Oral Glucose Ingestion Stimulates Cholecystokinin Release in Normal Subjects and Patients With Non–Insulin-Dependent Diabetes Mellitus

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The role of glucose in the regulation of plasma cholecystokinin (CCK) level was investigated in healthy control subjects and patients with non–insulin-dependent diabetes mellitus (NIDDM). Plasma CCK concentration was determined by a specific and sensitive bioassay and by a highly sensitive and reliable double-antibody radioimmunoassay using OAL-656 as an antiserum. In control subjects, ingestion of Trelan G-75 (1,200 mOsm/L, 225 mL), which is equivalent to 75 g glucose as metabolic products, caused a rapid and significant increase in plasma CCK bioactivity from 1.3 ± 0.2 to a peak of 5.8 ± 0.6 pmol/L and immunoreactive CCK concentration from 1.2 ± 0.1 to 4.6 ± 0.6 pmol/L. Ingestion of 75 g glucose in 225 mL water (33.3% solution) increased plasma CCK bioactivity to a similar degree to that observed following Trelan G-75 (peak response, 4.5 ± 0.4 pmol/L). The same volume of 0.9% NaCl solution or water failed to increase plasma CCK concentration. A smaller dose of glucose (50 g/150 mL water) increased plasma CCK concentration, although the peak level (3.0 ± 0.5 pmol/L) was less than that observed following 75 g glucose. In patients with NIDDM, Trelan G-75 ingestion increased CCK concentration, but the peak level was lower, albeit insignificantly, than that of normal subjects. When the maximal increment of plasma CCK above the basal value was compared between control and NIDDM subjects, the differences were statistically significant (NIDDM, 3.6 ± 0.1 pmol/L; control, 5.0 ± 0.4; $P < .01$). However, integrated CCK responses to Trelan G-75 in NIDDM (165.8 ± 15.5 pmol/120 min) were not significantly different from those in control subjects (189.8 ± 15.9 pmol/120 min). Peak CCK bioactivity occurred within 10 to 30 minutes of ingestion, preceding the increase in glucose and insulin. These results suggest a possible effect of CCK on insulin release in humans, and that the CCK secretory response to glucose in well-controlled diabetic patients is not significantly altered.

PERIPHERAL INSULIN concentrations are considerably higher following oral versus intravenous glucose, whether it is given in equal doses or at rates that provide equal serum glucose levels.[1,2] It is generally accepted that intestinal factors released after food ingestion sensitize B cells to the stimulant action of glucose or amplify the insulin secretory response to glucose via the enteroinsular axis.[3] This phenomenon is known as the incretin effect.[4] Several gastrointestinal hormones are thought to be involved in the enteroinsular axis. Although most attention has been focused on the role of gastric inhibitory polypeptide (also referred to as a glucose-dependent insulinotropic peptide),[4-6] accumulating evidence suggests that cholecystokinin (CCK) may play an important role in this phenomenon.[7,8]

CCK is a well-characterized gastrointestinal hormone released into the circulation from endocrine cells in the mucosa of the upper small intestine during meal intake,[9-11] and it regulates pancreatic enzyme secretion, gallbladder contraction, gastric emptying, and bowel motility.[10,12-15] In addition, both in vivo and in vitro studies in different animals have suggested that CCK stimulates release of insulin and other islet hormones.[6-8,16-19] Moreover, quantitative electron microscopic autoradiography demonstrated that ^{125}I-CCK specifically binds to B cells in the islets.[20] The existence of specific CCK receptors in pancreatic islets has also been shown by a binding study.[18] The finding of a complete suppression of CCK-stimulated insulin secretion by recently developed potent and specific CCK_A receptor (peripheral) antagonists[18,19,21,22] further supports a direct insulin-releasing effect of CCK. These findings strongly suggest that CCK plays an important role in insulin release by binding to its own receptor on B cells. However, whether CCK acts as an insulinotropic hormone in man is less clear. Furthermore, it is not clear whether CCK is released by glucose, a prerequisite for a gut peptide to qualify as an incretin.[4] Therefore, in the present study, we measured plasma CCK concentration after glucose ingestion in normal subjects and patients with non–insulin-dependent diabetes mellitus (NIDDM) using a sensitive bioassay based on amylase release from isolated rat pancreatic acini,[9,11,23] and also by a recently developed highly sensitive and reliable radioimmunoassay.[24]

SUBJECTS AND METHODS

Materials

The following materials were purchased: synthetic CCK octapeptide (CCK-8) from Peptide Research Institute (Osaka, Japan); soybean trypsin inhibitor (type 1-S) from Sigma Chemical (St Louis, MO); chromatographically purified collagenase from Worthington Biochemicals (Freehold, NJ); minimal Eagle's medium amino acid supplement from Grand Island Biological (Grand Island, NY); atropine sulfate, cycloheximide, and HEPES from Nakarai Tesque (Kyoto, Japan); bovine plasma albumin fraction V from Armour Pharmaceutical (Phoenix, AZ) and Miles Laboratories (Elkhart, IN); octadecylsilysilica cartridges (Sep-Pak C-18) from Waters Associates, Millipore (Milford, MA); Trelan G-75, which consists of 34% glucose, 14% polysaccharide, 36% maltose

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plus isomaltose, and 16% oligosaccharide and is equivalent to 75 g glucose as metabolic products, from Shimizu Pharmaceutical (Shizuoka, Japan); CCK-8 *N*-terminus specific rabbit antiserum OAL-656 from Otsuka Assay Laboratories, Otsuka Pharmaceutical (Tokushima, Japan); [^{125}I]-Bolton Hunter–labeled CCK-8 ([^{125}I]-CCK-8, specific radioactivity, 81.4 TBq/mmol) from Du Pont, Biotechnology System (Wilmington, DE); the glucose kit, Glucose-E reagent, from International Reagents (Kobe, Japan); and the insulin kit, ShionoRIA Insulin, and blue-dyed starch polymer, Amylase Test A, from Shionogi Pharmaceutical (Osaka, Japan).

CCK_A receptor antagonist loxiglumide (CR 1505) was a generous gift from Kaken Pharmaceutical (Tokyo, Japan).

Subjects

Ten healthy control subjects with no family history of diabetes mellitus and seven NIDDM patients on oral hypoglycemic agents participated in the study. The male to female ratio was 4:6 in controls and 3:4 in NIDDM patients. The mean age was 55 years (range, 44 to 63) for NIDDM patients and 40 years (range, 29 to 53) for normal subjects. The mean body mass index (weight in kilograms divided by height in meters squared) was 22.8 ± 0.7 in control subjects and 24.9 ± 1.4 in NIDDM patients. NIDDM patients tended to be older and heavier than normal subjects, but the differences were insignificant. All NIDDM patients received oral hypoglycemic agents (glibenclamide 0.625 to 7.5 mg/d), which were withheld for 24 hours before the study. It has been demonstrated that the biological half-life of orally administered glibenclamide is 5 hours, with no accumulation for a dosage interval of 24 hours.[25] Clinical features of the NIDDM patients are summarized in Table 1.

Experimental Design

The study protocol was approved by the Institutional Ethics Review Committee for Human Experimentation, and informed consent was obtained from each subject. After an overnight fast, patients and control subjects ingested Trelan G-75 (225 mL), which is equivalent to 75 g glucose as metabolic products and is widely used for the oral glucose tolerance test in Japan. Blood samples for measurements of serum glucose, serum insulin, and plasma CCK levels were withdrawn from the antecubital vein at specified time points after ingestion of Trelan G-75. Blood samples for determination of CCK bioactivity were obtained in iced heparinized tubes.

Five control subjects underwent three additional studies in random order separated by a minimum of 1 week, and concentrations of blood glucose and serum insulin and plasma CCK bioactivity were determined serially after oral ingestion of (1) 75 g D-glucose in 225 mL water (33.3%), (2) 50 g D-glucose in 150 mL water (33.3%), or (3) 225 mL of 0.9% NaCl solution.

Because CCK bioactivity in the blood may depend on more than one peptide with CCK-like activity and since possible interactions with CCK of other agents present in plasma extracts cannot be excluded, we determined plasma CCK concentrations using a specific and sensitive radioimmunoassay.[24] For this purpose, blood samples were placed into tubes containing 500 KIU aprotinin and 1.2 mg EDTA per 1.0 mL blood from control subjects before and after ingestion of 225 mL Trelan G-75 or water.

Bioassay of Plasma CCK

Plasma CCK bioactivity was measured using a highly specific and sensitive bioassay as described previously.[11,23] This assay is based on the ability of CCK to stimulate amylase release from isolated rat pancreatic acini. The method is sensitive for detection of plasma CCK levels as low as 0.17 pmol/L. Recoveries of 3 and 10 fmol CCK-8 added to CCK-free plasma were 93.3% ± 3.3% and 90.5% ± 4.2%, respectively. The coefficient of variation between assays was 10.1%, and within assay, 9.1%.

To confirm that changes in measured bioactivity were due to changes in CCK only, two additional experiments were performed. First, serial dilutions of plasma obtained 10 minutes after ingestion of Trelan G-75 were compared with the dose-response curve of CCK-8–stimulated amylase release. Second, CCK_A receptor antagonist loxiglumide (100 μmol/L),[26] muscarinic receptor antagonist atropine (100 μmol/L), or glibenclamide (0.1 μmol/L) were added to the plasma extracts from the Sep-Pak C-18 cartridge to examine the possibility that plasma obtained after Trelan G-75 contains factors other than CCK that may stimulate amylase release from isolated rat pancreatic acini.

Radioimmunoassay of Plasma CCK

Plasma CCK concentration was measured by double-antibody radioimmunoassay using OAL-656 as an antiserum,[27] [^{125}I]-CCK-8 as a tracer, and CCK-8 as a standard. Antiserum OAL-656 is specific for the CCK-8 aminoterminus and thus recognizes all biologically active forms of CCK. The antibody does not bind to CCK-4, nonsulfated CCK-8, or nonsulfated gastrin-17. Cross-reactivity with sulfated gastrin-17 was 0.18%.[28] CCK was extracted from 1.5 mL plasma by adsorption onto Sep-Pak C-18 cartridges and eluted with 3.0 mL 80% acetonitrile containing 0.5% trifluoroacetic acid into dichlorodimethylsilane-coated tubes. Eluants were dried under a nitrogen stream at 40°C and reconstituted in assay buffer to the original volume before assay. Recoveries of 4.5 and 15 fmol CCK-8 added to CCK-free plasma were 85% and 105%, respectively. The detection limit of the assay with 95% confidence was 0.67 pmol/L. Intraassay and interassay coefficients of variation were 6.1% and 8.9%, respectively. Preliminary results indicated that glibenclamide at a concentration of 0.1 μmol/L had no influence on the assay system.

Table 1. Clinical Features of Patients With NIDDM

Patient No.	Age/Sex	BMI	HbA_{1c} (%)	Duration (yr)	Medication	FBG (mg/100 mL)	Plasma CCK (pmol/L)	
							Basal	Peak
1	44/F	25.9	8.0	4	SU	134	0.7	5.8
2	48/F	27.9	8.0	10	SU	118	0.7	4.0
3	63/F	24.0	8.0	1	SU	128	1.6	3.7
4	56/M	22.6	7.4	18	SU	155	1.3	4.6
5	57/M	26.6	7.0	11	SU	178	1.1	5.2
6	60/M	18.1	7.9	5	SU	135	1.0	4.7
7	60/M	29.3	9.8	6	SU	101	1.5	4.9
Mean ± SE	55.4 ± 2.6	24.9 ± 1.4	8.6 ± 3.0	7.9 ± 2.1		135.6 ± 9.4	1.1 ± 0.1	4.7 ± 0.3

NOTE. No subjects had an abnormal Schellong test or retinopathy and nephropathy.

Abbreviations: BMI, body mass index (kg/m^2); HbA_{1c}, hemoglobin A_{1c} (normal range, 4.1% to 6.0%); SU, sulfonylurea; FBG, fasting blood glucose.

Other Assays

Amylase activity was measured using a chromogenic method with the Amylase Test A.[29] Blood glucose levels were determined by the glucose oxidase method[30] using the glucose kit, and serum concentrations of immunoreactive insulin were measured with a radioimmunoassay using a double-antibody technique.[31]

Data Analysis

The data are expressed as the mean ± SE. The integrated CCK output was calculated according to the method of Stern and Walsh.[32] It represents the area under the curve formed by the stimulated values minus the basal output over the time of the stimulation period investigated. Statistical analysis was performed using Student's *t* test for unpaired samples. Differences with *P* less than .05 were considered statistically significant.

RESULTS

Normal Subjects

Ingestion of Trelan G-75 (225 mL, 1,200 mOsm/L) by control subjects increased mean serum concentrations of glucose and insulin from basal values of 88.3 ± 2.0 mg/100 mL and 8.1 ± 1.7 μU/mL, respectively, to respective peaks of 157.2 ± 1.7 mg/100 mL and 41.7 ± 5.7 μU/mL (Fig 1A and B). Furthermore, plasma CCK bioactivity increased from a basal level of 1.2 ± 0.2 pmol/L (CCK-8 equivalent) to a mean peak value of 5.8 ± 0.6 pmol/L at 5 minutes and then gradually decreased to the basal value (Fig 1C).

Serial dilutions of plasma obtained 10 minutes after ingestion of Trelan G-75 paralleled the dose-response curve of CCK-8–stimulated amylase release. In addition, glibenclamide had no influence on amylase release from isolated rat acini (data not shown). Since the CCK receptor antagonist loxiglumide completely blocked CCK bioactivity in plasma extracts, it is unlikely that CCK bioactivity in the blood was overestimated due to the presence of more than one peptide with CCK activity or to interactions between CCK peptides and other peptides that can affect the exocrine response of the pancreas (Table 2). In addition, plasma CCK concentrations determined by radioimmunoassay[24] were similar to those determined by bioassay (Fig 2).

Results of radioimmunoassay also demonstrated a significant increase in plasma CCK level from 1.2 ± 0.1 pmol/L to a peak level of 4.6 ± 0.6 pmol/L within 5 minutes of ingesting Trelan G-75 (Fig 3). Ingestion of the same volume (225 mL) of water induced a small and insignificant increase in plasma CCK level (basal, 1.5 ± 0.3 pmol/L; peak, 2.5 ± 0.4 pmol/L; NS). Based on these observations, plasma CCK concentrations in the following study were determined by bioassay.

To examine whether ingestion of glucose had a similar effect on CCK release compared with that of Trelan G-75, 75 g D-glucose in the same volume (225 mL as 33.3% glucose solution) was administered to the same control subjects. D-Glucose caused a rapid and significant increase in CCK bioactivity from a fasting value of 1.0 ± 0.1 pmol/L to a peak of 4.5 ± 0.4 pmol/L. These changes were comparable to those induced by Trelan G-75 (Fig 4 *v* Fig 1C).

To examine the effect of different doses of glucose on

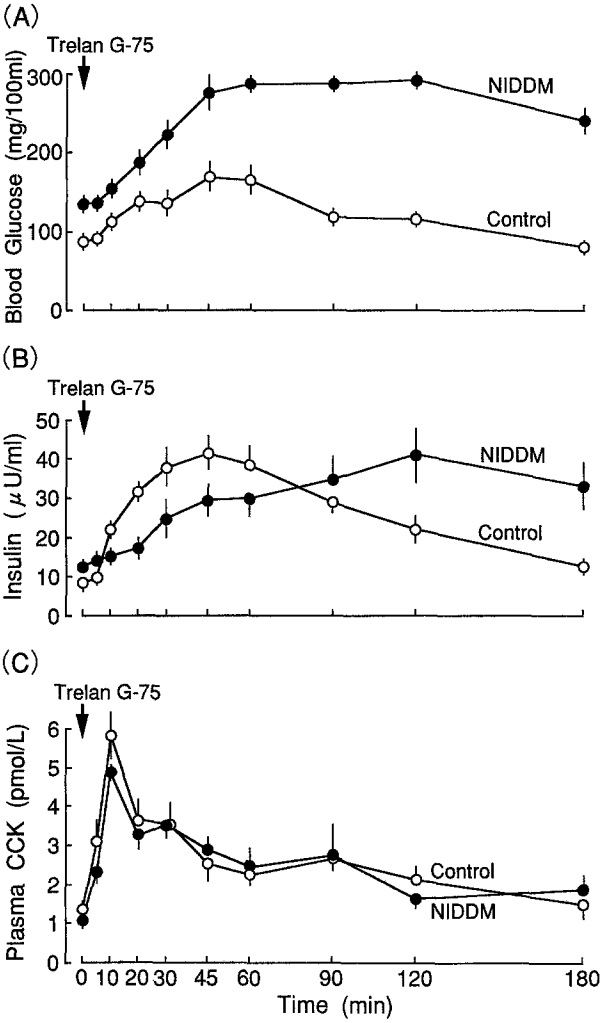

Fig 1. Serum concentrations of glucose (A) and insulin (B) and CCK bioactivity in plasma (C) before and after ingestion of Trelan G-75 in healthy control subjects and patients with NIDDM. After an overnight fast, 6 control subjects and 7 NIDDM patients received 225 mL Trelan G-75, which is equivalent to 75 g glucose. Each value is the mean ± SE.

CCK release, the same concentration of D-glucose solution (33.3%) but in a smaller amount (50 g D-glucose in 150 mL) was used in the next series of experiments. Ingestion of 50 g glucose induced a threefold increase in plasma CCK concentrations (basal, 1.0 ± 0.1 pmol/L; peak, 3.0 ± 0.5 pmol/L; $P < .01$). Furthermore, the peak and integrated increment of CCK release over 60 minutes in response to 50

Table 2. Effects of the Muscarinic Receptor Antagonist, Atropine, or the CCK Receptor Antagonist, Loxiglumide, on CCK Bioactivity in Plasma Extracts

	Without Antagonists	Plus Atropine (100 μmol/L)	Plus Loxiglumide (100 μmol/L)
CCK bioactivity (pmol/L)	5.2 ± 0.6	5.5 ± 0.6	UD

NOTE. Pancreatic acini were incubated with 2 mL plasma extracts in the presence or absence of either 100 μmol/L atropine or 100 μmol/L loxiglumide. Results are the mean ± SE of 4 determinations.

Abbreviation: UD, undetectable (<0.17 pmol/L).

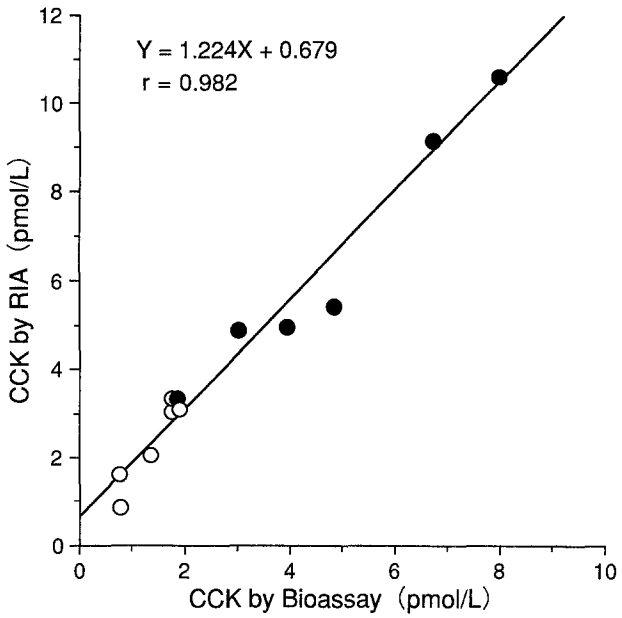

Fig 2. Relationship between plasma CCK concentrations determined by bioassay and radioimmunoassay (RIA) methods. Determinations were made on plasma samples from control subjects in the fasting state (○) and after glucose ingestion (●).

g glucose were significantly less than for 75 g glucose (Fig 5). Physiological saline in a volume of 225 mL had no influence on plasma bioactive CCK concentrations (basal, 1.2 ± 0.2; peak, 1.7 ± 0.3 pmol/L; NS).

Patients With NIDDM

We also examined plasma CCK response to Trelan G-75 in patients with NIDDM. Serum glucose and insulin responses to Trelan G-75 in these patients were delayed as compared with those in healthy control subjects, reaching peak levels 60 to 120 minutes after ingestion. Serum glucose concentrations were significantly higher at all time points as compared with control values. On the other hand, the early insulin response (10 to 45 minutes) to Trelan G-75 was significantly low, although the late response (120 to 180 minutes) was significantly higher, as compared with those in

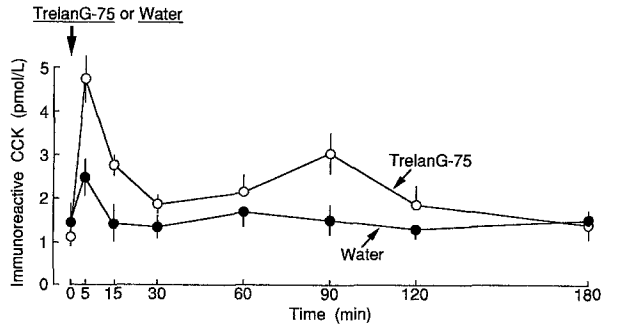

Fig 3. Plasma concentrations of immunoreactive CCK before and after ingestion of Trelan G-75 (○) or water (●). After an overnight fast, 4 control subjects were given Trelan G-75 in a volume of 225 mL or the same volume of water. Each value is the mean ± SE.

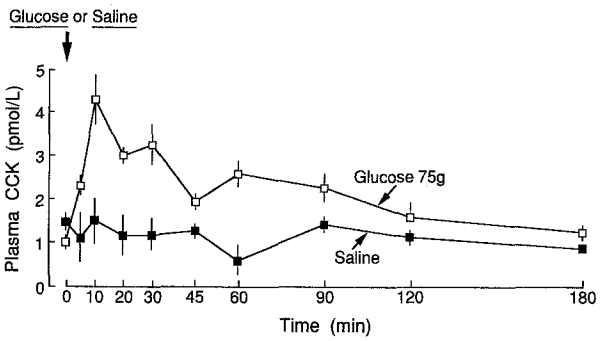

Fig 4. CCK bioactivity in plasma before and after ingestion of 75 g D-glucose (□) or 0.9% saline (■). After an overnight fast, 5 control subjects were given 75 g D-glucose in a volume of 225 mL (33.3%) or the same volume of 0.9% NaCl solution. Each value is the mean ± SE.

control subjects at the corresponding time points (Fig 1A and B). The peak value of serum glucose was significantly higher than that of control subjects (NIDDM, 308.3 ± 12.7 mg/100 mL; control, 157.2 ± 1.7 mg/100 mL; $P < .001$), whereas that of serum insulin was similar to the control value (NIDDM, 45.1 ± 10.1 μU/mL; control, 41.7 ± 5.7 μU/mL; NS). Similar to control subjects, plasma CCK bioactivity in diabetic patients increased rapidly from a mean basal value of 1.1 ± 0.1 pmol/L to a peak of 4.9 ± 0.3 pmol/L 5 minutes after Trelan G-75 ingestion (Fig 1C). Although differences did not reach statistical significance, the mean peak CCK response tended to be lower in patients with NIDDM than in normal subjects. The maximal increment of plasma CCK above the basal value was significantly lower in NIDDM subjects (3.6 ± 0.1 pmol/L) as compared with controls (5.0 ± 0.4 pmol/L, $P < .01$). However, the integrated increment of CCK release over 60 minutes in response to Trelan G-75 in NIDDM patients was similar to that in control subjects (Fig 5). Both basal and Trelan G-75–stimulated peak CCK responses in diabetic patients did not correlate with body mass index, duration of diabetes, diabetic complications, hemoglobin

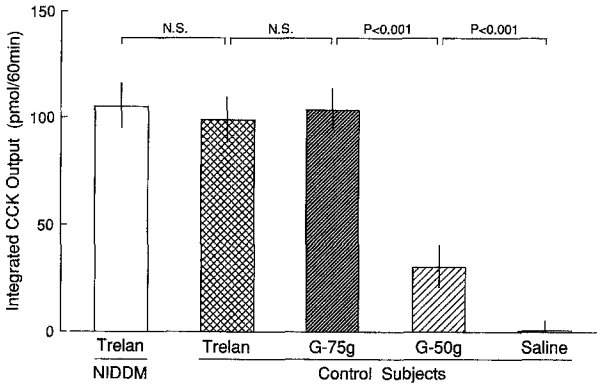

Fig 5. Integrated bioactive CCK output in patients with NIDDM and healthy control subjects 60 minutes after ingestion of Trelan G-75 or different doses of glucose. G-75 g, 75 g D-glucose in 225 mL water (33.3%); G-50g, 50 g D-glucose in 150 mL water (33.3%); saline, 225 mL 0.9% NaCl solution. Integrated CCK output represents the area under the curve calculated by the stimulated values minus basal output over 60 minutes. Each value is the mean ± SE.

A_{1c} levels, and fasting blood glucose concentrations (Table 1).

DISCUSSION

The interaction of enteric factors can explain the greater insulin release after oral versus intravenous administration of glucose.[1-4] It is well established that gastric inhibitory peptide[5,33] and glucagon-like peptide-1(7-36) amide play a role as incretins in humans.[34,35] Although CCK has also been postulated as an incretin because of its insulinotropic effect in experimental animals[6-8,16-19] and the presence of specific CCK receptors on B cells,[18,20] several previous studies failed to verify the glucose-dependent insulinotropic effect of CCK in humans.[5,36-38] Moreover, it remains uncertain whether CCK is released after glucose ingestion.

The recent development of a specific and sensitive bioassay for CCK using isolated rat pancreatic acini[9-12,23] and a sensitive radioimmunoassay using antiserum OAL-656 specific for the CCK-8 aminoterminus[24,27,28] made it possible to estimate fasting and stimulated plasma CCK levels exactly. Our results demonstrated that an oral dose of 75 g D-glucose and Trelan G-75 caused a fourfold to fivefold increase in plasma CCK concentrations in both control subjects and patients with NIDDM. Three separate types of evidence indicate that biologically active CCK was being measured in the present assay. First, serial dilutions of plasma paralleled the dose-response curve of CCK-8–stimulated amylase release. Second, bioactivity of plasma was completely inhibited by the specific CCK receptor antagonist, loxiglumide. Third, plasma CCK levels determined by bioassay paralleled those determined by radioimmunoassay using an antibody against the aminoterminus of CCK-8. It is thus unlikely that the plasma of our subjects contained factors other than CCK that could have interfered with the bioassay and overestimated CCK concentrations.

Liddle et al[9] were the first to report increased plasma CCK bioactivity in response to an oral dose of 100 g glucose in 400 mL water (25%) in humans, but they failed to detect any increase in CCK concentration in their subsequent study using 60 g glucose in 400 mL water (15%).[10] Recently, Fried et al[39] have also reported an increase in plasma CCK to 2.8 ± 0.6 pmol/L within 20 minutes after ingestion of glucose 100 g/500 mL. Consistent with the above observations, our results demonstrated a rapid increase of plasma CCK in response to glucose and Trelan G-75 ingestion. Moreover, low-dose glucose (50 g) caused a smaller but significant increase in CCK levels. Since the volume effect was excluded in these studies, the higher glucose concentrations in our study (33.3%, 1,852 mOsm/L) and in earlier studies by Liddle et al[9] (25%, 1,389 mOsm/L) and Fried et al[39] (20%, 1,190 mOsm/L) as compared with the other study by Liddle et al[10] (15%, 833 mOsm/L) may explain this discrepancy. Indeed, our preliminary results indicated that oral ingestion of Trelan G-75 solution with a lower osmolality (400 mL, 675 mOsm) was less effective on plasma CCK release as compared with that in a volume of 225 mL (1,200 mOsm) (peak CCK level determined by radioimmunoassay, 2.9 ± 0.5 *v* 4.6 ± 0.6 pmol/L, $P < .05$).

Meerof et al[40] were the first to recognize that duodenal infusion of a hyperosmolar solution increases duodenal output of trypsin and bilirubin. More recently, Owyang et al[41] clearly reported that intraduodenal infusion of 500 mOsm/L NaCl solution stimulates chymotrypsin output to the same extent as that obtained after intravenous infusion of 20 ng/kg/h CCK-8. Both groups of investigators demonstrated, using a bioassay, that CCK is not released during duodenal infusion of hypertonic saline. This increase in enzyme secretion induced by hyperosmolar solutions in the duodenum can be abolished by atropine, but not by trypsin.[41] Due to the rapid increase in CCK within 5 to 10 minutes after ingestion in the present study, it seems unlikely that the increase in plasma CCK represents a response to duodenal distension or to hypertonic solution. In the present study, we adjusted different doses of glucose solutions to the same concentration to minimize the osmolality effect on CCK levels. Therefore, the smaller increase in CCK at low-dose glucose suggests that the CCK response to glucose is dose-related and that osmolality has a small effect, if any.

In the present study, plasma CCK increased rapidly and reached a peak value within 30 minutes (mostly at 10 minutes) of ingestion, whereas serum glucose and insulin levels reached their peaks at a later time (30 to 60 minutes). The increases in plasma CCK concentrations preceding the increases in blood glucose and serum insulin concentrations suggest some physiological roles for CCK in glucose homeostasis. In this regard, infusion of CCK-8, giving rise to plasma concentrations of 4.5 to 8.2 pmol/L, potentiates amino acid–induced insulin release.[36,42] Moreover, a bolus injection of CCK-8 or CCK-33 at pharmacologic doses stimulates basal and meal-stimulated insulin release.[6] These results suggest a possible effect for CCK on insulin release in man. However, it is not known whether specific CCK receptors exist on human B cells. Furthermore, a selective inhibition of CCK action by CCK_A receptor antagonists failed to identify a direct incretin effect of CCK on B cells in humans.[42,43] However, there is a possibility that gastrointestinal peptides other than CCK may compensate for the lack of CCK influences during CCK receptor blockade, since several gastrointestinal peptides with insulinotropic properties may be released together with CCK and synergistically increase postprandial insulin release. Taken together, it is conceivable that the increase in CCK preceding the increase in glucose may sensitize or prime B cells to induce sufficient insulin response to subsequent glucose stimulation, as has been demonstrated with in vitro experiments.[16,44,45] Thus, it is possible that CCK may be physiologically important under normal conditions or when the influences of other incretin factors are eliminated.

Another interesting finding in the present study is the CCK response to glucose in patients with NIDDM. Previous study has demonstrated higher plasma immunoreactive CCK levels after a test meal in patients with NIDDM versus normal subjects.[46] In contrast, the maximum plasma CCK response to Trelan G-75 in our patients tended to be lower than that of normal subjects. However, our NIDDM patients were older than the controls, although the difference

was not statistically significant. Taken together with the report by Khalil et al,[47] who demonstrated an age-dependent increase in fasting and fat-stimulated plasma CCK levels, it is possible that the secretory response of CCK to glucose ingestion in diabetic patients is decreased as compared with that in healthy subjects. Further investigation in patients with different metabolic states is needed.

In conclusion, the results of the present study demonstrate that glucose stimulates CCK release in a dose-dependent manner and that Trelan G-75 causes CCK release in well-controlled diabetic patients comparable to that in control subjects. An increase in plasma CCK concentration before elevation of serum glucose and insulin levels suggests a physiological role for CCK in glucose homeostasis in humans. Furthermore, these results suggest that CCK is still a possible candidate as an incretin in humans.

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