Supramaximal CCK-58 does not induce pancreatitis in the rat: role of pancreatic water secretion

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1Department of Physiology, University of Texas Health Science Center at San Antonio, San Antonio, Texas; and 2CURE: Digestive Diseases Research Center, Veterans Affairs Greater Los Angeles Healthcare System and 3Digestive Diseases Division, School of Medicine, University of California, Los Angeles, California

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Yamamoto M, Reeve JR, Jr, Green GM. Supramaximal CCK-58 does not induce pancreatitis in the rat: role of pancreatic water secretion. Am J Physiol Gastrointest Liver Physiol 292: G964–G974, 2007. First published January 4, 2007; doi:10.1152/ajpgi.00338.2004.—In contrast to supramaximal CCK-8 or caerulein, acute or prolonged supraphysiological levels of endogenous CCK-58 do not cause pancreatitis. Compared with CCK-8, CCK-58 is a much stronger stimulant of pancreatic chloride and water secretion, equivalent to maximally effective secretin, but with a chloride-to-bicarbonate ratio characteristic of acinar fluid. Because supraphysiological endogenous CCK does not cause pancreatitis and because coinjection of secretin ameliorated caerulein- or CCK-8-induced pancreatitis, coincident with restoring pancreatic water secretion, we hypothesized that supramaximal CCK-58 would not induce pancreatitis. Conscious rats were infused intravenously with 2 or 4 nmol·kg−1·h−1 of CCK-8 or synthetic rat CCK-58 for 6 h, and pancreases were examined for morphological and biochemical indexes of acute pancreatitis. A second group was treated as above while monitoring pancreatic protein and water secretion. CCK-8 at 2 nmol·kg−1·h−1 caused severe edematous pancreatitis as evidenced by morphological and biochemical criteria. CCK-58 at this dose had minimal or no effect on these indexes. CCK-58 at 4 nmol·kg−1·h−1 increased some indexes of pancreatic damage but less than either the 2 or 4 nmol·kg−1·h−1 dose of CCK-8. Pancreatic water and protein secretion were nearly or completely abolished within 3 h of onset of CCK-8 infusion, whereas water and protein secretion were maintained near basal levels in CCK-58-treated rats. We hypothesize that supramaximal CCK-58 does not induce pancreatitis because it maintains pancreatic acinar chloride and water secretion, which are essential for exocytosis of activated zymogens. We conclude that CCK-58 may be a valuable tool for investigating events that trigger pancreatitis.

pancreatic fluid secretion; cholecystokinin 8; cholecystokinin 58; acinar fluid secretion; pancreatic chloride secretion.

INJECTION OR INFUSION OF SULFATED CCK-8 or its amphibian analog caerulein at doses 4–10 times the maximally effective dose for pancreatic protein secretion induces edematous pancreatitis in rats and mice, with many similarities to human acute pancreatitis (11, 20). Doses in this range (termed “supramaximal”) inhibit or abolish pancreatic fluid and enzyme secretion, disrupt the acinar cytoskeleton, and activate stress responses such as nuclear factor (NF)-κB (1, 14, 20, 30).

Although many of the pancreatic intracellular events associated with this model have been elucidated, several distinctive phenomena are still unexplained. Particularly puzzling is the observation that, while CCK-8 and bombesin both stimulate protein secretion by mobilizing intracellular calcium and both strongly stimulate pancreatic enzyme secretion, supraphysiological doses of bombesin do not cause pancreatitis or inhibit pancreatic secretion (12, 44). Also unexplained is the observation that prolonged unphysiologically high levels of endogenous CCK do not induce pancreatitis in rats (28, 34), nor do high endogenous CCK levels exacerbate experimental pancreatitis after it is initiated (18, 29, 37, 39, 42). We recently reported that CCK-58 is the only detectable endocrine form of CCK in the rat (32) and the most abundant endocrine form of CCK in humans (7) and dogs (9). Therefore, it is important to determine whether endogenous CCK, i.e., CCK-58, differs from CCK-8 in important ways. We previously reported that not only was CCK-58 a much more efficacious stimulant of pancreatic fluid and chloride secretion than CCK-8, but that pancreatic fluid, protein, and electrolyte secretion stimulated by CCK-8 was highly aberrant in pattern and quantity of secretion, and in regulation, compared with CCK-58 and endogenous CCK (45). This aberrant pattern was manifested at all doses tested, including threshold, submaximal, maximal, and supramaximal doses. These results, taken together with the possibility that CCK-8 is not a circulating form of CCK in the rat (32), indicated that the actions of CCK-8 on pancreatic secretion at all doses are pharmacological, not physiological.

Two distinctive characteristics of CCK-58-stimulated pancreatic secretion, compared with CCK-8-stimulated secretion, suggested clues that might help understand CCK-8-induced acute pancreatitis (45). The most remarkable was that CCK-58 was a potent, efficacious, and dose-dependent stimulant of pancreatic chloride and fluid secretion, equivalent in volume to maximally effective doses of synthetic rat secretin, but with higher chloride and lower bicarbonate concentrations. This difference in chloride-to-bicarbonate ratio compared with secretin-stimulated secretion suggested that CCK-58-stimulated pancreatic fluid secretion might be predominately acinar in origin. The fluid secretory response to CCK-58 was unaffected by secretin antiserum but was abolished by the CCK-A receptor antagonist MK-329. In contrast, CCK-8 did not signifi-
cantly stimulate pancreatic chloride or fluid secretion. Second, the pancreatic protein secretory response to CCK-8 was distinctly different in pattern from that to CCK-58. Whereas pancreatic protein secretion stimulated by continuous intravenous infusion of CCK-58 peaked then reached a significantly elevated steady-state plateau, with CCK-8 infusion protein secretion returned to basal levels after maximal output was attained, despite continued infusion of the peptide. These differences between CCK-58 and CCK-8 in pancreatic secretion, taken together with the observation that prolonged high endogenous CCK levels did not cause pancreatitis in the rat (28), led us to test the hypothesis that supramaximal doses of CCK-58 would not induce pancreatitis. The results reported here show that at doses at which CCK-8 causes severe edematous pancreatitis (2 and 4 nmol·kg\(^{-1}\)·h\(^{-1}\)), effects of CCK-58 treatment were almost indiscernible from controls (2 nmol·kg\(^{-1}\)·h\(^{-1}\)), or the changes were much less dramatic (4 nmol·kg\(^{-1}\)·h\(^{-1}\)).

**MATERIALS AND METHODS**

**Materials**

Rat synthetic sulfated CCK-58 was obtained from the University of California Los Angeles (UCLA) Peptide Synthesis Facility (Dr. J. R. Reeve, Jr., Director). Rat CCK-58 was synthesized using an Applied Biosystems Peptide Synthesizer (Foster City, CA) and purified to >90% (32). Sulfated CCK-8 was purchased from Peninsula (San Carlos, CA).

**Animal Preparation**

Two types of experiments with separate groups of rats were performed. The objective of the first type was to induce pancreatitis by intravenous infusion of supramaximal doses of CCK-8 (2 and 4 nmol·kg\(^{-1}\)·h\(^{-1}\)) for 6 h and compare the morphological and biochemical changes with those induced by administration of equimolar doses of CCK-58. In the second type, the objective was to measure pancreatic fluid and enzyme secretion in conscious, cannulated rats subjected to the same dose rate and infusion protocol as used in the first set.

Male Wistar rats were obtained from Harlan Sprague Dawley (Indianapolis, IN). For the first set of experiments, rats weighing 180–200 g were anesthetized with isoflurane (Aerene; Fort Dodge Animal Health) and prepared with an external jugular vein cannula for infusion of CCK peptides or saline. After surgery, rats were placed in Bollman-type restraint cages modified to minimize discomfort. Rats were fed laboratory diet before surgery and during recovery. Experiments were carried out on the 4th day postoperative after an overnight fast.

For the second set of experiments, male Wistar rats weighing 250–300 g were anesthetized with isoflurane and prepared with Silastic cannulas (12 in. long, 0.02 in. ID × 0.037 in. OD), which drained pancreatic juice and bile, and with two additional cannulas in the duodenum for reintroducing bile and pancreatic juice. Rats were also prepared with an external jugular vein cannula for infusion of CCK peptides. Before closure, cannulas implanted in the abdominal cavity and in the jugular vein were routed under the skin to exit on the back. After closure, rats were placed in modified Bollman-type restraint cages. Rats were fed laboratory chow before surgery and had access to food during recovery and between experiments. During recovery and between experiments, pancreatic juice and bile were collected and continuously returned to the intestine by a servomechanism (13). In the second set of experiments, only one study was performed in each rat. The Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio approved the protocols in this study.

**Experimental Protocol**

Stock solutions of peptides were kept at −80°C and diluted in saline containing 0.1% BSA immediately before use. The purity of CCK-58 was evaluated by HPLC and high-pressure capillary electrophoresis at the UCLA Peptide Synthesis Facility. Purity was >90%.

In the first set of experiments, CCK-8 or CCK-58 was infused intravenously at 2 or 4 nmol·kg\(^{-1}\)·h\(^{-1}\) for 6 h. Controls received intravenous saline containing 0.1% BSA. Next, the whole pancreas was rapidly removed, blotted on filter paper, and weighed, and blood was taken from the abdominal aorta for the measurement of serum amylase levels. Tissue samples were stored at −80°C for subsequent assays. For all pancreatic tissue measurements except water content and histological score, the values were expressed per total pancreas (i.e., normalized to total pancreas content). Pancreatic water content. For quantification of pancreatic edema, a small piece of tissue was resected, trimmed of fat, blotted dry, weighed, desiccated for 48 h at 105°C, and weighed again (dry weight). Pancreatic water content was determined by calculating the wet weight-to-dry weight ratio.

Morphology. CCK-58- and CCK-8-induced morphological changes in the pancreas were assessed by a pathologist who was blinded to the identity of the specimens. A portion of the pancreatic tissue for light microscopic examination by hematoxylin and cosin staining was fixed overnight in 4% buffered neutral paraformaldehyde solution, embedded in paraffin, and deparaffinized by standard procedures. Another portion was examined by immunohistochemistry for interleukin-6 (IL-6) using goat anti-mouse IL-6 antibody in 1:10 dilution (Santa Cruz Biotechnology, Santa Cruz, CA). The primary antibody was visualized using the labeled streptavidin-biotin method available as a commercial kit (Dako, Carpinteria, CA) according to the instructions provided by the manufacturer.

Histological evaluation. The extent of vacuolization of acinar cells as well as edema and inflammatory infiltrate of interstitial space were quantified using a previously described scoring system (43). Two nonoverlapping fields per pancreatic specimen of five pancreases in each experimental group were examined. According to the severity of tissue damage, a score from one to three was given for each parameter.

Myeloperoxidase activity. Neutrophil sequestration in pancreatic tissue was quantitated by measuring myeloperoxidase (MPO) activity as described by Bhatia et al. (3). Tissue samples were thawed, homogenized in 20 mM phosphate buffer (pH 7.4), and centrifuged (10,000 g, 10 min, 4°C). The resulting pellet was resuspended in 50 mM phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide (Sigma). The suspension was subjected to four cycles of freezing and thawing and was subsequently disrupted by sonication (40 s). The sample was then centrifuged (10,000 g, 5 min, 4°C), and the supernatant was used for the MPO assay. The reaction mixture consisted of the supernatant, 1.6 mM tetramethylbenzidine (Sigma), 80 mM sodium phosphate buffer (pH 5.4), and 0.3 mM hydrogen peroxide. This mixture was incubated at 37°C for 110 s, the reaction was terminated with 2 M H2SO4, and the optical density (OD) was measured at 450 nm. This absorbance was then corrected for the weight of the pancreas, and activity (OD) was expressed per total pancreas.

Pancreatic trypsin and trypsinogen content. A splenic portion of the pancreas was homogenized in a 0.15 N NaCl solution by using a glass homogenizer with a motor-driven Teflon pestle. The homogenates were filtered through three layers of gauze and then sonicated for 1 min. The aqueous phase was used for trypsin and trypsinogen assay. Tryptosinogen was determined as tryptic activity after activation with enterokinase using the method of Erlanger et al. (8). Tryptsin was

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determined by the same method as trypsinogen but without activation using enterokinase.

Serum amylase. Blood samples from each rat in the first set of experiments were centrifuged at 2,500 g for 10 min at 4°C. Serum amylase levels were determined using a standard chromogenic assay (Phadebas; Pharmacia & Upjohn, Uppsala, Sweden).

In the second set of experiments, pancreatic secretory responses were carried out after the fourth postoperative day when the rats had fully recovered from surgery as judged by normalization of bile and pancreatic secretion and food intake. Pancreatic secretion rate was monitored for 6 h during intravenous infusion of CCK-8 or CCK-58 at 2 or 4 nmol·kg⁻¹·h⁻¹. The volume of pancreatic juice was measured by Hamilton syringe, and a 10-μl sample was taken for protein assay. Pancreatic protein concentration was estimated by determining OD at 280 nm of samples diluted 1:600 in 0.05 M Tris hydroxymethyl aminomethane buffer (pH 7.8). Purified bovine trypsinogen (Worthington Biochemical, Freehold, NJ) was used as a standard. In control rats, saline containing 0.1% BSA was infused intravenously in place of CCK peptides. Pancreatic juice and bile were collected during 1-h intervals and returned to the duodenum continuously during the subsequent 60-min period. Experiments with CCK peptides were performed only one time in each rat.

Statistical Analysis

Results are expressed as means ± SE. Comparisons between groups after a significant F-test (1-way ANOVA) were analyzed by Duncan’s multiple-comparison test. Differences were considered significant at P < 0.05.

RESULTS

Histological Changes in the Pancreas

Histological examination revealed marked interstitial edema, inflammatory cell infiltration, and intracellular vacuolization in the pancreas of rats treated with CCK-8 at 4 nmol·kg⁻¹·h⁻¹ (Fig. 1A). In addition, all acinar cells appeared contracted, resulting in a crowded appearance. In the 2 nmol·kg⁻¹·h⁻¹ CCK-8 group, significant interstitial edema, inflammatory cell infiltration, and intracellular vacuolization were noted (Fig. 1B), although these findings were less severe than in the 4 nmol·kg⁻¹·h⁻¹ CCK-8 group. In contrast, these alterations were mild in the 2 nmol·kg⁻¹·h⁻¹ CCK-58 group (C). Although most portions of the pancreas remained intact, these alterations were slightly noted in some portions in the 2 nmol·kg⁻¹·h⁻¹ CCK-58 group (D). Histological findings showed all portions of the pancreas remained intact in the control group (E). Original magnification, ×100.
CCK-58 and Pancreatitis in the Rat

In the 2 nmol·kg\(^{-1}\)·h\(^{-1}\) CCK-58 group, these alterations were slightly noted in some portions of the pancreas, but most portions of the pancreas remained intact (Fig. 1D). In the control group, these alterations were minimal (Fig. 1E).

Immunohistochemistry

Immunoreactivity for IL-6 was detected in inflammatory cells in 4 (Fig. 2A) and 2 (Fig. 2B) nmol·kg\(^{-1}\)·h\(^{-1}\) CCK-8 groups. More IL-6-positive cells were detected in the 4 nmol·kg\(^{-1}\)·h\(^{-1}\) CCK-8 group than in CCK-8 2 nmol·kg\(^{-1}\)·h\(^{-1}\) CCK-8 group. In contrast, although some inflammatory cells infiltrated in the pancreas in CCK-58 4 (Fig. 2C) and 2 (Fig. 2D) nmol·kg\(^{-1}\)·h\(^{-1}\) CCK-58 groups, IL-6-positive cells were rarely detected. In the control group, inflammatory cells were rarely noted, and IL-6-positive inflammatory cells were not detected (Fig. 2E).

MPO Activity

MPO activity in CCK-8-treated rats was significantly increased more than twofold compared with the same dose level of CCK-58. MPO activity in the 4 nmol·kg\(^{-1}\)·h\(^{-1}\) dose of CCK-58 was significantly increased compared with controls, whereas MPO activity was unchanged compared with CCK-58 at the 2 nmol·kg\(^{-1}\)·h\(^{-1}\) dose (Fig. 3).

Pancreatic Water Content

The ratio of wet weight to dry weight, indicative of edema, is illustrated in Fig. 4. Compared with vehicle controls, CCK-8 more than doubled the wet-to-dry ratio (102 and 122% increases for 2 and 4 nmol·kg\(^{-1}\)·h\(^{-1}\), respectively). In contrast, the 2 nmol·kg\(^{-1}\)·h\(^{-1}\) dose of CCK-58 was almost without effect (17% increase over controls). The 4 nmol·kg\(^{-1}\)·h\(^{-1}\) dose of CCK-58 increased the wet-to-dry ratio by 49%, although this did not reach statistical significance.

Histological Evaluation

The combined histology score for three parameters (edema, vacuolization, and inflammatory infiltration) is shown in Fig. 5. Compared with CCK-58-treated rats, the histological score for CCK-8-treated rats was essentially doubled at both doses. It is noteworthy that the score for the higher dose of CCK-58 was
significantly less than for the lower dose of CCK-8. All treatment groups had significantly higher scores than controls.

**Serum Amylase**

In rats treated with 2 nmol·kg⁻¹·h⁻¹ of CCK-8, serum amylase levels were significantly increased >283% compared with controls, whereas serum amylase levels were virtually unchanged by the 2 nmol·kg⁻¹·h⁻¹ dose of CCK-58. The 4 nmol·kg⁻¹·h⁻¹ dose of CCK-58 significantly increased serum amylase by 269% compared with saline-treated controls. However, this value (269% increase) in CCK-58-treated rats was significantly lower compared with those treated with 4 nmol·kg⁻¹·h⁻¹ of CCK-8 (400% increase; Fig. 6).

**Fig. 3.** Myeloperoxidase (MPO) activity in pancreas of rats treated with sulfated CCK-8 (CCK-8s) or sulfated CCK-58 (CCK-58s). MPO activity is expressed as total activity per pancreas and was significantly increased at both doses of CCK-8 compared with vehicle controls and compared with CCK-58-treated rats. Compared with vehicle controls, MPO activity was not significantly increased by CCK-58 at 2 nmol·kg⁻¹·h⁻¹ but was significantly increased by CCK-58 at 4 nmol·kg⁻¹·h⁻¹. †Significantly different from vehicle group. *Significantly different from CCK-8-treated rats at the same dose. The higher dose of CCK-58 produced significantly less MPO activity than the lower dose of CCK-8. The increase in MPO activity caused by doubling the dose of CCK-58 was not statistically significant. Values are expressed as means ± SE of 6 rats.

**Fig. 4.** Pancreatic water content (wet-to-dry ratio of pancreatic tissue) of rats treated with CCK-8 or CCK-58. CCK-8 at 2 and 4 nmol·kg⁻¹·h⁻¹ markedly increased water content compared with controls and CCK-58-treated rats. Water content was nonsignificantly increased by 49% by CCK-58 at 4 nmol·kg⁻¹·h⁻¹. Results are for the same rats as in Fig. 3. Symbols as in Fig. 3. The increase in the wet-to-dry ratio caused by doubling the dose of CCK-58 was not statistically significant.

**Fig. 5.** Total histological score for three parameters. The combined histological score for edema, vacuolization, and inflammatory infiltrate was significantly increased 2-fold by CCK-8 compared with the same dose of CCK-58. The higher dose of CCK-58 produced a significantly lower score than the lower dose of CCK-8. All groups were significantly increased compared with vehicle control. Results are for the same rats as in Fig. 3. Symbols as in Fig. 3. The increase in histological score caused by doubling the dose of CCK-58 was not statistically significant.

**Fig. 6.** Serum amylase in rats treated with CCK-8 or CCK-58. CCK-8 at each dose significantly increased serum amylase compared with corresponding doses of CCK-58 and compared with the vehicle group. CCK-58 at 2 nmol·kg⁻¹·h⁻¹ did not increase serum amylase compared with vehicle, whereas CCK-58 at 4 nmol·kg⁻¹·h⁻¹ significantly increased serum amylase by 3-fold compared with vehicle and CCK-58 at 2 nmol·kg⁻¹·h⁻¹. Results are means ± SE for 7–8 rats/group. Symbols as in Fig. 3.
Pancreatic Trypsin and Trypsinogen Content

Total trypsin and trypsinogen content of pancreases are shown in Figs. 7 and 8, respectively. Compared with CCK-58, trypsin activity in CCK-8-treated rats was doubled at each dose (Fig. 7). Trypsin activity in CCK-58-treated rats at the 2 nmol·kg\(^{-1}\)·h\(^{-1}\) dose was slightly elevated compared with controls, but this was not statistically significant.

Trypsinogen content was much lower in CCK-58-treated rats compared with CCK-8-treated rats (Fig. 8). This result is consistent with greater secretion, or export, of zymogen from pancreatic tissue in CCK-58-treated rats, in contrast to the retention of trypsinogen in CCK-8-treated rats in which protein secretion was nearly or completely abolished (Fig. 9). Trypsinogen content in rats stimulated by CCK-58 at 2 nmol·kg\(^{-1}\)·h\(^{-1}\) was much lower than in vehicle-treated controls as well, which can be explained by the transient inhibitory effect on protein synthesis caused by strong hormonal stimulation of the pancreas, simultaneous with increased secretion (23). In contrast, the much higher trypsinogen content, compared with vehicle controls, in rats treated with 4 nmol·kg\(^{-1}\)·h\(^{-1}\) of CCK-8 can be accounted for by the inhibition of basal (constitutive) and regulated protein secretion. Trypsinogen content of CCK-58-treated rats was significantly higher at the 4 nmol·kg\(^{-1}\)·h\(^{-1}\) dose compared with the 2 nmol·kg\(^{-1}\)·h\(^{-1}\) dose. This is consistent with the greater inhibition of fluid secretion with the higher dose of CCK-58 (Fig. 9), reflecting an inverse relationship between pancreatic fluid output and pancreatic trypsinogen content in CCK-58-treated rats.

Pancreatic Protein and Fluid Secretion

Pancreatic fluid secretion, and consequently protein secretion, was nearly abolished after 3 h infusion of CCK-8 at 2 nmol·kg\(^{-1}\)·h\(^{-1}\) (Fig. 9B, left and right) and was completely abolished by the 4th h of infusion at 4 nmol·kg\(^{-1}\)·h\(^{-1}\) (Fig. 9C, left and right). In contrast, in CCK-58-treated rats, pancreatic fluid secretion (Fig. 9, B and C, left) was maintained at or near pretreatment values and control (Fig. 9A, left) values, and protein output was at or above pretreatment values and control (Fig. 9A, right) values during the entire 6 h of infusion. Figure 9D shows the average output during the final 2 h of treatment, by which time the rate of secretion appears to have achieved steady state. There was no significant difference between the control (vehicle) and CCK-58 groups, but the CCK-8 groups were significantly different from all other groups.

DISCUSSION

In this study, we demonstrated that stimulation with supramaximal doses of CCK-58 caused far less injury to the pancreas compared with that caused by equimolar doses of CCK-8. Of a variety of indexes supporting this conclusion, the finding of IL-6 positive inflammatory cells in both CCK-58 groups, but not in either of the CCK-8 groups, is especially noteworthy. The proinflammatory cytokine IL-6 is elevated during experimental acute pancreatitis and correlates well with the severity of pancreatic damage (26). For CCK-8, where indexes of pancreatitis were elevated above controls, the magnitude observed at the highest dose was less than the magnitude of that index observed for CCK-8 at the lowest dose. At doses used to produce pancreatitis, CCK-8 stopped fluid secretion, whereas fluid flow was maintained with CCK-58. We suggest that this fluid flow ameliorates CCK-induced pancreatitis in the rat.

Although we propose here that the differences in pancreatic damage between CCK-58 and CCK-8 represent fundamental qualitative differences between the bioactivities of the two
Fig. 9. Pancreatic protein and fluid secretion in rats during 6 h iv infusion of CCK-8, CCK-58, or vehicle control. Studies were carried out in conscious rats after the 4th day postoperative. Fluid output in μL/h is shown on left, and protein output in mg/h is shown on right. Vehicle control; CCK-8; CCK-58. Results are means ± SE for 7–8 rats/group. There was no statistically significant difference in protein or fluid output caused by doubling the doses from 2 to 4 nmol·kg⁻¹·h⁻¹ with CCK-8 or CCK-58. D: average rate of secretion achieved during the final 2 h of treatment in which there was no significant difference between controls (vehicle) and CCK-58 groups, but CCK-8 groups were significantly different from all other groups.
forms (such as inability to stimulate chloride secretion with CCK-8), other interpretations should be considered. One such interpretation could be that the circulating or tissue concentrations of CCK-8 were substantially higher than for CCK-58 and therefore that the higher molar concentrations of CCK-8 compared with CCK-58 accounted for the greater pancreatic damage. This cannot be ruled out yet, because we did not measure plasma levels of CCK in CCK-8- and CCK-58-treated rats during peptide infusion and therefore cannot be sure that their circulating molar concentrations were equivalent, in spite of equivalent molar rates of infusion. This is a highly unlikely explanation, however, because the circulatory half-life of CCK-58 (4.4 ± 0.6 min) is substantially greater than that of CCK-8 (1.3 ± 0.1 min; see Ref. 15). This difference in half-lives would suggest that circulating concentrations of CCK-8 might be one-third less than for CCK-58 when the two forms are infused at the same molar dose rate. Furthermore, CCK-58 is more stable than CCK-8 to peptidases likely to be encountered in pancreatic interstitial fluid (33). Therefore, it is more likely that the circulating concentrations of CCK-58 and the concentrations of CCK-58 at the pancreatic receptor were greater than those of CCK-8.

A second alternative explanation could be that the differences in pancreatic damage between CCK-8 and CCK-58 could be a dosing effect, that is, supramaximal CCK-58 may be less damaging to the pancreas than equivalent circulating molar concentrations of CCK-8, but sufficiently high doses of CCK-58 might nevertheless produce the same effects as CCK-8. This cannot be ruled out and is consistent with the observation that, for most indexes of pancreatitis, the 4 nmol·kg⁻¹·h⁻¹ dose of CCK-58 produced a greater effect than the 2 nmol·kg⁻¹·h⁻¹ dose. On the other hand, in five out of six of these indexes (Figs. 3–5 and 7), the differences in effects between the two doses of CCK-58 were not statistically significant. Serum amylase (Fig. 6) was an exception, although serum amylase values may not correlate accurately with degree of pancreatic damage (25). The absence of IL-6-positive cells at either dose of CCK-58, but detected at both doses of CCK-8 (Fig. 2), is also not consistent with a simple dose-response relationship with supramaximal CCK-58. Finally, if CCK-8-induced pancreatitis is related to a major, fundamental difference in bioactivity of the two forms (further discussed below), reflected in the failure of CCK-8 to stimulate fluid and chloride secretion at any dose (45), then a simple dose-response relationship might not be expected for supramaximal CCK-58-induced pancreatic changes.

The most striking functional difference between CCK-8 and CCK-58 in the present studies was the maintenance of pancreatic juice flow and protein output with CCK-58 compared with a virtual shutdown of flow and protein output with CCK-8. Although the pancreatic fluid secretory response to 2 and 4 nmol·kg⁻¹·h⁻¹ of CCK-58 in the present study, and to 1 nmol·kg⁻¹·h⁻¹ in our previous study (45), was clearly inhibited compared with maximally effective doses of CCK-58, fluid output was nevertheless substantial. During the last 2 h of infusion when fluid output with 2 and 4 nmol·kg⁻¹·h⁻¹ of CCK-8 was virtually abolished, fluid output with 2 and 4 nmol·kg⁻¹·h⁻¹ of CCK-58 was still maintained at 449 ± 77 and 272 ± 87 μl/h, respectively, compared with control values of ~450 μl/h (Fig. 9).

Both CCK-8 and CCK-58 caused zymogen activation within the pancreas, but the content of activated trypsin was much less with CCK-58 than with CCK-8. This could be interpreted as a smaller extent of conversion of trypsinogen to trypsin with CCK-58 treatment. However, trypsinogen content of the pancreas was also much lower with CCK-58 treatment compared with CCK-8 treatment at each dose. Because the secretion studies (Fig. 9) showed that protein secretion was much greater with supramaximal CCK-58 than with CCK-8 (in which enzyme secretion ceased), the lower tissue content of trypsin and trypsinogen with CCK-58 treatment can be attributed to greater export of the activated enzymes and zymogens out of the pancreas. Therefore, the much lesser degree of pancreatic injury caused by CCK-58 may be because the activated zymogens are secreted from the cell and not retained within the cell.

The above interpretation is consistent with studies of Gorelick and coworkers (4, 12, 22) using isolated pancreatic acini. They examined the effects of supramaximal doses of caerulein, bombesin, and carbachol on zymogen activation and cellular injury in isolated rat pancreatic acini and explored the effects of increased intracellular cAMP in these processes. Zymogen activation occurred with hyperstimulatory doses of both caerulein and bombesin but was associated with cellular injury only with caerulein (12). Cellular damage with caerulein was associated with retention of activated zymogens within the acinar cells, whereas with bombesin-treated acini the activated zymogens were found in the media, indicating that activated zymogens were released (secreted) from the acinar cell. A similar phenomenon was seen when isolated pancreatic acini were exposed to supramaximal concentrations of carbachol in the presence or absence of cAMP or analogs (4). Directly increasing acinar cAMP using the cAMP analog 8-bromo-cAMP dramatically increased zymogen activation caused by supramaximal carbachol but simultaneously prevented cellular damage. This protective effect of cAMP was associated with enhanced secretion of activated enzymes from the acinar cell. The authors concluded that cAMP agonists, such as secretin and bombesin, might reduce acinar cell injury associated with activated zymogens by causing the secretion of active enzymes from the acinar cell. It is not known if cAMP plays a role in the lesser degree of pancreatic injury with CCK-58 or if the maintenance of fluid flow per se with CCK-58 has a direct role in maintaining enzyme secretion. However, both secretin and bombesin (40) share with CCK-58 the ability to strongly stimulate pancreatic fluid secretion in conscious, recovered rats in marked contrast to CCK-8. CCK-58 should be a valuable tool for evaluating the role cAMP on pancreatic injury caused by supramaximal doses of CCK-8.

Previously, CCK-8 was believed to be an important, if not major, circulating form of CCK in the rat and therefore assumed to be a physiological form of the peptide. Doses of CCK-8 that reproduced pancreatic secretory responses typical of a meal were considered “physiological.” The transition from physiological responses to submaximal “supraphysiological” responses was considered to distinguish CCK-8’s physiological effects from its pharmacological effects. However, we demonstrated that CCK-8 is probably not a physiological endocrine form of CCK (32), and compared with CCK-58 or endogenous CCK (e.g., camostat stimulated), its actions on the pancreas are highly aberrant at all doses, not just at supramaximal doses (45). In other words, CCK-8 is a pharmacological,
not physiological, agonist for pancreatic secretion. Therefore, in light of the relatively minor effect of supramaximal CCK-58 on the pancreas, we should consider whether CCK-8’s effects at doses that do not cause pancreatitis could yield clues as to the mechanism by which higher doses do cause pancreatitis. Unique among these aberrant effects of CCK-8 at doses ranging from threshold to supramaximal is its failure to stimulate chloride secretion.

Previous studies (45) comparing CCK-8 with CCK-58 at physiologically relevant doses (causing threshold to maximal protein output) demonstrated, for the first time, that CCK-8 does not significantly stimulate pancreatic chloride secretion in conscious, recovered rats, in marked contrast with CCK-58. This is shown in Fig. 10. The locus and mechanism of this failure to stimulate chloride secretion is unknown. Chloride in pancreatic juice is usually considered to come from two sources, the acinar cell cytoplasm and the duct (and centroacinar) cells, via cAMP- or calcium-activated chloride channels (24). A third source is chloride contained within the zymogen granule itself, which is transported (along with osmotically driven water) into zymogen granules via intracellular chloride channels in the zymogen granule membrane after fusion with the apical plasma membrane and pore formation (19).

We hypothesized that the chloride secretion stimulated by CCK-58 was of acinar origin because of the higher chloride-to-bicarbonate ratio of the secretion compared with secretin-stimulated secretion, because chloride output was tightly coupled to protein output (45), and because duct cells of the rat apparently lack CCK-1 receptors (38). Furthermore, the electrolyte pattern of CCK-58-stimulated secretion is unlike that in response to a combination of CCK-8 plus secretin, the latter of which produces a bicarbonate concentration intermediate between secretin alone and CCK-8 alone (35). Alternatively, electrolyte and fluid secretion in response to CCK-58 may be ductal in origin, reflecting stimulation of duct cells (as well as acinar cells) via a vago-vagal pathway (21). Although increased chloride secretion is considered indicative of stimulation of acinar cell secretion, recent reports that the permeability ratio of the cystic fibrosis transmembrane conductance regulator (CFTR) to bicarbonate and chloride can be regulated (31, 36) suggest that this generalization should be viewed with some caution. Therefore, the cellular source of CCK-58-stimulated fluid and electrolyte secretion is uncertain.

In contrast to CCK-8, CCK-58 strongly stimulates chloride output in a dose-related fashion (45). In epithelial cells, chloride secretion is the primary driving force for water secretion, and this was reflected by a strong correlation ($R^2 = 0.92$) between chloride output and fluid output in our previous study (45). Could a defect in chloride secretion, and consequently, water secretion contribute to the retention of activatedzymogens in rats treated with supramaximal CCK-8? This is suggested by our previous studies (45), which showed that not only chloride output, but protein output in response to CCK-8 was also aberrant, because it could not be sustained after reaching a maximum, but returned to basal values, whereas protein output with CCK-58 was always maintained well above basal levels. Because chloride output was tightly coupled to protein output in pancreatic secretion stimulated by CCK-58 ($R^2 = 0.92$) or by endogenous CCK (camostat, $R^2 = 0.94$), but not by CCK-8 ($R^2 = 0.01$), we suggest that defective chloride secretion with CCK-8 may inhibit exocytotic secretion of enzyme. This may contribute to the failure of CCK-8 to maintain stimulated protein secretion at physiologically relevant doses (45) and the retention of activatedzymogens at supramaximal doses. This hypothesis is supported by studies describing the critical role of chloride ion and intracellular chloride channels in exocytosis (6, 10, 17, 41).

Stimulation with the highest dose of CCK-58 did not result in edematous pancreatitis morphologically. However, we noted that pancreatic water content was modestly increased (not significantly), and the appearance of amylase in the blood was significantly increased compared with control rats. In addition, following supramaximal stimulation with CCK-58, some vacuolization in the pancreatic acini was observed. These findings suggest that, at the highest dose of CCK-58, some exocytosis at the lateral plasma membrane occurred, although to a much lesser extent than with the same dose of CCK-8. Also, while pancreatic fluid secretion did not fall below basal levels with
supramaximal CCK-58, it nevertheless was markedly inhibited compared with maximally effective doses (500 pmol·kg⁻¹·h⁻¹) of CCK-58. This suggests that the 4 nmol·kg⁻¹·h⁻¹ dose of CCK-58 may be damaging to the pancreas although not to the degree sufficient to cause pancreatitis. In this respect, CCK-58 may not be different from bombesin, which at supramaximal doses does not induce pancreatitis in rats but nevertheless significantly increases serum amylase at doses >1.0 nmol·kg⁻¹·h⁻¹ and produces some pancreatic histopathology at 40 nmol·kg⁻¹·h⁻¹ (44). A small amount of histopathology at the supramaximal doses of CCK-58 in the present study would be consistent with studies in rats in which endogenous CCK is chronically elevated for 2 wk by pancreatobiliary diversion. This causes severe subcellular pancreatic damage but not overt pancreatitis (34). Because CCK-58 may account for virtually all elevated endogenous CCK may have some deleterious effects on the pancreas and may sensitize it to induction of pancreatitis by other factors, such as ethanol.

Understanding the mechanisms accounting for the failure to induce pancreatitis in CCK-58-treated rats will be greatly helped if the phenomena described above could be studied using isolated pancreatic acini. Unfortunately, such preparations cause rapid degradation of 125I-labeled CCK-58 in spite of the phenomena described above could be studied using isolated pancreatic acini. Unfortunately, such preparations provide means of direct measurements of the cytoskeleton and activation of NF-κB, as occurs with supramaximal CCK-8/caerulein (14, 27). Because CCK-58 did not induce any migration of inflammatory cells in the pancreas (Fig. 2), some involvement of NF-κB might be expected. Finally, techniques are available to specifically label ion and water transport in pancreatic acinar cells and zymogen granule membranes, such as inhibition of the acinar cell CTFR (2, 46) and blocking of aquaporins in the acinar apical plasma membrane and zymogen granule membranes (5, 16). These blockers, CCK-58, and other tools may help clarify the role of pancreatic acinar fluid secretion in pancreatic disease.

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