Influence of Secretin–Cholecystokinin and Secretin–Pentagastrin on Pancreatic Secretion of Glycosidases[1] (35319)

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A recent publication from our laboratory (1) drew attention to the presence in canine pancreatic juice of the following glycosidases: β-*N*-acetyl-D-glucosaminidase (β-glucosaminidase), β-*N*-acetyl-D-galactosaminidase (β-galactosaminidase), α-D-mannosidase (α-mannosidase) and α-L-fucosidase (α-fucosidase). These enzyme systems probably originate from pancreatic tissue lysosomes since they have acid pH optima which closely follow those measured by others for lysosomal glycosidases. Because the activities of the glycosidases in pancreatic juice are low by comparison with those of other pancreatic enzymes such as trypsinogen or amylase, it is possible that their presence in pancreatic secretions is nothing but an incidental phenomenon resulting perhaps from entrainment with other enzyme proteins. On the other hand, if these glycosidases belong to the physiologic complement of enzyme protein in exocrine pancreatic secretions, their concentrations in pancreatic juice should increase in response to ecbolic stimuli. Attempts to clarify this point form the basis for our report. The data will show that pancreatic secretion of the 4 glycosidases named above increases in response to pancreatic stimulation by secretin–cholecystokinin and secretin–pentagastrin to the same degree as that of trypsinogen and amylase.

Materials and Methods. Collection of canine pancreatic juice. Duodenal fistulas were constructed in 2 dogs after the fashion described by Thomas (2). Pancreatic secretions were collected over 15-min periods via a small glass probe introduced into the major pancreatic duct and connected by plastic tubing to a precalibrated graduated cylinder immersed in ice. Volumes were recorded to the nearest 0.1 ml. Each 15-min collection was dialyzed against 0.15 *M* NaCl for 15 hr at 4°.

Pancreatic stimulation. All experiments were carried out over a 3-hr period during which the dogs received a continuous iv injection of secretin[2] at a dose of 1 unit/kg/hr during the experiments with cholecystokinin–pancreozymin or of 2 units/kg/hr when the effects of pentagastrin were studied.

During the second hour of each experiment, the dogs received (in addition to the continuous injection of secretin) a continuous injection of cholecystokinin–pancreozymin[2] (CCK) at a dose of 2, 4, and 8 Ivy dog units/kg/hr. Four experiments were carried out on each dog at each dose level. Secretin alone was given during the third hour of the experiment.

In another series of experiments the dogs received, during the second hour of each experiment, a continuous iv injection of pentagastrin[3] at a dose of 0.5, 1.0, 2.0, 4.0, and 16.0 μg/kg/hr. One experiment was carried out at each dose level. Secretin alone was given during the third hour.

Analytical methods. Protein concentration in pancreatic juice was estimated by measuring absorbance at 280 mμ after diluting the sample with phosphate buffer (pH 6.8). Bovine serum albumin was used as a standard

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[2] Secretin and cholecystokinin–pancreozymin were purchased from the Karolinska Institutet, Stockholm, Sweden.

[3] Pentagastrin was generously provided by the Ayerst Research Laboratories.

TABLE I. Influence of Secretin-CCK on Pancreatic Secretion of Protein and Enzymes.[a]

	Dog no.	Secretin[b] (0–60 min) $n = 48$	Cholecystokinin + secretin[b] (60–120 min) 2 units[b] $n = 16$	4 units[c] $n = 16$	8 units[c] $n = 16$	Secretin[b] (120–180 min)
Vol (ml/15 min)	1	12.3 ± 4.0	16.35 ± 4.81	17.59 ± 3.78	18.53 ± 2.90	16.00 ± 3.56
	2	13.9 ± 6.5	26.18 ± 8.12	24.80 ± 6.47	24.86 ± 4.67	21.84 ± 8.85
Protein (mg/15 min)	1	135.6 ± 73.9	343.90 ± 121.69	462.07 ± 135.06	643.90 ± 205.99	119.60 ± 68.86
	2	115.2 ± 83.3	320.89 ± 104.03	604.29 ± 182.63	701.16 ± 226.23	97.14 ± 65.17
Trypsinogen	1	28.6 ± 19.0	81.39 ± 27.44	107.27 ± 41.50	154.11 ± 61.99	25.87 ± 19.86
(μM/15 min)	2	24.6 ± 20.2	76.81 ± 28.11	119.56 ± 49.52	157.40 ± 63.62	20.14 ± 14.80
Amylase	1	10.4 ± 5.9	27.50 ± 8.91	36.39 ± 13.94	48.93 ± 17.19	8.74 ± 5.98
($\mu M \times 10^3$/15 min)	2	8.9 ± 6.9	24.28 ± 6.85	46.63 ± 13.64	59.34 ± 21.71	6.53 ± 5.21
β-D-Glucosaminidase	1	58.9 ± 51.4	167.46 ± 87.90	245.75 ± 127.48	327.72 ± 96.82	58.51 ± 39.67
($\mu M \times 10^{-3}$/15 min)	2	32.6 ± 33.0	78.44 ± 38.34	221.08 ± 65.60	243.90 ± 75.75	23.10 ± 16.26
β-D-Galactosaminidase	1	10.4 ± 8.7	29.08 ± 15.06	41.61 ± 22.28	56.13 ± 16.19	10.26 ± 6.62
($\mu M \times 10^{-3}$/15 min)	2	5.8 ± 5.4	13.18 ± 6.28	37.68 ± 11.93	40.41 ± 12.70	4.29 ± 2.84
α-D-Mannosidase	1	3.3 ± 2.0	8.47 ± 3.88	11.83 ± 3.78	14.57 ± 3.42	5.87 ± 1.89
($\mu M \times 10^{-3}$/15 min)	2	4.0 ± 2.1	8.58 ± 3.42	16.36 ± 3.64	20.89 ± 4.73	6.25 ± 2.64
α-L-Fucosidase	1	9.7 ± 5.3	26.77 ± 17.06	31.23 ± 11.49	38.71 ± 12.66	10.71 ± 5.93
($\mu M \times 10^{-3}$/15 min)	2	8.6 ± 6.5	26.50 ± 13.86	37.06 ± 12.60	43.29 ± 14.82	10.95 ± 5.49

[a] All values represent average ± SD of n 15-min collection periods.

[b] Secretin was administered at the rate of 1 unit/kg/hr.

[c] Dose of CCK (units/kg/hr).

and results were expressed as milligrams per 15-min collection of pancreatic juice.

Amylase activity was estimated according to Bernfeld (3) using maltose as a standard.

Tryptic activity was assayed after activation of trypsinogen with enterokinase (Calbiochem) by incubating 0.1 ml of appropriately diluted pancreatic juice with 0.6 mg of enterokinase in 0.4 ml of 0.05 *M* sodium barbital buffer (pH 8.0) for 10 min at 25°. Tryptic activity was then estimated according to Erlanger (4) using BAPA (Mann Res. Lab.) as substrate.

β-Glucosaminidase, β-galactosaminidase, α-mannosidase, and α-fucosidase were estimated according to previously used methods (1). Results were calculated as micromoles of substrate hydrolyzed per minute per milliliter of pancreatic juice and were expressed as total output/15 min. The assays were carried out under conditions of zero-order kinetics and the degree of hydrolysis of substrate never exceeded 15%. Under these routine assay conditions, the coefficient of variation of the assay of 14 replicate samples of pancreatic juice for activities of β-glucosaminidase, β-galactosaminidase, α-mannosidase, and α-fucosidase were 1.68, 2.28, 2.31, and 1.57%, respectively. For the same 4 enzyme systems there was a linear relationship between enzyme activity and the volume of pancreatic juice assayed within the range from 0.2 to 0.8 ml of pancreatic juice.

To determine the influence of CCK and pentagastrin on the different variables measured in pancreatic juice, data from all 15 min periods for each dose level of CCK and pentagastrin for each dog were averaged. Similar data from all of the 15-min periods during the first and third hr of the experiment when secretin alone was administered were averaged separately. Dose–response curves for each variable studied were established for the experiments with CCK by plotting the percentage increase in the variable during the first 15 min of secretin–CCK (av of 4 expts. at each dose level) over the value measured during the last 15 min of the first hour of secretin administration (av of all 12 expts.) against the logarithm of that dose

TABLE II. Influence of Secretin-Pentagastrin on Pancreatic Secretion of Protein and Enzymes.[a]

	Dog no.	Secretin[a] (0–60 min) $n = 20$	Pentagastrin + secretin[b] (60–120 min) 0.5[c] $n = 4$	1.0[c] $n = 4$	2.0[c] $n = 4$	4.0[c] $n = 4$	16.0[c] $n = 4$	Secretin[b] (120–180 min) $n = 20$
Vol (ml/15 min)	1	15.0 ± 3.6	16.83	23.30	20.15	19.19	21.00	17.90 ± 6.32
	2	18.4 ± 6.8	27.40	34.02	28.80	30.38	28.40	24.77 ± 8.87
Protein (mg/15 min)	1	160.5 ± 108.8	241.68	406.59	398.62	363.76	315.86	208.00 ± 79.70
	2	88.2 ± 67.4	198.75	355.63	237.71	324.90	446.36	141.85 ± 94.68
Trypsinogen	1	38.4 ± 28.6	46.84	93.89	90.55	94.49	80.42	50.23 ± 20.74
(μM/15 min)	2	18.8 ± 14.9	49.50	113.54	54.01	79.48	117.38	33.19 ± 24.04
Amylase	1	12.7 ± 8.9	18.02	30.89	32.50	28.40	26.06	15.67 ± 9.01
($\mu M \times 10^3$/15 min)	2	7.0 ± 6.2	15.73	30.98	20.59	29.47	48.56	12.46 ± 9.58
β-D-Glucosaminidase	1	67.8 ± 45.6	106.76	153.91	196.08	210.04	124.00	105.45 ± 57.54
($\mu M \times 10^{-3}$/15 min)	2	25.8 ± 23.9	41.12	142.31	69.80	113.21	159.55	41.16 ± 32.24
β-D-Galactosaminidase	1	11.5 ± 7.4	17.90	36.43	32.54	34.52	23.66	16.14 ± 8.51
($\mu M \times 10^{-3}$/15 min)	2	5.0 ± 4.7	7.50	25.34	12.28	18.21	26.70	7.47 ± 5.60
α-D-Mannosidase	1	4.6 ± 2.5	6.60	12.21	15.34	8.97	17.47	8.49 ± 3.11
($\mu M \times 10^{-3}$/15 min)	2	4.3 ± 2.2	6.98	14.69	8.73	11.37	12.36	7.29 ± 3.08
α-L-Fucosidase	1	13.6 ± 12.0	21.19	27.52	39.76	32.91	25.85	18.06 ± 6.37
($\mu M \times 10^{-3}$/15 min)	2	9.0 ± 6.6	8.46	22.28	22.48	32.50	33.57	12.25 ± 7.15

[a] All values represent averages of n 15-min collection periods ± SD where appropriate.

[b] Secretin was administered at the rate of (2 units/kg/hr).

[c] Dose of pentagastrin (μg/kg/hr).

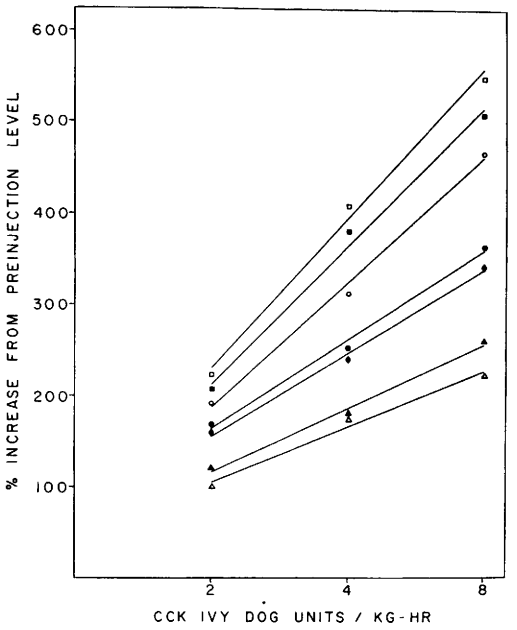

FIG. 1. Percentage increase in pancreatic output of protein and enzymes produced by CCK plotted as a function of log dose of CCK: (■) glucosaminidase ($p<.05$); (□) galactosaminidase ($p<.05$); (○) trypsinogen ($p<.025$); (●) amylase ($p<.05$); (◆) protein ($p<.025$); (▲) fucosidase ($p<.025$); (△) mannosidase ($p<.05$); p = significance of goodness of fit.

level. Regression lines were calculated for the 3 points and the goodness of fit of the lines to the actual data was determined according to Snedecor.

Results. The data are summarized in Tables I and II and in Fig. 1. The data indicate that the output of β-glucosaminidase, β-galactosaminidase, α-fucosidase, and α-mannosidase in pancreatic juice increased during pancreatic stimulation by secretin–cholecystokinin and by secretin–pentagastrin and that the magnitude of the response was similar to that of trypsinogen and amylase. Statistical analysis of the dose–response curves of percentage increase in enzyme output as a function of log dose of cholescystokinin demonstrated a significantly linear slope for all enzyme systems studied (Fig. 1). The data in Fig. 1 suggest that the log dose–response relationships of pancreatic glucosaminidase, galactosaminidase, fucosidase, and mannosidase are similar to those of trypsinogen and amylase.

Discussion and Summary. We had previously described the presence in canine pancreatic juice of several glycosidase enzyme systems. The present data indicate that the outputs of β-glucosaminidase, β-galactosaminidase, α-mannosidase, and α-fucosidase in pancreatic juice increase in response to pancreatic hormonal stimulation to the same degree as those of trypsinogen and amylase. This suggests that these glycosidase enzyme systems may belong to the physiologic complement of enzyme protein in pancreatic juice. It is, of course, important to recognize that the ability of a glycosidase to remove a glycosyl group from a synthetic substrate, which forms the basis of our enzyme assay, does not prove that the enzyme can release the same group from a naturally occurring large molecular substrate. However, the suggestion of a physiologic role for at least one of these enzymes, β-*N*-acetylglucosaminidase, is strengthened by our finding (to be reported) that this enzyme releases *N*-acetylglucosamine from ovomucoid.

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