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Release of Cholecystokinin by Acid (38562)

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In addition to its well known effect on stimulation of pancreatic bicarbonate secretion, acid in the intestine also causes contraction of the gallbladder (1) and stimulates pancreatic protein secretion to a greater degree than can be accounted for by secretin (2). Since these effects of acid on gallbladder contraction (1) and protein secretion (3) persist after nervous connections between gut and target organs have been cut, they are assumed to be mediated at least in part hormonally. Because cholecystokinin (CCK) can mimic these actions of acid, it is assumed that acid releases CCK, in addition to secretin, from the intestinal mucosa. Based on gallbladder response (4) or on pancreatic protein response (5), some have judged acid to be a powerful releaser of CCK, but from studies on pancreatic protein secretion we (6) classified acid as a weak releaser.

This study represents an attempt to estimate the amount of CCK released by acid. Since acid releases both secretin and CCK, and since both hormones have effects of both gallbladder contraction (7) and pancreatic protein secretion (8), it was necessary to devise a method to obviate the effects of secretin. This was done by giving a large background dose of secretin and allowing pancreatic secretion to reach a steady level before introducing acid into the intestine. Under these circumstances the endogenous secretin released by acid would be expected to have no effect in the face of the already supramaximal dose of exogenous secretin, so any effect on pancreatic protein secretion could be ascribed solely to release of CCK by acid. Under the same conditions, doses of CCK were given to determine how much was needed to match the effect of acid. Additional studies without secretin background were also done. The findings support our earlier conclusion (6) that acid is a weak releaser of CCK.

Methods. Animals. Four mongrel dogs (19–26 kg) were prepared with chronic pancreatic fistulas (9). In addition, a Thomas cannula (10) was placed in the dependent part of the stomach.

Test procedure. Tests were started no sooner than 2 wk after surgery. At least 48 hours intervened between tests. Food but not water was withheld for 18 hr before each test. At the beginning of each study, through the Thomas cannula of the pancreatic fistula, a polyvinyl tube 2 mm in diameter was introduced 15 cm into the duodenum for intestinal perfusion of test substances. Throughout each study 0.15 *M* NaCl was infused continuously into a leg vein at 60 ml/hr; intravenously administered hormones were added as needed.

Tests with secretin background. Basal pancreatic secretion was collected for four 15-min periods. Pure synthetic porcine secretin (gift of Dr. M. Ondetti, Squibb Institute) in a dose of 1300 ng/kg/hr was then given by continuous intravenous infusion during the remaining 4 hr of the test with 15-min collections of pancreatic secretion throughout. During the third hour of secretin infusion HCl or 0.15 *M* NaCl as control was infused into the duodenum from calibrated peristaltic pumps. The concentration of HCl or rate of perfusion was varied as shown in Table I. The osmolality of the perfusate was kept constant by reciprocal variation of NaCl and HCl concentrations. Only one concentration of acid and one rate of perfusion was used on any one day. Perfusates contained phenol red and the collections from the gastric fistulas, which were kept open during all tests, were monitored for appearance of the indicator; only occasional traces were seen. In some tests exogenous hormones were given in place of duodenal infusion. Either the dose of secretin was doubled from 1300 to 2600 ng/kg/hr or various doses of 10% pure porcine CCK (Gastrointestinal Hormone Research Unit, Karolinska Institutet, Stockholm, Sweden) were given.

Tests without secretin background. After

TABLE I. INCREMENT IN PROTEIN OUTPUT IN RESPONSE TO INFUSION OF HCl INTO THE INTESTINE OR TO INTRAVENOUS INJECTION OF CCK DURING CONTINUOUS INTRAVENOUS INFUSION OF SECRETIN. MEANS OF EIGHT TESTS ON FOUR DOGS.

Stimulus			Increment in protein output (mg/min)	P
HCl infused into intestine				
ml/hr	*M*	mmole/hr	mean ± SE	
100	0.005	0.5	0.09 ± 3.2	>0.05
100	0.01	1	3.7 ± 3.1	>0.05
100	0.02	2	7.3 ± 2.2	<0.05
100	0.04	4	6.4 ± 3.1	<0.05
100	0.08	8	5.3 ± 1.7	<0.05
50	0.16	8	6.1 ± 2.0	<0.05
100	0.16	16	12.6 ± 3.3	<0.05
200	0.16	32	20.9 ± 7.5	<0.05
0.15 *M* NaCl infused into intestine				
100			3.1 ± 2.7	>0.05
CCK intravenously				
unit/kg hr				
0.5			12.4 ± 4.2	<0.05
1.0			27.8 ± 6.0	<0.05
Secretin intravenously				
μg/kg hr				
1.3			−0.8 ± 2.4	>0.05

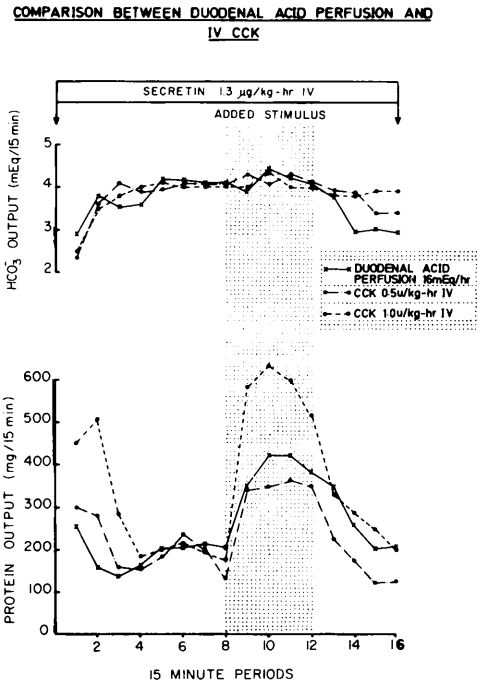

FIG. 1. Pancreatic bicarbonate and protein secretion in response to intraduodenal infusion of acid or intravenous infusion of CCK during continuous background stimulation by a large dose of secretin.

four basal 15-min collections from the pancreatic fistula, the pancreas was stimulated for 2 hr by: (a) Continuous duodenal perfusion with 0.16 *M* HCl at 12.5, 25, 50, 100, or 200 ml/hr; (b) continuous intravenous infusion of synthetic secretin in doses of 62.5, 125, 250, or 500 ng/kg-hr; or (c) continuous intravenous infusion of a fixed ratio combination of synthetic secretin and CCK, the lowest dose being 62.5 ng/kg-hr secretin plus 0.125 Ivy dog unit/kg/hr CCK, and the other doses being two, four, and eight times these. Only one of these stimuli was given on any one day and the order of tests was randomized.

Analyses on pancreatic juice. Volume was measured to the nearest 0.1 ml. Bicarbonate was measured by adding 1.0 ml 0.1 *M* HCl to 0.5 ml sample, boiling, and then back-titrating with 0.2 *M* NaOH to pH 7.0 using an automatic titrator (Radiometer, Copenhagen). Protein concentration was estimated by comparing absorbance at 280 nm with standards of bovine serum albumin.

Statistical analysis. The significance of differences between the pretest and the test periods was -estimated by Wilcoxon's matched-pairs, signed-ranks test (11).

Results. Tests with secretin background. The output of bicarbonate and protein reached a steady level during the second hr of secretin infusion (Fig. 1). Increments in secretion were calculated by subtracting the mean values of the last three 15-min periods before the added stimulus from those of the last three 15-min periods of the hr of added stimulation. The mean (±SE) outputs in the 45 min before the added stimulus were: 2.1 ± 0.1 ml/min, 272 ± 11 μEq/min bicarbonate, and 13.3 ± 1.4 mg/min of protein. None of the added stimulants caused a significant change in bicarbonate output so these values are not reported. Neither infusion of saline into the intestine nor doubling the dose of secretin caused a significant change in protein output (Table I). Infusion of 0.5 or 1 mmole/hr of HCl did not cause a significant increase in protein output but

TABLE II. PANCREATIC SECRETORY RESPONSES TO ACID INFUSED INTO THE INTESTINE, SECRETIN INTRAVENOUSLY, OR A MIXTURE OF SECRETIN PLUS CCK INTRAVENOUSLY. MEANS OF EIGHT TESTS ON FOUR DOGS.

		Volume μl/min	Bicarbonate μEq/min	Protein mg/min
HCl infused into intestine (meq/hr)				
2		247 ± 22	15 ± 2.7	7.3 ± 0.98
4		510 ± 20	57 ± 3.3	12.2 ± 0.87
8		1060 ± 51	134 ± 7.3	18.1 ± 0.99
16		1749 ± 43	235 ± 2.0	24.1 ± 0.94
32		1738 ± 45	228 ± 9.3	24.8 ± 1.04
		Secretin intravenously		
(ng/kg-hr)				
62.5		343 ± 24	31 ± 2.7	7.6 ± 1.23
125		390 ± 37	36 ± 4.7	8.0 ± 1.07
250		865 ± 41	111 ± 6.0	12.2 ± 0.77
500		1801 ± 15	240 ± 4.7	20.9 ± 0.68
		Secretin + CCK intravenously		
(ng/kg-hr)	(unit/kg-hr)			
62.5	0.125	293 ± 39	23 ± 4.0	10.0 ± 1.46
125	0.25	603 ± 41	67 ± 5.3	13.3 ± 1.03
250	0.5	1419 ± 20	184 ± 2.0	21.0 ± 1.13
500	1.0	2057 ± 23	275 ± 4.7	26.1 ± 1.00

doses of 2–32 mmole/hr did produce significant increases (Table I). The responses to 2, 4, and 8 mmole/hr did not differ significantly and only with the highest doses, 8, 16, and 32 mmole/hr, were graded responses seen. The increment in protein output caused by 16 mmole/hr of HCl was about equal to that seen in response to 0.5 unit/kg-hr of CCK.

Tests without secretin background. Samples obtained during the last six 15-min periods were used. Each of the stimulants, duodenal acid, secretin alone, or secretin plus CCK, produced graded bicarbonate and protein responses (Table II). A plot of bicarbonate output versus protein output (Fig. 2) shows that the response to acid is mimicked more closely by the mixture of secretin plus CCK than by secretin alone.

Discussion. These studies allow us to estimate the amount of CCK that must be given to mimic the action of acid in the intestine on pancreatic protein secretion. By interpolation in the nearly parallel portions of the log dose versus response lines from the data in Tables 1 and 2, the response to 16 mmole/hr HCl in the studies with secretin background was matched by 0.5 unit/kg-hr of CCK and in the studies without secretin background it was matched by 1.4 unit/kg-hr secretin and 0.7 unit/kg-hr CCK. By contrast, other studies (6) have shown that strong CCK releasers such as amino acids or fatty acids at similar molar rates of infusion into the intestine caused protein secretion rates equivalent to 3–5 unit/kg-hr CCK.

Only the highest acid loads, 16 and 32 mmole/hr, produced distinct stimulation of protein output. It is unlikely that acid loads of this magnitude ever reach the intestine during normal digestion. Although the peak rate of gastric acid secretion in response to a meal is in the range of 16–32 mmole/hr, that acid is strongly buffered by the protein in the meal and is partially neutralized by pancreatic bicarbonate which, in the present studies, was diverted to the exterior. The peak rate of secretin release by acid during a meal has been estimated to be about 0.5 unit/kg-hr (12). Assuming that acid releases secretin and CCK in a fixed ratio, as suggested by some of the findings of the present study, then the peak release of CCK in response to a meal would be about 0.25 unit/kg-hr, which is about a threshold dose.

Release of secretin by acid is dependent on

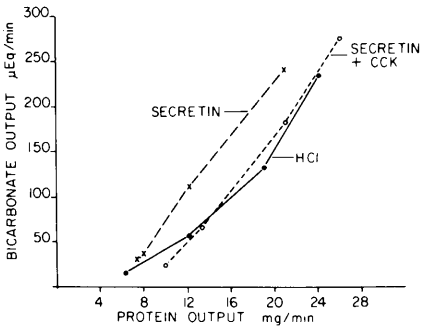

FIG. 2. Protein output versus bicarbonate output with various doses of secretin alone, secretin plus CCK, or duodenal HCl. The response to HCl is mimicked by the secretin-CCK mixture.

load (mmole/hr) and not on acid concentration provided that pH is three or less (13). Because the pancreatic protein responses to low acid loads are so small, it is difficult to assess the relative importance of load and acid concentration for CCK release. However, it is clear that CCK release is at least in part load dependent as shown by the greater protein output seen when 0.16 *M* HCl was given at 32 mmole/hr than at 16 mmole/hr.

Konturek and coworkers (5) found that on instillation into duodenal loops HCl and amino acids produced similar protein outputs although bicarbonate output was greater with acid. Because of the equal protein outputs, they concluded that acid released as much CCK as amino acids. Amino acids release only CCK and not secretin (8) whereas acid releases both. Also, stimulation of protein secretion by CCK is augmented in the presence of secretin (8). If the protein response to acid is ascribed to CCK alone rather than to secretin plus CCK, the CCK-releasing action of acid will be overestimated.

The effectiveness of acid in contracting the gallbladder despite its apparent weakness as a CCK releaser may depend on enhancement by secretin of CCK's gallbladder contracting action (7).

Berry and Flower (4) introduced various substances into the duodenum of anesthetized cats and measured the CCK content of the blood by an *in vitro* bioassay using rabbit gallbladder. They found that only HCl was a strong releaser of CCK, peptone and fat gave just detectable responses. Since strong stimulation of pancreatic protein secretion (6) and of gallbladder contraction (14) is regularly seen in unanesthetized animals and man when amino acids or fatty acids are introduced into the intestine, perhaps anesthesia interferes with CCK release by these agents.

Pure secretin has a potency of four clinical units per microgram and pure CCK has a potency of three Ivy dog units per microgram (15). The molecular weight of secretin is 3055 and of CCK 3918. Therefore one clinical unit of secretin is 82 pmol and one Ivy dog unit of CCK is 85 pmol. The D50 of secretin for bicarbonate secretion is 101 pmol/kg-hr and the D50 of CCK for protein secretion is 245 pmol/kg-hr (6). Assuming that acid releases a fixed ratio of 1 unit secretin for each 0.5 unit CCK, the ratio of secretin to CCK in this mixture is 1.9 on a molar basis and 4.7 on the basis of fractions of their respective D50s.

Although the effect of acid can be mimicked by exogenous CCK, it is possible that acid is releasing some other unidentified agent that stimulates pancreatic protein secretion. When immunoassay for CCK in blood is perfected it may help to answer this question (16).

Summary. In dogs with pancreatic fistulas, the amount of CCK released by acid in the intestine was estimated by determining how much exogenous CCK had to be given to mimic the pancreatic protein response. Duodenal infusion of 16 mmole/hr of HCl with a high background dose of intravenous secretin stimulated pancreatic protein secretion to about the same degree as 0.5 unit/kg-hr of exogenous CCK; the same acid load without secretin background gave a response equivalent to about 0.7 unit/kg-hr of CCK. Expressed as fractions of their respective doses for half maximal response, acid releases almost five times more secretin than CCK.

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