PROXIMAL AND DISTAL GUT HORMONE SECRETION IN IRRITABLE BOWEL SYNDROME

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ABSTRACT

Background: Sensory and motor dysfunction of the gut are both important characteristics of irritable bowel syndrome (IBS). Several gut peptides contribute to the regulation of gastrointestinal function but little is known on gut hormone secretion in IBS.

Methods: We evaluated perceptual thresholds and fasting and postprandial plasma levels of proximal (cholecystokinin (CCK), motilin) and distal (peptide YY) gut peptides up to 1 hour after ingestion of a high caloric meal in 99 IBS patients and 40 age and sex matched healthy controls.

Results: Fasting plasma CCK levels were significantly elevated in patients (1.2 ± 0.8 pM) compared to controls (0.8 ± 0.7 pM, \( P = 0.006 \)), as was the incremental postprandial CCK response (72 ± 73 versus 40 ± 42 pM·60 min, respectively; \( P = 0.003 \)). No differences in fasting and postprandial motilin or PYY levels were found. The postprandial PYY response was significantly increased in hypersensitive compared to normosensitive patients (215 ± 135 versus 162 ± 169 pM, \( P = 0.048 \)). Patients with a diarrhoea predominant bowel habit had higher fasting motilin levels compared to constipated patients or alternating type IBS patients (82.1 ± 36.5 versus 60.8 ± 25.1 versus 57.5 ± 23.9 pM, one-way ANOVA \( P = 0.003 \)).

Conclusion: IBS patients have increased fasting and postprandial plasma levels of CCK. Changes in plasma levels of motilin and PYY may contribute to the clinical expression of IBS, such as the presence of visceral hypersensitivity or predominant bowel habit.
INTRODUCTION

Irritable Bowel Syndrome (IBS) is a frequently occurring disorder that has received much attention over the last decades. However, its pathophysiology remains poorly understood. Disturbances at different levels of the brain-gut-axis have been proposed in symptom generation, including low-grade chronic intestinal inflammation\textsuperscript{1}, immune system alterations\textsuperscript{2}, autonomic dysfunction\textsuperscript{3}, and altered central processing of afferent sensory input\textsuperscript{4}. In particular, enhanced visceral perception is considered to be important as it has been reported that up to 94\% of patients are hypersensitive to rectal balloon distension\textsuperscript{5}. There is also evidence pointing to altered gut motility in IBS\textsuperscript{6}. IBS patients exhibit abnormal postprandial colonic motor activity\textsuperscript{7} and reduced perception thresholds for gas, discomfort and pain\textsuperscript{8} after a meal. Symptoms often deteriorate postprandially\textsuperscript{9}.

Several gut peptides are known to be involved in the regulation of gastrointestinal motor and sensory function. For instance, cholecystokinin (CCK) is a proximal gut hormone, released upon fat and protein ingestion, that delays gastric emptying\textsuperscript{10} and stimulates contraction of the gallbladder\textsuperscript{11} and exocrine pancreatic secretion\textsuperscript{12}. Studies in healthy individuals have shown that infusion of CCK stimulates colonic motility and increases rectal sensitivity to balloon distension\textsuperscript{13,14}. Motilin is also released from the proximal intestine and is involved in the regulation of interdigestive motility of the stomach and small intestine\textsuperscript{15}, but also affects colorectal motor function\textsuperscript{16}. Peptide YY (PYY) is a distal gut peptide that has been shown to delay proximal gastrointestinal motility\textsuperscript{17}. Spiller et al. recently showed that the number of PYY-containing colonic enteroendocrine cells is increased in IBS patients who develop symptoms after an acute infectious gastroenteritis\textsuperscript{18}.

Little is known about gut hormone secretion in patients with IBS. We hypothesize that changes in gut hormone secretion may contribute to the observed alterations in gut motor and sensory function in IBS. Therefore, we studied plasma levels of gut peptides released from the upper (CCK and motilin) and lower (PYY) small intestine under fasting and postprandial conditions in a large cohort of IBS patients. In addition, the influence of age, gender, IBS subtype and visceral hypersensitivity on gut hormone secretion was evaluated.

METHODS

Participants

Between March 2001 and July 2002, IBS patients between 18 and 65 years of age were invited to participate in a large clinical trial on psychological therapy, which in-
cluded assessment of psychological function, autonomic nerve function, postprandial gut hormone secretion, rectal barostat measurements, and evaluation of the efficacy of relaxation training for the treatment of IBS. This study reports on postprandial gut peptide response tests.

Patients were recruited through a tertiary referral centre (the outpatient department of Gastroenterology of the Leiden University Medical Centre (LUMC)) and through local advertisement. Healthy volunteers were recruited through advertisement. Eligible participants were screened by one of the investigators (PvdV). All patients met Rome II criteria for IBS19. Exclusion criteria were organic disease, previous abdominal surgery (except cholecystectomy and appendectomy), and pregnancy. Use of antispasmodics, bulking agents, laxatives, and occasional use of analgesics was permitted. Informed consent was obtained from each participant. The LUMC ethics committee had approved the study protocol.

Hypersensitivity testing

An electronic barostat (Synectics Visceral Stimulator, Synectics Medical, Stockholm, Sweden) was used to assess visceral hypersensitivity. This device is able to maintain constant pressure within a highly compliant, polyethylene bag tied to the end of a multilumen tube, as described elsewhere20. Perception of rectal pain was quantified on a 100-mm Visual Analog Scale (VAS) at every even pressure, with end points ranging from ‘none’ to ‘unbearable’. Pain thresholds were defined as the first pressure level at which perception scores exceeded 10 mm. Hypersensitivity to rectal balloon distension was defined as a pain threshold that was 2 SD or more below the mean threshold in healthy controls.

Subjects were permitted a small standardized breakfast at 8.00 AM and arrived at our department at 10.00 AM. A tap water enema was used to evacuate the rectum and the barostat bag was positioned as described previously21. Barostat recordings commenced after 30 min. The experimental protocol consisted of a slow ramp distension to assess rectal compliance. Intrabag pressure was increased at a rate of 1 mmHg/min, from 5 to 30 mmHg. At all even pressures (6, 8...30 mmHg), patients rated the urge to defecate and pain using the 100-mm VAS scale. After the experiment had ended, the rectal balloon was removed.

Meal

Fifteen minutes after the barostat experiment, an intravenous canula was inserted in the antecubital vein of one arm and a fasting blood sample was obtained (t=0). At 13.00 AM, patients were offered an 800 kcal solid meal, consisting of 2 slices of brown bread, 10 g of margarine, 1 slice of fat cheese, 1 slice of cooked ham, 350 ml of semi-skimmed milk, 1 boiled egg, 300 ml of yoghurt, and 10 g of honey (44
g of protein, 46 g of fat and 69 g of carbohydrates). Additional blood samples were obtained at t = 15, 30, 45 and 60 min.

**Plasma peptide assays**

Blood samples were collected in ice-chilled tubes containing 2 g/L EDTA. All samples were centrifuged at rate of 3000 rpm for 15 min at a constant temperature of 4 °C and stored at -20 °C until peptide levels were determined. Plasma CCK was measured by a sensitive and specific RIA as described previously22. Levels of PYY were determined using antiserum generated in rabbits by intracutaneous injections of synthetic human PYY (BACHEM AG, Bubendorf, Switzerland). PYY was labelled with 125I using chloramine T. There is no cross-reactivity with pancreatic polypeptide or vasoactive intestinal peptide. The detection limit is 10 pM and both PYY (1-36) and PYY (3-36) bind to the antibody in dilutions up to 1:250,000. Plasma motilin concentrations were determined using a sensitive and specific radioimmunoassay as described previously23.

**Statistical analysis**

All statistical analyses were carried out using SPSS for Windows, version 11.0.1 (SPSS Inc., Chicago IL, USA). An incremental postprandial response was computed for each peptide by calculating the incremental area under the curve. Linear mixed model analysis was used to detect overall differences in plasma peptide levels between groups over time. Plasma peptide level, subject group and the interaction were analysed as separate contributors to the model. Patient numbers were used to indicate repeated measurements. Demographical characteristics were compared between groups by Student-t or Mann-Whitney analysis and chi square analysis where appropriate. Between-group differences in plasma peptide concentrations were compared by Mann-Whitney or ANOVA with post-hoc Tukey’s correction for multiple group-wise comparisons. Within-group changes relative to fasting were analysed using Wilcoxon Signed Ranks Tests. Data are expressed as mean ± SD. The level of statistical significance was set at P<0.05.

**RESULTS**

**Subject characteristics**

We screened 130 patients and 40 healthy volunteers. Twenty-six patients did not meet Rome II criteria for IBS19. Blood sampling was unsuccessful in 5 patients, so that 99 patients and 40 healthy controls were included in the final analysis. All provided informed consent. Thirty-one patients (31%) were recruited through the
outpatient department, and 68 patients (69%) and all healthy controls were recruited through advertisement. Demographical and clinical characteristics are listed in Table 1. Mean age and gender distribution was comparable between groups.

### Table 1. Baseline characteristics of IBS patients and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>IBS patients (n=99)</th>
<th>Controls (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>41.9 ± 14.0</td>
<td>39.7 ± 15.0</td>
</tr>
<tr>
<td>Females n (%)</td>
<td>71 (72)</td>
<td>25 (62)</td>
</tr>
<tr>
<td>Bowel habit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>diarrhoea</td>
<td>34</td>
<td>0</td>
</tr>
<tr>
<td>constipation</td>
<td>34</td>
<td>0</td>
</tr>
<tr>
<td>alternating</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>not specified (IBS)/normal (controls)</td>
<td>9</td>
<td>40</td>
</tr>
</tbody>
</table>

IBS, irritable bowel syndrome; n, number of patients or controls.

**Plasma CCK**

Fasting and postprandial plasma CCK levels are shown in Figure 1. Fasting plasma CCK concentrations were significantly higher in patients compared to controls (Table 2). The postprandial plasma CCK response was significantly different between patients and controls (CCK concentration by group interaction, $P<0.001$). Plasma CCK concentrations increased significantly in patients and controls from 15 min onward ($P<0.001$ for all time points in both groups), reaching a peak at $t=30$ min in both

![Figure 1](image.png)

**Figure 1.** Fasting and postprandial plasma CCK concentrations in IBS patients (closed triangles) and controls (open triangles).

* $P<0.001$ compared to fasting. † $P<0.001$ compared to controls.
Gut hormone secretion in IBS

The incremental postprandial CCK response was significantly increased in patients compared to controls (Table 2).

Plasma motilin

Fasting and postprandial motilin levels are shown in Figure 2. Fasting plasma motilin levels were not different between patients and controls. Plasma motilin concentra-

![image]

Figure 2. Fasting and postprandial plasma motilin concentrations in IBS patients (closed triangles) and controls (open triangles).

* P<0.001 compared to fasting.
tions decreased significantly after the meal in both groups. The postprandial motilin response was similar in both groups (plasma motilin concentration by group interaction $P=0.49$) (Table 2).

**Plasma PYY**

Figure 3 illustrates fasting and postprandial PYY levels in patients and controls. Fasting PYY concentrations were similar in patients and controls. The overall plasma PYY response was similar in both groups (PYY concentration by group interaction, $P=0.80$). Plasma PYY concentrations increased significantly in both groups from 15 min to 60 min ($P<0.001$). The incremental postprandial PYY response was not significantly different between patients and controls (Table 2).

**Influence of age and gender**

Fasting CCK levels were significantly correlated with age in the whole group ($r=0.33$, $P<0.001$), but the postprandial CCK response was not ($r=0.05$, $P=0.58$). A small but significant correlation was found between age and fasting levels of motilin for the whole group ($r=0.19$, $P=0.03$). Age was neither correlated with the postprandial motilin response, nor with baseline nor postprandial levels of PYY.

A significant gender effect was found for fasting plasma levels of CCK (one-way ANOVA, $P=0.001$), which were significantly elevated in female IBS patients compared to male IBS patients (Tukey’s $P=0.012$) and female controls (Tukey’s $P=0.009$) (Table 2 and Figure 4). This was not accounted for by age, as mean age was similar between groups. Linear regression analysis showed that both age and gender were
Gut hormone secretion in IBS independently correlated with fasting plasma CCK concentration ($P=0.007$ for gender and $P<0.001$ for age).

Figure 4 shows that postprandial CCK levels were also significantly increased in female IBS patients compared to the other subgroups. While the ANOVA indicated an overall difference in the incremental CCK response between these subgroups ($P=0.05$), no significant differences were found between female patients compared to male patients and female controls after adjustment for multiple comparisons (Table 2).

Fasting levels of motilin were significantly higher in male IBS patients compared to female patients (Tukey $P=0.046$). No differences were found between female and male control subjects (Table 2). Neither postprandial motilin levels nor fasting and postprandial levels of PYY were different between males and females (Table 2).

**IBS subgroups**

Fasting plasma levels of CCK and PYY were not different between the three IBS subgroups, but basal plasma concentrations of motilin were significantly increased in IBS-D compared to IBS-C and IBS-A (Table 3). No differences were found with respect to the incremental postprandial responses of CCK, PYY or motilin.
Visceral hypersensitivity and gut peptides

Two of 99 patients declined to participate in the barostat study. Thirty-two of the remaining 97 patients (33%) were classified as hypersensitive to rectal balloon distension. No differences between hypersensitive and normosensitive patients were found for fasting plasma levels of CCK, PYY and motilin or postprandial responses, apart from an increased plasma PYY response in hypersensitive patients ($P=0.048$) (Table 4).

Table 3. Fasting plasma concentrations and incremental postprandial responses of CCK, PYY and motilin in IBS subgroups according to predominant bowel habit

<table>
<thead>
<tr>
<th></th>
<th>IBS-D (n=34)</th>
<th>IBS-C (n=34)</th>
<th>IBS-A (n=22)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>43.3 ± 13.0</td>
<td>39.0 ± 15.3</td>
<td>42.4 ± 14.1</td>
<td></td>
</tr>
<tr>
<td>Females n (%)</td>
<td>20 (59)</td>
<td>27 (79)</td>
<td>19 (86)</td>
<td></td>
</tr>
<tr>
<td>CCK</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fasting</td>
<td>1.1 ± 0.9</td>
<td>1.3 ± 0.7</td>
<td>1.4 ± 0.8</td>
<td>0.364</td>
</tr>
<tr>
<td>AUC meal</td>
<td>63 ± 66</td>
<td>80 ± 66</td>
<td>68 ± 67</td>
<td>0.577</td>
</tr>
<tr>
<td>Motilin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fasting</td>
<td>82.1 ± 36.5†</td>
<td>60.8 ± 25.1</td>
<td>57.5 ± 23.9</td>
<td>0.003</td>
</tr>
<tr>
<td>AUC meal</td>
<td>-655 ± 1390</td>
<td>-547 ± 774</td>
<td>-495 ± 867</td>
<td>0.846</td>
</tr>
<tr>
<td>PYY</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fasting</td>
<td>17.6 ± 7.7</td>
<td>17.5 ± 3.7</td>
<td>18.3 ± 6.4</td>
<td>0.897</td>
</tr>
<tr>
<td>AUC meal</td>
<td>191 ± 168</td>
<td>185 ± 175</td>
<td>164 ± 123</td>
<td>0.829</td>
</tr>
</tbody>
</table>

Table 4. Fasting plasma concentrations and incremental postprandial responses of CCK, PYY and motilin in hypersensitive and normosensitive IBS patients

<table>
<thead>
<tr>
<th></th>
<th>hypersensitive (n=32)</th>
<th>normosensitive (n=65)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>41.3 ± 12.8</td>
<td>42.5 ± 14.8</td>
<td></td>
</tr>
<tr>
<td>Females n (%)</td>
<td>23 (72)</td>
<td>48 (74)</td>
<td></td>
</tr>
<tr>
<td>CCK</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fasting</td>
<td>1.2 ± 0.9</td>
<td>1.2 ± 0.8</td>
<td>0.908</td>
</tr>
<tr>
<td>AUC meal</td>
<td>73 ± 71</td>
<td>72 ± 75</td>
<td>0.214</td>
</tr>
<tr>
<td>Motilin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fasting</td>
<td>73.2 ± 30.1</td>
<td>67.0 ± 31.6</td>
<td>0.155</td>
</tr>
<tr>
<td>AUC meal</td>
<td>-496 ± 1150</td>
<td>-678 ± 1000</td>
<td>0.710</td>
</tr>
<tr>
<td>PYY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fasting</td>
<td>18.9 ± 7.4</td>
<td>16.9 ± 5.1</td>
<td>0.220</td>
</tr>
<tr>
<td>AUC meal</td>
<td>215 ± 135</td>
<td>162 ± 169</td>
<td>0.048</td>
</tr>
</tbody>
</table>

AUC, Area Under the Curve. Fasting concentrations are expressed as pM. AUC is expressed as pM•60 min. * P-value for overall difference between subgroups (chi-square or one-way ANOVA) † P=0.011 versus IBS-C, P=0.009 versus IBS-A.
DISCUSSION

Our study demonstrates that both fasting plasma CCK concentrations and the postprandial CCK response are significantly increased in IBS patients compared to healthy controls. In contrast, neither fasting plasma levels of peptide YY and motilin nor the postprandial responses of these peptides are different between patients and controls.

The effects of CCK on gastrointestinal function are well-known and include increased sensitivity and motor activity of the distal gut. Previous studies in patients with IBS have pointed to disturbed CCK release and altered organ sensitivity to CCK. Infusion of CCK in IBS patients leads to excessive intestinal motor activity, reduced pain thresholds, and increased gallbladder smooth muscle sensitivity. A study by Sjölund et al. indicated that the release of CCK after ingestion of emulgated maize oil was higher in IBS patients compared to healthy controls. Our findings in a large cohort of IBS patients confirm that postprandial CCK secretion is exaggerated in IBS. Additionally, we found that fasting levels of CCK were elevated in IBS patients. This was not observed by Sjölund et al., possibly due to the smaller sample size in that study (n=18).

One could argue whether the relatively small difference in postprandial plasma CCK concentrations between patients and controls (i.e., approximately twofold increase in IBS) is sufficient to contribute to exaggerated sensorimotor responses in IBS. Niederau et al demonstrated that only infusion of pharmacological doses of cerulein, a CCK agonist, resulted in significantly increased colonic motor activity. Similarly, Sabate and colleagues showed decreased rectal sensory thresholds to balloon distension during CCK infusion at pharmacological but not physiological levels. Unfortunately, these experiments were carried out only in healthy individuals. We previously demonstrated decreased rectal sensory thresholds during CCK infusion in IBS patients. It is possible that increased sensitivity to CCK together with twofold increased postprandial plasma levels are, in part, responsible for altered gastrointestinal sensory and motor function in IBS.

Infusion of CCK has been shown to increase rectal pain sensitivity in IBS. One could hypothesize that elevated plasma levels of CCK may contribute to the pathophysiology of visceral hypersensitivity. Yet, our finding that neither fasting nor postprandial CCK levels were different between hypersensitive and normosensitive patients renders a contribution of changes in CCK secretion to the pathogenesis of enhanced visceral perception unlikely. However, increased CCK release after a meal may well be involved in the exaggerated postprandial colonic motor response in IBS patients. There is also evidence to suggest that CCK infusion aggravates symptom severity in patients with functional abdominal pain syndromes, including
IBS. Therefore, CCK antagonists such as loxiglumide are considered to have clinical potential in IBS.

Plasma levels of CCK correlated significantly with age, which confirms previous findings. Interestingly, the elevated fasting and postprandial plasma CCK levels in IBS patients were almost completely attributable to female patients. This was not accounted for by age, as mean age was similar between groups. Our finding is particularly interesting in view of the female predominance in IBS. Thus far, no studies on gender differences with respect to CCK secretion in humans have been published. One animal study, however, demonstrated gender differences in sphincter of Oddi sensitivity during CCK infusion, evidenced by a greater change in phasic wave amplitude in female compared to male dogs. CCK probably does not play a role in IBS subtypes, as fasting and postprandial CCK levels were not different between patient subsets divided by bowel habit.

Fasting and postprandial plasma levels of motilin were comparable between IBS patients and controls. Similar results have been reported by others, although increased and decreased motilin secretion after a meal has also been observed in IBS. Remarkably, plasma motilin levels decreased after meal ingestion in both groups. One should realise that motilin contributes to motility in the interdigestive and not in the digestive state, and is involved in triggering phase III of the migrating motor complex (MMC). Motilin levels fluctuate in accordance with the various phases of the MMC. Fasting motilin levels may have been obtained during phase III in some individuals, yielding higher mean plasma motilin concentrations, while in the first hour after meal ingestion phase III is suppressed, which may explain the observed decrease in plasma motilin concentrations. Furthermore, fasting motilin levels were significantly elevated in patients with diarrhoea predominance compared to those with constipation and alternating bowel habits. These findings may be clinically important as motilin is known to stimulate human colonic motility in vitro and in vivo and may therefore play a role in the accelerated colonic transit that has been demonstrated in diarrhoea predominant IBS.

Fasting and postprandial plasma peptide YY levels did not differ between IBS patients and controls, a finding that is in line with a previous study. Others have more specifically studied the density of PYY secretory cells in the distal gut mucosa of IBS patients. One study suggested that local tissue levels of PYY in the descending colon are reduced in IBS patients compared to controls. In contrast, another study showed increased numbers of PYY-containing enteroendocrine cells in rectal biopsy specimens of patients who developed IBS symptoms after an episode of acute dysenteric illness. The latter findings point to a role for PYY in the pathophysiology of post-infectious IBS and visceral hypersensitivity. Our observation that patients...
who were hypersensitive to rectal balloon distension have a greater PYY response supports this hypothesis.

Finally, it should be recognized that plasma hormone levels do not necessarily represent efficacy at target organ level. Peptides may act via endocrine, but also through paracrine and neurocrine pathways.

It is concluded that 1) fasting plasma motilin levels are significantly increased in diarrhea subtype IBS patients, 2) postprandial PYY secretion is significantly increased in patients with visceral hypersensitivity, and 3) fasting and postprandial CCK levels are significantly increased in (female) IBS patients. The observed changes in gut hormone secretion, especially of CCK, support a role for gut peptides in the pathophysiology of IBS.

ACKNOWLEDGEMENTS

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REFERENCES
