Water and enzyme secretion are tightly coupled in pancreatic secretion stimulated by food or CCK-58 but not by CCK-8

M. Yamamoto,1 J. R. Reeve, Jr.,2 D. A. Keire,2 and G. M. Green1

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

Submitted 8 September 2003; accepted in final form 3 November 2004

Yamamoto, M., J. R. Reeve, Jr., D. A. Keire, and G. M. Green. Water and enzyme secretion are tightly coupled in pancreatic secretion stimulated by food or CCK-58 but not by CCK-8. Am J Physiol Gastrointest Liver Physiol 288: G866–G879, 2005. First published November 18, 2004; doi:10.1152/ajpgi.00389.2003.—Pancreatic secretion of protein, water, chloride, and bicarbonate under basal conditions and in response to intravenous and intraduodenal stimuli were studied in awake rats fully recovered from surgery. During the basal phase of pancreatic secretion, protein output and water output were weakly correlated or uncorrelated, consistent with separate regulation and distinct cellular origin of enzyme (acinar cells) and water (duct cells), referred to as the two-component paradigm of pancreatic secretion. When pancreatic secretion was stimulated physiologically, water and protein output abruptly became strongly and significantly correlated, suggesting that protein secretion and water secretion are tightly coupled or that protein secretion is dependent on water secretion. The apparent function of this coupling is to resist or prevent increases in protein concentration as protein output increases. This pattern of secretion was reproduced by intravenous infusion of the CCK-58 form of cholecystokinin, which strongly stimulates pancreatic water and chloride secretion, but not by CCK-8, which only weakly stimulates water and chloride secretion in a non-dose-dependent manner. The remarkably tight association of water and protein secretion in food-stimulated and CCK-58-stimulated pancreatic secretion is consistent with a single cell type as the origin of both water and enzyme secretion, i.e., the acinar cell, and is not consistent with the two-component paradigm of pancreatic secretion. Because CCK-58 is the only detectable endocrine form of cholecystokinin in the rat and its bioactivity pattern is markedly and qualitatively different from CCK-8, actions previously recorded for CCK-8 should be reexamined.

cholecystokinin; secretin; electrolyte secretion; bicarbonate secretion; chloride secretion

THE GASTROINTESTINAL HORMONE cholecystokinin is widely reported to exist in multiple molecular forms in a variety of species, including humans. Some of the reported differences in potency or efficacy among forms have been shown to be due to variable tendencies for peptides to be lost from solution (59). In addition, because the CCK-58 form accounts for virtually all the cholecystokinin immunoreactivity in the circulation of the rat (48), questions regarding the physiological roles of other forms of cholecystokinin in the circulation may be moot. Furthermore, several reports have shown that CCK-58 differed qualitatively in bioactivity compared with shorter forms (18, 64). With the successful synthesis of rat sulfated CCK-58, we were able to definitively demonstrate a different pattern of bioactivity between CCK-8 and CCK-58 in the anesthetized rat; CCK-58 caused a marked increase in the volume of bile-pancreatic secretion compared with CCK-8, whereas enzyme (amylase) output was unchanged (49).

These experiments had some limitations. For example, because pancreatic juice and bile were not collected separately in the latter study, we could not be certain that the increased volume of bile-pancreatic juice was due to stimulation of pancreatic juice output. In addition, the study was conducted in acutely operated, anesthetized rats, which markedly affects both quantitative and qualitative characteristics of pancreatic secretion (38, 44). To address these issues, we carried out studies to characterize the pancreatic secretion of protein, water, bicarbonate, and chloride in response to CCK-8 and CCK-58 in conscious rats fully recovered from surgery. The results show that CCK-58 stimulates a remarkably different pattern of pancreatic secretion compared with CCK-8, which reveals a tight linkage in secretion between the enzyme and aqueous components of pancreatic juice, the significance of which has not been fully appreciated.

MATERIALS AND METHODS

Materials

Sulfated CCK-58 and secretin (rat sequences) were synthesized at the University of California, Los Angeles Peptide Synthesis Facility and infused intravenously in a vehicle of 0.1% bovine serum albumin (BSA) containing 0.15 M NaCl. Rat CCK-58 was synthesized using an Applied Biosystem peptide synthesizer (Foster City, CA) and purified to >90% as determined by high-performance capillary electrophoresis. The synthesis of rat CCK-58 is described in detail elsewhere (49). Rat secretin was synthesized and characterized as previously described for canine secretin and analogs (58). CCK-8 was purchased from Sigma (St. Louis, MO). Rabbit secretin antiserum (antiserum R402) was a generous gift from Dr. Travis Solomon. The CCK-A receptor antagonist MK-329 was obtained from ML Laboratories PLC (Warrington, UK). The synthetic protease inhibitor camostat (FOY-305) was obtained from Ono Pharmaceutical (Osaka, Japan). Sodium taurocholate was purchased from Sigma. Purified bovine trypsin and purified casein (Hammersten) were purchased from Worthington Biochemical (Freehold, NJ).

Animal Preparation

Adult male Wistar rats were obtained from Harlan Sprague Dawley (Indianapolis, IN) and weighed between 270–300 g when used in the pancreatic secretion studies. Rats were anesthetized with isoflurane
and prepared with Silastic cannulas (12 in. long, 0.020 in. ID × 0.037 in. OD), which drained pancreatic juice and bile, and with two additional cannulas (12 in. long, 0.025 in. ID × 0.042 in. OD) in the duodenum, 0.5–1 cm proximal to the ampulla of Vater. Rats were also prepared with an external jugular vein cannula for infusion of peptides. Cannulas from the abdominal cavity were routed under the skin to exit on the back. After closure, rats were placed in Bollman-type restraint cages modified to minimize the degree of restraint. Rats were fed laboratory chow before surgery, during recovery, and between experiments. During recovery and between experiments, pancreatic juice and bile were collected and continuously returned via the intestinal cannulas to the duodenum by a servomechanism (22). The Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio approved all animal studies.

Assays

Experiments on pancreatic secretory responses were performed on days 4–7 postoperatively. Pure pancreatic juice was collected at 30-min intervals. The volume of pancreatic juice was measured to the nearest 0.005 μl using a Hamilton syringe, a 10-μl sample was taken for protein assay, and two 25-μl samples were taken for chloride and bicarbonate assays. Protein output in pancreatic juice was estimated by determining optical density at 280 nm of samples diluted 1:600 in 0.05 M tris(hydroxymethyl)aminomethane (Tris) buffer (pH 7.8). Purified bovine trypsinogen (Worthington) was used as a standard. Chloride concentration in pancreatic fluid was determined using the method of Shaules and Shaules (54), in which pancreatic juice samples (25 μl) are titrated with 0.02 M Hg(NO3)2 and compared with standard chloride ion (NaCl) solutions. Bicarbonate concentration was determined by acidification of samples with HCl immediately after collection followed by back-titration using the DST800 multitrillation system (Radiometer, Copenhagen, Denmark).

Collection of Pancreatic Secretion

In previous studies on pancreatic secretion in conscious rats, we collected all the pancreatic juice and bile, retained only 10 μl for protein determination, and returned the remainder of pancreatic juice and all bile to the duodenum. Because we planned to measure several additional components in the present studies, i.e., bicarbonate and chloride concentrations as well as protein concentration, it was necessary to retain a larger volume of pancreatic juice for assay (total of 60 μl out of an average 30-min collection of 200–400 μl). Therefore, to prevent stimulation of cholecystokinin release that might occur with diversion of 60 μl of pancreatic juice, we infused purified bovine trypsin (10 mg/ml in 0.15 M NaCl containing 0.01 N HCl) via one duodenal cannula. Because we also retained all bile secreted, we infused sodium taurocholate (40 μmol/ml in 0.1 M sodium bicarbonate) via the second intraduodenal cannula to prevent interruption of the enterohepatic cycle. This enabled diversion of all pancreatic juice for multiple assays while preventing the spontaneous stimulation of cholecystokinin release and pancreatic secretion that is caused by diversion of bile and pancreatic juice from the duodenum (20). Neither CCK-8 nor CCK-58 affected bile secretion.

Experimental Protocols

Effect of intravenous infusion of CCK-8 and CCK-58 on pancreatic protein, fluid, and electrolyte secretion. To determine the efficacy and potency of CCK-58 compared with that of CCK-8, we monitored basal secretion every 30 min for 1.5 h during intravenous vehicle infusion (0.1% BSA in 0.15 M NaCl) and for 3 h during peptide infusion at five dose rates: 62.5, 125, 250, 500, and 1,000 pmol·kg⁻¹·h⁻¹. All intravenous solutions were infused at 1 ml/h via syringe pump (Harvard Apparatus).
Pancreatic secretion stimulated by CCK-58 and CCK-8

G868

Pancreatic Secretion Stimulated by CCK-58 and CCK-8

RESULTS

Dose-Response Relationship for CCK-8 and CCK-58 on Pancreatic Protein and Fluid Output

Figure 2 illustrates the pattern of pancreatic protein and fluid output stimulated by CCK-8 and CCK-58 at doses doubling from 62.5 to 1,000 pmol·kg⁻¹·h⁻¹ over a 3-h period. Results are presented as increments above basal. The increment was calculated by subtracting the third 30-min basal value (the last collection before stimulants were administered) from each 30-min value during stimulant infusion. The average value for the last 30-min basal period for all experiments described (n = 172) was 200 ± 9.6 μl/30 min for fluid, 6.25 ± 0.3 mg/30 min for protein, 27.3 ± 1.15 μmol/30 min for chloride, and 6.72 ± 0.28 μmol/30 min for bicarbonate. The dose-response patterns for protein output with CCK-8 and CCK-58 were similar to each other in that they followed the same rank order, and significance differences occurred at the same doses for submaximal, maximal, and supramaximal responses, i.e., 250, 500, and 1,000 pmol·kg⁻¹·h⁻¹, respectively. Although total incremental protein output during the 3-h infusion was not significantly different between CCK-8 and CCK-58 at any dose (Table 1), protein output with CCK-8 was distinct from CCK-58 in that output with CCK-8 returned to values not significantly different from basal values by the end of the 3 h of infusion, whereas protein output remained significantly elevated compared with basal in CCK-58-treated rats. Pancreatic fluid secretion, however, was markedly different in both volume and in the pattern of secretion between CCK-8 and CCK-58. With CCK-58, pancreatic fluid secretion increased in a dose-related fashion up to 500 pmol·kg⁻¹·h⁻¹. In contrast, pancreatic fluid output with CCK-8 was not only significantly lower than with CCK-58, but the pattern was distinct in that there was no tendency for pancreatic juice volume to increase with increasing dose of CCK-8, and output was much more dramatically inhibited at the supramaximal dose than was the case with CCK-58.

Figure 3 shows the changes in protein concentration in pancreatic juice stimulated by CCK-58 and CCK-8 over a 3-h period, calculated using the same data shown in Fig. 2. With CCK-58, protein concentration showed no significant increase at any dose except the supramaximal dose of 1,000 pmol·kg⁻¹·h⁻¹, despite large increases in protein output. In marked contrast, with CCK-8 there were large, dose-related increases in protein concentration, reaching a maximum concentration of ~90 mg/ml (compared with basal of ~24 mg/ml) at the dose of 1,000 pmol·kg⁻¹·h⁻¹.

equation for the line of best fit through bivariate data is desired. Statistical analysis for significance of the regression in Model 2 is performed using ANOVA. The coefficient of determination ($R^2$) in linear regression analysis by Model 2 is interpreted as indicating the proportion of variability in the y observations that can be accounted for by variability in the x observations, and vice versa, and is not an index of significance (16). For analysis of dose-response relationships for CCK-8 and CCK-58 vs. pancreatic secretion, the conventional Model 1 regression was performed using the program contained in Microsoft Excel, with dose as the independent variable.
Comparison Among CCK-8, CCK-58, Secretin, and Camostat on Pancreatic Fluid Secretion and on Concentrations of Bicarbonate and Chloride

Figure 4 illustrates the total volume of pancreatic juice (bottom) and the corresponding changes in bicarbonate and chloride concentrations (top). Data are from the same study shown in Figs. 1 and 2. Secretin and camostat were infused at their maximally effective doses of 250 pmol kg⁻¹ h⁻¹ and 5 mg/h respectively, and CCK-8 and CCK-58 were infused at their maximally effective dose (for protein output) of 500 pmol kg⁻¹ h⁻¹. Controls received intravenous vehicle (0.15 M NaCl with 0.1 M bovine albumin). CCK-58, secretin, and camostat significantly increased pancreatic fluid secretion. The total volume of pancreatic juice stimulated by CCK-58 was equivalent to that caused by maximally effective doses of secretin and camostat. However, the pattern of chloride and bicarbonate concentrations in secretin-stimulated secretion was unique. Secretin caused characteristic and significant reciprocal changes in bicarbonate and chloride concentrations, which was not the case with CCK-8 or camostat. Fluid output with CCK-8 was not significantly increased at any 30-min collection period. The time-paired control group (n = 8) revealed no time-dependent changes in any parameter. Similarly to secretin, CCK-8 showed significant reciprocal changes in chloride and bicarbonate concentrations in pancreatic juice at all but the lowest dose, although the changes in concentrations were much less than was the case with secretin.

When calculated as incremental output (average output/30 min during the 3-h period of peptide infusion minus output in the last 30-min basal period), there were no significant differences in fluid output among CCK-58, secretin, and camostat groups (Table 1). In contrast, because of the large increase in bicarbonate concentration caused by secretin, bicarbonate output was substantially and significantly increased in the secretin group compared with the CCK-58 and camostat groups (Table 1).

Role of CCK-A Receptor, Cholinergic Pathways, and Secretin in Pancreatic Secretory Response to CCK-8

The distinctly different pattern for bicarbonate and chloride concentrations between secretin and CCK-58 argues for an important difference between CCK-58 and secretin in stimulation of pancreatic fluid secretion. To further characterize the pancreatic fluid and protein secretory response to CCK-8, we infused the maximally effective dose of CCK-58, 500 pmol kg⁻¹ h⁻¹, in the presence of atropine (100 μg·kg⁻¹ h⁻¹ iv), or the CCK-A receptor antagonist MK-329 (1 mg·kg⁻¹ h⁻¹ iv) or secretin antiserum (100 μl, bolus iv). Results are shown in Fig. 5.

MK-329 abolished pancreatic protein and fluid secretion in response to CCK-8. Atropine had no inhibitory effect on either protein or fluid output stimulated by CCK-8, instead
slightly increasing both (not statistically significant). The significantly greater stimulation of fluid secretion by CCK-58 compared with CCK-8, shown in Fig. 4 and Table 1, was preserved in the presence of atropine (not shown). Under atropine, the incremental fluid output stimulated by CCK-8 and CCK-58 was 55.8 ± 12.9 and 183 ± 40.8 μl/30 min, respectively (n = 6 each). The effects of MK-329 and atropine on pancreatic secretion were also tested at the lowest dose of CCK-58. MK-329 abolished and atropine had no significant effect on protein or fluid secretion stimulated by 62.5 pmol·kg⁻¹·h⁻¹ of CCK-58 (not shown). The absence of inhibitory effects of atropine on pancreatic secretion stimulated by exogenous or endogenous cholecystokinin in conscious, recovered rats is well established (6, 33, 41). Surprisingly, MK-329 had no effect on the pancreatic fluid secretion caused by 500 pmol·kg⁻¹·h⁻¹ of CCK-8, while abolishing protein output (not shown). Secretin antiserum had no inhibitory effect on the pancreatic fluid or protein secretory response to CCK-58 at 500 pmol·kg⁻¹·h⁻¹. Preliminary studies showed that this dose of secretin antiserum was sufficient to abolish the pancreatic fluid and bicarbonate response to a supraphysiological dose of synthetic rat secretin (Fig. 1 and MATERIALS AND METHODS). Secretin antiserum also had no effect on bicarbonate secretion stimulated by 500 pmol·kg⁻¹·h⁻¹ of CCK-58 (not shown).

Linear Regression Analysis of Relationship Between Protein and Fluid Output in Basal Pancreatic Secretion and Secretion Stimulated by CCK-8, CCK-58, or Camostat

Figure 6 shows the relationship between protein and fluid output (top) and between protein and chloride output (bottom) in CCK-8- and CCK-58-stimulated pancreatic secretion compared with time-paired controls (left). The time period analyzed is the first 60 min of peptide infusion and the corresponding period in time-paired controls.¹ Results for CCK-8 (n = 30) and CCK-58 (n = 29) are for all doses (62.5–500 pmol·kg⁻¹·h⁻¹) except for the supramaximal dose (1,000 pmol·kg⁻¹·h⁻¹). For CCK-58, the regression reveals a strong linear relationship for protein vs. volume and for protein vs. chloride output. In contrast, the same analysis shows that there is no significant linear relationship for CCK-8-stimulated pancreatic secretion or for the controls. Preliminary experiments showed that rat synthetic CCK-33 produced the same protein-fluid relationship as CCK-8 (unpublished observations).

The strong correlation between protein and fluid output with CCK-58 and with camostat was not dependent on the rate of fluid secretion. With CCK-58, even at the lowest dose of 62.5 pmol·kg⁻¹·h⁻¹, the correlation between protein and water output was strong and statistically significant. Coefficients of determination ($R^2$) for protein vs. volume at each dose level of CCK-58 were 0.80, 0.68, 0.94, and 0.74 for dose levels of 62.5, 125, 250, and 500 pmol·kg⁻¹·h⁻¹, respectively, and regressions were statistically significant at each dose level. Corresponding $R^2$ values for CCK-8 were 0.16, 0.21, 0.001, and 0.001, with none of the regressions achieving statistical significance ($P > 0.30$). Thus, even though protein output and fluid output changed in parallel at the 62.5 pmol·kg⁻¹·h⁻¹ dose with both CCK-8 and CCK-58, only with CCK-58 were protein output and fluid output significantly correlated at this low dose. Likewise, in studies reported elsewhere (30), when camostat was infused intraduodenally at submaximal doses of 0.05 and 0.2 mg/h and at the maximally effective dose of 0.5 mg/h, the $R^2$ value at each dose was ≥0.95 (Table 2).

Figure 7 shows the time course of pancreatic protein and fluid secretion in rats infused intraduodenally with either casein (10% solution, 5 ml/h) or camostat (5 mg/h in saline) and the results of linear regression analysis (Model 2) of protein vs. fluid output. Both casein and camostat stimulated parallel secretion of protein and fluid (Fig. 7, left). Regression analysis of protein vs. fluid output for the basal periods shows that there was no linear relationship between protein and fluid output (Fig. 7, middle). Upon stimulation with casein or camostat, the relationship between protein output and fluid output immediately became strongly linearly correlated, with the $R^2$ value equal to 0.9 or greater. The periods analyzed for stimulated secretion were the first 30 min with casein and the first 60 min

¹ Correlation between fluid and protein output with all stimulants (except CCK-8) was very strong up to the time of maximal protein output, regardless of whether maximal output occurred at the first, second, or third 30-min period, which prevented or minimized increases in protein concentration. Beyond peak protein output, the correlation weakened as fluid output often continued to rise while protein output plateaued or declined (see Fig. 2).
with camostat, corresponding to the time required to reach maximal protein output. Chloride output in basal and stimulated pancreatic juice also was measured in camostat experiments, and the regression of chloride vs. protein output was almost identical to that of water vs. protein output, i.e., $R^2 = 0.04$ for basal and 0.94 for stimulated secretion (not shown). Compared with basal, camostat caused a significant increase in protein output ($F = 2.79$, $Fcrit = 2.15, P = 0.01$) but did not significantly increase protein concentration in pancreatic juice ($F = 0.37, Fcrit = 2.15, P = 0.93$), similar to the pancreatic protein secretory response to CCK-58.

The ratio of bicarbonate output ($\mu$mol/30 min) to protein output (mg/30 min) was calculated for secretin, CCK-58, and camostat groups to determine whether it revealed any effect of secretin release in the effects of camostat on pancreatic secretion. Ratios were $6.07 \pm 0.71$, $2.19 \pm 0.77$, and $0.76 \pm 0.17$ for secretin ($n = 18$), camostat ($n = 6$), and CCK-58 ($n = 24$), respectively, with each group significantly different from the others. The greater bicarbonate/protein ratio of camostat-stimulated pancreatic secretion compared with CCK-58 was expected, because camostat stimulates release of both CCK-58 (48) and secretin (68).

**Relationship of Bicarbonate and Chloride Secretion to Secretion of Protein and Water in Pancreatic Secretion Stimulated by CCK-58**

Regression analysis of protein vs. fluid produced $R^2$ values that were nearly identical to those obtained with regression analysis of protein vs. chloride in CCK-58- and camostat-stimulated pancreatic secretion. This is not surprising, because chloride ion secretion is considered to be necessary for water secretion by secretory epithelia (17). Regression of water output with chloride output in all of the studies reported in this study showed a strong linear correlation under both basal and stimulated conditions, with $R^2$ values of 0.88–0.99 under all experimental conditions (>1,000 data points). Figure 8 shows the relationship of water output vs. chloride output (Fig. 8, left), water output vs. bicarbonate output (Fig. 8, middle), and water output vs. the sum of chloride plus bicarbonate output (Fig. 8, right) in all studies with rats stimulated by CCK-58 ($n = 41$). Each point in the scattergram is the average rate of secretion (above basal) during the entire 3-h period of peptide infusion.

Water output vs. chloride output showed a strong linear relationship, with an $R^2$ value of 0.92. The relationship of water secretion vs. bicarbonate secretion also showed a significant linear relationship, but the relationship was not nearly as strong, as indicated by the more widely scattered points and an $R^2$ value of only 0.55. When water output vs. the sum of chloride and bicarbonate output was analyzed, the result was a virtually perfect linear relationship ($R^2 = 0.99$). These results strongly indicate that both chloride and bicarbonate secretion drive water secretion in the exocrine pancreas stimulated by CCK-58 but with chloride secretion apparently having the major role. Regression analysis for these same parameters during stimulation by CCK-8, secretin, and camostat and during basal secretion is summarized in Table 3.

Because of the very strong correlation between fluid output vs. the sum of chloride and bicarbonate output described above, we further analyzed the relationship between protein output and the output of water, chloride, bicarbonate, and the sum of chloride and bicarbonate output. We chose the data from CCK-58 infused at the submaximal dose of 250 pmol·kg$^{-1}$·h$^{-1}$, because this dose yielded the strongest correlation of the doses tested and because it was more likely to be a physiological dose given that it stimulated protein output similar to that stimulated by camostat. The results show that the strongest correlation ($R^2$) for protein output was with water output and with the sum of chloride plus bicarbonate output (Fig. 9), with a lesser, but still very strong, correlation with chloride output alone and bicarbonate output alone. Taken together, the results shown in Figs. 8 and 9 indicate that both chloride and bicarbonate secretion drive pancreatic water secretion and that the acinar cell may be the source of both chloride and bicarbonate secretion stimulated by CCK-58.
The lack of substantial or significant stimulation of fluid secretion by CCK-8 or caerulein in conscious rats is well documented (32, 37, 39, 40, 44). In contrast, in acutely operated, anesthetized rats, CCK-8 has been shown to significantly increase pancreatic juice volume to an even greater degree than secretin (44). This effect may be a consequence of the greatly suppressed basal pancreatic secretion caused by operative trauma rather than anesthesia itself, because reanesthetization after recovery from surgery did not cause significant inhibition of basal secretion (12), or the inhibition was much less severe (70). The strong stimulatory effect of CCK-8 or caerulein on volume of pancreatic juice in acutely operated, anesthetized rats may be secondary to improved blood flow to the pancreas (38), which would be expected to increase both acinar and ductule fluid secretion.

The different efficacies for stimulating pancreatic fluid secretion between CCK-8 and CCK-58 are unlikely to be due to differences in half-life in the circulation. The potencies for CCK-8 and CCK-58 were the same for stimulation of protein output, resulting in the same doses for maximal stimulation and supramaximal inhibition. In dogs, the half-life of canine CCK-58 was 4.4 ± 0.6 min compared with 1.3 ± 0.1 min for CCK-8 (27).

Although we have not done the analogous studies for rat CCK-58 and CCK-8, the major difference in effects between the two forms is qualitative, i.e., one form stimulates fluid secretion in a dose-related fashion, and the other does not, and this cannot be explained by differences in circulating half-lives.

Although CCK-8 did not significantly increase pancreatic water output at any 30-min collection period, bicarbonate and chloride concentrations were significantly changed in response to CCK-8, in contrast to CCK-58 and camostat. However, the lack of a dose-related effect of CCK-8 on fluid secretion and the secretin-like pattern of chloride and bicarbonate concentrations suggest that the fluid and electrolyte responses to CCK-8 may be pharmacological rather than physiological. This conclusion is supported by the failure to detect CCK-8 in rat blood (48), which suggests that CCK-8 may not be a physiological form of cholecystokinin. Also, we observed in these studies (not shown) that the pancreatic fluid and electrolyte response to CCK-8 was unaffected by MK-329, in contrast to the complete blockade of the same responses with CCK-58 and suggesting that CCK-8 may be acting in a nonphysiological manner on duct cells, as duct cells apparently have no CCK-A receptors (55).

The finding that the endogenous form of cholecystokinin (CCK-58) is a strong stimulant of pancreatic water secretion may explain the observation of parallel inhibition of pancreatic protein and water secretion by CCK-A receptor antagonists in rats in which pancreatic secretion is stimulated by food or diversion of pancreatic juice from the intestine. In those studies, protein output was sharply decreased, but because water output was proportionally decreased, protein concentration changed little (6, 62, 63).

A widely accepted model proposes that the bulk of pancreatic water secretion arises from duct cells under the influence of secretin (14, 31). We therefore compared the patterns of pancreatic water secretion and corresponding concentrations of chloride and bicarbonate caused by synthetic secretin (rat sequence), CCK-58, CCK-8, and camostat. Figure 4 shows that secretin infused at the maximally effective dose of 250
Rats were infused intravenously with CCK-8 or CCK-58 at 62.5, 125, 250, and 500 pmol·kg⁻¹·h⁻¹ for 3 h during collection of pancreatic juice. Results shown are incremental (basal subtracted) protein, fluid, and chloride output for all doses (n = 7–8 at each dose level) during the initial 60 min (to peak protein output) of peptide infusion and total output during the corresponding period in time-paired controls. Numbers of animals studied were 8 control, 30 CCK-8, and 28 CCK-58 rats. Regression of fluid output vs. protein output and chloride output vs. protein output for rats treated with CCK-8 showed strong linear relationships of peptide infusion and total output during the corresponding period in time-paired controls.

**Fig. 6.** Linear regression analysis of pancreatic protein vs. fluid secretion and protein vs. chloride secretion in rats treated with CCK-8, CCK-58, and controls. Rats were infused intravenously with CCK-8 or CCK-58 at 62.5, 125, 250, and 500 pmol·kg⁻¹·h⁻¹ for 3 h during collection of pancreatic juice. Results shown are incremental (basal subtracted) protein, fluid, and chloride output for all doses (n = 7–8 at each dose level) during the initial 60 min (to peak protein output) of peptide infusion and total output during the corresponding period in time-paired controls. Numbers of animals studied were 8 control, 30 CCK-8, and 28 CCK-58 rats. Regression of fluid output vs. protein output and chloride output vs. protein output for rats treated with CCK-58 showed strong linear relationships ($R^2 = 0.83$ and 0.82, respectively) that were highly significant ($P < 10^{-11}$ and $10^{-12}$, respectively). In contrast, rats treated with CCK-8 showed no correlation between protein output vs. fluid output or vs. chloride output ($P > 0.1$). Likewise, there was no correlation between fluid and protein or chloride and protein during the corresponding time period in time-paired controls.

pmol·kg⁻¹·h⁻¹ stimulated water output equivalent in volume to that caused by 500 pmol·kg⁻¹·h⁻¹ of CCK-58 and by 5 mg/h of intraduodenal camostat but much greater than that caused by CCK-8. However, the pattern of chloride and bicarbonate concentrations in secretin-stimulated secretion was unique compared with that caused by CCK-58 and camostat. Secretin caused characteristic reciprocal changes in bicarbonate and chloride concentrations typical of stimulation of pancreatic duct cells (14), which was not the case with CCK-58 or with camostat. The distinctly different pattern for bicarbonate and chloride concentrations between secretin and CCK-58 suggests that the stimulation of electrolyte secretion by CCK-58 is independent of secretin and that stimulation of bicarbonate secretion by synthetic secretin in these studies may have occurred at the duct cells. Immunoneutralization of circulating secretin with secretin antiserum did not inhibit pancreatic water, bicarbonate, or protein secretion stimulated by CCK-58, but the strong stimulation of water output, as well as protein output, by CCK-58 was abolished by the CCK-A receptor antagonist MK-329 (Fig. 5). These observations further strengthen the view that effects of CCK-58 on pancreatic secretion are independent of secretin.

The sharp contrast (shown in Fig. 7 and Table 2) between basal secretion, in which there was no correlation between protein and water output, and stimulated secretion, in which there was an immediate strong correlation of protein and water output, is noteworthy. This is all the more remarkable considering that basal or interdigestive protein and water secretion constitutes from one-third to two-thirds of the total protein and water output in maximally stimulated pancreatic secretion in conscious rats. This contrast between basal and stimulated pancreatic secretion must be due to different mechanisms.
Fig. 7. Pancreatic protein and fluid output in rats stimulated by intraduodenal casein and intraduodenal trypsin inhibitor (camostat). Rats were infused continuously intraduodenally for 2 h with saline at 5 ml/h. At time 0, they were infused for 2 h with 5 ml/h of 10% casein (n = 7) or for 3 h with 5 mg/h of camostat (n = 6). Protein (●) and fluid (○) output vs. time are shown at left. Results of linear regression analysis (Model 2) of fluid and protein output data during basal (pre-stimulated) secretion are shown at middle. Results of linear regression analysis of incremental (basal subtracted) fluid output and protein output during the initial 30 min (to peak protein output) of casein infusion and the initial 60 min (to peak protein output) of camostat infusion are shown at right. Regressions were significant for casein- and camostat-stimulated secretion (P < 0.001 and P < 10^-8, respectively, ANOVA). Regressions for basal secretion were not statistically significant (P > 0.6).

Implications of These Studies for the Two-Component Paradigm for Pancreatic Secretion

The “two-component” paradigm has dominated thinking about the source, composition, and regulation of pancreatic secretion for 65 years (35). In this paradigm, pancreatic enzyme, on one hand, and the bulk of pancreatic fluid on the other, arise from distinct cell types, acinar and duct cells, respectively (31). The two-component model also specifies that these secretions be regulated separately, with acinar cell secretion predominately regulated by cholecystokinin or acetylcholine and duct cell secretion predominately regulated by secretin. Therefore, during a meal, pancreatic digestive enzymes mobilized in acinar cells are “washed out” in a small volume of plasma-like acinar fluid into the duct system, where they are diluted by a much larger volume of bicarbonate-rich fluid to form the final secretion. In the two-component model, under physiological conditions one would expect to see widely varying concentrations of protein (depending on strength of stimulus at the acinar cell) in a final solution in which the predominant anion is bicarbonate from the duct cell.2

A modification of the two-component paradigm may be justified on the basis of results of the present study, which suggest that the acinar cell rather than the duct cell is the origin of water in pancreatic secretion stimulated by physiological agonists. This finding is based on the very strong correlation between protein and water output in pancreatic secretion stimulated by CCK-58 compared with secretin.

We suggest that the strong correlation between protein output and water output under stimulated conditions either unmasks a tight intracellular linkage between protein and water output or is evidence of a dependence of protein secretion on water secretion. The evidence for these propositions is as follows: in cannulated, awake rats fully recovered from surgery, pancreatic protein and fluid output changed in highly parallel fashion when stimulated by various treatments that increase either endogenous cholecystokinin release (6, 21, 36, 40) or vagal efferenst discharge (1, 11, 23) such that protein concentration changed little despite wide variations in protein output. Furthermore, when pancreatic secretion of protein (or enzyme) vs. pancreatic secretion of water is analyzed in a variety of models, including humans, the two appear to be strongly correlated, again suggesting either an intracellular linkage between protein and water output or a dependence of protein output on water output (26, 28, 52). The occurrence of such parallelism under the two-component paradigm would require very close coordination in release of acinar cell/enzyme-stimulating factors (cholecystokinin and acetylcholine) with release of duct cell/water-stimulating factors (secretin) and the simultaneous occupation of respective receptors on acinar and duct cells, yielding approximately the same degree of stimulation for both acinar and duct cells. Although there is

\[\text{[In species]}\]
Table 3. Relationship between volume of pancreatic juice and output of chloride and bicarbonate during basal conditions and during stimulation

| Treatment          | Volume vs. Cl<sup>-</sup> | Volume vs. HCO<sub>3</sub><sup>-</sup> | Volume vs. Cl<sup>-</sup> + HCO<sub>3</sub><sup>-</sup>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CCK-8 (62.5–500 pmol·kg&lt;sup&gt;–1&lt;/sup&gt;·h&lt;sup&gt;–1&lt;/sup&gt; iv)</td>
<td>y = 6.93x + 18.5</td>
<td>R² = 0.90</td>
<td>y = 18.7x − 13.8</td>
</tr>
<tr>
<td>Secretin (62.5–250 pmol·kg&lt;sup&gt;–1&lt;/sup&gt;·h&lt;sup&gt;–1&lt;/sup&gt; iv)</td>
<td>y = 9.39x + 61.7</td>
<td>R² = 0.88</td>
<td>y = 12.6x + 64.0</td>
</tr>
<tr>
<td>Camostat (5 mg/h id)</td>
<td>y = 8.48x − 0.63</td>
<td>R² = 0.98</td>
<td>y = 23.6x + 34.5</td>
</tr>
<tr>
<td>Basal</td>
<td>y = 7.05x + 19.3</td>
<td>R² = 0.97</td>
<td>y = 23.2x + 51.6</td>
</tr>
</tbody>
</table>

Linear regression analysis (Model 2) was performed to examine the relationship between volume of pancreatic secretion vs. output of chloride, vs. output of bicarbonate, and vs. the sum of chloride and bicarbonate output. Values analyzed were the average incremental (basal subtracted) outputs (volume of chloride in µl/30 min or bicarbonate in µmol/30 min) during the entire 3 h of stimulant infusion. For basal, values were the total output/30 min during the initial 90-min period before stimulant infusion for all experiments with CCK-8 and CCK-58. For CCK-8, n = 37; for secretin, n = 18; for camostat, n = 6; and for basal, n = 78. All regressions were statistically significant (ANOVA). In equations, y = volume in µl/30 min and x = µmol/30 min.

Fig. 8. Relationship between volume of pancreatic juice and output of chloride and bicarbonate ions in secretion stimulated by CCK-58. Linear regression analysis (Model 2) examined the relationship between volume of pancreatic secretion vs. output of chloride, vs. output of bicarbonate, and vs. the sum of chloride and bicarbonate output. Values analyzed were the average incremental (basal subtracted) outputs (volume of chloride in µl/30 min or bicarbonate in µmol/30 min) during the entire 3 h of stimulant infusion. All regressions were statistically significant (P < 10<sup>−7</sup>, ANOVA). In equations, y = volume in µl/30 min, x = µmol/30 min. Results indicate that pancreatic water secretion is driven by both chloride and bicarbonate secretion. Identical analyses were performed for pancreatic secretion stimulated by CCK-8, secretin, and camostat and for basal secretion and are shown in Table 3.
index of duct cells. The authors concluded that the time course of proliferating acinar cells and the high chloride concentration of the juice indicated that acinar cells were the source of the fluid hypersecretion.

Finally, the concept of the acinar cell as a major source of water in stimulated exocrine secretions should not seem unfamiliar given that the acinar cells of the salivary glands are the sole source of water in saliva. The duct cells of the salivary modify the primary (acinar) secretion by modification of electrolyte concentrations, but they add no water (8). Also, similar to the discovery in the pancreas is the finding of CFTR-dependent chloride channels in the acinar cells of the submandibular gland (73). Salivary fluid secretion is driven by a calcium signal that stimulates apically located chloride channels, analogous to the role of calcium in stimulation of enzyme secretion by rat pancreatic acinar cells (42). However, whereas fluid secretion is considered to be a primary function of salivary and lacrimal acinar cells (referred to as fluid-secreting acinar cells), fluid secretion is not considered to be a primary function of the pancreatic acinar cell (25). The belief that fluid secretion is not a primary function of the pancreatic acinar cell does not rest on evidence that directly compares the volume of pancreatic acinar vs. pancreatic ductal fluid secretion but is accepted largely because of the wide acceptance of the two-component model for pancreatic exocrine secretion. We suggest that the acinar cell of the pancreas should be considered a “fluid-secreting” acinar cell, analogous to the salivary and lacrimal glands, and that, in the rat, the acinar cell is the predominant source of stimulated pancreatic water secretion.

Role of Secretin in Regulation of Pancreatic Water Secretion

The trypsin inhibitor camostat releases both cholecystokinin and secretin. Pancreatic water and protein secretion stimulated by camostat are markedly inhibited by blocking the action of either secretin or cholecystokinin separately and are abolished by blocking the action of both secretin and cholecystokinin simultaneously (68). This observation is consistent with many studies showing potentiation between secretin and cholecystokinin in exocrine pancreatic function (57). The phenomenon of potentiation between cholecystokinin and secretin in pancreatic secretion has long been recognized, but the locus of this interaction is not known (5, 69). Others have proposed that the concept of potentiation requires that the potentiating stimuli act on different membrane receptors triggering distinct cellular pathways, but implicit in this concept is that the different receptors and distinct pathways are located in the same cell (31).

Does the cholecystokinin-secretin interaction occur at the acinar cell or the duct cell? Camostat-stimulated water (and chloride) secretion was tightly linked to protein secretion (Fig. 7, bottom), suggesting that the increased water secretion came from the same cell type as the protein secretion, i.e., the acinar cell. Furthermore, the likelihood of cholecystokinin stimulation of pancreatic water secretion by acting on duct cells is doubtful, because rat pancreatic duct cells apparently do not possess CCK-A receptors (55). Furthermore, purified porcine CCK-33 had no effect on secretin-stimulated pancreatic fluid output or bicarbonate output (i.e., no potentiation of secretin-stimulated water or bicarbonate secretion) in rats in which the acinar cells had been selectively destroyed by feeding a copper-deficient diet with penicillamine (14). These observations, together with our understanding of potentiation as it applies to regulation of secretion at the cellular level (56), cannot easily be reconciled with cholecystokinin and secretin interacting through separate cell types, but they are compatible with secretin and cholecystokinin acting through separate receptors on the same cell type, the acinar cell.

Whether potentiation of secretin-stimulated bicarbonate secretion by cholecystokinin can occur at the acinar cell is unknown. The acinar cell has not been considered to be an important source of pancreatic bicarbonate secretion. However, fluid collected from the acini of rabbit pancreas contains bicarbonate at concentrations four times that of extracellular fluid (4). The discovery that CFTR may also act as a bicarbonate channel (47) and the finding of CFTR in rat and mouse pancreatic apical acinar membrane (73) opens the possibility that the undetermined locus at which cholecystokinin, as well as acetylcholine, potentiates secretin-stimulated bicarbonate...
secretion may be the acinar cell. This hypothesis is further strengthened by our finding in the present study of a strong correlation between protein output and the summed output of chloride and bicarbonate.

Secretin receptors are found on pancreatic acinar cells of several species, including rat and guinea pig (29, 66). These observations suggest that synergistic interaction of secretin and cholecystokinin in stimulation of pancreatic water secretion occurs at the acinar cell, where acinar cell fluid secretion is integrated with protein secretion via as yet uncharacterized interaction of the second messengers cAMP and cellular calcium (67). It is not known whether secretin receptors are present on human pancreatic acinar cells, but the possibility is indicated by studies of Susini et al. (61), who reported that isolated human pancreatic acini responded to secretin and VIP with an increase in cellular cAMP.

**Mechanism for Proposed Coupling of Protein Secretion With Water Secretion in Pancreas**

A molecular basis for the hypothetical coupling of protein and water in pancreatic secretion evidenced in the present study is provided by studies of Park et al. (42) in which they demonstrated the presence of calcium-activated chloride channels in the apical, but not basolateral, plasma membrane of the mouse pancreatic acinar cell. These channels respond to the same pattern of intracellular calcium release that is associated with cholecystokinin-stimulated or acetylcholine-stimulated enzyme mobilization and discharge. In agreement with this finding, output of chloride with CCK-58 and camostat in the present studies was strongly correlated with protein output. Park et al. concluded that linkage of pancreatic water secretion and protein secretion in the acinar cell occurs via a common event, the increase of intracellular calcium, which causes enzyme mobilization and also activates apical chloride channels. This scenario could also account for the strong correlation between protein output and chloride/water output in rats treated with camostat (which releases both cholecystokinin and secretin), because cAMP and calcium have been shown to interact synergistically on apical chloride conductance in T84 colonic epithelial cells (67). Whether cAMP mediates the increased bicarbonate secretion associated with CCK-58 treatment remains to be determined.

**What Can Account for the Aberrant Actions of CCK-8?**

Although CCK-8 also increases intracellular calcium that, in turn, mobilizes enzyme secretion, CCK-8 fails to stimulate pancreatic water and chloride secretion and abolishes all pancreatic water secretion at high supramaximal doses (19). One explanation for the failure of CCK-8 to stimulate water secretion could be that CCK-8 uncouples chloride secretion from increased intracellular calcium and, consequently, uncouples water secretion from protein secretion. An uncoupling of increased cellular calcium and calcium-activated apical chloride channels has been described in cell lines originating from intestinal epithelia (17) and more recently in a human cystic fibrosis pancreatic ductal cell line (3). Another explanation rests on the observation that CCK-8 has a greater tendency to elicit global calcium transients than does acetylcholine (43, 46). This globalization may reduce sodium gradient-driven chloride uptake across the basal membrane (45), thereby inhibiting apical chloride and fluid secretion. If CCK-58 affects calcium transients differently than CCK-8 (CCK-58 behaving more like acetylcholine), this may explain the greater ability of CCK-58 to stimulate chloride and water secretion. Studies to elucidate these possibilities await the comparison of CCK-8, CCK-58, and acetylcholine in isolated pancreatic acinar cells. Attempts to investigate actions of CCK-58 by using dispersed pancreatic acinar cell preparations have been inconclusive, because all such preparations caused rapid degradation of $^{125}$I-labeled CCK-58, despite addition of a wide spectrum of protease inhibitors to the assay media (unpublished observations).

**Pathophysiological Implications of CCK-58 Effects on Pancreatic Protein and Water Secretion**

These studies may shed light on exocrine pancreatic diseases such as alcoholic pancreatitis and cystic fibrosis. In both diseases it is believed that a diminution in pancreatic ductule fluid secretion leads to obstruction of secretion, both acinar and ductule (50, 53). If a deficit in pancreatic acinar water secretion is also involved in these diseases, it would suggest that an additional target for intervention would be electrolyte and water transport by the acinar cell. The supramaximal CCK-8/caerulein model for acute pancreatitis may be a model of pancreatic acinar fluid insufficiency, because, in a preliminary report, we showed that in contrast to CCK-8, large supramaximal doses of CCK-58 (2-4 nmol·kg$^{-1}$·h$^{-1}$) cause minimal or no pancreatitis in the rat, which was associated with maintenance of pancreatic water secretion (71).

The studies reported in the present study and elsewhere indicate that CCK-58 stimulates actions that are qualitatively different from those stimulated by CCK-8 (18, 64). Together with the demonstration that CCK-58 is the only circulating form of cholecystokinin in the rat and considering that this is probably true in humans as well (13), these results suggest that actions attributed to cholecystokinin, but that were based on studies with CCK-8, may need to be reexamined. Nevertheless, actions of CCK-8 appear to mimic pathological processes, and therefore CCK-8 will continue to be a valuable tool for investigating pancreatitis and possibly cystic fibrosis. It is also conceivable that CCK-8 may occur in some pathological states in which breakdown of cholecystokinin is enhanced in blood or interstitial fluid.

Other peptide hormones have been shown to exist in more than one molecular form (e.g., peptide YY). The question therefore becomes, what are the actual endocrine forms and levels of these peptide hormones? Do they exist as identified, or are they artifacts of degradation during processing of blood? Furthermore, as shown in the present report with cholecystokinin, do the various forms of a given hormone have qualitative differences in bioactivity?

**ACKNOWLEDGMENTS**

We appreciate the expertise of F. J. Ho for the synthesis and purification of rat CCK-58.

Present address of M. Yamamoto: University of Occupational and Environmental Health, Japan, School of Medicine, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan (E-mail: m-yamamoto@med.uoeh-u.ac.jp).

**GRANTS**

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-33850 (to J. R. Reeve, Jr.) and DK-37482 (to...
REFERENCES

PANCREATIC SECRETION STIMULATED BY CCK-58 AND CCK-8


