Plasma Cholecystokinin Response to Oral Fat in Patients with Billroth I and Billroth II Gastrectomy

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The present study was undertaken to determine whether bypassing the duodenum in patients with Billroth II gastrectomy affects plasma cholecystokinin (CCK) release in response to ingestion of fat. Plasma CCK concentrations were measured by radioimmunoassay using two antibodies; antibody 1703 binds to all carboxl-terminal CCK-peptides containing at least 14 amino acid residues, while antibody T204 is specific for the sulphated tyrosine region of CCK. There were no significant differences among fasting plasma CCK concentrations in seven patients with Billroth II gastrectomy (1.3 ± 0.4 fmol/ml, antibody 1703; 2.6 ± 0.4 fmol/ml, antibody T204), six patients with Billroth I gastrectomy (0.6 ± 0.3 fmol/ml, antibody 1703; 2.9 ± 0.5 fmol/ml, antibody T204), and nine normal subjects (0.7 ± 0.1 fmol/ml, antibody 1703; 1.9 ± 0.3 fmol/ml, antibody T204). Ingestion of 250 ml 20% Intralipid induced similar increases in plasma CCK in patients with Billroth II gastrectomy (11.2 ± 2.0 fmol/ml, antibody 1703; 10.1 ± 2.4 fmol/ml, antibody T204) as in patients with Billroth I gastrectomy (11.8 ± 2.0 fmol/ml, antibody 1703; 8.4 ± 1.1 fmol/ml, antibody T204). However, the increments in plasma CCK in patients with gastrectomy (11.5 ± 1.4 fmol/ml, antibody 1703; 9.3 ± 1.4 fmol/ml, antibody T204) were significantly ($p < 0.01$) greater than those in normal subjects (4.7 ± 0.8 fmol/ml, antibody 1703; 4.1 ± 0.7 fmol/ml). Similarly, the integrated plasma CCK secretion in patients with Billroth II gastrectomy (510 ± 58 fmol/ml · 120 min, antibody 1703; 458 ± 69 fmol/ml · 120 min, antibody T204) and in patients with Billroth I gastrectomy (457 ± 143 fmol/ml · 120 min, antibody 1703; 365 ± 61 fmol/ml · 120 min, antibody T204) were significantly ($p < 0.05$) greater than in normal subjects (230 ± 49 fmol/ml · 120 min, antibody 1703; 162 ± 24 fmol/ml · 120 min, antibody T204). It is concluded that the plasma CCK response to oral fat is significantly greater in patients with partial gastrectomy than in normal subjects, and that patients with Billroth I and Billroth II gastrectomy have similar increases in plasma CCK after ingestion of fat.

CHOLECYSTOKININ (CCK) is a polypeptide hormone, isolated from the mucosa of the upper small intestine, which stimulates gallbladder contraction and pancreatic enzyme secretion.[1] Before the development of reliable radioimmunoassays for CCK in plasma, it was generally accepted that the actions of intestinal stimuli for gallbladder contraction and pancreatic enzyme secretion were mediated by the release of CCK into the circulation. In fact, gallbladder contraction and pancreatic enzyme secretion were used as bioassay systems for the measurements of CCK in plasma.[2,3] It is well known that it is very hard to develop a reliable radioimmunoassay for CCK.[4] Several factors contribute to difficulties in developing a radioimmunoassay for CCK, such as difficulties in preparing immunoreactive CCK-labels,[5] cross-reactivity of antibodies with gastrin because CCK and gastrin share the same five COOH-terminal amino acid residues,[6,7] suspected species differences in the chemical structure of CCK,[8,9] absence of purified or synthetic human CCK, and availability of only limited amounts of pure porcine CCK. We have developed radioimmunoassays for CCK sufficiently sensitive to measure the low concentrations of CCK in human plasma.

Cholecystokinin is produced by endocrine cells in the mucosa of the upper small intestine. Since the highest concentrations of CCK are found in the duodenal mucosa,[10] it is likely that food bypassing the duodenum will result in impaired CCK release and decreased stimulation of gallbladder and pancreatic enzyme secretion. The higher incidence of gallstones in patients with Billroth II gastrectomy has been attributed to impaired stimulation of gallbladder contraction due to the low postprandial plasma CCK concentrations in such patients.[11-14] Furthermore, impaired postprandial release of CCK in patients with Billroth II gastrectomy has been demonstrated by *in vitro* gallbladder bioassay.[15]

In the present study we have measured plasma CCK concentrations before and after ingestion of fat in patients with Billroth I gastrectomy and Billroth II gastrectomy. The results were compared to those obtained in normal subjects.

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Subjects and Methods

Thirteen patients with gastrectomy were studied. All patients had been operated upon for peptic ulcer. Six of them (four male, two female; mean age 45 years, range 27–59 years) had Billroth I anastomosis, and seven (six male, one female; mean age 51 years, range 35–66 years) had Billroth II anastomosis. In addition, nine normal subjects (eight male, one female; mean age 40 years, range 25–62 years) were studied. After an overnight fast the subjects ingested 250 ml 20% Intralipid (Kabi Vitrum, Stockholm, Sweden) within 10 minutes. Blood samples for measurement of CCK were obtained at −5, 0, 10, 20, 30, 40, 50, 60, 75, 90, 105, and 120 minutes.

Plasma CCK concentrations were measured by radioimmunoassay using two antibodies with different specificities.[16] Antibody 1703 binds to all COOH-terminal CCK peptides containing at least 14 amino acid residues and does not show any cross-reactivity with gastrin (Table 1). Antibody T204 binds to all CCK-peptides containing the sulphated tyrosine region. This antibody shows low binding to sulphated gastrins but it does not bind to unsulphated gastrins (Table 1). The antibodies do not bind to structurally unrelated regulatory peptides, including insulin, glucagon, pancreatic polypeptide, somatostatin, secretin, gastric inhibitory polypeptide, vasoactive intestinal polypeptide, bombesin, or neurotensin. Measurement of the eluate of a Sephadex G50 column to which an aqueous-acid extract of human upper small intestine was applied showed that both antibodies bind to component I CCK, CCK33-CCK39, and intermediate CCK, while antibody T204 binds also to CCK8.[16] CCK33 coupled to ^{125}I-hydroxyphenylpropionic acid succinimide ester (Bolton Hunter reagent) was used as label.[16] Ninety-nine per cent pure porcine CCK33 was used as standard. 0.05 mol/l sodium phosphate buffer pH 7.4 containing 0.08 mmol/l human serum albumin and 0.06 mmol/l sodiumethylmercurithiosalicylate was used as assay buffer. A nonequilibrium system was used with 72 hours preincubation followed by 24 hours incubation after addition of the labelled peptide. Separation between free and antibody-bound hormone was performed by adsorption of the free peptide to plasma-coated charcoal. The 50% inhibition dose (ID_{50}) was 2.8 fmol/ml for antibody 1703 and 3.3 fmol/ml for antibody T204. Plasma samples were extracted in 96% ethanol, dried under nitrogen at 37 C and reconstituted in assay buffer to the original volume before the assay. Recovery of CCK33 and sulphated CCK8 added to hormone-free plasma was 85.4 ± 2.0% (n = 11) and 89.8 ± 1.7% (n = 7), respectively. The detection limit of both assays was about 0.5 fmol/ml plasma. The intra-assay variation in the working range of the standard curve was between 4.6 and 11.5%, and the interassay variation ranged from 11.3 to 26.1%. Dilution curves of plasma samples containing a high concentration of endogenous CCK were parallel to the standard curve.

TABLE 1. *Relative Potencies of CCK-Peptides and Gastrins to Antibody 1703 and Antibody T204*

	Antibody 1703	Antibody T204
CCK39	0.91	1.09
CCK33	1.00	1.00
CCK20-33	0.62	1.69
CCK22-33	<0.01	1.71
CCK24-33	<0.01	0.78
CCK26-33 sulphated	<0.01	0.65
CCK26-33 unsulphated	<0.01	<0.01
CCK30-33	<0.01	<0.01
CCK1-21	<0.01	<0.01
CCK1-15	<0.01	<0.01
CCK10-20	<0.01	<0.01
CCK16-27	0.01	0.01
CCK20-27	0.01	0.02
gastrin 34 sulphated	<0.01	0.02
gastrin 34 unsulphated	<0.01	<0.01
gastrin 17 sulphated	<0.01	0.02
gastrin 17 unsulphated	<0.01	<0.01

Results were expressed as the mean ± 1 SEM. The integrated CCK secretion after ingestion of fat was determined by calculating the area under the curve after subtraction of the basal value. Statistical analysis was done by Student's t-test for paired and unpaired results. Informed consent was obtained from all subjects studied.

Results

Fasting plasma CCK concentrations in seven patients with Billroth II gastrectomy (1.3 ± 0.4 fmol/ml, antibody 1703; 2.6 ± 0.4 fmol/ml, antibody T204) were not significantly different from those in six patients with Billroth I gastrectomy (0.6 ± 0.3 fmol/ml, antibody 1703; 2.9 ± 0.5 fmol/ml, antibody T204) and from those in nine normal subjects (0.7 ± 0.1 fmol/ml, antibody 1703; 1.9 ± 0.3 fmol/ml, antibody T204). In normal subjects ingestion of fat induced increases in plasma CCK, which reached statistical significance ($p = 0.0001$–$p < 0.05$) over basal at 10, 20, 30, 40, 50, 60, 75, and 105 minutes when measured with antibody 1703, and at 10, 20, 30, 40, 50, 60, and 75 minutes when measured with antibody T204 ($p = 0.0005$–$p < 0.05$; Fig. 1). Ingestion of fat in gastrectomized patients resulted in significant increases in plasma CCK over basal value in all postprandial samples when measured with antibody 1703 ($p < 0.0001$–$p < 0.05$) and at 10, 20, 30, 40, 50, 60, 75, 90, and 105 minutes when measured with antibody T204 ($p < 0.0001$–$p < 0.05$; Fig. 1). The plasma CCK response to oral fat in the gastrectomized patients was significantly greater than in the normal subjects at 10, 20, 30, and 40 minutes when measured with antibody 1703 and in all postprandial samples when measured with antibody T204 ($p < 0.005$–

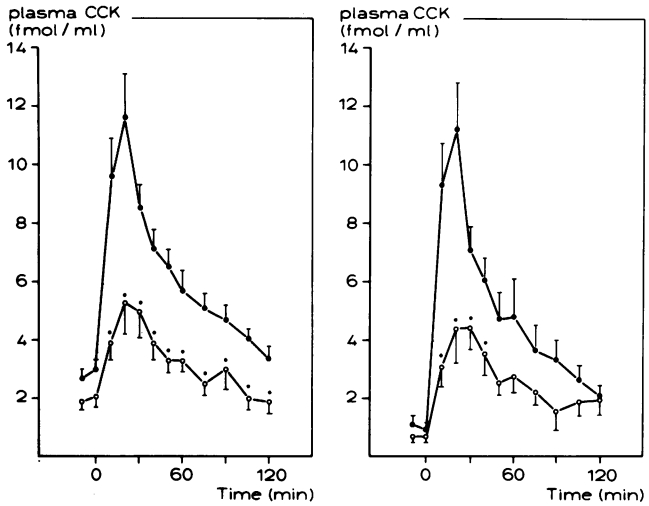

FIG. 1. Plasma cholecystokinin concentrations before and after oral ingestion of 250 ml 20% Intralipid in 13 patients with partial gastrectomy (● —— ●) and in 9 normal subjects (O —— O). The left panel represents the results as measured with antibody T204 and the right panel with antibody 1703. Asterisks indicate significant differences between patients with gastrectomy and normal subjects.

$p < 0.05$; Fig. 1). The peak increments in plasma CCK in gastrectomized patients (11.5 ± 1.4 fmol/ml, antibody 1703; 9.3 ± 1.3 fmol/ml, antibody T204) were significantly ($p < 0.01$) greater than those in normal subjects (4.7 ± 0.8 fmol/ml, antibody 1703; 4.1 ± 0.7 fmol/ml, antibody T204). Similarly, the integrated plasma CCK secretion after oral fat in gastrectomized patients (486 ± 70 fmol/ml · 120 min, antibody 1703; 415 ± 47 fmol/ml · 120 min, antibody T204) was significantly ($p < 0.05$) greater than in normal subjects (229 ± 49 fmol/ml · 120 min, antibody 1703; 162 ± 24 fmol/ml · 120 min, antibody T204; Fig. 2).

Ingestion of fat induced significant increases in plasma CCK in patients with Billroth II gastrectomy at 10, 20, 30, 40, 50, 60, 75, 90, and 105 minutes when measured with antibody 1703 ($p < 0.0001$–$p < 0.05$) and at 10, 20, 30, 40, 50, 60, 75, and 90 minutes when measured with antibody T204 ($p < 0.0001$–$p < 0.05$; Fig. 3). In patients with Billroth I gastrectomy postprandial plasma CCK concentrations were significantly increased over basal value at 10, 20, 30, and 40 minutes when measured with antibody 1703 ($p < 0.005$–$p < 0.05$) and at 10, 20, 30, 40, 50, 75, 90, and 105 minutes when measured with antibody T204 ($p = 0.0001$–$p < 0.05$; Fig. 3). The postprandial CCK concentrations were significantly ($p < 0.05$) greater in patients with Billroth II gastrectomy compared to patients with Billroth I gastrectomy 30 and 40 minutes after ingestion of the liquid fat meal as measured with both antibodies (Fig. 3). However, the peak increments in plasma CCK in patients with Billroth II gastrectomy (11.2 ± 2.0 fmol/ml, antibody 1703; 10.1 ± 2.4 fmol/ml, antibody T204) and in patients with Billroth I gastrectomy (11.8 ± 2.0 fmol/ml, antibody 1703; 8.4 ± 1.1 fmol/ml, antibody T204) were not significantly different. Similarly, the integrated plasma CCK secretion after ingestion of fat in patients with Billroth II gastrectomy (510 ± 58 fmol/ml · 120 min, antibody 1703; 458 ± 69 fmol/ml · 120 min, antibody T204) did not significantly differ from that in patients with Billroth I gastrectomy (457 ± 143 fmol/ml · 120 min, antibody 1703; 365 ± 61 fmol/ml · 120 min, antibody T204; Fig. 2).

Discussion

The present study confirms our previous finding that basal plasma CCK concentrations in normal subjects are

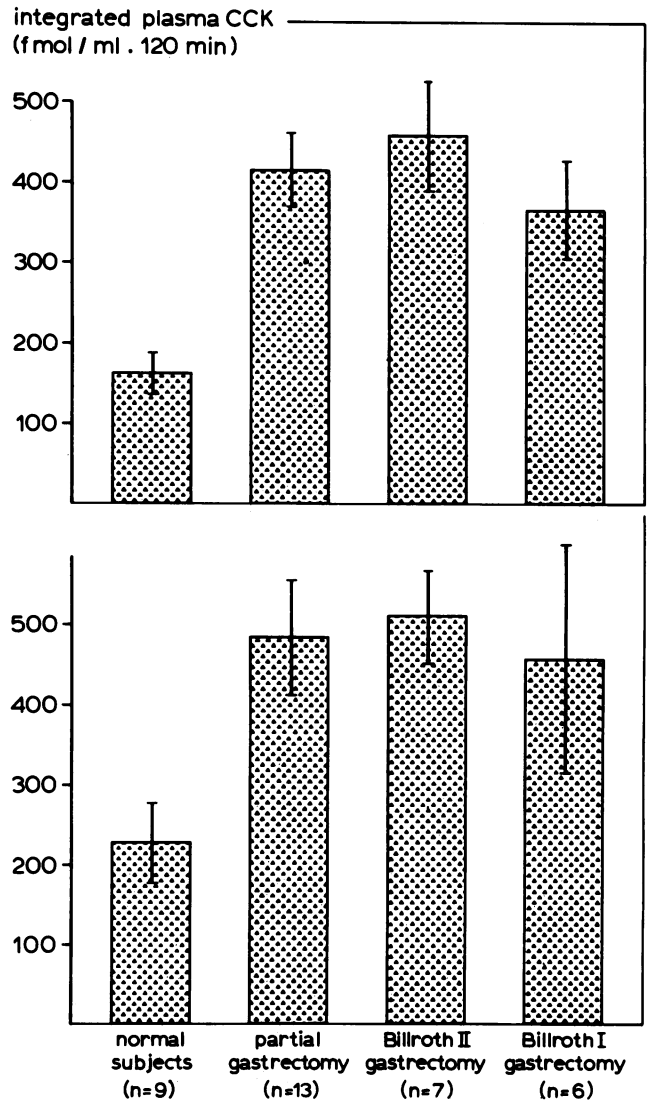

FIG. 2. Integrated plasma cholecystokinin secretion after oral fat in normal subjects and in patients with gastrectomy as measured with antibody T204 (upper panel) and antibody 1703 (lower panel).

very low.[16] Patients with partial gastrectomy had similarly low plasma CCK concentrations. As expected, ingestion of fat was a potent stimulus for CCK release in both normal subjects and patients with gastrectomy. However, the plasma CCK response to oral fat was significantly greater in gastrectomized patients than in normal subjects. The mechanisms for this exaggerated plasma CCK response in patients with gastrectomy is unknown, but it may be related to the rapid gastric emptying in such patients.[17] A surprising finding was that the plasma CCK secretion after oral fat was similar in patients with Billroth II and Billroth I gastrectomy. This finding indicates that large amounts of CCK can be released from the jejunum, and that bypassing the duodenum does not affect the CCK secretion after ingestion of fat. It is not known whether the high postprandial plasma CCK concentrations in gastrectomized patients induce clinical symptoms. Infusion of high doses of CCK stimulates small intestinal motility.[18] It may be possible that abdominal cramps and diarrhea after feeding in some gastrectomized patients result, at least in part, from the high circulating CCK concentrations. In fact, most gastrectomized patients in the present study complained of abdominal cramps after ingestion of fat. Furthermore, it has been reported that patients with gastrectomy have an increased incidence of gallstones.[11-14] The present study shows that this is not due to an impaired postprandial CCK secretion, as has been suggested previously.[12-15] However, it has been reported that parenteral administration of CCK with meals induces an increase in cholesterol saturation of gallbladder bile in man.[19] In analogy, it may be possible that the high concentrations of endogenous CCK in postprandial plasma from gastrectomized patients are involved in the higher incidence of gallstones in such patients.

There is considerable controversy relating to the molecular forms of CCK in human plasma. It has been suggested that a small molecular form, possibly CCK8, is the predominant form of CCK released after feeding.[6,7] However, in the present study we found large increases in plasma CCK after oral fat when measured with antibody 1703. Since antibody 1703 binds to COOH-terminal CCK peptides containing at least 14 amino acid residues, it is evident that large or intermediate forms of CCK are released after oral fat. It has recently been shown by high pressure liquid chromatography combined with radioimmunoassay that both large and small forms of CCK are released after ingestion of a liquid fat meal.[20] Furthermore, it has been reported that large and intermediate forms of CCK are the predominant molecular forms of CCK released during infusion of bombesin into humans.[21] The presence of large molecular forms of CCK in the circulation is of physiological relevance, since it has recently been shown that, in contrast to previous

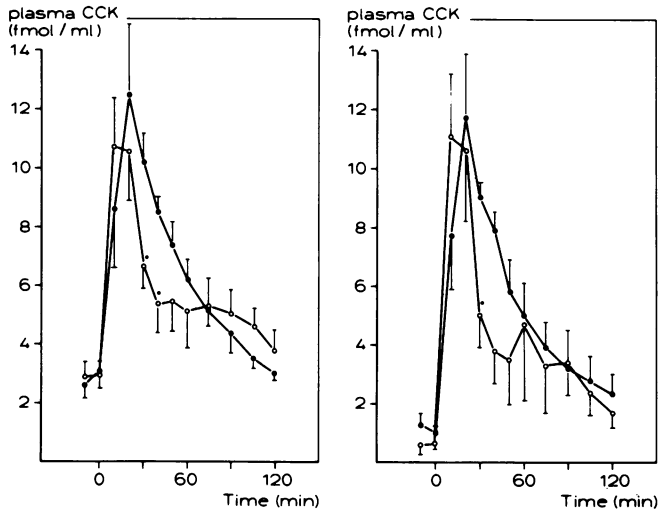

FIG. 3. Plasma cholecystokinin response to oral fat in seven patients with Billroth II gastrectomy (● —— ●) and in six patients with Billroth I gastrectomy (○ —— ○) as measured with antibody T204 (left panel) and antibody 1703 (right panel). Asterisks indicate significant differences between the two groups.

reports, CCK33 and CCK8 have similar potencies in contracting the gallbladder and stimulating pancreatic enzyme secretion.[22,23]

It is concluded that patients with partial gastrectomy show an exaggerated plasma CCK response to ingestion of fat and that the plasma CCK response in patients with Billroth II gastrectomy is similar to that in patients with Billroth I gastrectomy.

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