Meal-related changes in plasma CCK bioactivity in patients with chronic pancreatitis

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Abstract

In order to clarify whether there is a negative feedback mechanism for CCK secretion, we investigated plasma CCK bioactivity in patients suffering from chronic pancreatitis (CP) according to the characteristics of their pancreatic disease.

Basal, meal-stimulated, and integrated release of plasma cholecystokinin (CCK) bioactivity was measured in 24 patients with CP and in 12 healthy controls. The values obtained were compared between the healthy control group and the CP group, and between subgroups of CP patients established on the basis of the presence/absence of several parameters : abnormal gastric emptying, abdominal pain, steatorrhea, pancreatic calcification, insulin-requiring diabetes mellitus, and impairment of pancreatic exocrine functions as indicated by secretin test. A bioassay method using pancreatic acini was used to measure plasma CCK bioactivity. In the control group, plasma CCK bioactivity increased from a basal value of 1.6 ± 0.7 pmol/L to a maximal increase of 6.6 ± 4.1 pmol/L, and the integrated CCK release following a test meal was 37.7 ± 19.3 pmol/L · 150 min. In the CP group, plasma CCK bioactivity increased from 1.6 ± 0.9 pmol/L to a maximal increase of 8.2 ± 8.7 pmol/L, and the integrated release of CCK was 43.0 ± 37.7 pmol/L · 150 min. None of the differences between them were significant. No significant differences in basal value, maximal increase, or integrated plasma CCK release were noted according to any of the parameters of the CP patients and the control group. Nor was there any correlation between impairment of pancreatic exocrine function and plasma CCK bioactivity. These results provide no evidence of a negative feedback mechanism between pancreatic exocrine dysfunction and CCK secretion. (Acta gastroenterol. belg., **1998**, 61, **400-406**).

Key words : chronic pancreatitis, plasma CCK, gastric emptying, pancreatic pain, steatorrhea, diabetes mellitus, pancreatic exocrine function.

Introduction

The relationship between CCK and the various stages of chronic pancreatitis (CP) has often been studied. Plasma CCK levels have been found to be elevated in CP patients (1,2), in CP patients with abdominal or back pain (3,4), and in CP patients who have moderate impairment of pancreatic exocrine function. Conversely, plasma CCK levels are shown to be normal or reduced in patients with painless or advanced pancreatic exocrine insufficiency (4-11). In CP patients with increased plasma CCK levels, a feedback mechanism appears to exist between CCK secretion and tryptic activity in the small intestine. However, it has also been reported that there is no correlation between the severity of impairment of pancreatic exocrine function and CCK secretion (7-11). No agreement has yet been reached on certain points, such as the regulation of CCK secretion in relation to particular stages of chronic pancreatitis, or whether a correlation exists between CCK secretion and the development or aggravation of pathologic changes in this disease. To identify the determinants of basal and meal-stimulated plasma CCK bioactivity, we subdivided the CP patients into groups according to the presence/absence of seven parameters : abnormal gastric emptying, abdominal pain, steatorrhea, pancreatic calcification, insulin-requiring diabetes mellitus, and impairment of pancreatic exocrine function, including maximal bicarbonate concentration and amylase output according to the results of a secretin test.

Subjects and methods

A total of thirty-six subjects — 24 CP patients and 12 normal controls with no gastrointestinal disease or diabetes mellitus — were included in this study. CP was diagnosed on the basis of the following criteria : 1) dilatation and/or stenosis of the main pancreatic duct, as determined by ERCP and 2) abnormal pancreatic exocrine function, as determined by a secretin test. A maximal bicarbonate concentration of 86 mEq/L ≦, and a total amylase output of 1000 U/kg/hr ≦ are defined as normal (12). Table I shows the clinical

Table I. — **Clinical features of chronic pancreatitis patients**

No. of Patients, Sex	24, all male	
Age	54.3 ± 11.1 (37-73) yr	
Pancretic calcification	present	16
	absent	8
Insulin-requiring diabetes mellutus	present	13
	absent	11
Abdominal pain	present	8
	absent	16
Steatorrhea	present	9
	absent	15
Delayed gastric emptying	present	9
	absent	15
Pancreatic exocrine function		
max. HCO_3^-	< 30% of normal (< 32.4 mEq/L)	9
	≥ 30% of normal (≥ 32.4 mEq/L)	14
Amylase	< 30% of normal (< 456 U/kg/hr)	9
	≥ 30% of normal (≥ 456 U/kg/hr)	14

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characteristics of the CP patients. All of the patients were male, and their mean age was 54.3 ± 11.1 years (mean ± SD). High alcohol intake (at least 80 g of pure ethanol daily for more than 10 years) was the only causative factor of CP in 20 of them. The duration of the patients' chronic pancreatitis was 6.3 ± 3.5 years. Fifteen patients had concomitant diabetes mellitus, and 13 of them were on insulin therapy (17.1 ± 7.8 U/day). HbA1, an index of glycemic control in diabetes mellitus (normal : < 8.0%), was 9.6 ± 1.1%, showing fair control. Four patients had undergone surgery for relief of pancreatic pain (distal pancreatectomy in 3, and pancreatojejunostomy in 1). Eight patients had abdominal pain at the time of this study. Mean 72-hour fecal fat excretion was 7.0 ± 7.6 g/day. Steatorrhea (defined as daily fecal fat excretion of 5.0 g or more) (13,14) was noted in 9 patients, and the mean fat excretion of these 9 patients was 14.2 ± 8.2 g/day.

The CP patients were subdivided into groups according to the presence or absence of seven parameters (Table I) : 1) gastric emptying rate determined by acetaminophen level at 45 min (4.6 μg/ml ≧ normal) (15) ; 2) abdominal pain ; 3) steatorrhea ; 4) pancreatic calcification ; 5) insulin-requiring diabetes mellitus ; 6) maximal bicarbonate concentration below 30% of the value in the normal controls (< 32.4 mEq/L : severe impairment) ; and 7) amylase output below 30% of normal controls (< 456 U/kg/hr : severe impairment) in a secretin test (12).

During the examination period, which lasted 4-7 days, all medications, such as digestive enzymes, gastrokinetics, bile acid preparations and other drugs, were discontinued. After an overnight fast, the CP patients and controls ingested the test meal. Blood samples were obtained in heparinized tubes in the fasting state and at 10, 20, 30, 45, 60, 90, 120, and 150 min after the test meal. The blood samples were rapidly centrifuged at 4°C to separate the plasma.

The plasma was extracted by a previously described method (6,16). Briefly, 0.5 to 3 ml of plasma was added to a Sep-Pak C-18 cartridge (Nippon Waters, Tokyo, Japan) activated with acetonitrile, ethanol, and water. The mixture was then washed with water, and CCK was eluted with a mixture of acetonitrile : water (1:1 v/v). The extract was dried under a stream of nitrogen.

Isolated pancreatic acini were preincubated for 30 minutes in a 20 mM HEPES-buffered Ringer's solution containing 11.1 mM glucose, 0.01% trypsin inhibitor, 0.5% bovine albumin, essential amino acids, and 300 μM cycloheximide. Following preincubation, 1 ml of isolated pancreatic acini suspension was added to polycarbonate vials containing eluate from plasma or a known quantity of CCK-8, and the mixtures were incubated at 37°C for 30 min. Amylase activity in the supernatant from the suspension of isolated pancreatic acini was determined using Blue Starch (Amylase test A, Shionogi Pharmaceutical Co., Ltd., Osaka, Japan) in order to obtain the amylase release rate (17). Plasma CCK bioactivity was determined from a calibration curve for CCK-8 obtained using the amylase release rate for a known amount of CCK-8, and recorded as CCK equivalents.

The test meal consisted of 2 medium-sized boiled eggs and 400 ml of milk. The meal included a total of 26 g of fat and 420 Kcal and contained acetaminophen 20 mg/kg to assess the gastric emptying rate.

Plasma CCK levels to be compared with bioactivity values were also assayed by radioimmunoassay using an OAL-656 antibody by the previously described method (7).

The coefficient of correlation between the radioimmunoassay and bioassay values was $r = 0.932$ ($n = 13$, $p < 0.01$). Recovery rate of the CCK bioassay was 90.5 ± 4.2% with an intra-assay coefficient of variation of 9.9% and an interassay coefficient of variation of 10.1% (6).

Statistical analyses for non-parametric values were performed by the Mann-Whitney test, and statistical analyses among the three groups were performed by ANOVA. Values are expressed as means ± SD. A P value of 0.05 was considered significant.

Results

1. *Basal plasma CCK bioactivity* (Fig. 1)

The mean basal plasma CCK bioactivity obtained in the healthy controls was 1.6 ± 0.7 pmol/L. Basal plasma CCK bioactivity in the CP patients was 1.6 ± 0.9 pmol/L. The difference between them was not significant. Basal plasma CCK bioactivity in the CP subgroups was as follows : 1) 1.4 ± 0.9 pmol/L in patients with normal gastric emptying ($n = 15$), and 1.9 ± 0.7 pmol/L, in those with delayed gastric emptying ($n = 9$) ; 2) 1.9 ± 0.7 pmol/L in patients with pain ($n = 8$), and 1.5 ± 1.0 pmol/L in painfree patients ($n = 16$) ; 3) 1.5 ± 0.8 pmol/L in the patients with steatorrhea ($n = 9$), and 1.7 ± 1.0 pmol/L in those without steatorrhea ($n = 15$) ; 4) 1.7 ± 1.0 pmol/L in the patients with calcification ($n = 16$), and 1.3 ± 0.6 pmol/L in those without calcification ($n = 8$) ; 5) 1.7 ± 1.1 pmol/L in the patients on insulin therapy ($n = 13$) and 1.5 ± 0.6 pmol/L in those not on insulin ($n = 11$) ; 6) 1.5 ± 1.1 pmol/L in the patients with severe impairment of bicarbonate secretion ($n = 9$), and 1.6 ± 0.7 pmol/L in those with mild impairment ($n = 14$) ; and 7) 1.6 ± 1.3 pmol/L in the patients with severe impairment of amylase output ($n = 9$), and 1.5 ± 0.5 pmol/L in those with mild impairment ($n = 14$). There were no significant differences between basal plasma CCK bioactivity in any of the CP subgroups and the controls.

2. *Maximal increase in plasma CCK bioactivity* (Fig. 2)

The maximal increase in plasma CCK bioactivity in the healthy controls was 6.6 ± 4.1 pmol/L. The maximal increase in plasma CCK bioactivity in the CP

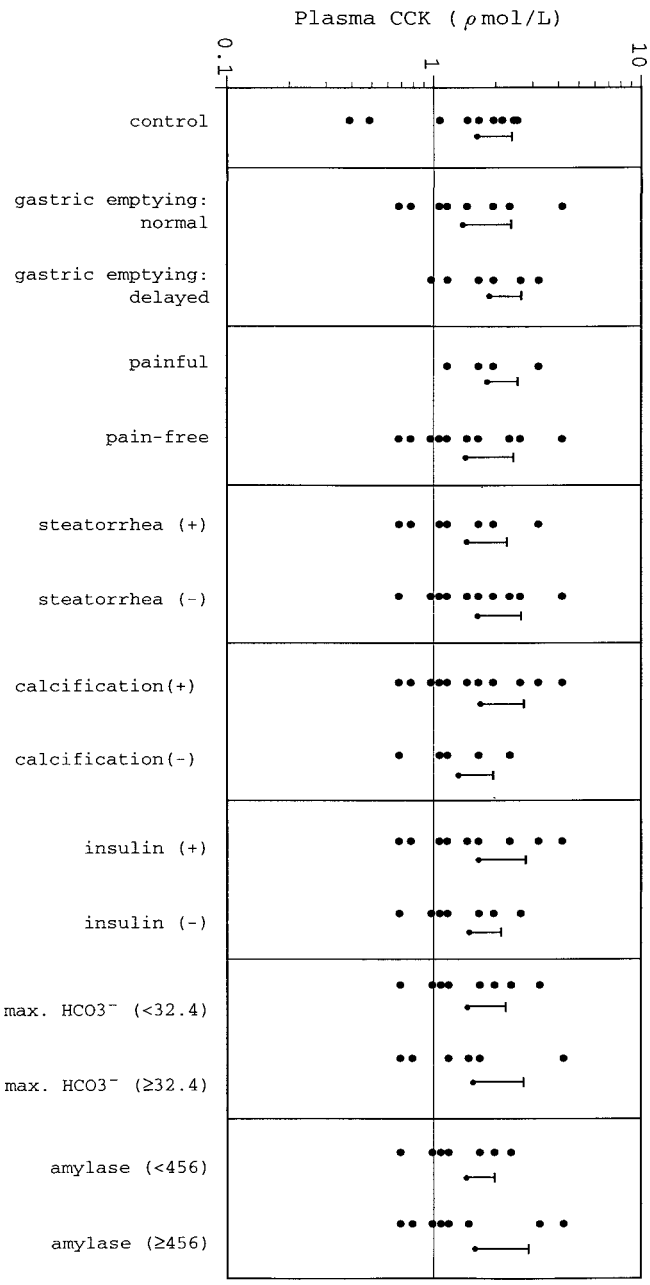

Fig. 1. — Basal plasma CCK bioactivity in chronic pancreatitis patients.

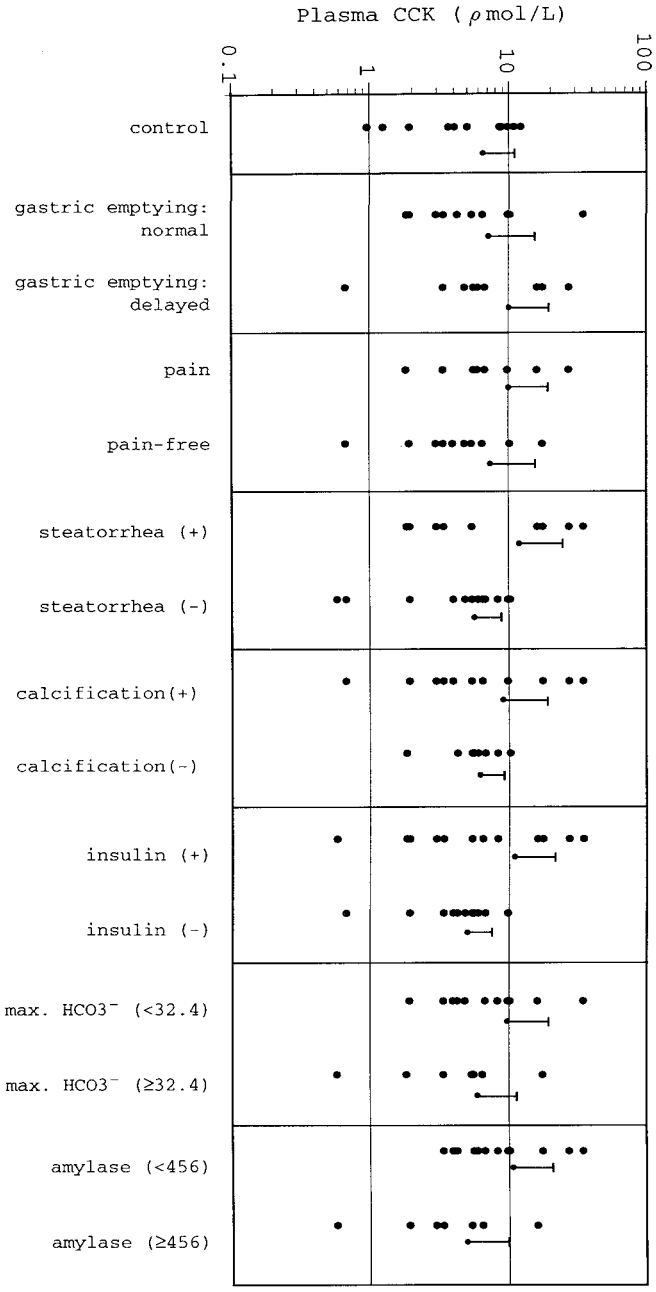

Fig. 2. — Test meal stimulated CCK (maximal increase in plasma CCK) bioactivity in chronic pancreatitis patients.

patients as a whole was 8.2 ± 8.7 ρmol/L. The difference between them was not significant. The following values were obtained in the CP patients : 1) 7.0 ± 8.6 ρmol/L in patients with normal gastric emptying, and 10.2 ± 9.0 ρmol/L in those with delayed gastric emptying ; 2) 10.0 ± 8.7 ρmol/L in patients with pain, and 7.3 ± 8.8 ρmol/L in the pain-free patients ; 3) 11.9 ± 12.3 ρmol/L in patients with steatorrhea, and 5.5 ± 3.1 ρmol/L in those without steatorrhea ; 4) 9.1 ± 10.4 ρmol/L in the patients showing calcification, and 6.3 ± 2.6 ρmol/L in those without calcification ; 5) 10.9 ± 10.9 ρmol/L in patients on insulin therapy, and 5.0 ± 2.5 ρmol/L in those not on insulin ; 6) 5.7 ± 5.1 ρmol/L in patients with severe impairment of bicarbonate secretion, and 10.3 ± 10.1 ρmol/L in those with mild impairment ; 7) 5.1 ± 4.8 ρmol/L in patients with severe impairment of amylase output and 10.4 ± 10.1 ρmol/L in those with mild impairment. There were no significant differences in the maximal increase in plasma CCK bioactivity between any of the CP patients subgroups and the control group.

3. *Integrated release of CCK* (Fig. 3)

The integrated release of CCK following stimulation with a test meal was 37.7 ± 19.3 ρmol/L · 150 min

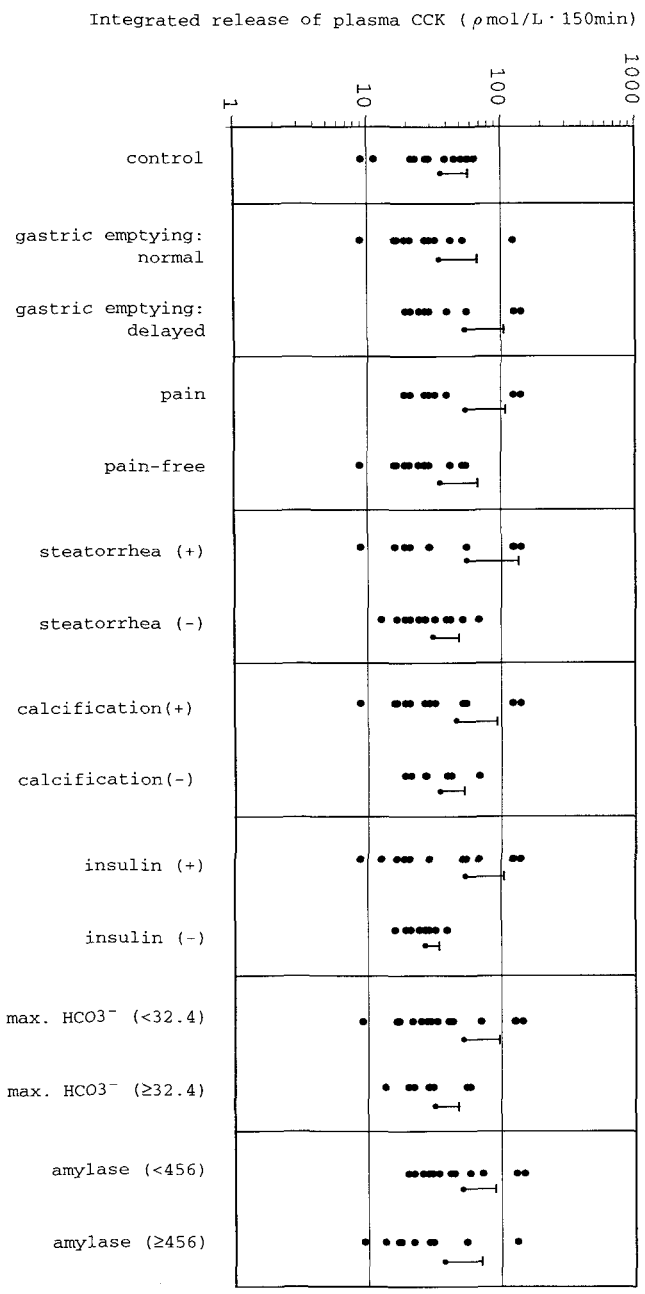

Fig. 3. — Integrated CCK release (CCK pmol/L · 150 min) following the test meal in chronic pancreatitis patients.

in the healthy controls. In the CP patients, the integrated release of CCK in response to the test meal was 43.0 ± 37.7 pmol/L · 150 min. The difference between them was not significant. The following values were obtained in the CP patients (all units pmol/L · 150 min) : 1) 35.8 ± 29.7 in the patients with normal gastric emptying, 54.9 ± 47.7 in those with delayed gastric emptying ; 2) 55.7 ± 50.4 in the patients with pain, 36.7 ± 29.3 in the pain-free patients ; 3) 58.2 ± 53.3 in patients with steatorrhea, 32.1 ± 15.4 in those without steatorrhea ; 4) 47.0 ± 44.7 in patients showing calcification, 34.9 ± 16.6 in those without calcification ; 5) 56.4 ± 47.5 in patients on insulin therapy, 27.1 ± 6.5 in those not on insulin ; 6) 28.9 ± 16.5 in patients with severe impairment of bicarbonate secretion and 53.8 ± 45.2 in those with mild impairment ; 7) 35.2 ± 37.2 in patients with severe impairment of amylase output, 47.7 ± 38.5 in those with mild impairment. There were no significant differences in the integrated release of plasma CCK among any of the subgroups of patients and the control group.

4. *Correlation between plasma CCK bioactivity and pancreatic exocrine function*

The correlation coefficients between plasma CCK bioactivity and maximal bicarbonate concentration in the secretin test were r = 0.360 for basal CCK bioactivity, r = 0.234 for maximal increase of CCK, and r = 0.409 for integrated release of CCK bioactivity, with no significant differences.

Correlation coefficients between plasma CCK bioactivity and pancreatic amylase output in the secretin test were r = 0.139, r = 0.090 and r = 0.231, respectively, and again there were no significant differences.

Discussion

Ever since a test to measure plasma CCK levels was established, the system regulating CCK secretion has been extensively studied in rats, and has been shown in an increase in CCK secretion of experimental pancreatic atrophy induced in rats (18), suggesting a that a negative feedback mechanism exists between intestinal trypsin activity and plasma CCK levels.

In regard to the presence of a regulatory system for CCK secretion in humans, however, no consensus has yet been reached as to whether CCK secretion is regulated by intestinal trypsin activity. Phenylalanine-stimulated increases in plasma CCK levels have been shown to be suppressed by intraduodenal infusion of 0.5-1.0 g/l trypsin in healthy volunteers (19). Slaff *et al.* (4) have reported that the administration of large doses of digestive enzymes to CP patients with pain significantly decreases both pain and basal CCK levels.

Conversely, the plasma CCK levels of patients with severe pancreatic exocrine insufficiency and steatorrhea have been shown to be normal (7-9) or reduced (6,11,20). These findings suggest that the basal CCK levels of CP patients are affected by the reserve capacity of pancreatic exocrine function.

All the reports have concerned the plasma CCK response to a test meal from a single standpoint, such as pancreatic pain, pancreatic exocrine insufficiency (steatorrhea), etc. We have investigated the meal-related plasma CCK response of CP patients taking a variety of factors into consideration. That is, we investigated plasma CCK bioactivity according to many determinants of CCK secretion in CP patients, in order to clarify whether there is a negative feedback mechanism for CCK secretion.

There have been a few reports concerning the relation between CCK release and gastric emptying function, and delivery to the duodenum of the major part of a liquid diet and a small amount of a solid diet has been found to be capable of inducing near-maximal stimulation of CCK release (21). In the present study, plasma CCK bioactivity was not influenced by gastric emptying function. Therefore, CCK release is thought to be induced when a small amount of diet (probably liquid) is emptied into the duodenum.

The severity of pancreatic exocrine insufficiency, as judged by the presence of steatorrhea, abdominal/back pain, development of concomitant diabetes mellitus, and the degree of delay in gastric emptying, influence the secretion of CCK bioactivity in CP patients. In the present study, basal levels and maximal increases of plasma CCK bioactivity following the ingestion of a test meal were measured in 24 CP patients by bioassay, and the results were analyzed according to factors including gastric emptying, pain, pancreatic exocrine function including steatorrhea, and insulin-requiring diabetes mellitus.

Patients with pancreatic pain have been shown to have elevated plasma CCK levels both basally and after the ingestion of a meal (3,4), and the administration of a large dose of a pancreatic enzyme preparation has been reported to decrease both basal CCK levels (4) and pain (4,22,23,24). These findings suggest that a negative feedback mechanism may exist between intestinal trypsin activity and CCK release into the blood. Furthermore, the suppression of CCK secretion by intraduodenal trypsin as well as the patient's own bile-pancreatic juice has been observed. Based on these findings, Ihse *et al.* (25) hypothesized that trypsin in the upper part of the intestine regulates pancreatic secretion in humans by means of a negative feedback mechanism. In support of this hypothesis, patients with non-calcified pancreatitis have been shown to have higher plasma CCK levels than either normal subjects or patients with calcified pancreatitis (6). In the present study, however, no significant differences were found between the plasma CCK levels determined by bioassay in patients with painful pancreatitis and patients with non-painful pancreatitis or between those with calcified and non-calcified pancreatitis. In particular, no significant differences in either basal or maximal increase in CCK bioactivity were noted between patients in the above groups and the healthy controls. Thus our findings appear to indicate the absence of any correlation between plasma CCK bioactivity and pancreatic pain due to pancreatitis. Both the basal plasma CCK levels and levels following the ingestion of a test meal were found to be normal or below normal in patients with advanced pancreatitis. There are two possible explanations for these findings. First, neutral fats in the meal are not hydrolyzed to fatty acids in the duodenum because of severe impairment of pancreatic exocrine function, and thus CCK release is not stimulated (8,11,20). Second, exhaustion of CCK-releasing cells as a result of the long-term stimulation of CCK release may explain the decreased plasma CCK levels in patients with advanced pancreatitis (6).

CCK secretion is thought to be reduced in CP patients who have developed diabetes mellitus (5,26), and Nakano *et al.* (5) have shown that patients with diabetes mellitus as a complication of chronic pancreatitis have lower plasma CCK levels (both basal and post-ingestion) than those without diabetes mellitus. In contrast, we could not find any significant differences in plasma CCK bioactivity between patients on insulin therapy and those who were not. Nor were any significant differences found in basal and meal-induced CCK bioactivity between patients with severe pancreatic exocrine insufficiency with steatorrhea and those without steatorrhea, or between those with severe impairment of bicarbonate concentration and amylase output and mild impairment in a secretin test in this study.

The method of measuring CCK, bioassay or radioimmunoassay (RIA), is thought to be one of the principle factors responsible for the variation in plasma CCK levels. Nearly all researchers have used Sep-Pak C-18 cartridges for extraction/purification of CCK in plasma in the bioassay. And with this method plasma basal CCK levels of healthy volunteers have been found to range between 0.75 and 1.3 ρmol/L (6,8,19,27). Our values for plasma basal CCK bioactivity were comparable to those reported in previous studies using similar methods. When RIA methods are used, plasma CCK levels vary significantly depending upon the type of antibodies employed. For example, when the CCK level is calculated as the difference between the value obtained with antibody 06, which reacts with both gastrin and CCK, and 56-02 antibody, which reacts with gastrin alone, the plasma basal CCK is found to be 14.3 fmol/ml (ρmol/L) (4). In Europe and the United States, where antibody G-160 has been used, large variations (1.2 - 8.0 ρmol/L) in values have been noted even in healthy volunteers (9-11,28). In Japan, Funakoshi *et al.* (3,5,30) have measured plasma CCK levels with antibody OAL-656 (29), which reacts with the N-terminal residues of CCK and have reported levels of 9.2-12.9 ρg/ml. Thus, the assay method is an important determinant of measured CCK concentrations. Nevertheless, we obtained a close correlation between plasma CCK levels measured by plasma CCK bioassay and by the RIA method. Thus, both methods of CCK measurement appear to be accurate.

The type and the composition of the test meal is also an important factor in determining plasma CCK levels. Cantor *et al.* (9) have reported similar peak plasma CCK levels in healthy volunteers irrespective of whether liquid or solid food is ingested. However, liquid food, which is emptied earlier than solid food, is thought to induce a more rapid increase in the plasma CCK level. In addition, the fat content of the test meal is thought to have a significant influence on CCK release. Patients and normal controls are given 30 g of fat in clinical studies performed in Europe and the

United States (11,28). In our study the subjects received 26 g of fat. The latter value is approximately one-third the daily fat intake of Japanese, whose daily intake is approximately 60 g (14,31,32), and thus considered to be suitable for fat loading. Funakoshi *et al.* (3,20) have given 5.2 g of fat to patients. This amount of fat may be too small to induce CCK release, although fat is known to be as powerful a stimulant of CCK release in humans as amino acids (33). In fact, the peak CCK levels in the healthy volunteers in their study were only 1.5-2.4 times higher than the basal levels.

In contrast, our results for maximal increase in CCK were 4.1 fold in healthy controls and 5.1 fold in CP patients. These data are similar to those reported by other researchers (6,8,11,19).

In conclusion, our findings suggest that none of the various factors analyzed, including pancreatic pain, steatorrhea, pancreatic exocrine function (directly evaluated by a secretin test), diabetes mellitus as a complication of pancreatitis, or delayed gastric emptying, individually affected basal plasma CCK levels or levels following the ingestion of a test meal. However, interactions between some of these factors may have affected CCK secretion. Thus, no evidence was found of a negative feedback mechanism between CCK secretion and pancreatic exocrine function. Layer *et al.* (34) has reported that luminal protease-mediated feedback regulation of pancreatic secretion may be operative in healthy humans, since it is not affected by the stimulation of plasma cholecystokinin level. Further study of the relationship between cholinergic mechanisms or bile salts in the duodenum and a feedback mechanism of CCK release other than pancreatic exocrine insufficiency is necessary (35).

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