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Lysosomal Enzyme Secretion into Pancreatic Juice in Rats Injected with Pancreatic Secretagogues and Augmentd Secretion after Short Term Pancreatic Duct Obstruction

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Summary

To examine the possible secretion of lysosomal enzymes into the pancreatic juice in rats stimulated with pancreatic secretagogues under both physiological and pathological conditions, we investigated the changes in the secretion of cathepsin B, as a lysosomal enzyme, into pancreatic juice with stimulation of 5 different doses (0.1, 0.2, 0.5, 1.0, and 1.5 μg/kg・hr) of caerulein. Control rats had only pancreatic duct cannulation. In other rats, the pancreatic duct was obstructed for 3 hours and secretin was infused (0.2 CU/kg・hr). Caerulein stimulated the secretion of cathepsin B into the pacreatic juice in a dose-dependent manner, as in amylase secretion, and caerulein in higher doses (1.0 and 1.5 μg/kg・hr) inhibited cathepsin B output as it did amylase output. There was a significantly high positive correlation between cathepsin B output and amylase output after stimulation with caerulein. The secretion of several other lysosomal enzymes was also stimulated by caerulein. Blockage of the pancreatic duct for 3 hours caused a significant but moderate rise in serum amylase levels. Redistribution of cathepsin B activity in the pancreatic subcellular fractions was noted with an increase in the amount of cathepsin B recovered from zymogen-rich pellets after 15 min of centrifugation at 1300 × g. These changes after temporary pancreatic duct obstruction are very similar to those previously noted during the early stage of diet-and caerulein-induced experimental pancreatitis and suggest colocalization of lysosomal enzymes and digestive enzymes. In addition, in ductobstructed rats, the secretion of cathepsin B and other lysosomal enzymes stimulated by caerulein was significantly greater than in animals with free-flowing pancreatic juice.

These results indicate that lysosomal enzymes are secreted into pancreatic juice after stimulation by gut hormones in the same manner as classical pancreatic digestive enzymes such as amylase. Moreover, lysosomal enzymes which colocalize with zymogen granules in rats with short-term pancreatic duct obstruction are also secreted into pancreratic juice together with digestive enzymes after stimulation with gut hormones. These findings suggest that lysosomal enzymes are present in zymogen granules under normal conditions and that they may play pathophysiological roles in pancreatic juice. They also contribute to an understanding of thepathogenesis of pancreatitis, since cathepsin B can activate trypsinogen.

Key words: Amylase, Caerulein, Cathepsin B, Pancreatic duct obstruction, Redistribution of lysosomal enzyme.
索引用語: アミラーゼ, セルレイン, カテプシンB, 膵管閉塞, ライソゾーム酵素再分布

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Introduction

Both pancreatic digestive enzymes and lysosomal hydrolases are synthesized in ribosomes attached to the endoplasmic reticulum. These two types of enzymes are co transported to the Golgi complex where the lysosomal hydrolases are glycosylated with mannose-6-phosphate and bound to mannose-6-phosphate specific receptors, and lysosomal enzymes are transported to lysosomes[15,26,31]. The digestive enzyme zymogen is packed in condensing vacuoles which mature into zymogen granules as they migrate towards the luminal plasmalemma where they discharge their contents into the ductal space[23]. Thus, these two enzymes are separated in the normal physiological state in the pancreatic acinar cells for the maintenance of normal cellular organization, and the mixture of these two enzymes is potentially dangerous to the acinar cells. Although there seems to be almost no possibility for lysosomal enzymes to be secreted into pancreatic juice after stimulation by pancreatic secretagogues, there have been reports of secretory profiles of lysosomal enzymes in many cell lines[9,12,30,36], and of their presence in pancreatic juice[24] as well as of the possible secretion of lysosomal enzymes into pancreatic juice in normal subjects and in patients with chronic calcifying pancreatitis[7]. These studies suggest that lysosomal enzymes have physiological and pathological roles in biological fluids[17]. It has been shown in both morphological and biochemical studies that the early stage of two forms of experimental pancreatitis, diet-induced in mice[13,21] and secretagogue-induced in rats[27,28,29,35] share the common attribute of co-localization of digestive enzymes with lysosomal hydrolases within large cytoplasmic vacuoles[33], and that redistribution of lysosomal enzymes tatkes place from the lysosome-rich to the zymogen granule-rich fraction. Since cathepsin B, as a lysosomal enzyme, can activate trypsinogen[8,10,11,25] and trypsin can activate the other panceratic digestive enzymes, the co-localization of digestive enzymes with lysosomal hydrolases could lead to the activation of intracellular digestive enzymes and increased lysosomal fragility as an important triggering event in the development of acute apncreatitis[32] within the acinar cells. In contrast to the reversibility that characterizes these secretagogues-induced and rabbit pancreatic duct obstruction models of mild pancreatitis, diet-induced pancreatitis may be characterized by hemorrhagic necrosis of the gland. It is not known whether these observation can be extrapolated to pancreatitis in humans, since the clinical disease obviously does not result from ethionine ingestion and is unlikely to be caused by supramaximal secretagogue stimulation.

On the other hand, gallstone pancreatitis, which is the most common clinical form of acute pancreatitis[1], seems to be triggered by the passage through or incarceration of a stone in the terminal bile duct. It has been suggested that such a stone might obstruct the pancreatic duct, but the mechanism whereby pancreatic duct obstruction might induce pancreatitis has not been clarified. Our present model of pancreatic duct obstruction in rats seems to have much merit in the study of the mechanism of pancreatitis due to duct obstruction. We report here the results of experiments on the secretion of lysosomal enzyme into pancreatic juice after stimulation with pancreatic secretagogue and the effect of short-term obstruction of the pancreatic duct. The possible secretion of digestive enzyme colocalized with lysosomal enzyme following stimulation with 2 different pancreatic secretagogues, secretin and caerulein, is also discussed.

Materials and Methods

Male Wistar rats, weighing 350–375 g, purchased from Shizuoka Experimental Animals

(Shizuoka, Japan) were housed in light-dark cycle regulated light (5:00–17:00) and air-conditioned (23±3°C) animal quarters and fasted for 16 hours prior to each experiment. Caerulein and secretin were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), CBZ-arginyl-arginine-β-naphthylamide from Bachem Biosciences (Philadelphia, PA, U.S.A) and β-naphthylamide from Sigma Chemical. Leucine-2-naphthylamide, 4-methylumbelliferyl sulfate, 4-methylumbelliferyl-N-acetyl-β-D-galactosaminide, and 4-methyl-umbelliferyl-N-acetyl-β-D-glucosaminide were all purchased from Sigma Chemical. All other reagents were of the highest purity available commercially.

Animal preparation

All the experiments were started at 9:00 AM to rule out the circadian rhythm of the rat exocrine pancreas. Rats were anesthetized with intraperitoneal sodium pentobarbital (25 mg/kg · hr initially, supplemented by periodic doses of 10 mg/kg as needed) and a cannula (PE 50) was introduced into the superior vena via the right external jugular vein. After upper abdominal midline laparotomy, the pylorus was ligated and a drainage cannula placed in the stomach (PE 70). The common pancreatic-billiary duct was cannulated (PE 50) extraduodenally, adjacent to the duodenum, and another cannula (PE 10) was introduced into the common hepatic duct near the hepatic hilum for a biliary bypass to the duodenum. After the placement and exteriorization of the various cannulas, the abdominal wound was closed. Core temperature was maintained with a heating pad (American Medical Systems, Cincinnati, OH, U.S.A.) and over-head lamp. In-vivo secretion of cathepsin B into the pancreatic juice was stimulated by caerulein. To wash out the bile from the pancreatic duct, secretin was infused for the first hour through the intra venous catheter at a rate of 0.2 CU/kg · hr, and then caerulein was infused at 5 different rates (0.1 to 1.5 μg/kg · hr) for the next 2 hours at an infusion speed of 0.58 ml/hr of heparinized saline (30 U/ml) with an infusion pump (Harvard Apparatus, Millis, MA, U.S.A.). Pancreatic juice was collected hourly into preweighed ice-chilled ependorf tubes. Amylase and cathepsin B activity output was measured in each fraction and expressed as U/kg · hr.

Caerulein-stimulated secretion of other lysosomal enzymes

In the fractions from rats stimulated by 0.2 and 0.5 μg/kg · hr of caerulein, other lysosomal enzymes (leucine naphthylamidase, N-acetyl-β-D-galactosidase, N-acetyl-β-D-glucosaminidase and sulfatase) were also assayed, the output of each enzyme was expressed as U/kg · hr.

Effect of pancreatic duct obstruciton on serum amylase, pancreatic water content, and cathepsin B distribution subcellular fractions

In other rats prepared by the procedure described above, secretin (0.2 CU/kg · hr) was infused at a speed of 0.58 ml/hr. The pancreatic duct was obstructed for 3 hours, after an initial 30 min period for stabilization, by raising the ductal cannula to a vertical position and allowing the column of secreted juice to rise unitl it reached a steady-state level (usually 15–24 cm above the mid-axillary line). In non-obstructed animals, the cannula was kept horizontal to permit the free-flow of juice for 3 hours. In both groups, blood sampling (0.4 ml) was done through the intra venous catheter before and 1, 2 and 3 hours after obstruction or stabilization for the determination of serum amylase. After blood removal, the same volume of saline was injected. After the completion of each experiment, the rats were sacrified with a large dose of intravenous pentobarbital and the pancreas was removed and trimmed of fatty tissue. One part of the pancreas was used for the estimation of the pancreatic water content by a comparison of the wet weight immediately after removal with the dry weight after desiccation at 150°C for 48°C in a desiccator (Isotemp®, Fisher Scientific, Fair Lawn, NJ, U.S.A.).

Another part of the pancreas was used for subcellular fractionation. The pancreas was rinsed in

cold homogenization buffer (MOPS buffer: 5 mM MOPS, 250 mM sucrose, and 1 mM $MgSO_4$) (pH 6.5) and divided into small fragments, which were homogenized in 6 ml of cold MOPS buffer with 3 full up-and-down strokes of a Dounce homogenizer (Wheaton, Millville, NJ, U.S.A.). The homogenate was centrifuged (150 × g for 15 min at 4°C) to remove unbroken cells and debris, and the resulting supernatant was fractionated by differential centrifugation. The protocol employed was that of Tartakoff and Jamieson[34] with some recent modifications suggested by DeLisle et al[5]. Briefly, this supernatant was centrifuged (1300 × g for 15 min at 4°C) and yeilded a "zymogen granule" pellet and supernatant. The latter was harvested and centrifuged (12,000 × g for 12 min at 4°C) to obtain the "lysosome-mitochondria" pellet and a 12,000 g supernatant, which is considered to be the microsomal and soluble fraction. The various pellets described above were resuspended individually in 2 ml of MOPS buffer prior to the measurement of cathepsin B activity. The cathepsin B activity in each fraction was expressed as % of total activity as an index of cathepsin B distribution in the acinar cells.

Effect of pancreatic duct obstruction on pancreatic amylase and cathepsin B content

The remainig portion of the pancreas was homogenized in a Brinkman Polytron (Brinkman Instruments, Inc., Westbury, NY, U.S.A.) in 5 ml of cold phosphate-buffered (pH 7.4) saline containing 0.5% Triton X-100. After low speed centrifugation (150 g for 15 min at 4°C), the resulting supernatant was tested for amylse activity, cathepsin B activity and DNA concentration. Both the amylase and cathepsin B activity in this supernatant were expressed as U/mg DNA as an index of pancreatic tissue content. Another small part of the pancreas was stained with hematoxylin-eosin for microscopic examination.

Effect of pancreatic duct obstruction on caerulein-stimulated secretion of cathepsin B

After a 30 min period for stabilization following duct cannulation and the start of secretin stimulation, the ductal cannula was raised to a vertical position in the "obstructed" group and left in the horizontal position in the "free-flowing" group. Secretin (0.2 CU/kg · hr) was infused for an additional 3 hours in all animals. Then, the vertically placed cannula of the "obstructed" group was returned to the horizontal position, and juice was collected twice at hourly intervals, while the animals received either secretin (0.2 CU/kg · hr) (fractions OS_1 and OS_2) or caerulein (0.2 μg/kg · hr) (fractions OC_1 and OC_2). In the "free-flowing" animals, after 3 hours of secretin infusion, caerulein (0.2 μg/kg · hr) was infused for 2 hours. Hourly samples of pancreatic juice in the first 3 hours (S_1, S_2 and S_3) and in the next 2 hours (FC_1 and FC_2) were collected. The amylase and cathepsin B activity in each fraction was expressed as U/kg · hr.

Effect of pancreatic duct obstruction on the secretion of other lysosomal enzymes

Bacause of the limitations of sample volumes, only the following other lysosomal enzymes were assayed: leucine naphthylamidase, N-acetyl-β-D-glucosaminidase and aryl sulfatase. Output was expressed as U/kg · hr.

Effect of caerulein administration on redistribution of cathepsin B in rats with short-term obstruction of the pancreatic duct and secretin infusion

After 2 hours of caerulein infusion (0.2 μ/kg · hr), the animals in the duct "obstructed" group were sacrificed by a large dose of pentobarbial, and the pancreas was removed, and used for subcellular fractionation to demonstrate the distribution of cathepsin B in the acinar cells.

Assays

Amylase activity was measured by the method of Bernfeld[3]. One unit of amylase activity was defined as that which releases 1 mg equivalent of maltose from 1% soluble starch in 1 min at 30°C.

Cathepsin B activity was measured fluorometrically with CBZ-arginly-arginine-β-naphthylamide as the substrate, as described by McDonald and Ellis[19]. Deoxyribonucleic acid (DNA), was measured by the method of LaBarca and Paigen[16] with calf thymus DNA as the standard. Leucine-naphthylamidase, N-acetyl-β-D-galactosamidase, N-acetyl-β-D-glucosaminidase and aryl sulfatase were assayed as described by Rinder Knecht, et al[24], with leucine-2-naphthylamide, 4-methylumbelliferyl-N-acetyl-galactosaminide, 4-methylumbelliferyl-N-acetyl-β-D-glucosamidase, annd 4-methylumbelliferyl-sulfate as the respective substrates.

One unit of cathepsin B activity was defined as that which liberates 1 n mole of β-naphthylamine in 1 min at 37°C. One unit of leucine naphthylamidase activity was defined as that which liberates 1 n mole of substrate hydrolyzed in 20 min at 25°C. One unit of β-D-glucosaminidase activity was defined as that which liberates 1 n mole of substrate hydrolyzed in 30 min at 25°C. One unit of aryl sulfatase and β-D-galactosaminidase activity was defined as that which liberates 1 n mole of substrate hydrolyzed in 30 min at 37°C.

Presentation of data

The results reported in this communication represent the mean±SEM values for n determinations each from a different animal. The significance of differences was evaluated by ANOVA with Tukey method a p value of <0.05 was considered to be significant.

Results

In-vivo cathepsin B secretion into pancreatic juice after stimulation with caerulein

The infusion of secretin (0.2 CU/kg · hr) alone caused very little amylase and cathepsin B secretion. The addition of the CCK analogue caerulein to the infusate resulted in a dose-dependent increase of both amylase and cathepsin B output (Figs 1a and 1b), which reached a peak when 0.5 μg/kg · hr of caerulein was infused (34±4 U/kg · hr) and was lower with caerulein infusions of 1.0 (21±4 U/kg · hr) or 1.5 μg/kg · hr (14±3 U/kg · hr). There was close correlation ($p<0.001$) between the stimulation of amylase secretion and the stimulation of cathepsin B secretion induced by caerulein in doses of 0.1–1.5 μg/kg · hr (Fig. 2). There was also a dose-dependent relationship between the volume of pancreatic juice and the concentration of caerulein as with amylase and cathepsin

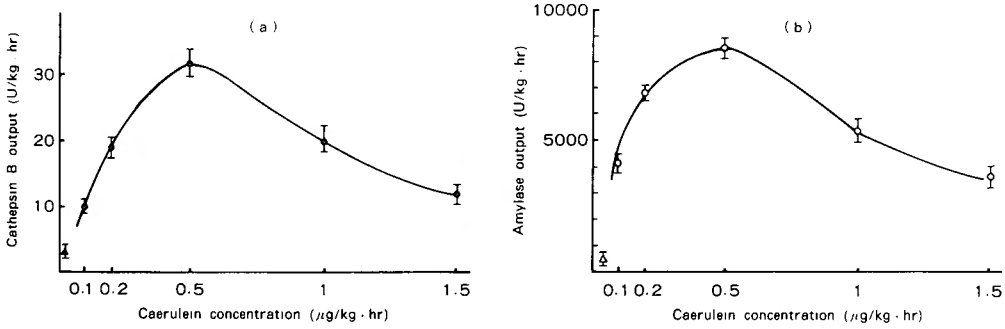

Fig. 1 Cathepsin B (a) and amylase output (b) stimulated by 5 different doses of caerulein (0.1, 0.2, 0.5, 1.0, and 1.5 μg/kg · hr)
Four rats were given each dose and one secretin (0.2 CU/kg · hr) and two caerulein fractions were obtained from each animal. The values are expressed as mean±SEM. (○, amylase output; ●, cathepsin B output; △, amylase output in the secretin fraction; ▲, cathepsin B output in the secretin fraction)

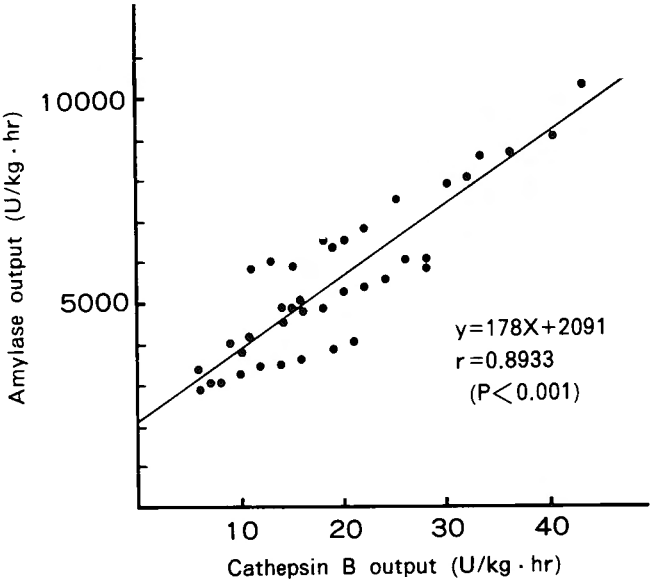

Fig. 2 Correlation between amylase and cathepsin B output stimulated by 5 different doses of caerulein (0.1–1.5 μg/kg · hr)
In the 40 fractions tested, there was a highly significant positive correlation between amylase and cathepsin B output ($r = 0.8933$, $p<0.001$).

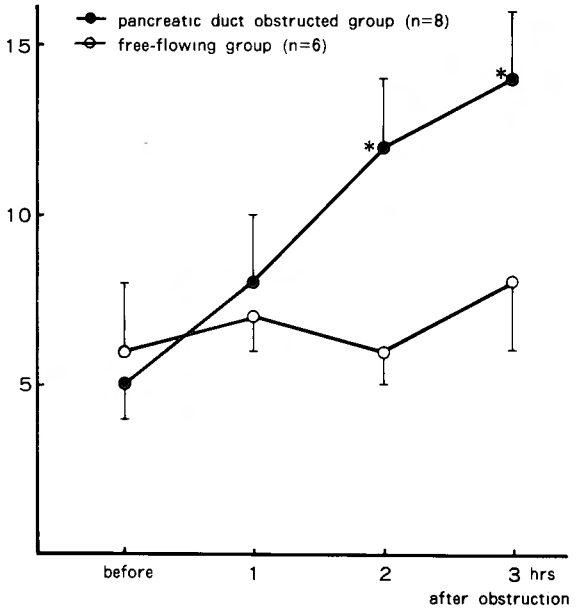

Fig. 3 Effect of short-term pancreatic duct obstruction on serum amylase levels
There were 8 animals in the duct obstructed group and 6 in the free-flowing group. The values are expressed as mean±SEM. (●, obstructed group; ○, free-flowing group; *, $p<0.05$)

B output (Table 1).

Stimulation of secretion of other lysosomal enzymes by caerulein

Infusion of caerulein in concentrations of 0.2 and 0.5 μg/kg · hr also stimulated secretion of several other lysosomal hydrolases: leucine naphthylamidase, N-acetyl-β-D-galactosidase, N-acetyl-β-D-glucosaminidase, and aryl sulfatase (Table 2), In each instance, secretin caused only mild enzyme secretion. The addition of 0.2 μg/kg · hr of caerulein caused a roughly 4-fold increase of secretion, and that of 0.5 μg/kg · hr caused a roughly 7-fold increase of secretion of each of the lysosomal enzymes tested.

Table 1 Effect of secretin (0.2 CU/kg · hr) and 5 different concentrations of caerulein on volume of pancreatic juice.

Caerulein concentration (μu/kg · hr)	n	Pancreatic juice volume (ml/kg · hr) Pancreatic juice fraction		
		S	C_1	C_2
0.1	4	0.23±0.03 (4)	0.85±0.07 (4)	0.96±0.08 (4)***
0.1	4	0.28±0.05 (4)	1.04±0.09 (4)	1.12±0.06 (4)+
0.5	4	0.25±0.03 (4)	1.26±0.11 (4)*	1.33±0.09 (4)++
1.0	4	0.29±0.04 (4)	1.13±0.12 (4)**	0.95±0.10 (4)***
1.5	4	0.21±0.03 (4)	0.74±0.08 (4)	0.64±0.12 (4)

Four rats received each concentration of caerulein and one secretin fraction (S), and two caerulein fractions (C_1 and C_2) were obtained from each animal. The values are expressed as mean±SEM for n determinations. (*, $p<0.02$ compared with 0.1 and 1.5; **, $p<0.05$ compared with 0.1 and 1.5; +, $p<0.02$ compared with 1.5; ++, $p<0.01$ compared with 1.5, $p<0.02$ compared with 0.1 and 1.0 $p<0.05$ compared with 0.2; ***, $p<0.05$ compared with 1.5; ( ), number of fractions.

Table 2 Secretion of other lysosomal enzymes after stimulation by secretin (0.2 CU/kg · hr) and caerulein (0.2 μg/ka · hr).

Lysosomal enzymes	n	Enzyme output (U/Kg · hr) Pancreatic juice fraction		
		S	C0.2	C0.5
Leucine naphthyamidase	4	3±1 (4)	9±2 (8)*	20±4 (8)+
N-acetyl-β-d-galactosaminidase	4	4±1 (4)	15±2 (8)**	29±3 (8)++
N-acetyl-β-d-glucosaminidase	4	5±2 (4)	14±3 (8)*	32±4 (8)++
Aryl sulfatase	4	3±1 (4)	8±2 (8)*	24±3 (4)++

Four rats received each concentration of caerulein and one secretin fraction (S), and 2 caerulein fractions (C0.2 and C0.5) were obtained from each animal. (n, number of animal; S, secretin (0.2 CU/kg · hr) fraction; C0.2, caeruelin (0.2 CU/kg · hr) fraction, C0.5, caerulein (0.5 CU/kg · hr) fraction; ( ), number of fractions; *, $p<0.05$ compared with S, +, $p<0.01$ compared with S and $p<0.02$ compared with C0.2, **, $p<0.01$ compared with S, ++, $p<0.001$ compared with S and $p<0.01$ compared with C0.2)

Table 3 Effect of pancreatic duct obstruction on pancreatic water content.

group	n	Pancreatic water content (%)
Pancreatic duct obstructed group	8	80±3
free-flowing group	6	77±2
normal rats	5	75±2

The values are expressed as mean±SEM for n determinations. There were no significant differences among these three groups.

Effects of pancreatic duct obstruction on serum amylase, pancreatic water content, and cathepsin B distribution in subcellular fractions

After obstruction of the pancreatic duct, the serum amylase levels were significantly higher ($p<0.05$) at 2 hours (12 ± 2 U/ml) and at 3 hours (15 ± 2 U/ml) than in the rats with free-flowing pancreatic juice (at 2 hours, 6 ± 1 U/ml; and at 3 hours, 8 ± 2 U/ml) (Fig. 3). The pancreatic water content in the duct-obstructed group was slightly greater than that in the free-flowing group but not significantly (Table 3). Three hours of duct obstruction caused redistribution of cathepsin B in the subcellular fractions. Cathepsin B activity in the zymogen pellet was $52.4\pm2.8\%$ and that in the lysosomal pellet was $26.4\pm2.4\%$, significantly higher and lower respectively than in the free-flowing

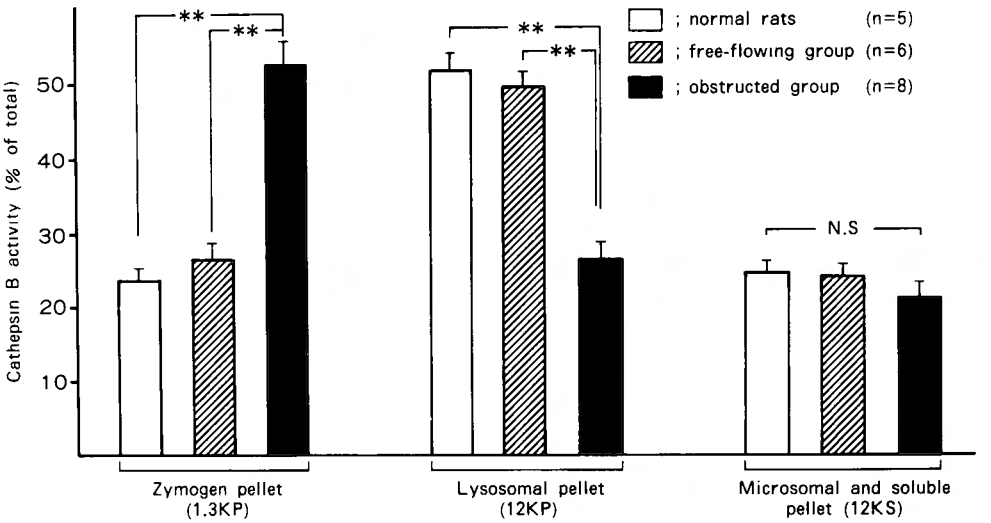

Fig. 4 Effect of short-term pancreatic duct obstruction on distribution of cathepsin B in subcellular fractions in acinar cells
Cathepsin B activity in each fraction was expressed as % of total activity; values are expressed as mean±SEM **, $p<0.01$).

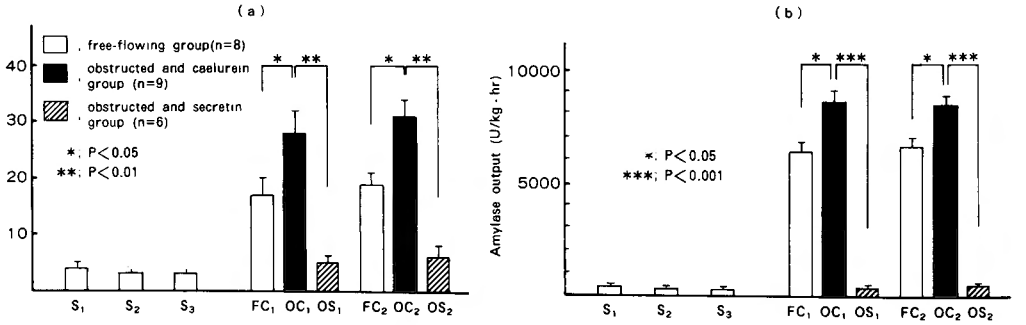

Fig. 5 Effect of short-term pancreatic duct obstruction on cathepsin B (a) and amylase output (b) before and after stimulation by secretin (0.2 CU/kg · hr) or caerulein (0.2 μg/kg · hr)
The values are expressed as mean±SEM. S_1, S_2, S_3; secretin fractions in free-flowing group; FC_1, FC_2; caerulein fractions in free-flowing group; OC_1, OC_2, caerulein fractions in obstructed group; OS_1, OS_2; secreting fractions in obstructed gorup; *, $p<0.05$; **, $p<0.02$, ***, $p<0.01$)

group (zymogen pellet, 26.7±1.9%; lysosomal pellet, 49.3±2.2%) or in normal rats (zymogen pellet; 23.8±1.2%; lysosomal pellet, 51.6±2.3%) (Fig. 4). These results showed the shift of lysosomal enzyme from the lysosomal pellet to the zymogen pellet.

Effects of pancreatic duct obstruction on pancreatic amlyase and cathepsin B content

The amylase content in pancreatic tissue after 3 hours of pancreatic duct obstruction was significantly higher than in the free-flowing or in the normal rats (Table 4). The cathepsin B content in pancreatic tissue after 3 horus of obstruction was not significantly different from that in the control groups. Histologically, 3 hours of obstruction caused no significant changes.

Effect of pancreatic duct obstruction on secretion of cathepsin B after stimulation with caerulein

In rats with free-flowing gpancreatic juice, there were a little cathepsin B activity (S_1, 4±1 U/kg・hr; S_2, 3±1 U/kg・hr; S_3, 3±1 U/kg・hr) and amylase activity (S_1, 423±38 U/kg・hr; S_2, 378±21 U/kg・hr; and S_3, 353±29 U/kg・hr) in the secretin fractions. After 3 hours of obstruction, cathepsin B output after 0.2 μg/kg・hr of caerulein was significantly higher (OC_1, 28±4 U/kg・hr and OC_2, 31±3 U/kg・hr) than in the free-flowing group (FC_1, 17.3±3 U/kg・hr and FC_2, 19.2±2 U/kg・hr) (Fig. 5a). Even after 3 hours of obstruction, secretin infusion alone did not raise cathepsin B output significantly (OS_1, 5±1 U/kg・hr and OS_2 6±2 U/kg・hr). After 3 hours of obstruction, amylase output after stimulation with 0.2 μg/kg・hr of caerulein was significantly higher (OC_1, 8723±398 U/kg・hr; OC_2, 8546±375 U/kg・hr) than in the free-flowing group (FC_1, 6458±316 U/kg・hr; FC_2, 6721±345 U/kg・hr) (Fig. 5b). Even in the obstructed group, secretin infusion alone did not cause a large amylase output (OS_1, 476±31; and OS_2, 519±63 U/kg・hr).

Effect of pancreatic duct obstruction on secretion of other lysosomal enzymes

Secretin increased the secretion of other lysosomal enzymes only slightly in rats with free-flow-

Table 4 Effect of pancreatic duct obstruction on pancreatic amylase and cathepsin B contents.

group	n	Pancreatic amylase content (%)	Pancreatic cathepsin B content (%)
Pancreatic duct obstructed group	8	653±34*	2239±381
free-flowing group	6	428±21	2067±467
normal rats	5	415±19	1953±489

There was a significant difference in the amylase content, but not in the cathepsin B content.
(*, $p<0.01$ compared with free-flowing group and normal rats)

Table 5 Effect of pancreatid duct obstruction on the secretion of other lysosomal enzymes other lysosomal enzymes folowed by stimulation by secretin (0.2 CU/kg・hr) adn caerulein (0.2 μg/ka・hr)

Lysosomal enzyme	S	Enzyme output (U/kg・hr) Pancreaticjuice fraction		OC
		FC	OS	
Leucine naphthyamidase	4±1 (8)	8±2* (16)	5±1 (12)	14±2+ (18)
N-acetyl-β-d-galactosaminidase	5±2 (8)	16±3** (16)	4±1 (12)	26±3+ (18)
N-acetyl-β-d-glucosaminidase	4±1 (8)	13±2*** (16)	6±1 (12)	22±2+ (18)
Aryl sulfatase	3±1 (8)	9±1* (16)	5±1 (12)	13±2+ (18)

There were 8 rats in the free-flowing group (S and FC) and 15 in the obstructed group (OS and OC). The values are expressed as mean±SEM for n determinations. (( )), number of fractions; S, Secretin fractions of free-flowing group; FC, caerulein fractions of free-flowing group; OS secretin fractions of obstruced group; OC, caerulein fractions of obstructed group; *, $p<0.05$ compared with S and OS; **, $P<0.01$ compared with S and OS; ***, $p<0.02$ compared with S and OS; +, $p<0.05$ compared with FC, and $p<0.01$ compared with S and OS)

ing pancreatic juice and even after 3 hours of obstruction of the pancreatic duct it caused onyl a slight increase in the secretion of lysosomal enzymes. On the other hand, the caerulein-stimulated output of the other lysosomal hydrolases was significantly increased after the pancreatic duct had been obstructed temporarily by a vertically positioned cannula (Table 5).

Effects of caerulein and of secretin infusion on the redistribution of cathepsin B induced by short-term obstruction of the pancreatic duct

The administration of 0.2 μg/kg・hr of caerulein after 3 hours of pancreatic duct obstruction, significantly improved the redistribution of cathepsin B in the subcellular fractionations of the acinar cells (zymogen pellet, 43.5±1.9%; and lysosomal pellet, 34.4±2.5%). The corresponding figures after the administration of secretin (0.2 CU/kg・hr) were: zymogen pellet, 49.6±2.3%; lysosomal pellet, 28.1±2.2%. The cathepsin B distribution after obstruction without caerulein or secrein stimulation was: zymogen pellet, 52.4±2.8% and lysosomal pellet, 26.4±2.4% (Fig. 6). These results indicate that some lysosomal enzyme-colocalized zymogen granules induced by short-term pancreatic duct obstruction may be secreted when stimulated by caerulein. This colocalization of lysosomal enzymes and digestive enzymes induced by short-term pancreatic duct obstruction was reflected in the increased cathepsin B/amylase output ratio following stimulation by caerulein (Fig. 7).

Discussion

There have been several reports describe in detail the cell biology of the two experimental forms of acute pancreatitis: diet-induced[13,21] and secretagogue-induced[27,28,29,35]. Remarkably similar col-

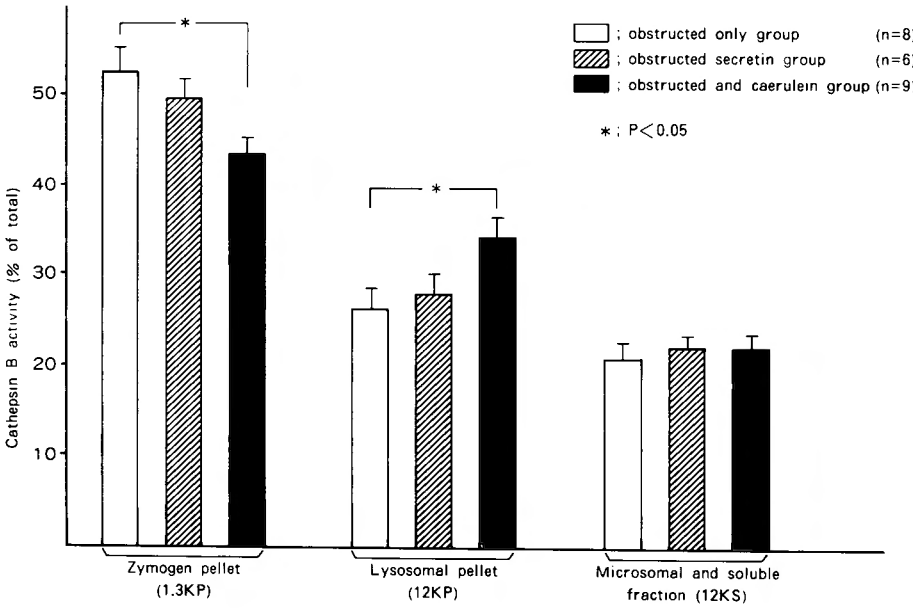

Fig. 6 Effect of caerulein (0.2 μg/kg・hr) administration on redistribtuion of cathepsin B induced by short-term pancreatic duct obstruction
Cathepsin B activity in each fraction was expressed as % of the total activity; values and expressed as mean±SEM.

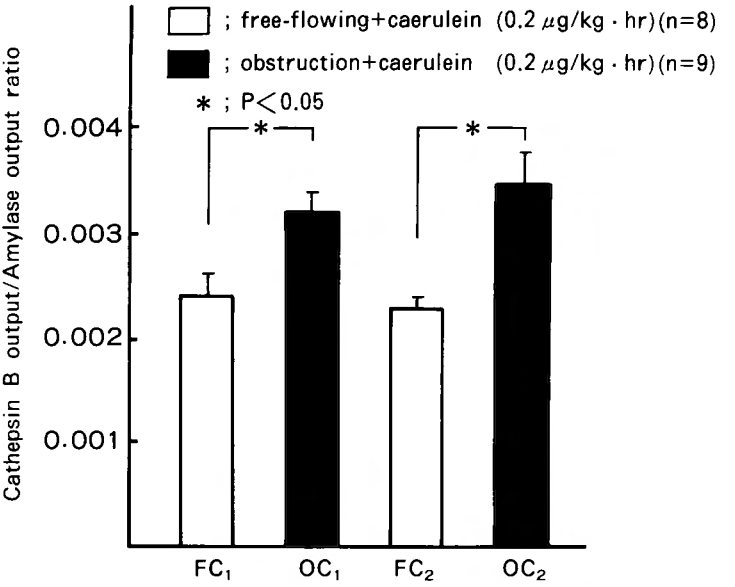

Fig. 7 Effect of short-term pancreatic duct obstruction and stimulation by caerulein (0.2 μg/kg · hr) on cathepsin B/ amylase output ratio

ocalization of lysosomal enzymes and digestive enzymes was noted during the early stages in these models, although the ultimate degree of pancreatic injury differed considerably. Although obstruction might be involved in the genesis of the two experimental models of pancreatitis, their relevance to clinical acute pancreatitis was not clear since in humans this disease that is frequently associated with biliary tract stones and does not appear to be associated with exposure to ethionine or excessive secretagogue stimulation. Rather, gallstone pancreatitis in humans appears to be precipitated by the passage of a stome through or its incarceration in the terminal portion of common bile duct[1]. The mechanism whereby such a stone might precipitate acute pancreatitis has been the subject of many studies and continues to be an issue of considerable controversy. The studies reported in this communication may provide an important clue to the understanding of the events leading to pancretitis. We have demonstrated that in the normal state, the pancreatic secretagogue caerulein can stimulate the secretion of lysosomal enzymes into pancreatic juice in a dose-dependent manner as it does the classical digestive enzymes, such as amylase. Although the mechanism by which gut hormones can stimulate secretion of lysosomal enzymes into pancreatic juice remains to be ellucidated, this bulk discahrge of lysosomal hydrolases into pancreatic juice is the most probable mechanism of release consistent with the maintenance of normal cellular organization. Furthermore, lysosomal enzymes may play some physiological roles in the pancreatic juice. It may also be that a considerable amount of cathepsin B in pancreatic juice could lead to the activation of pancreatic digestive enzymes in the pancreatic duct, since cathepsin B can activate trypsingogen[8,10,11,25], and trypsin can activate other digestive enzymes. Furthermore, there was a highly positive correlation between amylase and cathepsin B output, suggesting that lysosomal enzymes and digestive enzymes can exist in the same subcellular compartment (ie, zymogen granules) and can be secreted by exocytosis when stimulated by gut hormones in the normal physiological state. In fact, there have been a few reports on the localization of lysosomal enzymes, such as acid phophatase, in premature zymogen granules[18,20].

However, the amount of cathepsin B which can be secreted by almost maximal stimulation with caerulein (0.5 μg/kg・hr) was only a small percentage of the total activity in the acinar cells (data not shown). If there is a colocalization of lysosomal hydrolases and digestive enzymes within zymogen granules, it seems to involve only a small compared with amount of the total lysosomal enzyme content of the acinar cells. In general, pancreatic digestive enzymes and lysosomal hydrolases are transported separately from the Golgi apparatus to their own subcellular compartments, condensing vacuoles and lysosomes, and theoretically there seems to be no colocalization of these two types of enzymes in the acinar cells. However, at the beginning of their transport these two types of enzymes share common pathway from cytoplasmic reticulum attached ribosomes to the Golgi apparatus. This mixture of a small amount of lysosomal enzymes and digestive enzymes may be accidental, or it may represent some physiological roles of lysosomal enzymes in zymogen granules, such as the processing of digestive enzymes. In our rat model, the pancreatic amylase content increased after short-term pancreatic duct obstruction, indicating that although protein synthesis continues, newly synthesized digestive enzymes are not discharged, so the concentration of digestive enzymes within the acinar cells increases. In addition, our subcellular fractionation studied indicate that short-term pancreatic duct obstruction leads to a redistribution of cathepsin B activity and that, as a result, lysosomal hydrolases become localized within a fraction that is rich in digestive enzymes. This phenomenon of colocalization of these two enzymes observed in our present study may well be the result of crinophagy (ie, discharge of secretory granules into lysosomes) and a defect in the normal sorting events by which digestive enzymes and lysosomal hydrolases are segregated from each other as they pass throught the Golgi apparatus[6]. This localization phenomenon might be an important triggering event in the evolution of pancreatitis, because of the possible activation of trypsinogen by cathepsin B. This colocalization phenomenon could, under appropriate conditions, result in the intra-acinar cell activation of potentially dangerous digestive enzymes. Although pancreatic duct obstruction might stimulate crinophagy and thus lead to the colocalization of digestive enzymes and lysosomal hydrolases, these observations suggest an alternative explanation: diversion of lysosomal enzymes into the regulated secretory pathway. Another important finding in our present study was that lysosomal hydrolases colocalized with digestive enzymes during short-time pancreatic duct obstruction can be secreted when stimulated by caerulein and can still react to caerulein in this model of very mild pancreatitis. This finding was supported by the observation that the redistribution of lysosomal enzyme can be improved by caerulein and that the cathepsin B/amylase output ratio increases after temporary obstruction. This secretion of lysosomal hydrolases colocalized with digestivve enzymes into pancreatic juice and into the pancreatic duct system and the redistribution of lysosomal enzymes in the pancreatic acinar cells may be important in consideration of the etiology of gallstone pancreatitis in humans. Gallstone attacks are often repeated, and after the first obstruction induced by a gallstone, the secretion of pancreatic secretagogues, such as cholecystokinin and secretin, is stimulated by food intake, and colocalized digestive enzymes and lysosomal hydrolases are secreted into the pancreatic juice together. When the next obstruction is induced by another stone, or if edema of the sphincter of Oddi is still present, these digestive enzymes and lysosomal hydrolases cannot into the pancreatic juice, and, within the acinar cells, another redistribution of lysosomal enzymes takes place. In the normal physiological state, a connection between the pancreatic ductal space and the interstitial space has been reported[2,4], and pancreatic duct obstruction, with or without hypersecretion, seems to increase this connection, which facilitates the entry of digestive enzymes into the systemic circulation or the interstitium of the pancreas. Under these conditions, the

excrine pancreas is exposed to the activation of digestive enzymes by lysosomal hydrolases both within the acinar cells (lysosomal enzyme-colocalized zymogen granules) and outside the acinar cells (colocalization of lysosomal and digestive enzymes in the pancreatic duct system and pancreatic interstitium). These circumstances would lead to a ductal hypertension and damage to the protective barrier of the ductal epithelium[14], induced by simple mechanical pressure or by the reflux of infected biliary juice, somtimes found in chololithiasis, and the pancrease would become more susceptibel to autodigestion from both within and outside the acinar cells.

Although the factors responsible for triggering and worsening the pancreatitis, are not clearly understood, the bulk secretion of lysosomal enzymes, which can potentially activate the pancreatic digestive enzymes, into pancreatic juice following stimulation by pancreatic secretagogue seems to favor the progression from mild edematous pancreatitis to severe hemorrgagic and necrotic pancreatitis, and may be one of the triggers of pancreatitis in "the common channel" theory[22]. Our pancreatic duct obstruction model is the mildest type of pancreatitis in its broad spectrum and should be very useful in explain the very early events in acute pancreatitis. Many recently reported studies support the hypothesis that ductal obstruction is important in thepathogenesis of gallstone pancreatitis, but it is clear from these as well as many other studies that ductal obstruction alone is not Sufficient to cause the morphological changes of pancreatitis. Clearly, other events must occur if the changes induced by ductal obstruction are to lead to more severe pancreatic injury. It is important to identify and explain those events, because they are likely to be the ultimate determinants of the severity of pancreatitis. It is even more important to advance our knowledge of thepathogenesis and pathophysiology of this disease, so that therapy can be improved.

Our study does not explain clearly the significance of the secretion of lysosomal enzymes into pancreatic juice after stimulation by gut hormones, but our findings strongly suggest that exocytosis of lysosomal enzymes is induced by pancreatic secretagogues. Further details remain to be explored in the future.

Our model of controlled pancreatic duct obstruction seems to be of considerable value for furure studies designed to address these issues, since it provides a method of minimizing the severity of acute pancreatitis.

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和文抄録

ラット膵液中でのライソゾーム酵素と短期間膵管閉塞後の分泌動態について

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ラット膵液中へのライソゾーム酵素分泌動態を解明する目的にて、caerulein（0.1～1.5 ug/Kg・hr）の刺激下および3時間の膵管閉塞後の cathepsin B 分泌動態を検討した。caerulein 刺激下での cathepsin B 分泌動態は膵酵素である amylase とほぼ同様の分泌動態を示すとともに amylase 分泌量と高い正の相関関係を示した。3時間の膵管閉塞後にはコントロール群に比べ caerulein 刺激下、より大量の cathepsin B を含むライソゾーム酵素の存在を示すとともに、消化管ホルモンを介した膵液中へのライソゾーム酵素の分泌をも示すものであった。さらに短期間の膵管閉塞後には膵液中へのライソゾーム酵素の分泌が増加し、cathepsin B は trypsinogen を活性化し得ることより、胆石性膵炎等による膵管閉塞後には、膵管腔での膵消化酵素の活性化にも十分注意を払う必要があるものと考えられた。