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Food and Secretagogue Stimulation Decrease the Digestive Enzyme Content Remaining in the Rat Pancreas

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The aim of the study reported here was to investigate changes in the digestive enzyme content in the pancreas after food and secretagogue stimulation. Rats from which food had been withheld overnight were either fed (between 6 and 8 a.m.) or not before euthanasia and pancreatic excision (at 8 a.m.: 21 not fed and 21 fed) and at 4 (12 p.m.: six not fed and six fed) and 8 h later (4 p.m.: six not fed and six fed). Another 16 rats were anesthetized, fitted with jugular vein and pancreatic duct catheters, and infused with the secretagogues, CCK-33 and secretin, during 1.5 h of pancreatic juice collection before euthanasia and pancreatic excision. The pancreata were homogenized, and total soluble protein and individual enzyme (trypsin and amylase) tissue contents were analyzed. Results indicated lower amounts of protein and enzymes remaining in the pancreata of the fed, compared with non-fed rats. Enzyme values indicated recovery within four hours in fed rats, but non-fed rats also had increased values during daytime. High enzyme secretion during the high dose of hormonal stimulation was reflected in lower enzyme values remaining in the pancreas, compared with that in response to low-dose stimulation. Results indicated that stimulation of the pancreas, either by food ingestion or exogenous secretagogues, lowers the amounts of digestive enzymes remaining in the pancreas, and imply that stimulation and circadian rhythms influence the pancreatic enzyme content at euthanasia. This finding should be borne in mind in interpretation of data from pancreatic studies.

Exocrine pancreatic function has been investigated by analysis of enzyme activities in excised pancreatic tissue or in secreted pancreatic juice collected from animals with surgically implanted pancreatic duct catheters. Direct comparison between these two methods has, to our knowledge, not been done, but circumstantial evidence indicates that these principally different methods may not always yield consistent results. For example, in two studies from our laboratory concerning development of the pancreatic exocrine function in young pigs, Weström and co-workers (1) analyzed changes in pancreatic enzyme content, whereas Pierzynowski and co-workers (2) analyzed the secretion of pancreatic juice. Though results of both studies indicated a general increase in pancreatic function with age, the increase in individual enzyme activities in the two studies did not match temporally. Merchant and co-workers (3) reported that the pancreatic content of enzymes in newborn rat pups decreased soon after birth and concluded that the reason for this decrease was the initiation of suckling and, thus, pancreatic secretion, although actual secretion was not measured. These examples indicate a problem when interpreting results of studies of pancreatic tissue (i.e., that the state of stimulation preceding euthanasia might have influenced the secretion and, thus, the enzyme content remaining in the pancreas).

To be able to perform accurate measures of pancreatic function, it is essential to determine whether the pancreatic content of digestive enzymes at a particular time point is influenced by the amount secreted previously. Therefore, the purpose of the study reported here was to determine whether stimulation by feeding or secretagogues might influence the remaining amount of secretory proteins in the pancreas and whether the quantity of proteins secreted is correlated to the amounts remaining in the pancreas.

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Materials and Methods

Animals. The study was performed by use of conventionally raised male rats (*Rattus norvegicus*) of the Sprague-Dawley strain (Møllegaard Breeding & Research Ltd., Skensved, Denmark). Health monitoring of the rats in the departmental animal rooms was performed according to the FELASA protocol (4). The rats were kept under a controlled environment at a temperature of 20 ± 1°C, relative humidity of 50 ± 10%, and a 12-h day-night rhythm (lights on at 8 a.m. and off at 8 p.m.) for at least one week before the experiments were done. Rats were housed on a good laboratory practice (GLP) manufactured chopped wood bedding (Beekay, Sollentuna, Sweden) made from aspen trees in polycarbonate cages and with ad libitum access to rat chow (Altromin 1324, Brogaarden, Gentofte, Denmark) and tap water. The Lund University Ethical Review Committee for Animal Experiments approved the study.

Experimental procedure. Food was withheld from rats (300 ± 42 g) overnight (from 4 p.m. the preceding day), then to synchronize the feeding period, either rat chow was made available ad libitum during a restricted time period between 6 and 8 a.m. (n = 21, fed group), or not (n = 21, non-fed group) before euthanasia and pancreatic excision between 8 and 9 a.m. To investigate pancreatic recovery capacity, a separate experiment was performed where rats (250 ± 12 g) were either not fed or fed as described previously, then were euthanatized at 3 separate times

during the daytime between 8 and 9 a.m. (six not fed and six fed), between noon and 1 p.m. (six not fed and six fed) and between 4 and 5 p.m. (six not fed and six fed). All rats were euthanatized by administration of an overdose of pentobarbiturate (50 to 60 mg/kg of body weight, i.v.), and after laparotomy, the entire pancreas was removed by dissection immediately, washed in ice-cold (0°C) 0.9% NaCl, blotted dry, and weighed.

Another group of rats (n = 16), used for collection of pancreatic juice, were anesthetized with xylazine (Rompun, Bayer, Leverkusen, Germany; 0.8 mg/100 g) combined with ketamine (Ketalar, Parke-Davis, Barcelona, Spain; 10 mg/100 g) given subcutanously. Anesthesia was maintained with ketamine given i.v. (1 mg/100 g), when needed, during the experiment. Under aseptic conditions, a silicon (Silastic [i.d., 0.51 mm; o.d., 0.94 mm] Dow Corning, Midland, Mich.) tubing was implanted in the jugular vein. A ventral incision was made into the abdominal cavity, and the common biliary-pancreatic duct was catheterized close to its entry into the duodenum by use of silicon tubing (Silastic [i.d., 0.30 mm; o.d., 0.63 mm]). The bile duct was ligated to collect pure pancreatic juice.

After a 30-min stabilization period, the rats were given an intravenous infusion of cholecystokinin-33 (CCK, Ferring, Malmö, Sweden) with secretin (Secretin, Ferring) dissolved in 0.15*M* NaCl and 0.5% bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, Mo.), using a syringe pump at a flow of 10 ml/h. The secretagogues were given at a low dose of 25.4 pmol of CCK/h and 22 pmol of secretin/h and in a tenfold higher dose of 254 pmol of CCK/h and 220 pmol of secretin/h. The pancreatic juice was diverted completely and collected in plastic tubes during 1.5 h, and the volume was measured before freezing. Directly after the sampling period, the rats were euthanatized and the entire pancreas was removed by dissection and washed and weighed as described previously. The pancreatic juice samples and pancreata were frozen rapidly in liquid nitrogen and stored at -20°C until analysis.

Analyses. The pancreata were homogenized at 0°C in 0.2*M* Tris-HCl buffer containing 0.05*M* $CaCl_2$, pH 7.8, in the ratio 1:10 (wt./vol.), using a glass/glass homogenizer with motor-driven pestle. The homogenates were then centrifuged at 15,000 ×*g* for 1 h (4°C), and the supernatant was used for analysis of total soluble protein concentration and trypsin and amylase activities. Total protein concentration was analyzed, using the Lowry method (5), which modified for use of 96-well microtitration plates (2) and with BSA as a standard.

Trypsin activity was measured by use of a microtitration plate modification (2) of the original method by Fritz and co-workers (6), using the substrate N-α-benzoyl-DL-arginine-*p*-nitroanilide (Sigma Chemical Co.). Amylase activity was analyzed, using blue starch as a substrate, with the Phadebas Amylase Test (Pharmacia, Uppsala, Sweden) according to manufacturers instructions. Enzyme activities were expressed as units (U), with one unit defined as the amount of enzyme causing transformation of 1.0 μmol of substrate/min at 25°C.

Statistics. The results from the various groups of rats were compared statistically, using Student's *t* test or two-way analysis of variance (ANOVA) and Tukey's post hoc test (SigmaStat for Windows v2.0, SPSS Science, Chicago, Ill.).

Results

The pancreata from the synchronically fed (between 6 and 8 a.m.) rats contained significantly decreased total protein content, as well as individual enzyme (trypsin and amylase) activities, compared with values for the non-fed rats at euthanasia at 8 a.m. (Table 1). Pancreatic recovery of total protein and trypsin and amylase values was apparent within four hours (at noon) after feeding and appeared stable thereafter (at 4 p.m.) (Table 2). Also, the pancreata of rats from which food had been withheld overnight contained increasing total protein, trypsin, and amylase values during daytime, as measured at noon and 4 p.m. (Table 2).

Table 1. Mean (± SEM) pancreatic content of total soluble protein, trypsin, and amylase in rats from which food had been withheld overnight (non-fed) and then were either fed between 6 and 8 a.m. or not fed before euthanasia and pancreatic excision at 8 a.m.

	n	Protein (mg/kg)	Trypsin (U/kg)	Amylase (kU/kg)
Non-fed	21	255.0 ± 15.2	28.7 ± 1.7	60.4 ± 4.5
Fed	21	205.4 ± 12.4*	19.2 ± 1.7†	35.0 ± 2.5†

*$P < 0.01$ and †$P < 0.001$ indicate significant differences.

Table 2. Mean (± SEM) pancreatic content of total soluble protein, trypsin, and amylase in non-fed and fed rats

		n	Protein (mg/kg)	Trypsin (U/kg)	Amylase (kU/kg)
Non-fed	8 a.m.	6	299.3 ± 16.9^{a,b}	28.3 ± 3.0^{a,b}	41.7 ± 2.6^{a}
	Noon	6	307.8 ± 17.9^{b}	37.0 ± 3.3^{b,c}	61.7 ± 8.0^{b}
	4 p.m.	6	349.7 ± 20.4^{b}	40.4 ± 4.3^{c,d}	63.8 ± 7.9^{b}
Fed	8 a.m.	6	255.2 ± 18.6^{a}	21.3 ± 3.4^{a}	36.1 ± 5.5^{a}
	Noon	6	325.1 ± 8.9^{b}	42.7 ± 4.0^{d}	60.8 ± 5.6^{b}
	4 p.m.	6	342.6 ± 21.0^{b}	43.0 ± 4.9^{d}	61.8 ± 7.0^{b}

Different superscript letters between the values in the columns indicate significant differences at $P < 0.05$. Food was withheld from all rats overnight, then half were euthanatized at 8 a.m., noon, and 4 p.m., and the other half were fed between 6 and 8 a.m., followed by euthanasia at the same time points. Parameters for the various groups of rats were compared statistically by use of analysis of variance (ANOVA) and Tukey's post hoc test.

Infusion of the secretagogues, CCK and secretin, in low doses slightly increased the volume of pancreatic juice secreted, compared with baseline secretion, whereas protein output did not change during the 1.5 h of collection. High-dose stimulation by the secretagogues increased the volume secreted and the total protein output, compared with baseline secretion. The high quantity of trypsin and amylase secreted during 1.5 h of high-dose stimulation was reflected in significantly lower enzyme content in the pancreas, compared with that associated with low-dose stimulation (Fig. 1). However, this was not true for total protein content, which was associated with an equal amount in the pancreatic tissue, though tendency toward a greater amount was secreted and collected in the pancreatic juice.

Discussion

Results of this study indicated that stimulation of the exocrine pancreas by feeding rats, from which food had been withheld overnight, depleted the pancreas of secretory proteins for four postprandial hours. This was true not only for total soluble proteins, which mainly represent digestive enzymes, but also for the individual enzymes, trypsin and amylase. Results also indicated that pancreatic protein content had recovered within four hours after feeding and remained stable for another four hours. Thus, food stimulation lowered the pancreatic content of digestive proteins for a postprandial period of less than four hours. Feeding provoked the release of stored pancreatic secretory proteins from zymogen granules and stimulated new synthesis of

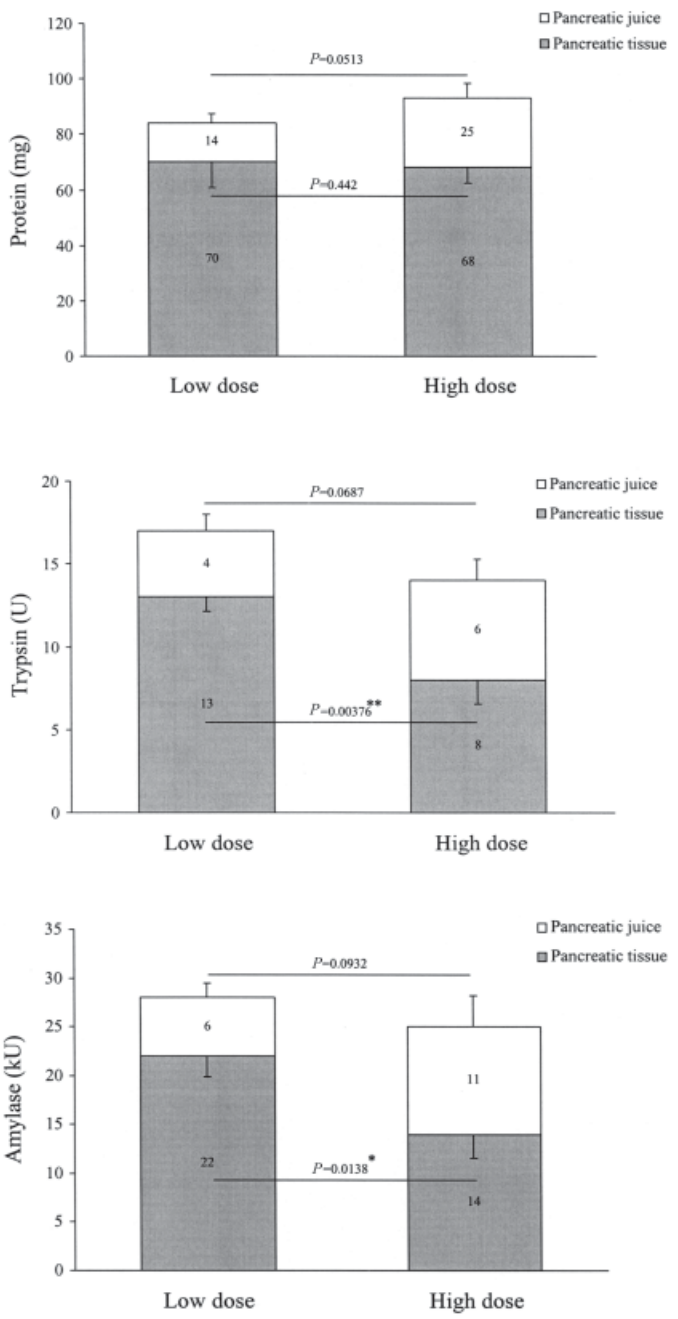

Figure 1. Mean (± SEM) amounts of protein, trypsin, and amylase secreted into pancreatic juice during 1.5 h (white part of the bar) and the remaining amount within the pancreas (gray part of the bar) after secretagogue stimulation. The secretagogues cholecystokinin (CCK) and secretin were infused into anesthetized rats (n = 8 in each category) at a low (25.4/22.0 pmol/h) or high (254/220 pmol/h) dose. $^{*}P < 0.05$ and $^{**}P < 0.01$ indicate significant differences for pancreatic tissue and juice between low- and high-dose secretagogue stimulation.

secretory proteins in the postprandial phase (7). In the later postprandial phase, with still slightly increased secretion, zymogen granules began to be reconstituted until the next feeding stimulus. The synthesis and transport of secretory proteins from the rough endoplasmic reticulum to the Golgi complex take about 20 to 30 min (8). These newly synthesized enzymes, as documented for amylase, lipase, trypsinogen, and chymotrypsinogen, are transported further at different rates through the acinar cells (9), indicating that the time from synthesis until secretion differs depending on the particular enzyme. Thus, the pancreas might be drained of secretory proteins following a stimulation period before particular enzymes have been resynthetized and transported to the secretory granules. In the early postprandial phase, one could expect parallel enzymatic secretion, because of a release of stored enzymes from the acinar granules, whereas non-parallel secretion might be expected during the late postprandial phase, because of differences in the synthesis and release of specific enzymes. Therefore, our observations might also enlighten the issue of parallel and non-parallel enzyme secretion, which explains the relations between synthesis and secretory rates for various secretory pancreatic enzymes (10, 11).

The amounts of pancreatic proteins in the non-fed rats during the day—period after four and eight hours were similar to the increased values seen in the fed rats at the same time intervals. This indicates release of pancreatic digestive enzymes, at least to some degree, independent of feeding, probably during the dark period, and recovery during four to eight hours thereafter in the daylight period. This pattern agrees with that of other studies of circadian rhythms concerning exocrine pancreatic secretion and pancreatic content. For example, in rats, circadian rhythms for the exocrine pancreas have been documented by Montaruli and co-workers. (12), who found peak cytoplasmic volume fraction of acinar cells at the end of the light period. Moreover, Maouyo and co-workers (13) reported that pancreatic juice secretion increased during the dark period and decreased during the light period. The pancreatic content of digestive enzymes is obviously not only dependent on prandial status, but also on a circadian rhythm of the exocrine pancreas.

To investigate the relationship between the amounts of secretory proteins remaining in the pancreas and the amount actually secreted after secretagogue stimulation, controlled stimulation with the secretagogues, secretin and CCK, was performed on catheterized and anesthetized rats. Feed intake increases the blood concentration of these stimulatory hormones and, thus, the pancreatic secretion of digestive enzymes in a diet-related way (7, 14). Cholecystokinin and CCK-like hormones (e.g., cerulein) also stimulate the synthesis of pancreatic enzymes in an enzyme type-specific manner (15). As expected, high-dose infusion of CCK and secretin stimulated high pancreatic secretion (i.e., fluid secretion and output of protein, trypsin and amylase). Interestingly, the secretory response was coupled to a decrease in enzyme content in the pancreas. This observation indicated that high stimulation of secretion depleted the pancreas of secretory proteins, and apparently, the synthesis rate of the secretory enzymes was not sufficient to maintain stable enzyme values. However, the experiment may, to some degree, have been influenced by anesthesia, resulting in depression of protein synthesis and secretion (16, 17). A higher combined quantity of total protein, secreted and remaining in the pancreas, could be detected after high-dose stimulation, indicating that the secretagogues, despite anesthesia, had stimulated protein synthesis (Fig. 1). Surprisingly, trypsin and amylase values had the opposite pattern, with lower quantities of combined secreted and remaining enzyme amounts after high-dose secretagogue stimulation. Taken together, this probably means that synthesis of other digestive enzymes within the pancreas, not measured during this study increased because of the high secretagogue stimulation.

Results of this study indicated that endogenous stimulation of the pancreas by food consumption and exogenous stimulation by hormone infusion temporarily lowers the amounts of digestive enzymes remaining in the pancreas. Thus, our results indicate that when performing studies on pancreatic tissue, with the aim of investigating pancreatic function and/or development, one must be aware of the state of pancreas stimulation during the immediate period preceding euthanasia and resection of the pancreas. Because the quantity of enzymes within the pancreas at a certain time point is a combination of what is secreted and what is synthesized, it is not possible to compare directly this content with what is actually secreted in the pancreatic juice over a period. The time point for and the composition of the feed (i.e., prandial status) and the phase of the circadian rhythm can, therefore, influence results of studies on pancreatic tissue. Compared with analysis of pancreatic juice secreted in vivo, which yields an integrated view over time, analysis of pancreatic tissue yields a steady-state view and may lack reliability when one is interested in pancreatic exocrine function and development. This implies that when performing functional studies on pancreatic tissue, the state of pancreatic stimulation must be taken into account.

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