Role of cholecystokinin in the intestinal phase of pancreatic circulation in dogs

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Nakajima, Morio, Satoru Naruse, Motoji Kitagawa, Hiroshi Ishiguro, Chunxiang Jin, Osamu Ito, and Tetsuo Hayakawa. Role of cholecystokinin in the intestinal phase of pancreatic circulation in dogs. Am J Physiol Gastrointest Liver Physiol 280: G614–G620, 2001.—The regulatory mechanisms of postprandial pancreatic hyperemia are not well characterized. The aim of this study is to clarify the role of cholecystokinin (CCK) in the intestinal phase of pancreatic circulation. Pancreatic, gastric, and intestinal blood flows were measured by ultrasound transit-time flowmeters in five conscious dogs. Pancreatic and gastric secretion and blood pressure were also monitored. Synthetic CCK octapeptide (CCK-8) or gastrin heptadecapeptide (gastrin-17) was infused intravenously, and milk was infused into the duodenum with or without loxiglumide, a specific CCK-A receptor antagonist. CCK-8 induced dose-related increases of pancreatic, but not gastric or intestinal, blood flow and protein secretion without affecting systemic blood pressure. Gastrin-17 did not affect pancreatic blood flow. An intraduodenal infusion of milk increased pancreatic and intestinal blood flows and pancreatic protein secretion. Loxiglumide completely inhibited pancreatic blood flow and protein responses to CCK-8 and milk but not the intestinal blood flow response. CCK is a potent and specific pancreatic vasodilator, with its effect mediated by CCK-A receptors. CCK plays an important role in the regulation of the intestinal phase of the pancreatic circulation in dogs.

Pancreatic blood flow; intestinal blood flow; postprandial hyperemia; loxiglumide

Pancreatic blood flow increases after an ingestion of a meal (6, 13). Postprandial pancreatic hyperemia results from the complex interplay of both intrinsic (tissue metabolism) and extrinsic factors (neurohumoral regulators) as well as general hemodynamics (18). A physiological contribution of each factor, however, has not been fully clarified yet. The pancreatic secretory response to a meal is arbitrarily divided into the cephalic, gastric, and intestinal phases. The intestinal phase is quantitatively the most important one for postprandial pancreatic secretion (29, 30). Two classic gastrointestinal hormones, secretin and cholecystokinin (CCK), are considered to be the major hormonal regulators of the intestinal phase of pancreatic secretion. Their involvement in the regulation of pancreatic circulation has been suggested, because both hormones can increase pancreatic blood flow in anesthetized animals (3, 18). However, conclusive evidence is still lacking owing to the anatomical complexities of pancreatic circulation and technical difficulties in pancreatic blood flow measurement in the conscious state.

Recently, we developed a method for a long-term measurement of pancreatic blood flow in conscious dogs and found that secretin, the most potent and efficacious stimulant of pancreatic fluid and bicarbonate secretion, is a weak pancreatic vasodilator (14, 22). The maximal dose of secretin for fluid and bicarbonate secretion increased pancreatic blood flow to only 10–15% of postprandial peak levels. Therefore, it is unlikely that secretin is a major regulator of pancreatic circulation. The aim of this study is to clarify the role of CCK, the most important humoral mediator of pancreatic enzyme secretion, in the pancreatic blood flow response to intestinal nutrients. In this study, we have measured pancreatic blood flow and secretion simultaneously and have found that CCK plays a dominating role in the control of the intestinal phase of pancreatic blood flow and secretion in conscious dogs.

MATERIALS AND METHODS

The following study was approved by the Ethical Committee of Nagoya University on Animal Use for Experiment and was conducted in conformity with the “Guiding Principles in the Care and Use of Animals” (1996) of the American Physiological Society.

Animal Preparation

Five beagle dogs (11.0–19.0 kg) of either sex (Oriental Yeast, Tokyo, Japan) were used. After an 18-h fast, following intravenous injections of thiamin (20 mg/kg; Kyorin, Tokyo, Japan) and atropine sulfate (0.5 mg; Sigma, St. Louis, MO), the abdomen was opened by a midline incision under N2O-O2-halothane anesthesia (using a ventilator). Saline was infused intravenously at the rate of 2 ml/min during surgery. Flow probes of ultrasound transit-time flowmeters (Transonic Systems, Ithaca, NY) were placed around a branch from the splenic artery to the pancreas and left gastric (LGA) and superior cranial mesenteric (SMA) arter-
cies, as described previously (14, 21, 22). The connectors of the probes were pulled out of the abdominal cavity through a subcutaneous tunnel and fixed at the neck. A stainless steel cannula was placed into the body of the stomach, and a Thomas-type duodenal fistula was provided to collect pancreatic juice (19). A catheter of the blood pressure sensor for the telemetry system (Data Science, St. Paul, MN) was placed inside the femoral artery. One month was allowed for recovery. The animals could move freely in individual cages and were allowed free access to water and food, except during the experiments.

**Blood Flow Measurement**

Flow probes of ultrasound transit-time blood flowmeters were calibrated before surgery. Because the calibration of the flow probe was not possible once it was implanted, the signal intensity and capacitance of the flow probe and the resting pulse and mean flow were monitored before each experiment. Pancreatic, LGA, and SMA flows at the start of the study were not significantly different from those at the end of the study. No significant drift of the flow levels at the end of cardiac diastole from the initial zero flow levels was observed during this period. A reliable measurement of pancreatic blood flow was possible for 6–12 mo (14).

**Experimental**

The experiments were conducted in the conscious state. Each dog was fasted for 18–24 h and then restrained in a Pavlov stand during the experiments. Saline was infused intravenously at 2 ml/min throughout the experiment. The gastric fistula was opened to drain gastric secretion. Pancreatic secretion was collected for 10-min periods by cannulation of the duct via the duodenal fistula (19, 20). The flow probes were connected to ultrasound flowmeters. Gastric and duodenal motility were measured by catheter-type pressure transducers (Millar, Houston, TX) placed in the stomach and duodenum via respective fistulae. Pancreatic, LGA, and SMA flows, gastroduodenal motility, blood pressure, and heart rate were measured via a polygraph system (NEC-SanEi, Tokyo, Japan) and recorded on a pen recorder (NEC-SanEi), a magnetic data recorder (TEAC, Tokyo, Japan), and a computer-controlled data acquisition system (MacLab system; Analog Digital Instruments, Castle Hill, Australia). Each animal was studied twice per week, and only one series of study was carried out on each day.

**Exogenous CCK.** Synthetic CCK octapeptide (CCK-8) and gastrin heptadecapeptide (gastrin-17) (Peptide Institute, Osaka, Japan) were dissolved immediately before use. Each dose of CCK-8 (25, 50, 100, and 200 ng·kg⁻¹·h⁻¹) or gastrin-17 (840 ng·kg⁻¹·h⁻¹) was infused intravenously for 1 h in a random order. To avoid the influence of periodic pancreatic secretion and blood flow changes associated with interdigestive gastrointestinal motor activity (19, 20, 23), each dose of CCK-8 or gastrin-17 was administered during the gastroduodenal motor quiescent phase.

**Endogenous CCK.** To investigate the effect of endogenous CCK, commercially available milk was infused into the duodenum via a duodenal fistula at a rate of 4 ml/min. The rate of milk infusion was comparable with gastric emptying of milk (15).

**CCK-A receptor antagonist.** Loxiglumide (5 mg/kg followed by 10 mg·kg⁻¹·h⁻¹; Tokyo Tanabe, Tokyo, Japan), a CCK-A receptor antagonist, was infused intravenously together with the intravenous infusion of CCK-8 (200 ng·kg⁻¹·h⁻¹) or the intraduodenal infusion of milk (4 ml/min). At this dose, this antagonist inhibited the pancreatic protein response to CCK-8 (200 ng·kg⁻¹·h⁻¹) almost completely (32).

**Analysis**

**Chemical.** Pancreatic protein concentration was estimated by spectrophotometry at 280 nm (Hitachi, Tokyo, Japan) with the use of bovine serum albumin as the standard (Sigma). Gastric acid secretion was measured by titration to pH 7.0 with 0.1 N NaOH by use of an autotitrator (RADIOMETER, Copenhagen, Denmark).

**Data.** The mean value during the fasting quiescent phase for 5 min served as a basal control. The vascular resistance (mmHg·ml⁻¹·min⁻¹) was calculated by dividing the mean systemic blood pressure (in mmHg) by blood flow (in ml/min). All data are presented as means ± SE, with n equal to the number of dogs. The dose-response data obtained at the 10- to 20-min periods and the integrated data for 30 min were used for analysis.

**Statistics.** Statistical analysis was carried out by analysis of variance followed by paired t-test for paired data and Dunnett’s procedure for multiple comparison. Regression analysis was carried out by the method of least squares. P < 0.05 was taken as the level of significance.

**RESULTS**

**Resting Hemodynamics**

The resting mean systemic blood pressure and heart rate were 143 ± 5 mmHg and 81 ± 8 beats/min, respectively. Blood flow in a branch of the splenic artery to the pancreas, which will hereafter be referred to as pancreatic blood flow, during the fasting quiescent phase was 3.7 ± 0.5 ml/min. Resting LGA and SMA blood flows were 32 ± 3 and 164 ± 11 ml/min, respectively.

**Exogenous CCK**

CCK-8 (25, 50, 100, and 200 ng·kg⁻¹·h⁻¹) induced dose-related increases of pancreatic blood flow and protein secretion (Fig. 1) without affecting the systemic blood pressure (147 ± 7 mmHg) and heart rate (87 ± 8 beats/min). CCK-8 decreased pancreatic vascular resistance in a dose-related manner but had little effect on SMA vascular resistance (Fig. 2). A significant (P < 0.05) increase of LGA resistance was observed when a high dose of CCK-8 (200 ng·kg⁻¹·h⁻¹) was administered (Fig. 2). Basal gastric acid secretion was very low (0.01 ± 0.01 mmol/10 min). Gastrin-17 (840 ng·kg⁻¹·h⁻¹) significantly (P < 0.05) increased gastric acid secretion (2.0 ± 0.5 mmol/10 min) but failed to increase pancreatic (Fig. 3), LGA, and SMA blood flows.

**Endogenous CCK**

An intraduodenal infusion of milk (4 ml/min) increased pancreatic blood flow to the peak of 19.1 ± 2.7 ml/min (Fig. 4A) as well as fluid (1.7 ± 0.3 ml/10 min) and protein (279 ± 24 mg/10 min) (Fig. 5) secretion in 32 ± 5 min. Pancreatic blood flow (Fig. 4A) and secretion then declined gradually but remained above the control levels during the infusion of milk. SMA blood flow continued to increase to 260% of the basal flow...
during the infusion of milk (Fig. 4B), whereas LGA blood flow remained at fasting levels (Fig. 4B).

CCK-A Receptor Antagonist

Loxiglumide (10 mg·kg⁻¹·h⁻¹) inhibited the pancreatic blood flow response to CCK-8 (200 ng·kg⁻¹·h⁻¹) to fasting levels (Fig. 3). The pancreatic, but not SMA or LGA, blood flow response to the intraduodenal infusion of milk was significantly (P < 0.05) suppressed by loxiglumide (Fig. 4). The integrated incremental pancreatic fluid, protein, and blood flow responses to CCK-8 in the 30-min period were significantly (P < 0.05) reduced by this CCK-A receptor antagonist by 77 ± 6, 90 ± 5, and 86 ± 7%, and those to milk were reduced by 93 ± 4, 88 ± 5, and 89 ± 4%, respectively (Fig. 5).

Relationship Between Pancreatic Blood Flow and Secretion

There was a significant (P < 0.05) linear correlation between pancreatic protein secretion and blood flow during the infusion of CCK-8 and milk (Fig. 6). The slopes and y-intercepts of two regression lines (CCK-8 and duodenal milk) were not significantly different.

DISCUSSION

Among various methods used for measuring splanchnic blood flow (7), only three methods have been successfully applied for measuring postprandial pancreatic hyperemia (6, 13, 14, 22). A microsphere technique can measure microcirculatory changes in a given part of the pancreas, but it provides only limited point estimates of flow because different radioisotopes have to be used in each measurement (6). The use of a thermocouple allows the monitoring of continuous changes of pancreatic blood flow, but the measurement is not quantitative (13). An ultrasound transit-time blood flowmeter can provide a continuous and quantitative measurement of pancreatic blood flow in the conscious state (14, 22). A small branch of the splenic artery to the left extremity of the splenic lobe of the pancreas was selected for measurement because it has no anastomoses with the extrapancreatic tissues (22).
Because there is no regional difference of blood flow within the pancreas (6), it is reasonable to assume that our measurements in this area of the pancreas reflect blood flow changes in other parts of the pancreas.

The present study has shown that CCK-8 is a potent stimulant of pancreatic blood flow in conscious dogs. The circulatory effect of CCK-8 appears specific to the pancreas, because CCK-8 in the dose range tested did not affect general hemodynamics and blood flow of the stomach and intestine (Fig. 2). In agreement with previous studies in anesthetized dogs (3), CCK-8 induced a dose-related increase of pancreatic blood flow as well as protein secretion (Fig. 1). The maximal protein response to CCK-8 was observed at doses of 400–450 ng·kg⁻¹·h⁻¹ or 350–400 pmol·kg⁻¹·h⁻¹ (16, 20), and the half-maximal effective dose (ED₅₀) for protein secretion was estimated to be 100–200 ng·kg⁻¹·h⁻¹. At this dose, CCK-8 induced a blood flow response comparable with that observed after the ingestion of milk or the intravenous administration of vasoactive peptides, such as vasoactive intestinal polypeptide and pituitary adenylate cyclase activating peptide (14). The peak pancreatic blood flow response to CCK-8 observed in this study was ~500% of the basal flow, which is much larger than that (180%) observed in anesthetized dogs (3). The use of anesthesia is known to decrease pancreatic blood flow by ~50% (13). Because protein secretion was also about one-fifth of that of the present study, it is likely that anesthesia...
reduced both circulatory and secretory response. The difference in the methods used in two studies, i.e., arterial blood flow measured by an ultrasound transit-time flowmeter versus capillary blood flow by a laser-Doppler flowmeter, may also cause differences.

Two types of CCK receptors, CCK-A receptor and CCK-B/gastrin receptor, have been cloned (35). CCK-A receptors mediate classic actions of CCK, whereas CCK-B receptors that are originally found in the brain prove to be identical to gastrin receptors that mediate gastric acid secretion. Both CCK-A and CCK-B receptors are present in pancreatic acini in dogs (5, 17). L-364718 and loxiglumide are selective antagonists of CCK-A receptors (28, 35). L-364718 inhibited both pancreatic blood flow and protein responses to CCK-8 and the 33-amino acid peptide (CCK-33) in anesthetized dogs (3). The present study confirmed this observation in conscious dogs; loxiglumide inhibited CCK-8-induced pancreatic blood flow and protein secretion almost completely (Fig. 3). Thus the pancreatic vasodilatory effect of CCK, as in enzyme secretion, appears to be mediated by CCK-A receptors in dogs. This conclusion is further supported by the experiment with gastrin; a supraphysiological dose of gastrin-17 stimulated gastric acid secretion but failed to affect pancreatic blood flow and protein secretion (Fig. 3).

In the present study, to minimize the effects of cephalic and gastric phases, gastric secretion was diverted to the exterior via the gastric fistula, and milk was infused directly into the intestine via the duodenal fistula at a rate comparable with the gastric emptying rate for milk (31). The infusion of milk, as expected, increased intestinal blood flow to levels observed after the oral ingestion of milk (31). However, the heart rate, systemic blood pressure, and gastric blood flow were unaffected, suggesting that the activation of cephalic and gastric phases was minimal (34). Under these conditions, the pancreatic blood flow and protein responses to milk were comparable with those to CCK-8 at 50–100 ng·kg\(^{-1}\)·h\(^{-1}\) (Figs. 1 and 4). Loxiglumide reduced both pancreatic blood flow and protein response to milk by ~90%, and the responses that remained after loxiglumide were not different from control levels of interdigestive pancreatic blood flow and secretion (Fig. 4). This finding strongly indicates that CCK is the major regulator of postprandial pancreatic circulation and enzyme secretion in the intestinal phase and that both effects are mediated by CCK-A receptors.

Although CCK was originally isolated as CCK-33, it is now known that the 55-amino acid peptide (CCK-58) is the major circulating form in dogs (4) and humans. Doi et al. (3) compared the effects of CCK-8 and CCK-33 on pancreatic blood flow and protein secretion in anesthetized dogs and could not detect differences between the two molecular forms. Raybould and Reeve (26) found that CCK-8 and CCK-58 were equipotent in their effects on pancreatic protein secretion and gastric motility in anesthetized rats. As depicted in Fig. 6, there was a linear relationship between pancreatic protein secretion and blood flow. The two regression lines obtained from data during the infusion of CCK-8 and milk are almost identical, which strongly suggests that endogenous CCKs have the same vascular effects as CCK-8 when their molar concentrations are equal. Thus it appears that there is little difference among the various molecular forms of CCK in their known biological actions, although the pancreatic vascular effect of CCK-58 remains to be studied.

A number of possible mediators have been proposed for CCK-induced hyperemia (11, 12, 18, 24), but it is not exactly known how CCK induces pancreatic vasodilation. There is evidence for specific binding sites of CCK-33 in the rat vascular endothelium, especially in the pancreatic islets but to a lesser extent in the exocrine pancreas (27). Pancreatic blood flow stimulated by CCK, but not by secretin, is inhibited by nitric oxide (NO) synthase inhibitor (24). CCK, therefore, may act on the pancreatic blood vessels by releasing NO. However, CCK-8 had no effect on the isolated pancreateicoduodenal arteries in dogs (33). Pancreatic tissue \(\text{O}_2\) tension decreased when secretion was stimulated by CCK-8 (9). A direct linear relationship between the rate of exocrine secretion and \(\text{O}_2\) consumption has been demonstrated in the salivary gland and pancreas, and the magnitude of the functional hyperemia is proportional to the increment in \(\text{O}_2\) consumption (18). Thus a linear relationship between pancreatic protein secretion and blood flow (Fig. 6) suggests that an increase in \(\text{O}_2\) consumption may be responsible for CCK-induced hyperemia. However, the relative contribution of metabolic factors cannot be deduced from the present study.

The profound inhibitory effect of loxiglumide on pancreatic blood flow is rather surprising, because intestinal nutrients stimulate not only CCK release but also nervous mechanisms that mediate the enteropancreatic reflex (29, 30). Konturek et al. (16) observed a similar inhibitory effect of CR-1409 (Iorlumide), another CCK-A receptor antagonist, on the pancreatic protein response to intraduodenal amino acids but a slightly lowered inhibition (~70%) to intragastric meal, in which both gastric and intestinal mechanisms were activated. In humans, loxiglumide inhibits the pancreatic secretory response to intraduodenal meals by ~60% (1, 10). We showed previously that ~90% of the pancreatic protein response to lower doses (~ED\(_{50}\)) of CCK-8 was mediated by nicotinic cholinergic mechanisms in dogs (Magee and Naruse, Ref. 20). Dependence of the physiological actions of CCK on cholinergic nerves was more evident in the human pancreas (1), where CCK-A receptor transcripts are absent (35). Thus the present observation is best explained if CCK, in addition to its actions mediated via CCK-A receptors on acini and pancreatic blood vessels, initiates or affects the enteropancreatic reflex. However, the neural mediators that are involved in CCK-induced hyperemia remain to be studied.

It was suggested that CCK might be a physiological humoral regulator of intestinal circulation, because CCK derived from natural sources was found to be a potent intestinal vasodilator (2). Studies using syn-
thetic peptides, however, suggest that contaminants in CCK preparations might have a vasodilatory action. An intra-arterial infusion of CCK-8 did increase intestinal blood flow, but the dose required was 100 times larger than postprandial CCK levels (25). In this study, CCK-8 failed to increase intestinal blood flow (Fig. 2), which is consistent with the finding that intestinal blood flow response to milk was not inhibited by loxiglumide (Fig. 4). Thus our study supports the conclusion that CCK is not of quantitative importance in the regulation of intestinal circulation (8).

Left gastric arterial blood flow increases only in the initial 10 min after the ingestion of a meal and remains at fasting levels for 2 h (15). It is not known why the flow remains unchanged despite the presence of food in the stomach. Postprandial hyperemia appears to be confined to the gastric mucosa (6). However, an increase in mucosal flow associated with acid secretion causes only a small increase in the total gastric blood flow. Neither pentagastrin (15) nor gastrin-17 (Fig. 4), which caused 50% of the maximal acid secretion, increased left gastric arterial blood flow. Thus metabolic demands of the mucosa-associated acid secretion may be met by redistribution of flow from the muscle layer to the mucosa. In this study, a large dose of CCK-8 induced a small but significant increase in gastric vascular resistance (Fig. 2), which was blocked by the CCK-A receptor antagonist. Because the intraduodenal infusion of milk did not affect gastric blood flow, the physiological significance of this finding may not be high.

In conclusion, CCK is a potent and specific pancreatic vasodilator. Pancreatic vasodilation appears to be mediated by CCK-A receptors. In the intestinal phase, CCK plays a dominating role in the control of pancreatic blood flow and secretion in dogs.

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REFERENCES