vagal nerve damage as a consequence of autonomic neuropathy.

In conclusion, the present study demonstrates that PP release in response to intravenous administration of ERY is to a large extent dependent on intact long vagal pathways. The diminished PP response to ERY in vagotomized patients is further reduced by antrectomy, suggesting that ERY activates long vago-vagal and to a lesser extent local gastropancreatic cholinergic reflexes. We speculate that ERY stimulates vagal sensory endings in the antrum by binding to motilin receptors.

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# Impaired pancreatic polypeptide response to erythromycin in patients with chronic liver disease

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## Abstract

Autonomic neuropathy of the gastrointestinal tract may alter vagal nerve integrity and frequently complicates the course of chronic liver disease. Because impaired PP secretion may reflect dysfunction of the autonomic nerve system of the gastrointestinal tract, the aim of this study was to compare plasma PP responses to intravenous injection of erythromycin and to modified sham feeding in 11 patients with cirrhosis of the liver (Group A) classified as Child A (n=8) or Child B (n=3) and 20 controls (Group B). In addition cardiovascular reflex tests (finapres) were performed in all patients with chronic liver disease.

Integrated plasma pancreatic polypeptide levels after stimulation with 0.4 and 1.2 mg/kg.15min of erythromycin were decreased in group A compared to group B (509 [121-2290] vs 1021 [326-2964] pM\*20min (p<0.1), and 776 [29-2670] vs 1228 [514-7608] pM\*20min (p<0.05), respectively). After modified sham feeding, integrated pancreatic polypeptide responses in group A (923 [65-4505] pM\*60min) tended to be lower than those in group B (1580 [473-5115] pM\*60min; n=8) but the results did not reach statistical significance.

It is concluded that the pancreatic polypeptide response to erythromycin but not to modified sham feeding is significantly decreased in patients with chronic liver disease classified as Child A or B.

## Introduction

Autonomic neuropathy (AN) frequently complicates the course of the disease in patients with diabetes mellitus, cerebrovascular diseases, spinal cord lesions, Parkinson's disease and Shy Drager syndrome. AN has also been described in chronic liver disease, where its presence may identify a subgroup of patients with a substantial dismal outlook<sup>1,2</sup>. Cardiovascular reflex tests, based on heart rate and blood pressure variability induced by forced breathing, standing up, the Valsalva manoeuvre and sustained handgrip are most commonly used to detect AN<sup>3</sup>. It is generally accepted that adequate assessment of autonomic nerve function should be based on a series of distinct tests rather than on a single investigation concerning one reflex system. The automated computerized method using "Finapres" for measuring cardiovascular reflexes has proven to be very useful for the detection of cardiovascular AN<sup>4</sup>. Whether the results obtained with this method may also predict AN of the gastrointestinal tract, remains to be established.

At the moment, several tests have been described to evaluate vagal nerve integrity of the gastrointestinal tract. The release of pancreatic polypeptide (PP) plays a central role in most of these tests because the secretion of PP is unique in the way that cholinergic, vagal stimulation is not only the most powerful stimulus, but also the key through which other mechanisms act<sup>5</sup>. Impaired PP secretion may therefore be used, as a sensitive indicator of AN of the gastrointestinal system. PP release by stimulation of cholinergic pathways can be achieved e.g. by insulin induced hypoglycaemia, modified sham feeding (MSF) or electrical stimulation of the vagal nerve<sup>5</sup>. It has been shown that intravenous injection of erythromycin (ERY), also stimulates the secretion of PP<sup>6,7</sup>. The effects of ERY on PP release can be blocked by atropine, but not by the specific cholecystokinin receptor antagonist loxiglumide, indicating the involvement of cholinergic pathways<sup>6</sup>. In a previous study we have demonstrated that ERY stimulated PP release is dependent on intact vagal-cholinergic pathways<sup>7</sup>.

Because impaired PP secretion may reflect dysfunction of the autonomic nerve system of the gastrointestinal tract, the aim of this study was to compare plasma PP responses to intravenous injection of erythromycin and to modified sham feeding between patients with cirrhosis of the liver and control subjects. In addition cardiovascular reflex tests (finapres) were performed in patients with chronic liver disease.



## Subjects and methods

The PP-response to stimulation with ERY was determined in 11 patients with cirrhosis of the liver (group A; 4F, 7M; median age 46 [26-65] years) and in 20 controls with abdominal complaints (group B; 9F, 11M; median age 45 [21-71] years). Median Quetelet-indexes of groups A and B were 24.6 [16.5-32.9] and 24.3 [16.9-42.8], respectively. Only patients with histologically proven cirrhosis of the liver or patients with biochemical and clinical evidence of chronic liver disease with oesophageal varices and/or splenomegaly, participated in the study. One patient had primary biliary cirrhosis, 1 had cirrhosis as a result of primary sclerosing cholangitis, 3 had alcoholic liver cirrhosis, 2 had autoimmune hepatitis with portal hypertension and 4 patients had cryptogenic cirrhosis of the liver. Three of the patients were classified as Child B (1F, 2M; age 66, 58, 26 year, respectively), while all other patients were classified as Child A. Six patients had been treated with sclerotherapy for variceal bleeding. The period between the last sclerotherapy treatment and the present study was at least 3 months. These patients were also included since it has been demonstrated that sclerotherapy does not affect vagal nerve integrity on the long term<sup>8</sup>. The control subjects were all on treatment in the outpatient clinic for mild abdominal complaints. Eight of these controls met the criteria of irritable bowel syndrome, 4 had proctitis and 3 had Crohn's disease (all in remission), 4 had non ulcer dyspepsia and one had colonic polyps. Patients with diabetes mellitus, recent alcohol abuse (3 months), previous upper abdominal surgery, neurological disease, pancreatic insufficiency, cardiovascular disease or subjects on medication that could possibly influence test results and that could not be stopped at least 3 days before the tests, were excluded from the study. All patients of group A but only 9 patients of group B gave informed consent to the MSF study.

After an overnight fast, blood samples for PP were taken at -5 min, immediately before and at 5, 10, 15, 20, 30, and 60 min after intravenous injection of 0.4 mg/kg.15min and 1.2 mg/kg.15min of ERY (Erythrocin<sup>®</sup>, Abbott Laboratories, Berks, England). ERY was injected over a 15 minute period. The interval between the ERY tests was at least 1 hour. One hour after the last ERY test, a standard meal consisting of 2 slices of buttered bread with 50 g of cheese, a boiled egg and 200 ml of tea with 10 g of sugar, was sham fed over a 20 min period by the chew and spit technique. During the MSF study subjects were encouraged not to swallow any of food. For further surveillance CCK was measured at regular intervals to monitor inadvertent swallowing of food. Two times before, during and after MSF, plasma PP and plasma CCK values were monitored for 1 hour at 10 min intervals. One hour after MSF, a meal was ingested over 20 minutes to investigate if PP cells were capable to produce and release PP. PP responses were determined subsequently at 10 min intervals for 40 minutes. Plasma PP and CCK were measured by sensitive and specific radioimmunoassays as previously described<sup>9,10</sup>.

Cardiovascular reflex tests were monitored by an automated computerized method using a Finapres device (FINger Arterial PRESsure; Finapres model 5; TNO, Amsterdam, The Netherlands)<sup>4</sup>. With this device, heart rate (beats/min) and blood pressure (mmHg) were continuously recorded. The principle of this instrument is based on servo-plethysmo-manometry, employing the volume clamp technique<sup>11,12</sup>. The digital signal of the Finapres was recorded using a personal computer. Subsequently, the test results were calculated automatically by a computer program, immediately after performing the manoeuvres<sup>4</sup>. The reproducibility of this program is slightly better than that of the conventional method, using a sphygmomanometer and electrocardiogram<sup>4</sup>. Normal values, corrected for age and sex, were obtained in 124 healthy volunteers. The Finapres cuff was wrapped around the middle finger of the non-dominant arm, which was fixed at heart level. Each subject was tested at the same time of the day. Before starting the tests, the manoeuvres were trained to perform them correctly, according to the time-clock of the computer program. All subjects refrained from smoking and caffeine- or alcohol-containing beverages for at least 12 hours. After 5 min supine rest, six consecutive forced

breathings were performed in one minute. The subjects stood up 5 minutes later in 2–3 seconds and remained in the upright position for 2 minutes. After 2 minutes rest in the sitting position, the Valsalva manoeuvre (expiratory pressure of 40 mmHg for 15 seconds) was performed three times, each after 1 minute rest. Finally, the subjects were asked to exert 30% of their previously determined maximum voluntary contraction for 3 minutes on a handgrip dynameter, 2 minutes after the last Valsalva manoeuvre. During forced breathing, the mean difference between the highest heart rate in inspiration and the lowest in expiration for the six breathing cycles was calculated (Inspiration-Expiration differences)<sup>13,14</sup>. During standing up, the program calculated the difference between the maximum heart rate after the manoeuvre and control heart rate (mean of 30 seconds) before (delta maximum heart rate) and also the quotient of maximum heart rate and minimum heart rate after standing up (T/B ratio). Furthermore, the difference in averaged diastolic blood pressure between 50 and 80 seconds in the standing position and during the control supine period is calculated (delta diastolic blood pressure). The highest Valsalva ratio of the three manoeuvres, defined by the lowest heart rate after the manoeuvre, was used to evaluate the heart rate variability during this test. During sustained handgrip, the highest increase in average diastolic blood pressure over 5 seconds (delta diastolic blood pressure) was calculated. The results were defined as abnormal if the test parameter was below the 5th percentile of normal. During the standing up test, heart rate variability was abnormal if delta maximal heart rate and T/B ratio were below the 5th percentile. Cardiovascular AN was defined as abnormal if 2 or more of the 5 test parameters were abnormal, because of poor predictive value of a single test parameter<sup>3,15</sup>.

The basal plasma PP level was taken as the mean of two unstimulated samples obtained with a 5 minute interval after an overnight fast of at least 12 hours. The integrated incremental PP response was determined by calculating the area under the plasma PP concentration–time curve after subtraction of the mean baseline value.

Statistical analysis was performed by analysis of covariance after logarithmic transformation with age as a covariate and by the rank sum test. Two tailed p values were calculated. Results are given as median and range, unless stated otherwise. The study was approved by the local ethics committee, and all subjects gave their informed consent.

## Results

Plasma PP concentration–time curves after intravenous stimulation with 0.4 mg/kg.15min of ERY, 1.2 mg/kg.15min of ERY or MSF are depicted in figures 1, 2 and 3, respectively. Mean baseline PP levels before stimulation with ERY, were decreased ( $p < 0.05$ ) in group A when compared to group B (27 [16–94] and 40 [25–149] pM, respectively; figure 4). The integrated plasma PP response in group A, after stimulation with 0.4 mg/kg.15min of ERY, was lower than in group B (509 [121–2290] pM\*20min vs 1021 [326–2964] pM\*20min, respectively), but this difference just failed to reach statistical significance ( $p < 0.1$ ; figure 5). However, after stimulation with 1.2 mg/kg.15min of ERY, integrated plasma PP responses in group A (776 [29–2670] pM\*20min) were significantly decreased ( $p < 0.05$ ), compared to group B (1228 [514–7608] pM\*20min)(figure 6). Analysis of covariance of PP responses with age as a covariate, revealed a significant ( $p = 0.06$ ) age effect and the differences between group A and B became even more apparent and significant for stimulation with both doses of ERY ( $p = 0.05$  for the 0.4 mg/kg.15min dose and  $p = 0.007$  for the 1.2 mg/kg.15min dose). In 5 patients of group A, the integrated PP response to 1.2 mg/kg.15min of ERY was below the lowest value in group B, but only in 2 patients of group A after 0.4 mg/kg.15min of ERY. The integrated PP response to MSF in group A was not significantly different (923 [65–4505] pM\*60min) from group B (1580 [473–5115] pM\*60min;  $p = 0.3$ ) (figure 7). In 3 patients of group A, the integrated PP response to MSF was

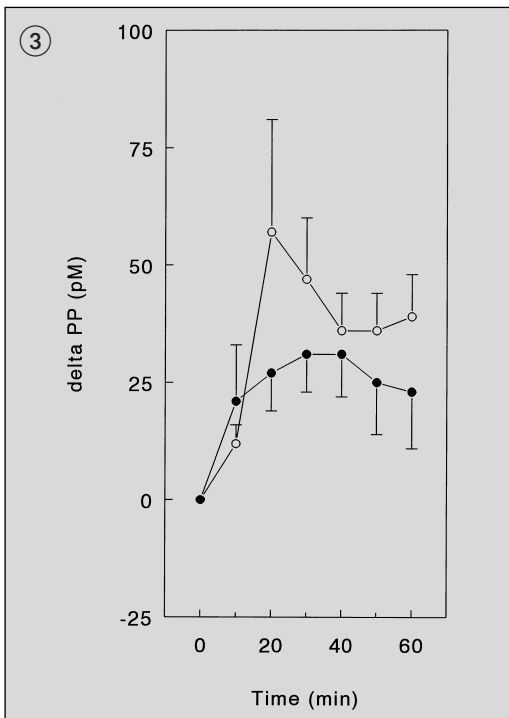
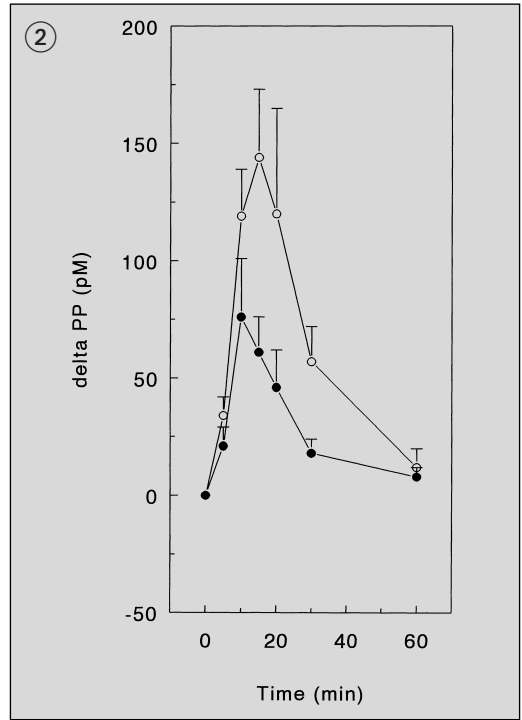
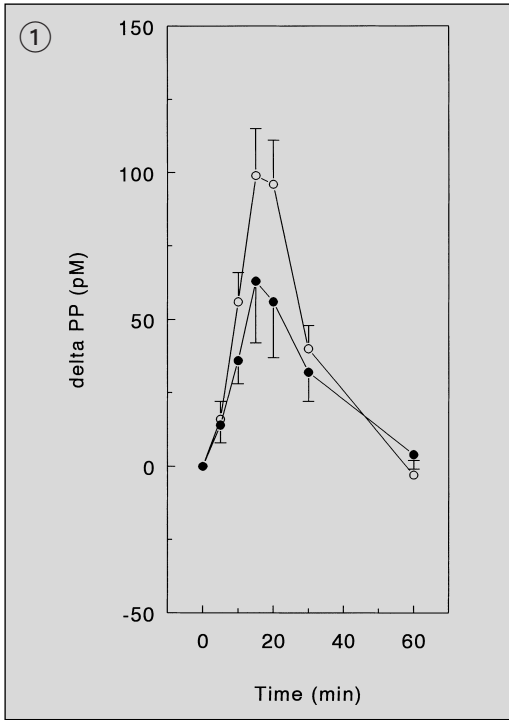


Fig 1: Delta PP time curves after stimulation with 0.4 mg/kg.15min ERY (i.v.) in 11 patients with chronic liver disease (group A; closed dots) and 20 controls (group B; open dots).

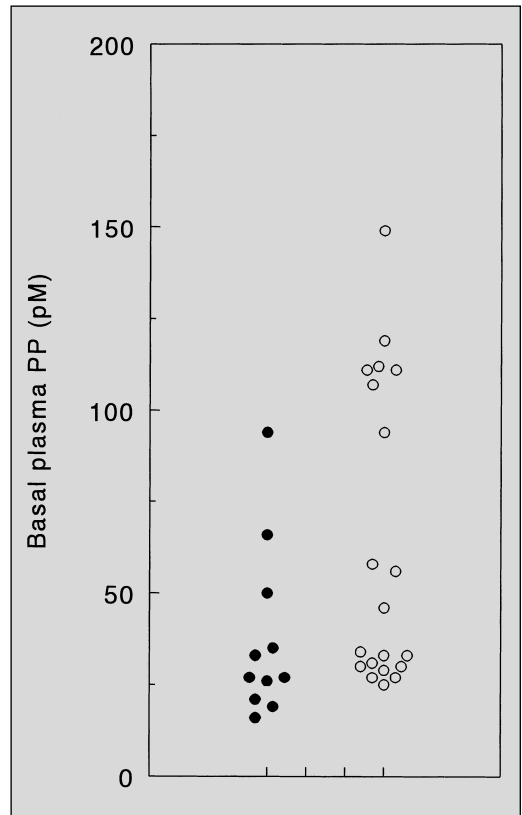
Fig 2: Delta PP time curves after stimulation with 1.2 mg/kg.15min ERY (i.v.) in 11 patients with chronic liver disease (group A; closed dots) and 20 controls (group B; open dots).

Fig 3: Delta PP time curves after stimulation with MSF in 11 patients with chronic liver disease (group A; closed dots) and 8 controls (group B; open dots).

Fig 4: Basal individual plasma PP levels in 11 patients with chronic liver disease (group A; closed dots) and 20 controls (group B; open dots).

below the lowest value of group B. In one patient of group B (F; 55 years), plasma CCK levels in response to MSF exceeded baseline levels by more than 1.3 pM suggesting swallowing of food<sup>16</sup>. For this reason she was excluded from this part of the study. In non of the other subjects of group A or B, CCK plasma levels exceeded baseline levels. Integrated plasma PP levels of the intestinal phase of the meal (30–40 min) were not significantly different between groups A and B (755 [185–2800] pM\*10min and 855 [-20–5595] pM\*10min, respectively).

Integrated PP-responses to ERY or MSF in sclerotherapy patients, were not different from non-sclerotherapy patients. Four (36%) of the patients with chronic liver disease (2 child A; 2 Child B) had an abnormal Finapres response. Integrated PP responses in the patients with cardiovascular AN were 598 [359–1463] pM\*20min, 1050 [29–2238] pM\*20min and 2149 [145–3405] pM\*60min, after stimulation with 0.4 mg/kg.15min of ERY, 1.2 mg/kg.15min of ERY and MSF, respectively. These PP responses were not significantly different from the values obtained in the patients with a normal cardiovascular reflex test (459 [121–2290] pM\*20min, 430 [170–2670] pM\*20min and 770 [65–4505] pM\*60min, respectively).



## Discussion

In the present study we have demonstrated that patients with chronic liver disease mainly classified as Child A, had a significantly lower PP response to intravenous ERY than age matched controls. Since ERY induced PP release is dependent on an intact vagal cholinergic nerve supply and since it has been demonstrated that patients with chronic liver disease may have vagal nerve damage as a consequence of AN<sup>1,2,17,18</sup>, our data may suggest that an impaired PP response to intravenous ERY reflects dysfunction of the vagal nervous system in patients with chronic liver disease. MSF as a test for the evaluation of vagal nerve integrity, did not reveal significant differences in PP response between patients with chronic liver disease and controls. Several studies have claimed that the PP response to MSF is safe and reliable to test vagal nerve integrity, because PP release to the cephalic phase of a meal is dependent on intact long vagal cholinergic pathways<sup>19,20,21,22</sup>. If performed properly, without swallowing any food, the PP response to MSF is specific but the sensitivity of the test to determine vagal cholinergic nerve integrity is rather low, because of low PP responses in normal subjects. In our MSF study one patient with a rise in plasma CCK concentration suggesting inadvertent swallowing of food was excluded

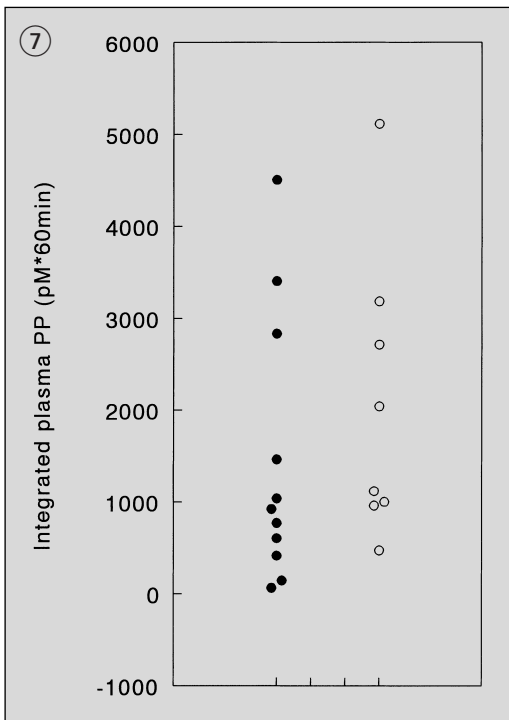
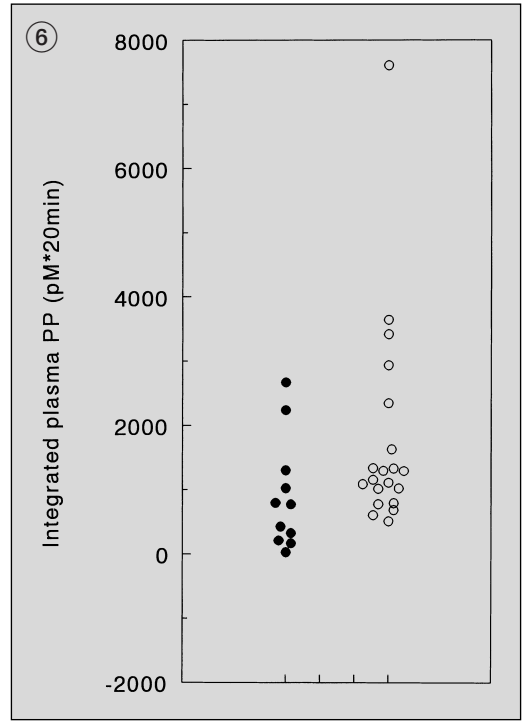
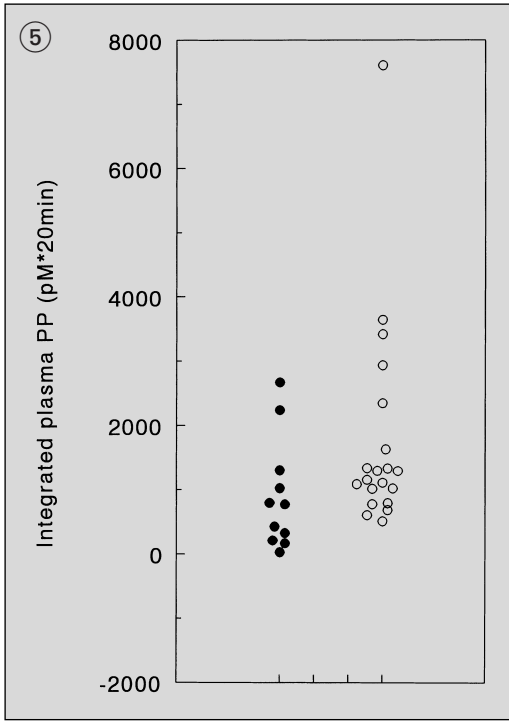


Fig 5: Integrated plasma PP responses over a 20 minute interval after stimulation with 0.4 mg/kg, 15min (i.v) in 11 patients with chronic liver disease (group A; closed dots) and 20 controls (group B; open dots).

Fig 6: Integrated plasma PP responses over a 20 minute interval after stimulation with 1.2 mg/kg, 15min (i.v) in 11 patients with chronic liver disease (group A; closed dots) and 20 controls (group B; open dots).

Fig 7: Integrated plasma PP responses over a 60 minute interval after stimulation with MSF in 11 patients with chronic liver disease (group A; closed dots) and 8 controls (group B; open dots).

from this part of the study<sup>16</sup>. The lack of differences in MSF stimulated PP release between patients with chronic liver disease and controls may on the one hand be the result of the low sensitivity of the test, but on the other hand be related to the stage of chronic liver disease in our patients, since the majority of our patients with chronic liver disease were classified as Child A. The PP response to insulin induced hypoglycaemia is probably the most appropriate test to assess vagal nerve function<sup>5,23,24</sup>. However, we did not perform this test in our patients with liver disease, since stimulation of PP release by insulin induced hypoglycaemia may be hazardous in such patients due to the risk of prolonged hypoglycaemia<sup>25</sup>.

The cardio-vascular responsiveness to standardized manoeuvres, may be diminished in patients with chronic liver disease<sup>1,2,17,18</sup>. However, even if multiple cardiovascular reflex tests are used for the evaluation of the autonomic nervous system, it is uncertain if these tests adequately assess autonomic nerve function of the gastrointestinal tract, since damage to peripheral, sympathetic, and parasympathetic nerves may occur independently of one another<sup>2</sup>.

In the present study 5 of the 11 patients with chronic liver disease had an abnormal PP response to ERY, and 4 of the 11 patients had an abnormal test result with the Finapres computer program. However, only one of the patients scored abnormal in both tests, indicating a poor relation between the PP response to ERY and the Finapres results. Therefore, it is suggested that gastrointestinal autonomic dysfunction may occur independent of cardiovascular autonomic dysfunction in patients with chronic liver disease. Vagal dysfunction of the cardiovascular system in patients with chronic liver disease identifies a subgroup of patients with a substantial dismal outlook<sup>1</sup>. The clinical implication of an abnormal PP response to ERY remains to be established.

The integrated PP release after ingestion of a meal was prominent, demonstrating that PP cells were intact and capable to release PP irrespective of AN in patients and controls. The difference in ERY stimulated PP response between groups A and B is therefore not explained by PP cell damage.

Baseline and stimulated plasma PP levels may increase progressively with age<sup>26,27</sup>. Therefore, we performed analysis of covariance on the data with age as a covariate. Correction for age resulted in even more pronounced differences between groups.

Decreased baseline PP levels have been reported in patients with obesity and autonomic neuropathy<sup>28,29</sup>. The significantly lower mean baseline PP levels in our patient group seem to be the result of AN, since no differences in Quetelet indexes were found between patients and controls. Yet, the baseline PP values were less discriminative than ERY stimulated PP values in our study, because the overlap between patients and controls was greater for baseline PP than for ERY stimulated values.

In conclusion, the present study demonstrates that integrated PP release in response to intravenous administration of ERY, is decreased in patients with chronic liver disease classified as Child A or B. The decreased PP response to ERY most probably reflects vagal-cholinergic nerve dysfunction, which may indicate AN of the gastrointestinal tract.

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# **Pancreatic polypeptide responses to erythromycin in patients with diabetes mellitus complicated by cardiovascular autonomic neuropathy**

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## Abstract

Pancreatic polypeptide release in response to intravenously administered erythromycin is dependent on intact vagal cholinergic pathways and requires an intact antrum. Since autonomic neuropathy of the gastrointestinal tract damages vagal nerve integrity and frequently complicates the course of diabetes mellitus, we have studied the pancreatic polypeptide response to erythromycin in 17 diabetics with abnormal cardiovascular reflex tests and with (n=8) or without (n=9) symptoms suspicious of autonomic neuropathy of the gastrointestinal tract. Age matched controls with atypical abdominal complaints (n=20) and patients with surgical vagal nerve damage (n=4) were also studied. The integrated pancreatic polypeptide responses to erythromycin were significantly lower in diabetics with gastrointestinal symptoms and in patients with surgical vagal damage, when compared to controls and diabetics without gastrointestinal complaints.

We conclude that in diabetics with complaints suspicious of gastrointestinal autonomic neuropathy, erythromycin stimulated pancreatic polypeptide release is decreased. It is speculated that this impaired pancreatic polypeptide response to erythromycin reflects autonomic neuropathy of the gastrointestinal tract.

## Introduction

Diabetes mellitus is frequently complicated by autonomic neuropathy, which may affect virtually all systems including the gastrointestinal tract. The late clinical manifestations of autonomic neuropathy are hypoglycaemic unawareness, spontaneous cardiac arrest and silent myocardial infarction<sup>1,2</sup>. Gastrointestinal manifestations of autonomic neuropathy are quite common and include constipation, anorexia, nausea, vomiting, early satiety, fullness, bloating, diarrhoea, anal incontinence and abdominal pain<sup>3,4</sup>.

Presently, a combination of 5 simple, non-invasive cardiovascular reflex tests are used as "golden standard" in the assessment of autonomic neuropathy<sup>5</sup>. These tests are developed to evaluate sympathetic and parasympathetic autonomic nerve function of the cardiovascular system. Autonomic neuropathy of the gastrointestinal tract can be investigated separately by testing the integrity of long vagal cholinergic pathways. This can be done by stimulating the vagal nuclei in the brain, either by insulin-induced hypoglycaemia or by modified sham feeding<sup>6,7,8</sup>. In the presence of an intact efferent vagal nerve system, these stimuli promote the release of pancreatic polypeptide (PP). It has been shown that intravenously administered erythromycin (ERY) also stimulates PP release by cholinergic pathways<sup>9</sup>. In a recent study we have demonstrated that PP release to ERY is dependent on intact vagal pathways<sup>10</sup>. It can therefore be speculated that ERY mediated PP release may be used to investigate vagal nerve integrity and to assess dysfunction of vagal cholinergic pathways as a consequence of autonomic neuropathy in patients with diabetes mellitus.

To test this hypothesis, we have studied the plasma PP response to ERY in diabetes mellitus with abnormal cardiovascular reflex tests with or without gastrointestinal symptoms suspicious of autonomic neuropathy of the gastrointestinal tract and we have compared the results of ERY stimulated PP release with those obtained in age matched controls and in patients with proven surgical vagal nerve damage.

## Patients and methods

The PP response to intravenous stimulation with 0.4 mg/kg.15 min and 1.2 mg/kg.15 min of ERY was measured in 17 diabetes mellitus patients (group A; 9F; 8M; median age 60 [30-79] yrs), in 4 patients with surgical vagal nerve damage (group B; 4M; median age 49 [28-68] yrs) and in 20 controls with abdominal complaints that fulfilled the exclusion criteria (group C; 9F; 11M; median age 45 [21-71] yrs). Eight of these controls met the criteria for irritable bowel syndrome, 4 had proctitis and 3 had Crohn's disease (all in remission), 4 had non ulcer dyspepsia and one had colonic polyps. The characteristics of the diabetes mellitus patients are shown in table 1. Three patients in group B had an accidental vagotomy after anti-reflux surgery and 1 patient had truncal vagotomy for peptic ulcer disease. Vagal nerve dysfunction in these patients was demonstrated by reduced PP release in response to insulin-induced hypoglycaemia (plasma glucose <2.5 mmol/l; incremental PP < 50 pM)<sup>11,12,13,14</sup>. Patients with chronic liver disease, alcohol abuse, previous upper abdominal surgery, neurological disease, pancreatic insufficiency, heart disease, hypertension, irregular heart rate or patients on medication known to affect the autonomic nervous system were excluded from the study. Polyneuropathy was assessed by clinical signs and symptoms and diodesiometry (Biomedical Instruments).

After an overnight fast, blood samples for PP and glucose were taken at 5 min intervals twice before and at +5, +10, +15, +20, +30, and +60 min during and after stimulation with 0.4 mg/kg.15 min and 1.2 mg/kg.15 min of ERY, respectively. Before the tests, blood was drawn to determine HbA1c levels. The patients were asked to take 50% of the prescribed dose of insulin. At the end of the test, the other 50% of the insulin dose was injected.

The cardiovascular reflex tests included 3 parasympathetic and 2 sympathetic tests. The parasympathetic tests were heart rate variation with deep breathing, supine/erect heart rate and Valsalva ratio, while the sympathetic tests were postural adjustment ratio and sustained handgrip. In one diabetes mellitus patient (M; 57 yrs) the cardiovascular reflex test were performed in the classical way. In all other diabetes mellitus patients, the test results were monitored using the FINger Arterial PRESSure method (FINAPRES)<sup>15</sup>. The principle of this instrument is based on servoplethysmomanometry, employing the volume clamp technique<sup>16,17</sup>. The finapres device (Finapres model 5; TNO, Amsterdam, The

Table 1: Characteristics of diabetes mellitus patients. N.P. = nefropathy; R.P. = retinopathy; P.N. =polyneuropathy; T.T.S = total symptom score.

NUMBER	AGE (years)	M/F	TYPE OF DM	DURATION OF DM (years)	Hba1c (mmol/l)	N.P.	R.P.	P.N.	FINAPRES (abnormal)	TSS	DEFAECATION (/week)	WEIGHT (kg)
1	57	M	I	55	9,9	+	+	+	4	161	½	60
2	45	M	I	36	7,5	+	+	+	4	39	18	72
3	50	F	I	42	11,8	-	+	+	3	18	7	71
4	30	F	I	18	10,1	-	-	+	5	60	3	54
5	67	M	II	15	8,0	-	+	+	3	69	½	97
6	43	F	I	29	7,9	+	+	+	5	36	3	47
7	60	F	I	34	9,0	-	+	+	4	21	3	58
8	37	F	I	31	9,8	+	+	+	3	202	3	72
<b>MEDIAN[RANGE]</b>	<b>48[30-67]</b>			<b>32.5[15-55]</b>	<b>9.4[7.5-11.8]</b>					<b>50[18-202]</b>	<b>3[0-18]</b>	<b>65.5[47-97]</b>
1	71	F	II	5	11,0	-	-	+	4	2	7	64
2	66	F	II	1	6,1	-	+	-	3	3	11	64
3	79	M	II	29	7,6	-	+	+	5	0	7	77
4	64	F	I	11	7,6	-	-	+	3	2	5	59
5	58	M	I	38	7,4	-	+	+	3	3	19	67
6	61	F	I	58	9,4	-	-	+	2	1	25	84
7	60	M	I	12	9,0	-	-	+	2	0	15	90
8	62	M	I	47	7,9	-	-	+	3	0	3	75
9	58	M	I	28	9,6	-	+	+	3	3	7	89
<b>MEDIAN[RANGE]</b>	<b>62[58-79]</b>			<b>28[1-58]</b>	<b>7.9[6.1-11]</b>					<b>2[0-3]</b>	<b>7[3-25]</b>	<b>75[59-90]</b>

Netherlands) was connected to an IBM-compatible computer using normal co-axial or shielded cable with a standard BNC connector. In Turbopascal 3.0 (Borland International Inc, Scotts Valley, CA, U.S.A.) a programme was written based on a timeclock. Before each test the clock was started and the recorded digital signal of the finapres was used to calculate the programmed test results. The manoeuvres were performed after rest and in the posture described by Wieling and co-workers<sup>18</sup>. Before the start of the test, the subjects were trained to perform the manoeuvres correctly. The finapres cuff was wrapped around the middle finger of the non-dominant arm, which was fixed exactly at heart level. Each subject refrained from smoking and caffeine- or alcohol-containing beverages for 12 hours before the test. Control systolic blood pressure, diastolic blood pressure, mean blood pressure and heart rate were calculated as the mean values during the 30 s before the onset of each manoeuvre. Cardiovascular autonomic neuropathy was defined as an abnormal score of 2 or more of the 5 tests<sup>15</sup>.

For the assessment of gastrointestinal symptoms, all diabetes mellitus patients were asked to keep a daily symptom score record list for one week. The total symptom score (TSS) was assigned to the severity and frequency of pyrosis, nausea, vomiting, ructus, fullness, bloating and retrosternal or abdominal pain using a scale from 0 to 3 (0 = absence of symptoms; 1 = mild symptoms noticed only when payed attention to; 2 = moderate, symptoms clearly noticed only without interfering with normal daily activities; 3 = severe, symptoms interfering with normal daily activities)<sup>19</sup>. Based on the total symptom score patients with diabetes mellitus were subdivided in group A1 (a score of > 10) and group A2 (a score of ≤ 10). A cumulative symptom score above 10 was considered abnormal. Other possible causes related to the symptoms like pyrosis, nausea, vomiting, ructus, fullness, bloating and retrosternal or abdominal pain (e.g. reflux oesophagitis, peptic ulcer disease or gallstone disease) were excluded by upper abdominal endoscopy or ultrasonography.

Plasma PP was measured by a sensitive and specific radioimmunoassay as previously described<sup>20</sup>. The basal plasma PP level was taken as the mean of two unstimulated samples. Integrated incremental PP secretion was determined by calculation of the area under the plasma PP concentration-time curve after subtraction of mean basal values. All values are expressed as median and range, unless stated otherwise. Statistical analysis was performed by Kruskal-Wallis one way analysis of variance and by the rank sum test. In addition, integrated PP responses after logarithmic transformation were tested for significance by analysis of covariance with age as a covariate. Two tailed probability (p) values were calculated. The study protocol was approved by the local ethics committee. All subjects gave their informed consent to participate in the studies.

## Results

One diabetes mellitus patient refused the 1.2 mg/kg, 15 min ERY dose. All diabetics had 2 or more abnormal finapres tests. Peripheral neuropathy was detected in all but one of the diabetes mellitus patients (Table 1).

Basal PP levels of group A1 and B (19.5 [9-48] pM and 27.5 [20-56] pM, respectively) were lower than those of group A2 and C (57 [23-111] pM and 54 [22-122] pM, respectively).

The delta PP concentration-time curves of the different groups are depicted in fig 1 and 2. The integrated PP responses to 0.4 and 1.2 mg/kg, 15 min of ERY in group A (=A1 + A2) (629 [-81-2996] pM\*20min and 1307 [123-4365] pM\*20min) were not significantly different from those of group C (1021 [326-2964] pM\*20min and 1228 [514-7608] pM\*20min). After stimulation with 0.4 mg/kg, 15 min of ERY, integrated PP responses in groups A1 and B (452 [-81-1513] pM\*20min and 372.5 [184-571]) were decreased (p<0.05) compared to groups A2 and C (1454 [321-2996] and 1021 [326-2964] pM\*20min, respectively) (fig 3). After stimulation with 1.2 mg/kg, 15 min of ERY, integrated PP re-

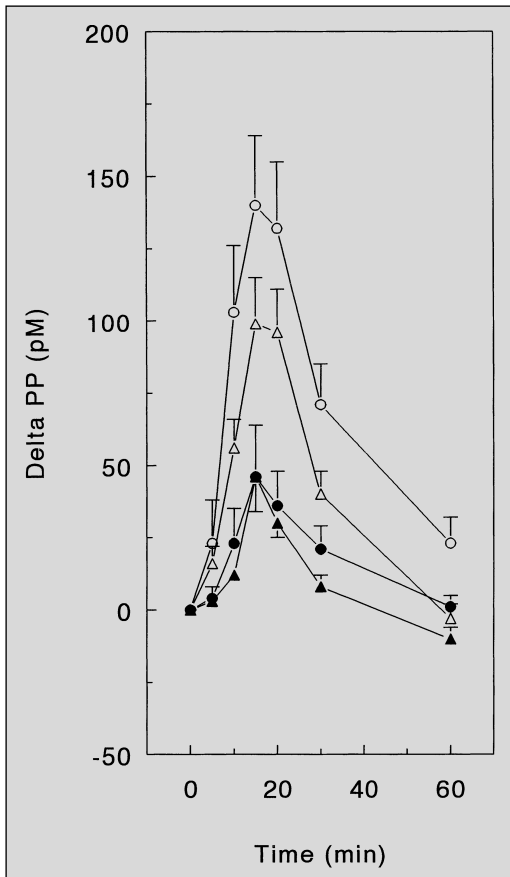


Fig 1: Delta PP concentration-time curves after intravenous stimulation with 0.4 mg/kg.15 min of erythromycin in 8 diabetics with gastrointestinal symptoms (group A1; closed dots), in 9 diabetics without gastrointestinal symptoms (group A2; open dots), in 4 patients with surgical vagal nerve damage (group B; closed triangles) and in 20 controls (group C; open triangles). Results are expressed as mean $\pm$ SEM.

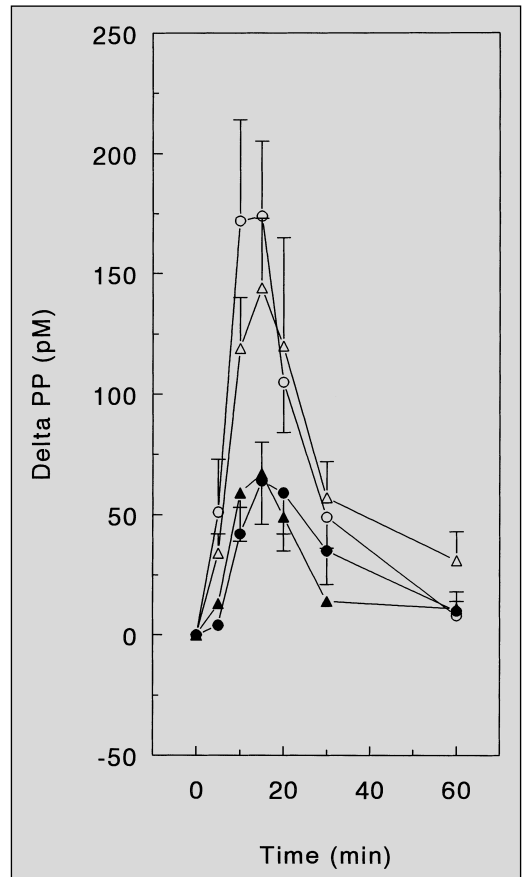


Fig 2: Delta PP concentration-time curves after intravenous stimulation with 1.2 mg/kg.15 min of erythromycin in 7 diabetics with gastrointestinal symptoms (group A1; closed dots), in 9 diabetics without gastrointestinal symptoms (group A2; open dots), in 4 patients with surgical vagal nerve damage (group B; closed triangles) and in 20 controls (group C; open triangles). Results are expressed as mean $\pm$ SEM.

sponses in group A1 was also markedly ( $P < 0.05$ ) reduced (608 [123-1310] pM\*20min) compared to groups A2 and C (2233 [130-4365] and 1228 [514-7608] pM\*20min, respectively) (fig 4). The integrated PP response of group B (660.5 [276-1680]) was lower than that of group A2 and C but this difference was not statistically significant ( $p = 0.1$ ). Comparable differences between groups were obtained when the data were tested by analysis of covariance with age as a covariate. Integrated plasma PP responses to both doses of ERY in groups A1 and B were not significantly different.

Mean HbA1c level in group A was 8.8 [6.1-11.8] mmol/l. There was no difference in HbA1c levels between groups A1 and A2 (9.4 [7.5-11.8] mmol/l vs 7.9 [6.1-11.0] mmol/l). The diabetes mellitus patients had comparable blood glucose levels throughout the study, not exceeding 15 mmol/l. Patients with gastrointestinal symptoms suffered from diabetes mellitus for a median period of 33

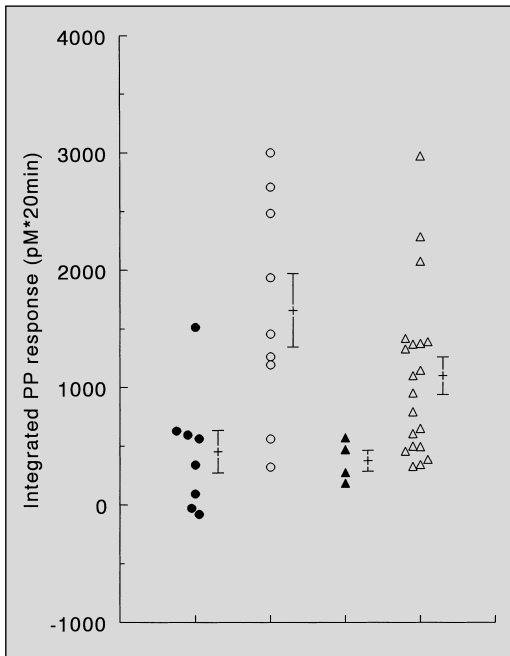


Fig 3: Integrated plasma PP responses over a 20 minute interval after intravenous stimulation with 0.4 mg/kg. 15 min of erythromycin in 8 diabetics with gastrointestinal symptoms (group A1; closed dots), in 9 diabetics without gastrointestinal symptoms (group A2; open dots), in 4 patients with surgical vagal nerve damage (group B; closed triangles) and in 20 controls (group C; open triangles). Results are expressed as mean $\pm$ SEM.

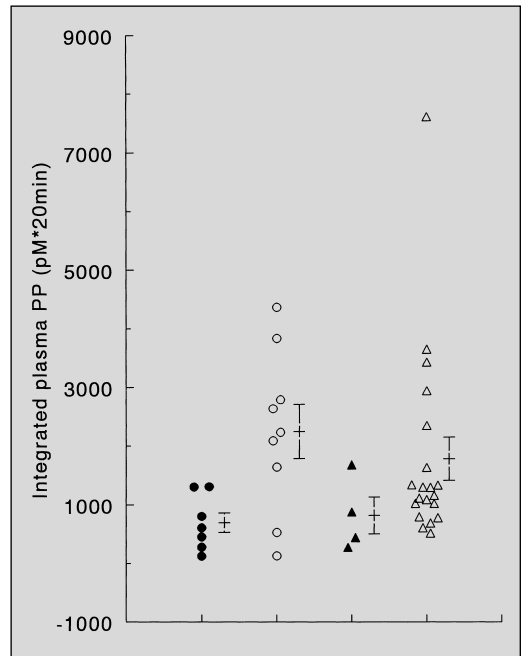


Fig 4: Integrated plasma PP responses over a 20 minute interval after intravenous stimulation with 1.2 mg/kg. 15 min of erythromycin in 7 diabetics with gastrointestinal symptoms (group A1; closed dots), in 9 diabetics without gastrointestinal symptoms (group A2; open dots), in 4 patients with surgical vagal nerve damage (group B; closed triangles) and in 20 controls (group C; open triangles). Results are expressed as mean $\pm$ SEM.

[15–55] years, while those without these symptoms had diabetes mellitus for 28 [1–58] years (not significant). Defaecation frequency was markedly lower ( $p < 0.05$ ) in the patients of group A1 (3 [0–18] times/week) compared to those of group A2 (7 [3–25] times/week).

## Discussion

In the present study we have demonstrated that the PP response to ERY is lower in diabetes mellitus patients with autonomic neuropathy and gastrointestinal symptoms than in controls and in diabetes mellitus patients with autonomic neuropathy but without gastrointestinal symptoms. In previous studies it has been demonstrated that the PP response to ERY in healthy subjects is abolished by atropine<sup>9</sup> and that ERY stimulated PP release is dependent on vagal nerve integrity<sup>10</sup>. Our data may therefore indicate that stimulation of PP release by ERY may be used to assess vagal nerve integrity in diabetics. Sensitive and specific tests to assess autonomic neuropathy of the gastrointestinal tract in diabetics are needed, since the tests presently used, like the insulin-induced hypoglycaemia test and the sham feeding test, are potentially dangerous and difficult to perform in diabetics or are not sensitive enough.



Based on abnormal PP responses to insulin induced hypoglycaemia in patients with normal cardiovascular reflex tests, it has been suggested that gastrointestinal autonomic neuropathy precedes cardiovascular neuropathy<sup>13,21,22</sup>. In our study all diabetes mellitus patients had autonomic neuropathy as assessed by cardiovascular reflex tests. However, only a subgroup of these patients had a decreased PP response to ERY. This suggests that cardiovascular reflex tests may either have a higher sensitivity to detect autonomic neuropathy than the ERY test, or that cardiovascular autonomic neuropathy precedes gastrointestinal autonomic neuropathy or that it occurs independently in diabetes mellitus patients. The correlation between ERY stimulated PP release and dyspeptic symptoms suggests that cardiovascular autonomic neuropathy precedes intestinal autonomic neuropathy or develops independently in our diabetes mellitus patients.

PP release is influenced by plasma glucose levels<sup>23,24,25</sup>. To minimize the effect of this factor, the patients were restrained from eating for 15 h prior to the tests, and blood glucose levels were measured at regular intervals during the tests. Blood glucose levels in our diabetes mellitus patient group ranged from 7 to 14 mmol/l, without differences between the patients with or without gastrointestinal symptoms. This excludes the possibility that the decrease of ERY stimulated PP release in diabetes mellitus patients with gastrointestinal symptoms was the result of hyperglycaemia<sup>25</sup>.

Jebbink et al have demonstrated that dysregulation and hyperactivity of the small intestine are early manifestations in diabetics with cardiac autonomic neuropathy<sup>19</sup>. Analogous to ERY stimulated PP release, these motor abnormalities correlated with gastrointestinal symptoms but not with the severity of cardiac autonomic neuropathy as assessed by standard cardiovascular reflex tests<sup>19</sup>. When compared to ERY stimulated PP release, the assessment of gastrointestinal motor disorders is difficult, time consuming and requires expertise.

Diarrhoea may be an important gastrointestinal problem in long standing diabetes mellitus and its prevalence has been underestimated<sup>4,26</sup>. On the other hand, constipation is one of the most bothersome gastrointestinal complaints in diabetics and appears to be related to parasympathetic autonomic neuropathy<sup>27,28</sup>. Chang et al postulated that diarrhoea in diabetics might be due to reduced electrolyte absorption, secondary to loss of normal sympathetic innervation<sup>29,30</sup>. In our diabetes mellitus patient group, constipation but not diarrhoea was a major problem. The diabetes mellitus patients with gastrointestinal symptoms had a significantly lower defaecation frequency than those without gastrointestinal symptoms. This suggests that in our patient group, autonomic neuropathy of the gastrointestinal parasympathetic system was more pronounced than autonomic neuropathy of the sympathetic system.

In interpreting ERY stimulated PP release, one must take into account that PP release is influenced by several disturbing factors, like age, circadian rhythm, obesity, pancreatic insufficiency, duodenal ulcer disease, stressful events and renal insufficiency<sup>20,31,32,33,34</sup>. In 4 diabetes mellitus patients with proteinuria, basal and stimulated PP levels did not differ from the other patients (results not shown). Furthermore, similar results were obtained when differences between groups were tested by analysis of covariance with age as a covariate. Obesity, duodenal ulcer disease, alterations induced by circadian rhythm and pancreatic insufficiency were also excluded as confounding factors in the present study.

We conclude that ERY stimulated PP release in diabetes mellitus patients is decreased in a subgroup of patients with gastrointestinal symptoms, suggesting that a decreased PP response to ERY reflects the presence of gastro-intestinal autonomic neuropathy. It is tempting to speculate that the PP response to ERY may be used to assess autonomic neuropathy of the gastrointestinal tract in diabetics.

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# Pancreatic polypeptide response to insulin induced hypoglycaemia in Parkinson's disease

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## Abstract

In order to assess vagal nerve integrity, we have studied pancreatic polypeptide responses to insulin induced hypoglycaemia in Parkinson's disease patients, with (group A1, n=7) or without (group A2, n=12) levodopa treatment and in 8 controls (group B). Patients kept a diary for one week to reveal symptoms suggestive of gastrointestinal autonomic dysfunction. Cardiovascular reflex tests were also performed. Integrated PP release in response to insulin hypoglycaemia was decreased in both, group A1 (4390 [1025-8400] pM\*30min) and group A2 (4362 [935-9465] pM\*30min) compared to group B (8587 [5205-18550] pM\*30min;  $p < 0.05$ ). Integrated PP response to insulin induced hypoglycaemia was significantly lower in patients with abnormal results of cardiovascular reflex tests ( $p < 0.05$ ) and abnormal gastrointestinal symptom score ( $p < 0.05$ ) when compared to patients with normal results. Conclusion. Pancreatic polypeptide responses to insulin induced hypoglycaemia are decreased in Parkinson patients. Decreased pancreatic polypeptide responses in these patients are related to abnormal cardiovascular reflex tests and symptoms suggestive of gastrointestinal autonomic neuropathy.

## Introduction

Neuropathy frequently complicates the course of Parkinson's disease<sup>1-6</sup>. In this process, damage to peripheral, sympathetic or parasympathetic nerves may occur independently of one another<sup>7-9</sup>. It is generally accepted that assessment of autonomic neuropathy should be based on a series of distinct tests, rather than on a single investigation of one reflex system. Recently, an automated program was developed, using a finapres device and a personal computer, to perform five cardiovascular reflex tests for the assessment of cardiovascular autonomic neuropathy in a simple and quick way<sup>10-12</sup>. Cardiovascular reflex tests are presently considered as the "golden standard" in the assessment of autonomic neuropathy<sup>13,14</sup>. In addition, several tests have been described to evaluate vagal nerve integrity of the gastrointestinal tract. The release of pancreatic polypeptide (PP) plays a key role in these tests because this hormone is completely under vagal cholinergic control<sup>15</sup>. Impaired PP secretion can therefore be used as a sensitive indicator for autonomic nerve dysfunction of the gastrointestinal system. Stimulation of vagal cholinergic pathways can be achieved by insulin induced hypoglycaemia, modified sham feeding or electrical stimulation of the vagal nerve<sup>15</sup>.

Most patients with Parkinson's disease are allocated to treatment with levodopa. Dopamine may decrease basal PP concentration and inhibit postprandial PP release in men via dopaminergic and adrenergic receptors<sup>16,17</sup>. The extensive dopaminergic innervation of the upper gastrointestinal tract<sup>18</sup> as well as the uptake and decarboxylation of levodopa by PP-producing cells<sup>19</sup> suggests that this mechanism may be of biological importance. The effect of levodopa on PP release in response to insulin induced hypoglycaemia is presently not known.

The aim of this study was to determine whether PP responses to insulin induced hypoglycaemia are disturbed in patients with Parkinson's disease. A second aim was to determine whether a relationship exists between PP responses to insulin induced hypoglycaemia and abnormal cardiovascular reflex tests and symptoms suggestive of gastrointestinal autonomic dysfunction.

## Patients

Nineteen ambulant patients (group A: 7F, 12M; median age 54 [39-79] yrs) with Parkinson's disease according to the criteria of the UK Parkinson's Disease Society Brain Bank and 8 ambulant control



patients on treatment for abdominal complaints (group B: 5F, 3M; median age 37.5 [22–59] yrs) were studied. Characteristics of the Parkinson’s disease patients are depicted in table 1. Five of the control patients met the criteria of irritable bowel syndrome. One had lymphangiectasia of the small bowel, one had proctitis, one had an extirpation of the spleen due to a traumatic rupture and one had unilateral paralysis of the diaphragm of unknown origin. Median body weight and length of the patients of group A and the control subjects of group B were 74 [57–104] kg/174 [157–188] cm and 69 [48–115] kg/169 [164–178] cm, respectively. In group A, the stages of Parkinson’s disease according to Hoehn & Yahr were I in 7, II in 4, III in 6 and IV in 2 patients. Seven patients with Parkinson’s disease used levodopa 400–700mg/24hr (with decarboxylase inhibitor) until 10 hours before the tests (group A1: 2F, 5M; median age 68 [47–79] yrs). All other Parkinson’s disease patients (group A2: 5F, 7M; median age 49 [39–65] yrs) used no medication (n=9) or discontinued their medication for more than 5 days before the tests (n=3). None of the patients or controls used anticholinergic medication or other medication known to affect PP responses. Patients with diabetes mellitus, recent alcohol abuse, oesophago-gastric surgery, other neurological disorders, cardiovascular disease or other factors that could influence test results, were excluded from the study. One patient with Parkinson’s disease was excluded (M, 56 yrs) because of insufficient insulin induced hypoglycaemia (defined as < 2.5 mmol/L).

## Methods

All tests were performed after an overnight fast. Blood samples for PP and glucose were taken in the fasting state and at 10 minute intervals for one hour after the intravenous administration of insulin (0,1 IU/kg).

To show that PP cells were intact and capable of producing the hormone, a standardized meal consisting of 2 slices of buttered bread with 50 gr of cheese, a boiled egg and 200cc tea with 10 gr of sugar, was ingested over a 20 minute period and blood samples for PP were obtained at 10 minute intervals after taking two unstimulated samples.

In addition 5 cardiovascular reflex tests were performed in all patients with Parkinson’s disease. During these tests, a finapres (FINger Arterial PRESsure) device recorded heart rate (beats/min) and blood pressure (mmHg) continuously from a finger. The principle of this instrument is based on ser-

*Table 1: Data and characteristics of all Parkinson’s disease patients.*

	with levodopa	without levodopa
Number of patients	7	12
Male/Female	5/2	7/5
Age (yrs)	68 [47-79]	49 [39-65]
Body mass index (kg/m <sup>2</sup> )	24.1 [15.5-25.2]	25.1 [19.3-34]
Total symptom score (arbitrary units)	28 [0-85]	9.5 [0-137]
Defaecation frequency (stools/week)	5 [3-13]	6 [2-8]
Patients with abnormal Finapres	2	8
Classification of Parkinson’s disease (n)		
Hoehn and Yahr I	3	4
Hoehn and Yahr II	0	4
Hoehn and Yahr III	2	4
Hoehn and Yahr IV	2	0

voplethysmomanometry, employing the volume clamp technique<sup>10,11</sup>. The digital signal of the Finapres was recorded by a personal computer. Immediately after the tests were performed, a special developed computer programme calculated the test results automatically. The finapres cuff (Finapres model 5 TNO, Amsterdam, The Netherlands) was wrapped around the middle finger of the non-dominant hand, and was fixed at heart level. Before starting the tests the manoeuvres were trained to perform them correctly, according to the timeclock of the computer programme.

After 5 minutes of supine rest, the subjects performed six consecutive maximal respirations in 1 minute. Five minutes later the subjects stood up and remained in the upright position for 2 minutes. After 2 minutes rest in the sitting position the Valsalva manoeuvre was performed three times, each after 1 minute rest. Finally the patients were asked to exert 30% of their previously determined maximum voluntary contraction for 3 minutes on a handgrip dynamometer, 2 minutes after the last Valsalva manoeuvre.

During deep breathing at 6 breaths/min, the mean difference between the highest heart rate in inspiration and the lowest in expiration for six consecutive breathing cycles was calculated<sup>12</sup>. During the standing up test, the programme calculated the difference between the maximum heart rate after the manoeuvre and the control heart rate before and the quotient of maximum heart rate and minimum heart rate after standing up<sup>20</sup>. Furthermore, the difference in averaged diastolic blood pressure between 50 and 80 seconds in the standing position and during the control period was calculated<sup>21</sup>. The highest Valsalva ratio of the three manoeuvres performed, stated the maximum heart rate variability during this test<sup>22</sup>. During sustained handgrip the highest increase in average diastolic blood pressure over 5 seconds was calculated. The test results were interpreted as abnormal if the testparameter was below the 5<sup>th</sup> percentiles of normal<sup>12</sup>. Autonomic neuropathy was defined as an abnormal score of 2 or more of the 5 tests<sup>12</sup>.

For the assessment of bowel movement frequency and gastro-intestinal symptoms, all Parkinson's disease patients were asked to keep a daily symptom score record list for a week<sup>23</sup>. Constipation was defined as a bowel movement of  $\leq 5$ /week. The total symptom score included symptoms like pyrosis, nausea, vomiting, ructus, fullness, bloating and retrosternal or upper abdominal pain using a scale from 0 to 3 (0 = absence of symptoms; 1 = mild, symptoms noticed only when payed attention to; 2 = moderate, symptoms clearly noticed only without interfering with normal daily activities; 3 = severe, symptoms interfering with normal daily activities). A cumulative symptom score above 10 was considered abnormal.

Plasma PP was measured by a sensitive and specific radioimmunoassay<sup>24</sup>. All values are reported as median and range. Plasma PP concentrations obtained at 30, 40, 50 and 60 minutes were used to determine integrated PP response to insulin. This integrated PP response was determined by calculating the area under the curve after subtraction of the baseline values.

Statistical analysis was performed by Kruskal Wallis one way analysis of variance followed by the rank sum test. All tests were two tailed. A difference was considered to be statistically significant if p-values were equal to or below 0.05. The study was approved by the local ethics committee and all subjects gave their informed consent to participate in the studies.

## Results

In all Parkinson patients that participated in the study, the PP cells were intact and capable of producing PP as demonstrated by an incremental PP release of more than 50 pM after the ingestion of a test meal<sup>15</sup>. In the control subjects the individual delta PP after stimulation with insulin hypoglycaemia always exceeded 250 pM.

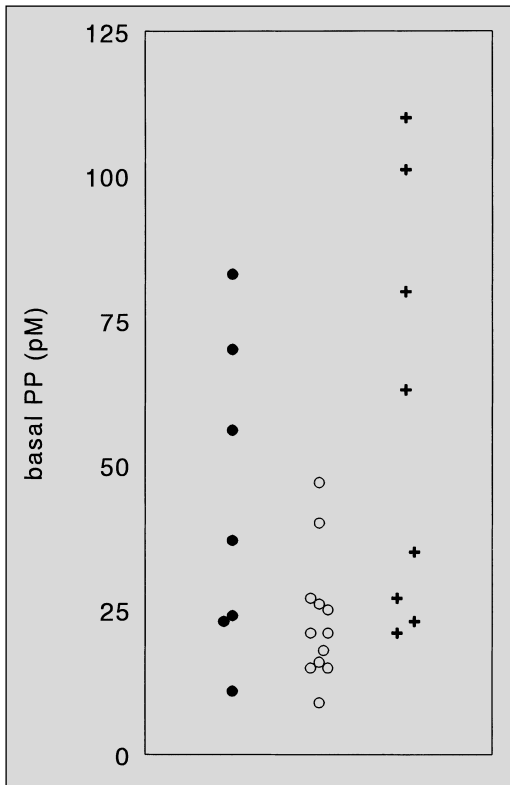


Fig 1: Individual basal plasma PP levels in 7 Parkinson patients on levodopa treatment (group A1: closed dots), 12 Parkinson patients without levodopa treatment (group A2: open dots) and 8 controls (group B: crosses).

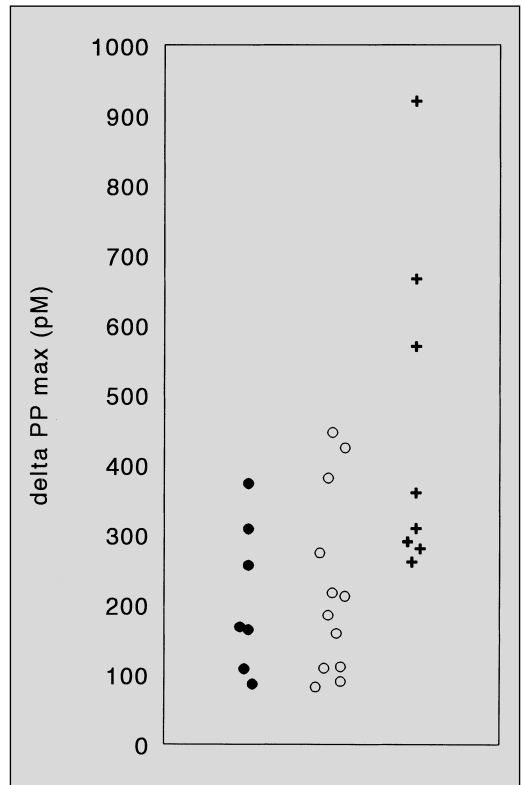


Fig 2: Individual incremental plasma PP levels after intravenous stimulation with insulin (0,1 IU/kg as a bolus) in 7 Parkinson patients on levodopa treatment (group A1: closed dots), 12 Parkinson patients without levodopa treatment (group A2: open dots) and 8 controls (group B: crosses).

The basal plasma PP levels of group A2 (21 [9-47] pM) but not those of group A1 (37 [11-83]) were lower ( $p=0.01$ ) than those of group B (49 [21-110]). Basal PP concentrations were equivalent or below the lowest value in the control group B in 1 of the patients of group A1 and in 7 of the patients of group A2. Individual basal plasma PP levels are shown in figure 1.

After insulin induced hypoglycaemia, the integrated PP response in the patients of group A1 and group A2 were decreased (4390 [1025-8400] pM\*30min,  $p=0.04$  and 4362 [935-9465] pM\*30min,  $p=0.006$  respectively) compared to that of group B (8587 [5205-18550] pM\*30min).

The integrated PP response to insulin induced hypoglycaemia of the Parkinson patients classified as stage I or II (N=11) and those classified as III or IV (N=8) according to Hoehn & Yahr were not statistically different from each other (4675 [975-8400] pM\*30min and 3790 [935-9465] pM\*30min, respectively).

Individual insulin stimulated plasma PP peak increments of group A1, A2 and B are depicted in figure 2. The peak incremental PP response to insulin induced hypoglycaemia was markedly decreased in the patients of group A1 (168 [86-373] pM;  $p=0.02$ ) and group A2 (199 [82-446] pM;  $p=0.02$ ) compared to the subjects of group B (335 [261-920] pM). The peak incremental PP response to in-

sulin induced hypoglycaemia was below the lowest value in the control subjects of group B in 5 of the patients of group A1 and in 8 of the patients of group A2. Basal and insulin stimulated PP were not significantly different among group A1 and A2 patients with Parkinson's disease.

All patients with Parkinson's disease that participated in the study even those with severe rigidity or tremor of the hands were able to perform the Finapres tests correctly. In 10 of the 19 Parkinson patients the cardiovascular reflex tests were abnormal (2 in group A1 and 8 in group A2). Integrated PP response to insulin induced hypoglycaemia was decreased ( $p=0.02$ ) in these Parkinson patients (2667 [935-8130] pM\*30min) compared to the Parkinson patients with normal cardiovascular reflex tests (6580 [3625-9465] pM\*30min).

In the Parkinson patients with a bowel movement of  $\leq 5$ /week ( $n=8$ ), the integrated PP response to insulin hypoglycaemia was not significantly decreased (3272 [975-9465] pM\*30min;  $p=0.3$ ) compared to the Parkinson patients with bowel movements of more than 5 times a week ( $n=11$ ; 4835 [935-8400] pM\*30min). Four of the Parkinson patients of group A1 and 6 of the patients of group A2 had a symptom score of  $\geq 10$ . In the Parkinson patients with a total symptom score of  $\geq 10$ , the integrated PP release to insulin induced hypoglycaemia was significantly ( $p=0.006$ ) decreased (3185 [935-6580] pM\*30min) when compared to Parkinson patients with a lower total symptom score (6825 [2590-9465] pM\*30min).

## Discussion

In previous experiments it was demonstrated that the PP response to insulin induced hypoglycaemia is a sensitive and specific marker for vagal dysfunction, for instance in case of autonomic neuropathy, provided that PP producing cells in the pancreas are intact<sup>15,25</sup>. Since autonomic neuropathy may frequently complicate the course of Parkinson's disease, we have performed the present study. We have related the results with a symptom score for autonomic gastrointestinal neuropathy and with cardiovascular reflex tests.

In the present study the PP response to insulin induced hypoglycaemia was significantly decreased in patients with Parkinson's disease when compared to controls. The differences in PP responses to insulin induced hypoglycaemia between controls and Parkinson patients could not be explained by treatment with levodopa. In previous studies it was demonstrated that dopamine decreased basal plasma PP concentrations and inhibited postprandial PP release in humans<sup>16,17</sup>. In our study, however, no difference in basal and integrated PP response to insulin induced hypoglycaemia was observed between Parkinson patients with or without levodopa. On the one hand, this may be explained by discontinuation of levodopa administration for at least 10 hours before the experiments in our study. On the other hand one may speculate that levodopa treatment has only little effect on PP response after insulin hypoglycaemia.

In previous studies it has been demonstrated that the PP response to insulin induced hypoglycaemia is more sensitive to detect autonomic neuropathy than basal PP levels<sup>15,26</sup>. In the present experiment, basal PP levels and the PP response to insulin induced hypoglycaemia were lower in Parkinson patients compared to controls. However, the overlap in basal PP levels between controls and patients was more pronounced than the overlap in PP responses to insulin induced hypoglycaemia. This supports previous findings that the PP response to insulin induced hypoglycaemia is probably a more sensitive marker to detect autonomic neuropathy than basal PP levels<sup>15</sup>.

A significant negative relation between PP responses to insulin induced hypoglycaemia and the severity of gastrointestinal symptoms in Parkinson patients was observed. However, no significant relation was found between the PP response to insulin induced hypoglycaemia and the severity of the

disease as indicated by the Hoehn & Yahr scale, indicating that autonomic neuropathy may complicate both, recent onset and advanced Parkinson's disease<sup>4,27</sup>.

Presently, non-invasive cardiovascular reflex tests are used as "golden standard" to detect autonomic neuropathy. A time consuming battery of cardiovascular reflex tests should be used because of the poor predictive value of a single test parameter<sup>13</sup>. With the automated computerized programme used in this study, the time required to elaborate all the test results can easily be overcome<sup>12</sup>. Furthermore, all the Parkinson patients tested were able to perform the tests, even those with severe tremor and rigidity. Satisfactory performance of these tests needs good co-operation and some of the tests are effort depending in these patients.

In clinical practice, the concordance between cardiovagal and abdominal vagal function tests remains controversial<sup>28</sup>. In our study, the PP response to insulin induced hypoglycaemia was decreased in Parkinson patients with abnormal cardiovascular reflex tests, or gastrointestinal symptoms.

Autonomic nerve function deteriorates with advancing age<sup>29,30</sup>. Basal and stimulated PP levels are also related to age<sup>29,31</sup>. Therefore, it is important to take the age into account when evaluating PP results. The control group used in our study was younger than our patient group. It may therefore be suggested that the PP response after insulin hypoglycaemia in age matched controls could even be more pronounced, resulting in more significant differences between the investigated groups.

We conclude that insulin stimulated pancreatic polypeptide release in Parkinson patients is decreased compared to controls. The results are not dependent on levodopa treatment. We speculate that pancreatic polypeptide release in response to insulin induced hypoglycaemia may be helpful in the assessment of gastrointestinal autonomic neuropathy in patients with Parkinson's disease.

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# Summary



Pancreatic polypeptide (PP) is a 36 amino-acid polypeptide localized in the secretory granules of endocrine cells in the pancreas. The secretion of PP is affected by several physiological stimuli, such as food intake and fluctuations in blood glucose concentrations. Vago-cholinergic mechanisms are involved in the cephalic, gastric, intestinal and postabsorptive phase of digestion. The cephalic phase of PP secretion, however, is selectively mediated by the vago-cholinergic system. For this special reason, PP release after vagal-cholinergic stimulation by various stimuli can be used in the assessment of autonomic neuropathy.

Modified sham feeding (MSF) has been demonstrated to activate central vagal activity resulting in PP release. This PP response to MSF is modest and dependent on the quantity of food and the duration of MSF. Although gustatory, olfactory, acoustic and visual signals are known to be involved in the cephalic phase of meal stimulation, no data were available on the stimulating effects of the different components of food when sham fed. In chapter 2 we therefore investigated the contribution of fat, protein and carbohydrates on the stimulation of vagal cholinergic activity on gallbladder contraction and in chapter 3 on PP release. Both, The gallbladder contraction and PP release in response to MSF appeared to be dependent on the composition of the meal. Gallbladder contraction and PP release were mainly the result of the amount of fat or protein in the meals. The same nutrients that stimulate gallbladder contraction during the intestinal phase also stimulate gallbladder contraction during the cephalic phase of the meal, although via different mechanisms, since real feeding but not MSF stimulates the release of CCK. There was a similar pattern of PP release in response to the cephalic and intestinal phase of the meal. It seems that the same type of chemoreceptors are triggered by nutrients throughout the intestine, but that the chemoreceptors subsequently may activate endocrine, or neurocrine mechanisms depending on their location in the intestinal tract.

Erythromycin (ERY), stimulates PP release. This effect can be blocked by atropine but not by loxiglumide (a cholecystokinin antagonist), suggesting the importance of cholinergic pathways in this process. It was not known whether the stimulation of PP cells by ERY is mediated centrally via the vagal nerve or locally by gastroenteropancreatic cholinergic reflexes. To determine the role of long vagal and local gastropancreatic reflexes in ERY-induced PP release, ERY was administered as an intravenous bolus injection to healthy volunteers and patients with impaired vagal function with or without gastric antrectomy. The results of this study, described in chapter 4, demonstrated that the effect of ERY on PP release is to a large extent dependent on intact long vagal pathways. The diminished PP response to ERY in vagotomized patients is further reduced by antrectomy, suggesting that ERY activates both long vago-vagal and to a lesser extent local gastropancreatic cholinergic reflexes.

During the 1950s and 1960s clinical descriptions were made on autonomic neuropathy and physiologists devised a variety of techniques to ascertain the patterns of autonomic nerve damage. This resulted in a series of cardiovascular reflex tests. These tests exhibit both, symptoms and prognosis and it can be assumed that cardiovascular reflex abnormalities indicate diffuse damage throughout the autonomic nervous system. However, damage to peripheral, sympathetic and parasympathetic nerves may occur independently of one another.

The release of PP to several different physiological stimuli can also be used in the assessment of autonomic neuropathy since the release of PP is selectively governed by vagal-cholinergic stimulation. For this reason, the secretion of PP can be used for studies of vagal control of endocrine systems at the afferent, central, and efferent levels of vago-vagal reflexes. In medical practice, stimulation of PP cells is achieved by sham feeding or acute insulin induced hypoglycaemia. Damage of the vago-cholinergic axis may result in a decreased or absent PP response. Although sham feeding by the 'chew and spit' technique is rather specific, it forms only a modest stimulation of vago-cholinergic activity resulting in approximately 20% negative results in normal subjects. Insulin induced hypoglycaemia is a reliable method to test vagal nerve integrity. This test, however, is time consuming, haz-

ardous and unpleasant for the patient. Furthermore, in diabetics, a rapid standardized hypoglycaemia is difficult to achieve.

To investigate if ERY stimulated PP release could be of help in the assessment of gastrointestinal autonomic neuropathy, we studied ERY stimulated PP release in patients with chronic liver disease (5) and diabetes mellitus (6) and insulin hypoglycaemia induced PP release in Parkinson's disease (7). The results of these tests were compared with cardiovascular reflex tests (5, 6 and 7) or MSF (5). We also studied the correlation between gastrointestinal symptoms and autonomic neuropathy as shown by PP and cardiovascular reflex tests (6 and 7).

In patients with chronic liver disease, integrated PP response to ERY, but not to MSF was significantly decreased compared to age matched patients with atypical abdominal complaints. Four of the patients with chronic liver disease had abnormal cardiovascular reflex tests. The stimulated PP response in these patients did not differ from those with normal cardiovascular reflex tests.

All patients with diabetes mellitus had abnormal cardiovascular reflex tests. In chapter 6 it was demonstrated that the integrated PP release after stimulation with ERY is significantly decreased in diabetics with complaints suspicious of gastrointestinal autonomic neuropathy and not in diabetics without gastrointestinal complaints.

Integrated PP release after insulin hypoglycaemia was also decreased in patients with Parkinson's disease compared to age matched control patients with atypical abdominal complaints. The integrated PP response to insulin hypoglycaemia was also decreased in Parkinson patients with abnormal cardiovascular reflex tests compared to those with normal cardiovascular reflex tests. The decreased PP test results in Parkinson patients correlated with symptoms suspicious of gastrointestinal autonomic neuropathy.

According to the results described in chapter 4,5,6 and 7, one may postulate that ERY stimulated PP release may be a valuable, supplementary test in the assessment of gastrointestinal autonomic neuropathy.

# Samenvatting



Het autonome zenuwstelsel van het maag-darmkanaal is een multifunctioneel systeem dat de motiliteit, de endo- en exocriene secretie en de microcirculatie coördineert. Het speelt ook een rol bij de regulatie van immuun- en ontstekingsprocessen.

Pancreas polypeptide (PP) is een 36 aminozuren tellend polypeptide met hormonale eigenschappen dat zich bevindt in de granula van de endocriene cellen in het pancreas. Deze zogenaamde "PP-cellen" bevinden zich zowel in de eilandjes van Langerhans als tussen de acinaire cellen, hoofdzakelijk in de kop van het pancreas. De secretie van PP wordt beïnvloed door verschillende fysiologische stimuli. Zo leidt eten tot een sterke stijging van de concentratie van PP in het bloed door een complexe interactie tussen neurogene- en hormonale factoren. Tijdens de stimulatie van PP in de cephale fase van een maaltijd speelt het vago-cholinerge systeem een essentiële rol. Zo kan de stimulatie van PP tijdens deze fase volledig worden afgevlakt door het gelijktijdig toedienen van atropine, een anticholinergicum. Hierdoor kan de toename van PP na stimulatie van het vago-cholinerge systeem een bijdrage leveren aan de diagnostiek van diverse aandoeningen, waarbij stoornissen van het autonome zenuwstelsel van het maag-darmkanaal, worden verondersteld. De cephale fase van de productie van PP wordt in de medische praktijk gestimuleerd door schijnvoeding of door insuline geïnduceerde hypoglycaemie.

Met schijnvoeding bedoelen we het laten ruiken en proeven van voedsel zonder dat dit mag worden doorgeslikt. De PP-secretie na stimulatie met schijnvoeding is afhankelijk van de hoeveelheid voedsel en de duur van de schijnvoeding. Verder is bekend dat smaak, geur, geluid en visuele factoren een rol spelen bij de stimulatie van de cephale fase door schijnvoeding. Het is echter niet bekend wat de invloed is van de samenstelling van de maaltijd die gebruikt wordt tijdens schijnvoeding. Daarom hebben we in hoofdstuk 2 onderzocht wat het effect is van de hoeveelheid vet, eiwitten en koolhydraten in schijnvoeding op de vago-cholinerge stimulatie van de galblaascontractie en in hoofdstuk 3 op de PP-secretie. Zowel de galblaascontractie als de productie van PP door schijnvoeding bleken afhankelijk van de samenstelling van de maaltijd. Galblaascontractie en PP-secretie waren vooral afhankelijk van de hoeveelheid vet en eiwit in de schijnvoeding. Dezelfde voedingsstoffen die de galblaascontractie stimuleerden tijdens de intestinale fase stimuleerden ook de galblaascontractie tijdens de cephale fase van de maaltijd. Dit gebeurt echter via verschillende mechanismen omdat schijnvoeding in tegenstelling tot doorslikken van voeding geen toename teweeg brengt van het hormoon cholecystokinine. Er was eenzelfde patroon in de concentratie-tijd curve herkenbaar tijdens de stimulatie van PP in de cephale en intestinale fase van de verschillend samengestelde maaltijden. Mogelijk dat dezelfde chemoreceptoren door het gehele maag-darmstelsel actief zijn, maar afhankelijk van de locatie in het maag-darmstelsel endocriene-, paracriene- of neurocristiene mechanismen stimuleren.

Erythromycine (ERY), een antibioticum uit de macrolide-groep stimuleert de productie van PP. Deze stimulatie kan worden geblokkeerd door atropine, maar niet door loxiglumide (een cholecystokinine antagonist). Dit geeft het belang aan van het cholinerge systeem bij de ERY gestimuleerde productie van PP. Het was echter niet bekend of deze stimulatie van ERY centraal verliep via de nervus vagus of lokaal door gastro-entero-pancreaticogene cholinerge reflexen. Om dit verder te onderzoeken werd ERY als een intraveneuze bolus toegediend aan gezonde proefpersonen en aan patiënten met een accidentele of bewuste vagotomie. De resultaten van deze studie zijn beschreven in hoofdstuk 4 en laten zien dat het effect van ERY op de productie van PP voor een belangrijk deel afhankelijk is van intacte lange nervus vagus takken. De afgenomen productie van PP na stimulatie met ERY in patiënten met een vagotomie, neemt verder af indien er ook een antrectomie is verricht. Dit doet vermoeden dat de productie van PP door ERY verloopt via de lange vago-vagale takken van de nervus vagus maar voor een deel ook via de lokale gastro-pancreaticogene cholinerge reflexen.

In de vijftiger- en zestiger jaren kreeg autonome neuropathie meer aandacht. Door klinici en fysiologen werden cardiovasculaire reflextesten ontwikkeld en gestandaardiseerd om meer inzage te



krijgen in het voorkomen van autonome neuropathie. Men nam aan dat deze reflex testen een weerspiegeling zouden zijn van een diffuse autonome neuropathie. Inmiddels is gebleken dat schade aan perifere-, sympathische- en parasymphatische zenuwen door autonome neuropathie onafhankelijk van elkaar kunnen voorkomen. De mate van toename van PP in het bloed na stimulatie van het vago-cholinerge systeem, met schijnvoeding of insuline geïnduceerde hypoglycaemie, zou kunnen fungeren als parameter voor autonome neuropathie van het maag-darmkanaal. Beide stimuli hebben echter hun nadelen. Ondanks het feit dat stimulatie van PP door schijnvoeding specifiek is, blijkt schijnvoeding in de praktijk moeizaam omdat patiënten enerzijds door hun slikreflex kleine hoeveelheden voedsel kunnen doorslikken waardoor de test niet meer betrouwbaar is, anderzijds geeft schijnvoeding slechts een milde stimulatie van de produktie van PP waardoor een vals-positieve uitslag kan volgen. Bij de door insuline geïnduceerde hypoglycaemie krijgt de patiënt op geleide van zijn lichaamsgewicht een bolus insuline intraveneus toegediend. Hierdoor probeert men een hypoglycaemie te verkrijgen van minder dan 2,5 mmol/l waardoor er een zeer sterke vago-cholinerge stimulatie van PP plaatsvindt. Het is met name bij diabetes mellitus patiënten moeilijk, bewerkelijk en gevaarlijk om de juiste mate van hypoglycaemie te verkrijgen. Stimulatie van de produktie van PP via het vago-cholinerge systeem door ERY heeft bovenbeschreven nadelen niet.

Om te beoordelen of ERY een rol zou kunnen spelen in het vaststellen van autonome neuropathie bij patiënten met een chronische leverziekte (hoofdstuk 5) onderzochten we de ERY- (0.4 mg/kg.15min en 1.2 mg/kg.15min) en de schijnvoeding gestimuleerde PP produktie. De resultaten werden vergeleken met die van patiënten met a-specifieke buikklachten. Het bleek dat de geïntegreerde PP-secretie na stimulatie met ERY, maar niet na stimulatie met schijnvoeding, significant was afgenomen in patiënten met een chronische leverziekte. Bij 4 patiënten met chronisch leverlijden werden afwijkende cardiovasculaire reflex testen gevonden. De geïntegreerde PP-secretie van deze patiënten na stimulatie met ERY bleek niet te verschillen van de leverpatiënten met normale cardiovasculaire reflex testen.

In hoofdstuk 6 werd de ERY gestimuleerde PP-secretie onderzocht in diabetes mellitus patiënten met en zonder maag-darmklachten passend bij autonome neuropathie. Alle diabetes mellitus patiënten hadden afwijkende cardiovasculaire reflex testen. De resultaten werden vergeleken met die van controle patiënten met aspecifieke buikklachten en patiënten met status na vagotomie. De geïntegreerde PP-secretie na stimulatie met ERY bleek significant verlaagd bij diabetes mellitus patiënten met maag-darmklachten passend bij autonome neuropathie en patiënten met een status na vagotomie in vergelijking met controle patiënten en diabetes mellitus patiënten zonder symptomen.

Om de integriteit van de nervus vagus te onderzoeken bij Parkinson patiënten werd in hoofdstuk 7 de geïntegreerde PP-secretie na stimulatie met insuline geïnduceerde hypoglycaemie onderzocht. Tevens hielden deze patiënten gedurende een week een klachtendagboek bij en ondergingen zij cardiovasculaire reflex testen. De resultaten werden vergeleken met die van patiënten met a-typische buikklachten. De geïntegreerde PP-secretie na insuline geïnduceerde hypoglycaemie bleek bij Parkinson patiënten afgenomen in vergelijking met de controles. Verder bleek de geïntegreerde PP-secretie na insuline geïnduceerde hypoglycaemie significant verlaagd in de Parkinson patiënten met afwijkende cardiovasculaire reflex testen en in Parkinson patiënten met maag-darmklachten passend bij autonome neuropathie in vergelijking met Parkinson patiënten met normale cardiovasculaire reflex testen en Parkinson patiënten zonder gastro-intestinale klachten.

Op grond van de resultaten beschreven in hoofdstuk 4,5,6 en 7 zou men kunnen veronderstellen dat de ERY gestimuleerde PP-secretie als een aanvullende test zou kunnen fungeren bij het in kaart brengen van autonome neuropathie van het maag-darmstelsel.

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# Curriculum Vitae

De schrijver van dit proefschrift werd geboren op 16 augustus 1958 te Leiden. In 1974 haalde het diploma M.A.V.O. en in 1977 het V.W.O. diploma aan het Leeuwenhorst college te Noordwijkerhout. Aansluitend studeerde hij geneeskunde aan de Rijksuniversiteit te Leiden waar hij in 1983 het doctoraal-, en in 1984 het artsexamen aflegde.

Van 1984-1985 werkte hij als vakantie-assistent op de afdeling Gastroenterologie en Hepatologie van het academisch ziekenhuis te Leiden (AZL). In 1985 begon hij de opleiding tot internist in het St. Elisabeth ziekenhuis te Leiderdorp (opleider: Dr. W.A. van Amstel). Deze opleiding werd in 1987 voortgezet in het AZL (opleiders: Prof. dr. L.A. van Es en Prof. dr. A.E. Meinders). Na inschrijving in het specialisten register in Mei 1990 was hij tijdelijk werkzaam op de afdeling Intensive Care (hoofd: Prof. dr. A.E. Meinders) en de afdeling Maag-, Darm- en Leverziekten (hoofd: Prof. dr. C.B.H.W. Lamers) van het AZL, waar hij tevens een aanvang maakte met basaal gastro-enterologisch onderzoek, dat uiteindelijk resulteerde in dit proefschrift. Van september 1991 tot september 1994 was hij werkzaam op de afdeling Maag-, Darm- en Leverziekten van het St. Radboud ziekenhuis (Opleiders: Prof. dr. J.H.M. van Tongeren en Prof. dr. J.B.M.J. Jansen). Hier vond tevens de opleiding tot gastro-enteroloog plaats en het promotieonderzoek werd afgerond. Vanaf 1 januari 1995 is hij als gastro-enteroloog werkzaam in het Gelderse Vallei ziekenhuis te Wageningen.











