Modulatory Effect of Glucose, Amino Acids, and Secretin on CCK-8-Induced Somatostatin and Pancreatic Polypeptide Release in Dogs

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SUMMARY

Protein- and fat-rich test meals elicit a strong stimulatory effect on postprandial somatostatin (SLI) and pancreatic polypeptide (PP) release, whereas carbohydrate-rich meals rather attenuate the response of both hormones. Since there is evidence that intestinal hormones might contribute to the postprandial SLI and PP response, it was the aim of the present study to determine in dogs the effect of low-dose cholecystokinin octapeptide (CCK-8) on basal hormone levels and also during a background infusion of amino acids or glucose. In a group of six conscious dogs, sulfated CCK-8 was infused intravenously (i.v.) via a hindleg vein at stepwise increasing infusion rates of 10, 30, and 50 pmol · kg^{-1} · h. The infusion of CCK was applied during a background infusion of saline (2 ml/min), glucose (0.2 g/min), or an amino acid mixture (8.5%, 2 ml/min). CCK-8 had no effect on plasma insulin and glucagon levels under all experimental conditions. Plasma SLI levels were significantly stimulated by all doses of CCK. This stimulatory effect was similar during background infusions of either saline, glucose, or amino acids, respectively.

Pancreatic polypeptide (PP) levels rose 200–300 pg/ml during CCK plus saline. This was slightly attenuated by glucose. During CCK plus amino acids, the PP response was augmented to 600–800 pg/ml.

Since secretin is also released after the ingestion of a meal and intraduodenal acidification is a potent stimulus not only of secretin but also of gastric and pancreatic SLI release, the effect of secretin was examined additionally. The infusion of secretin (0.03 CU · kg^{-1} · h) had no effect on basal levels of SLI, PP, insulin, or glucagon and abolished the stimulatory effect of CCK-8 on SLI and PP levels. Interestingly, the concomitant infusion of glucose or amino acids together with secretin restored the stimulatory effect of CCK-8 to those levels observed in the absence of secretin. In conclusion: (1) Postprandial SLI and PP release might in part be due to the stimulatory effect of CCK-8. (2) CCK-induced PP release is attenuated by elevated plasma glucose levels and augmented by elevated levels of circulating amino acids. CCK-induced SLI release remains unaffected by either glucose or amino acids. (3) Low-dose secretin abolishes CCK-8-induced SLI and PP release. Interestingly, circulating nutrients can restore the CCK effect via as yet unknown mechanisms. (4) CCK-8 at the low doses employed has no effect on insulin and glucagon release, suggesting that its primary target cells are the pancreatic PP cells and the gastric and pancreatic D-cells rather than A- and B-cells. DIABETES 1986; 35:523–29.

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Received for publication 12 July 1985 and in revised form 1 November 1985.

Somatostatin is located in gastric, pancreatic, and intestinal D-cells and it is released into the peripheral circulation after the ingestion of a meal in dogs and humans.[1–5] The postprandial increase of peripheral somatostatin levels can exert an inhibitory effect on gastric acid and gastrin secretion, gastric emptying, gall bladder emptying, pancreatic volume, bicarbonate and enzyme secretion, glucose and triglyceride absorption, and insulin and glucagon secretion (for review see ref. 6). While the presence of food in the stomach is a strong stimulus primarily for gastric somatostatin release, there is some evidence that intestinal factors such as GIP, CCK, and secretin contribute to the postprandial somatostatin release from both pancreas and stomach.[7–10]

The ingestion of a protein- or fat-rich meal is the strongest stimulus for somatostatin release in dogs and humans. Both nutrients are potent stimuli for the release of the intestinal hormone cholecystokinin (CCK), which has originally been isolated from the pig intestine as a 33-amino acid-containing peptide.[11] Recent advances in radioimmunologic measurements of CCK in plasma have shown that the octapeptide of CCK is released into the circulation in vitro and in vivo.[12–15]

This raises the possibility that CCK-8 might contribute to the postprandial somatostatin response after the ingestion of fat- or protein-rich meals.

Previous studies have already demonstrated a stimulatory effect of CCK-8 on somatostatin release in dogs; however, the doses employed in these experiments were presumably unphysiologically high.[7,9] It was the aim of the present study to determine the effect of low-dose CCK-8 on basal somatostatin release, and because the release of intestinal hormones is tightly coupled to the absorption of nutrients, the effect of CCK-8 was also examined during a background infusion of glucose or amino acids.

Another potent stimulus of somatostatin release is acid, which on the other hand is probably the most physiologic stimulus for secretin release. Secretin is released into the circulation after the ingestion of a meal in humans and dogs,[16–18] and experimental evidence exists that secretin is of substantial importance for the stimulation of postprandial pancreatic bicarbonate secretion.[19] Recent studies in humans have demonstrated that secretin and CCK have a potentiating effect on pancreatic exocrine function,[20] which might also be relevant for endocrine function and, therefore, the effect of this hormone was examined additionally.

In addition to measurements of somatostatin, simultaneous determinations of plasma insulin, glucagon, and pancreatic polypeptide (PP) were performed. These hormones are either influenced by endogenous somatostatin[21,22] or they contribute to the regulation of postprandial somatostatin release.[23–25] In addition, the inhibitory effect of PP on pancreatic exocrine function and bile acid output[26–30] might be complementary to the retarding effects of somatostatin. Thus, the activation of CCK and secretin stimulates, on the one hand, pancreatic exocrine function and, on the other hand, somatostatin and PP release, which in turn inhibit pancreatic exocrine function, thus establishing a feedback control.

MATERIALS AND METHODS

All experiments were performed in six conscious dogs (foxhounds, body wt 26–34 kg). After an overnight fast, the dogs received an infusion of saline (2 ml/min), glucose (0.2 g/min), or an amino acid mixture (Aminoplasmal, 8.5%, 2 ml/min). Thirty minutes later an infusion of CCK-8 sulfated (Peninsula, Belmont, California) was started at stepwise increasing doses of 10, 30, and 50 pmol/kg · h, respectively. Each dose was applied for 30 min via a hindleg vein. In other experiments, secretin was infused additionally at a rate of 0.03 CU · kg^{-1} · h. All peptides were dissolved in saline containing 0.2% bovine serum albumin (BSA). In the control experiments, the dogs received either saline-BSA, glucose, or amino acids without CCK, respectively. All experiments were carried out in random order with 2-day intervals between experiments, and each dog served as its own control.

Frequent blood samples were obtained before and during the infusion of CCK from a crural vein and transferred into chilled tubes containing 500 KIU/ml Trasylol and 3 mg/ml EDTA. The samples were kept chilled in an ice bath until centrifugation at 2000 rpm for 20 min at 4°C. The separated plasma was stored at −20°C until the time of assay. Plasma levels of insulin,[31] somatostatin-like immunoreactivity,[32] pancreatic polypeptide,[33] and glucagon[34] were determined as described elsewhere. Antiserum 80C for measurements of somatostatin-like immunoreactivity (SLI) and antiserum 30K for glucagon measurements were generously provided by Dr. R. H. Unger (Dallas, Texas). Standard human pancreatic polypeptide (hPP) and rabbit anti-hPP serum were a generous gift of Dr. R. E. Chance, Eli Lilly Research Lab., Indianapolis, Indiana. Blood glucose levels were determined by the glucose oxidase method employing the Technicon autoanalyzer.

Incremental values were calculated as the sum of the differences of each time point from the mean of the three values obtained during the 30-min period of saline, glucose, or amino acids that preceded the start of the CCK infusion. For the calculation of statistical significances, the values at each time point during CCK infusion were compared with baseline levels by analysis of variance for multiple comparisons and P-values of 0.05 or less were considered significant.

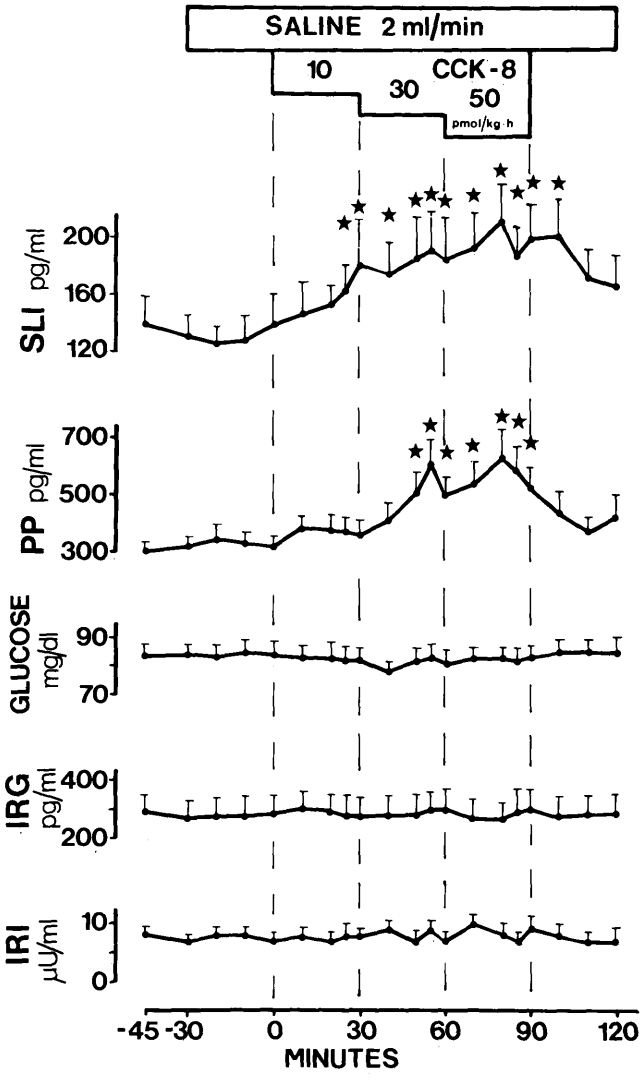

FIGURE 1. Effect of i.v. infused cholecystokinin octapeptide (CCK-8) during an i.v. background infusion of saline on peripheral venous plasma insulin (IRI), glucagon (IRG), glucose, somatostatin-like immunoreactivity (SLI), and pancreatic polypeptide (PP) levels in a group of six conscious dogs (mean ± SEM). *Indicates significant difference of $P < 0.05$ or less versus baseline levels.

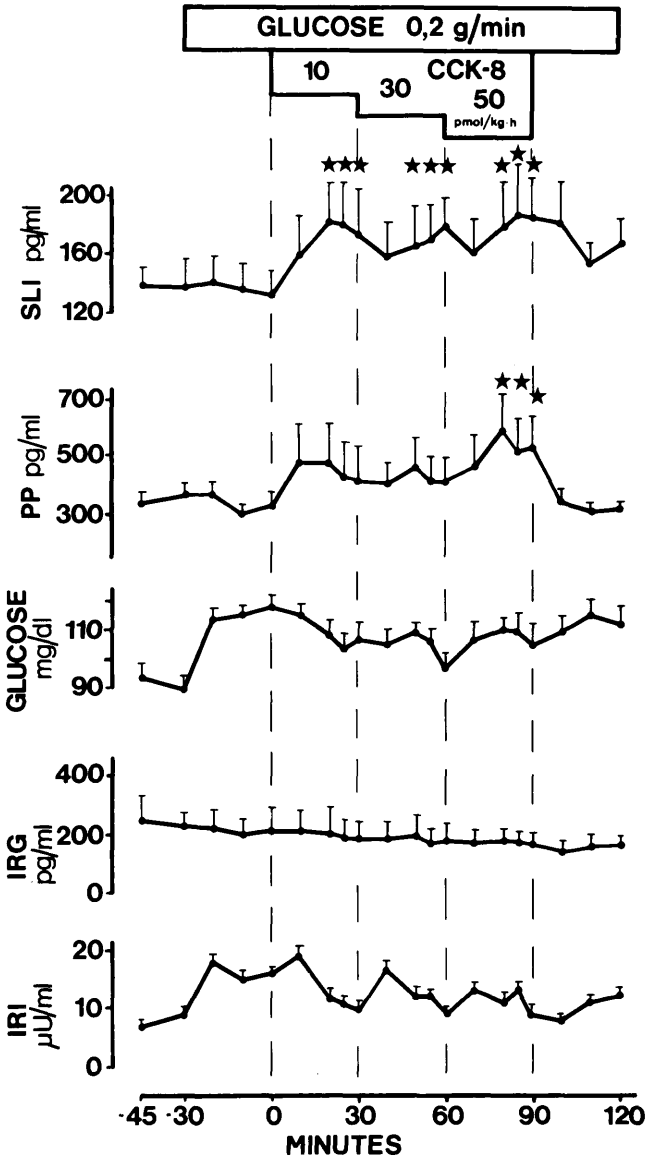

FIGURE 2. Effect of i.v. infused cholecystokinin octapeptide (CCK-8) during an i.v. background infusion of glucose on peripheral venous plasma insulin (IRI), glucagon (IRG), glucose, somatostatin-like immunoreactivity (SLI), and pancreatic polypeptide (PP) levels in a group of six conscious dogs (mean ± SEM). *Indicates significant differences of $P < 0.05$ or less versus baseline levels.

RESULTS

Effect of CCK-8 during i.v. saline. During the infusion of saline alone, no change of hormone levels was observed. During a background infusion of saline, CCK-8 elicited a significant stimulatory effect on plasma SLI levels at the end of the lowest dose of 10 pmol · kg^{-1} · h and at the higher doses employed (Figure 1). PP levels were significantly elevated by 30 and 50 pmol · kg^{-1} · h. Plasma insulin, glucagon, and glucose levels remained unchanged.

Effect of CCK-8 during i.v. glucose. The background infusion of glucose elicited a significant increase of plasma glucose levels by 20–30 mg/dl and a significant increase of plasma insulin levels. This was not changed by the infusion of CCK-8 (Figure 2). The stimulatory effect of CCK-8 on plasma SLI levels was similar to that in the saline experiments. With regard to PP levels, only the highest dose of CCK-8 elicited a small, significant stimulation (Figure 2). The infusion of glucose alone for the entire experimental period elicited a similar increase of plasma glucose and insulin levels and a slight decrease of plasma glucagon. Plasma SLI and PP levels did not change from baseline levels during i.v. glucose alone.

Effect of CCK-8 during i.v. amino acids. The infusion of the amino acid mixture elicited a significant stimulatory effect on plasma insulin and glucagon levels that was not influenced by the additional infusion of CCK-8 (Figure 3). The effect of CCK-8 on plasma SLI levels was similar to that in the glucose and saline experiments. The effect of CCK-8 on PP levels

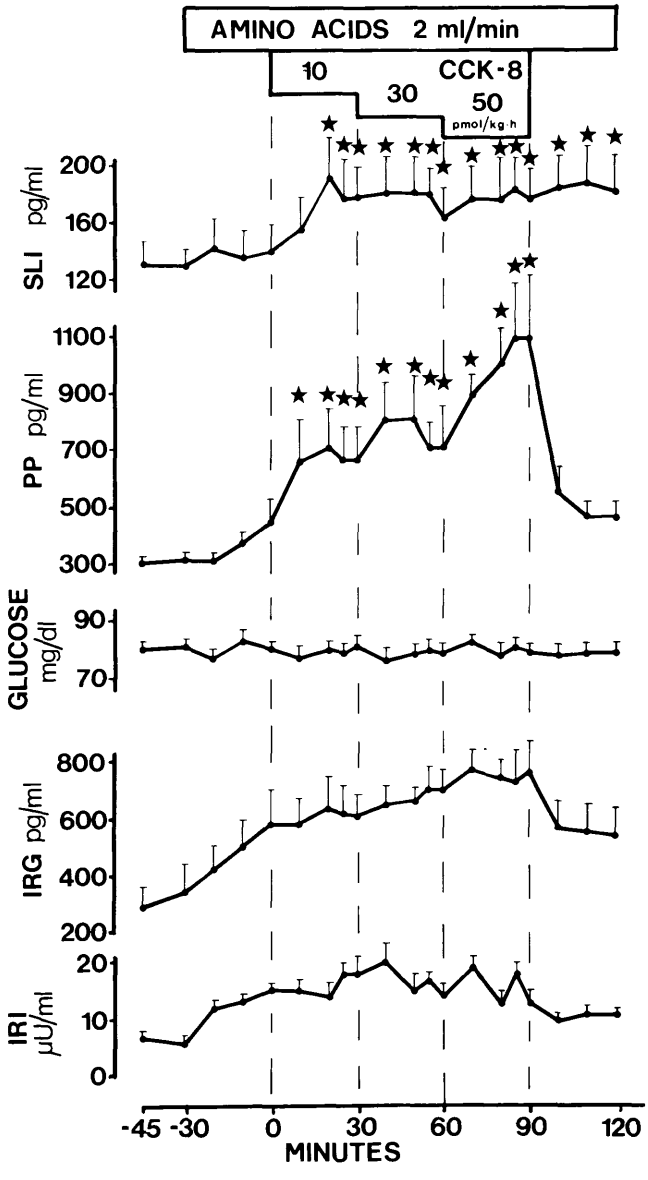

FIGURE 3. Effect of i.v. infused cholecystokinin octapeptide (CCK-8) during an i.v. background infusion of amino acids on peripheral venous plasma insulin (IRI), glucagon (IRG), glucose, pancreatic polypeptide (PP), and somatostatin-like immunoreactivity (SLI) levels in a group of six conscious dogs (mean ± SEM). *Indicates significant difference of $P < 0.05$ or less versus baseline levels.

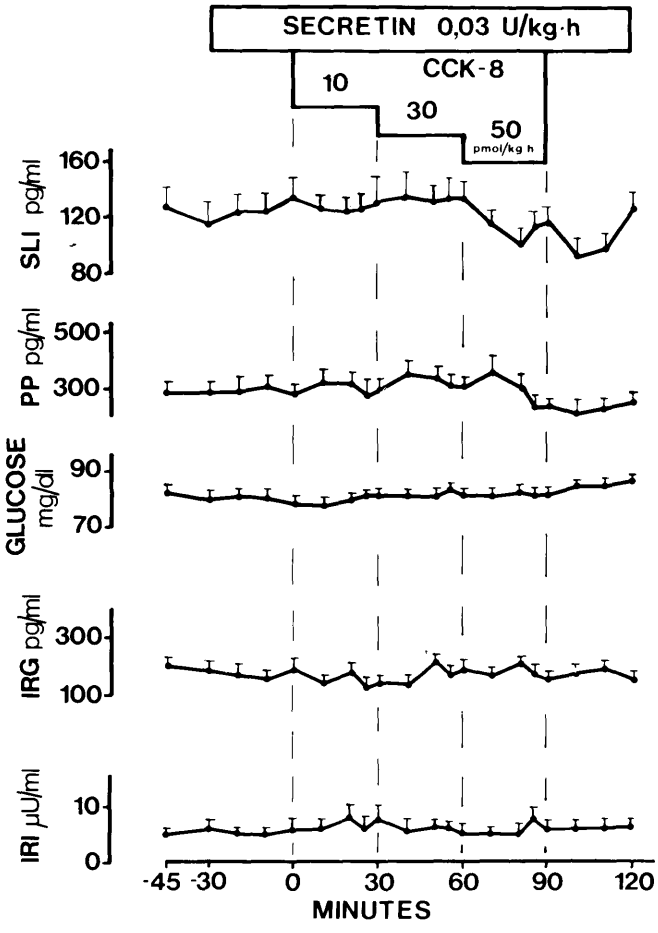

FIGURE 4. Effect of i.v. infused cholecystokinin octapeptide (CCK-8) during an i.v. background infusion of secretin on peripheral venous plasma insulin (IRI), glucagon (IRG), glucose, somatostatin-like immunoreactivity (SLI), and pancreatic polypeptide (PP) levels in a group of six conscious dogs (mean ± SEM).

was augmented by amino acids. The lowest dose of 10 pmol · kg^{-1} · h had already elicited a significant stimulatory effect, and the maximal response to the highest dose was 750 pg/ml above basal, compared with only 200–250 pg/ml during a saline or glucose background infusion. The infusion of i.v. amino acids for the entire experimental period without CCK-8 resulted in an identical increase of plasma insulin and glucagon levels, while SLI and PP levels remained unchanged from basal.

Effect of CCK-8 during i.v. secretin. During the background infusion of secretin, CCK-8 had no effect on plasma SLI, PP, insulin, glucagon, and glucose levels (Figure 4). The infusion of secretin alone over the entire experimental period had no effect on plasma insulin, glucagon, SLI, or PP levels. During a background infusion of i.v. glucose or amino acids, secretin had no effect on all four hormones measured compared with the changes observed during glucose or amino acids alone.

Effect of CCK-8 during i.v. secretin plus glucose. When CCK-8 was infused in addition to glucose plus secretin, a stimulatory effect on SLI and PP levels was observed at 30 and 50 pmol · kg^{-1} · h (Figure 5). Compared with CCK-8 plus secretin, the additional infusion of glucose prevented in part the inhibitory effect of secretin on the stimulatory effect of CCK-8 on plasma SLI and PP levels.

Effect of CCK-8 during i.v. secretin plus amino acids. During i.v. amino acids plus secretin, the effect of CCK-8 on PP levels was similar to that observed during CCK-8 plus amino acids without secretin. The effect of CCK-8 on plasma SLI was augmented. The lowest dose of 10 pmol · kg^{-1} · h of CCK-8 had already elicited an increase of plasma SLI levels by ~70 pg/ml (Figure 6). This elevation was maintained during the other infusion rates.

DISCUSSION

The present study demonstrates that low-dose infusion of CCK-8 stimulates basal somatostatin and PP release in dogs but has no effect on insulin and glucagon levels. The stimulatory effect on somatostatin levels remains unchanged during i.v. administration of glucose or amino acids but is abolished by low-dose secretin infusion. The stimulation of PP release is attenuated by i.v. glucose and augmented by i.v. amino acids. Similar to its effect on somatostatin levels, secretin abolishes the CCK-induced PP response. The effect

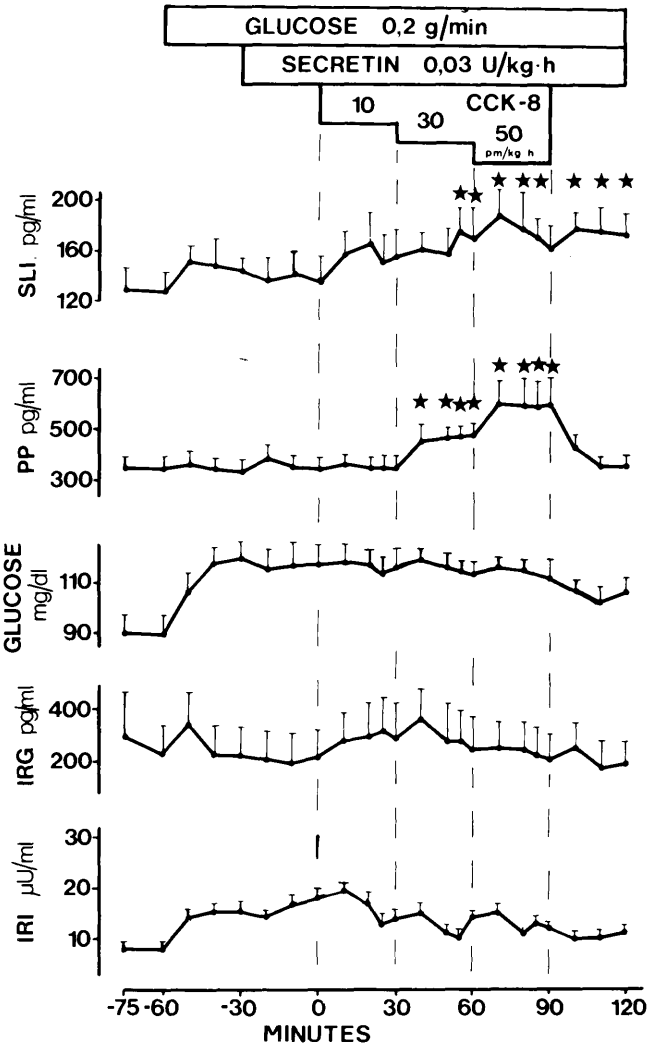

FIGURE 5. Effect of i.v. infused cholecystokinin octapeptide (CCK-8) during i.v. background infusions of glucose and secretin on peripheral venous plasma insulin (IRI), glucagon (IRG), glucose, somatostatin-like immunoreactivity (SLI), and pancreatic polypeptide (PP) levels in a group of six conscious dogs (mean ± SEM). *Indicates significant difference of $P < 0.05$ or less versus baseline levels.

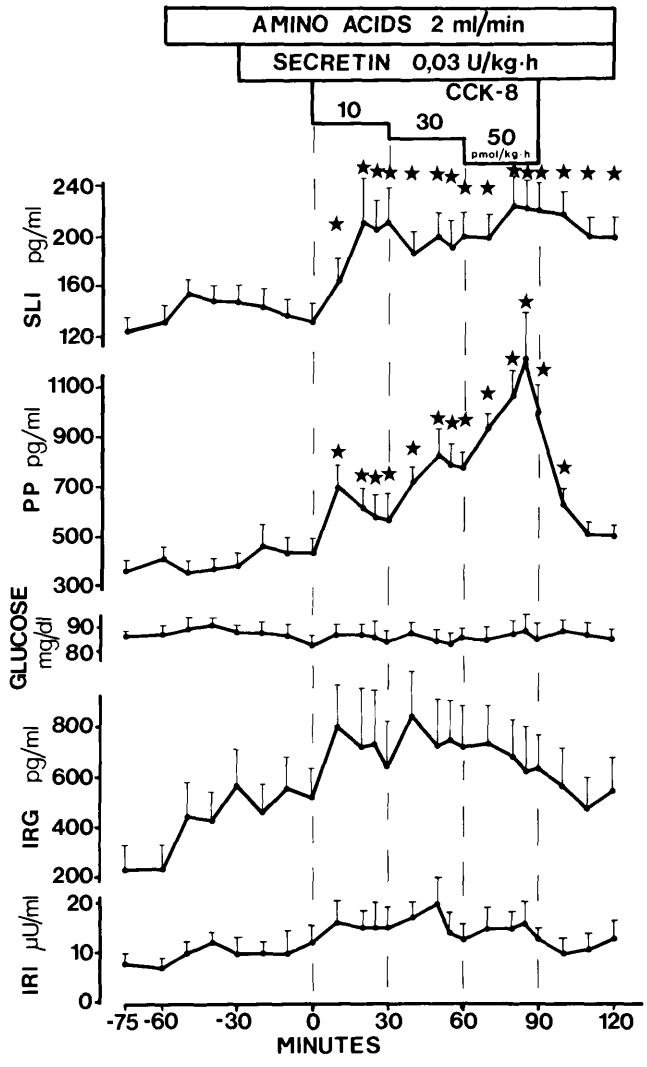

FIGURE 6. Effect of i.v. infused cholecystokinin octapeptide (CCK-8) during i.v. background infusions of amino acids and secretin on peripheral venous plasma insulin (IRI), glucagon (IRG), glucose, somatostatin-like immunoreactivity (SLI), and pancreatic polypeptide (PP) levels in a group of six conscious dogs (mean ± SEM). *Indicates significant difference of $P < 0.05$ or less versus baseline levels.

of CCK-8 on both hormones is restored when, in addition to secretin, glucose or amino acids are administered.

The ingestion of fat- and protein-rich test meals is followed by an increase of circulating SLI and PP levels.[2,3,35-38] Infusion studies with several intestinal hormones have shown a stimulation of SLI and PP release.[7,9,39-45] This suggests that intestinal hormones might contribute to the postprandial signal that activates gastric and pancreatic D-cells and also PP-cells.

Previous studies have shown that CCK-8 stimulates gastric and pancreatic SLI release in dogs; however, the doses employed were presumably unphysiologically high. The present study demonstrates that doses of CCK-8 that are at or even below those that elicit a minimal stimulation of pancreatic exocrine function in dogs[46] elicit an increase of peripheral vein plasma SLI levels. In addition, these experiments confirm previous studies that have already shown that low doses of CCK-8 stimulate basal PP release in dogs.[40]

The elevation of circulating glucose levels does not affect CCK-induced stimulation of plasma SLI levels. In contrast, the same glucose infusion rate attenuates the somatostatin response to protein- and fat-rich meals in dogs,[35,47] which suggests that other than CCK-mediated effects on SLI release are affected by physiologic elevations of plasma glucose levels. PP release is attenuated by elevated glucose levels, which is in agreement with previous studies in dogs and humans,[23,44-49] demonstrating that i.v. or oral glucose attenuates the PP response. The demonstration that CCK-induced PP but not CCK-induced SLI release is attenuated by glucose suggests that reduction of the postprandial response of both hormones by glucose is mediated via different mechanisms.

On the other hand, i.v. amino acids potentiate the PP but not SLI response to low-dose CCK-8, demonstrating for the first time that an interaction between circulating nutrients in the form of amino acids and CCK can be of potential importance for the strong postprandial PP response after the ingestion of a protein meal. Previous studies have shown, and the present experiments confirm these findings, that elevation of circulating amino acids has no effect on basal PP release.[37]

The addition of secretin abolishes the CCK-induced SLI and PP release. This finding is in sharp contrast to the potentiating action of these two hormones on pancreatic exocrine function.[20] Since somatostatin and pancreatic polypeptide exert inhibitory effects on pancreatic exocrine function, it can be speculated that the reduction of these two inhibitory factors favors the potentiation of the exocrine response. The exact mechanism for this inhibitory interaction cannot be determined from the present studies. It is noteworthy that, in a previous study, there was no effect of secretin plus cerulein on PP levels.[50] There are several other reports of somewhat unexpected interactions between gastrointestinal peptides. It has been shown that VIP stimulates basal pancreatic bicarbonate secretion but inhibits secretin-induced bicarbonate secretion.[51] In addition, a partially inhibitory interaction has been reported for the effects of GIP and CCK-8 on insulin secretion in mice.[52] These findings demonstrate that interactions between gastrointestinal peptides can produce substantially different results than expected from the effects of the individually tested peptides.

Interestingly, the effect of CCK-8 on SLI and PP levels is restored to control values when glucose or amino acids are given additionally to i.v. secretin. This indicates that circulating nutrients not only potentiate the effect of individual peptides but also of peptide combinations. Since secretin is released after ingestion of mixed meals, an increase of secretin without a concomitant elevation of plasma glucose and/or amino acid levels seems to be rather unlikely.

In all these experiments, insulin and glucagon release is not affected by CCK-8 and secretin. The lack of an effect of CCK-8 on insulin release would be in agreement with some[53-55] but not all[56] in vitro data, whereas in vivo it has been shown that low-dose CCK-33 elicits a glucose-dependent stimulation of insulin secretion in the rat.[57] If this represents a species difference or if this is due to the different molecular forms of CCK employed in these studies remains to be determined. Although GIP has a major role in postprandial glucose-induced insulin release,[58,59] there is evidence for other intestinal factors contributing to this "entero-insular axis."[60,61]

From the present data it seems unlikely that CCK-8 is a major candidate for the non-GIP-mediated entero-insular axis at least in the dog.

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