

Title	<Original Articles>Effect of 3-hour Pancreatic Duct Obstruction on Pancreatic Lysosomal and Digestive Enzymes in Rabbits
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Citation	日本外科宝函 (1992), 61(2): 125-133
Issue Date	1992-03-01
URL	http://hdl.handle.net/2433/203730
Right	
Type	Departmental Bulletin Paper
Textversion	publisher

Effect of 3-hour Pancreatic Duct Obstruction on Pancreatic Lysosomal and Digestive Enzymes in Rabbits

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Received for Publication, Nov. 11, 1991.

Summary

We studied the effect of short-term (3 hours) pancreatic duct obstruction (PDO) on the exocrine pancreas and on the secretion of lysosomal enzymes into the pancreatic juice of rabbits during stimulation by pancreatic secretagogues. The following evaluations were made: serum amylase levels, pancreatic water content, pancreatic amylase, trypsinogen and cathepsin B content, and output of pancreatic enzymes and lysosomal hydrolases when stimulated by secretin and caerulein as well as the distribution of cathepsin B in subcellular fraction.

PDO for 3 hours plus secretin infusion caused a significant rise in serum amylase levels, pancreatic water content, and pancreatic amylase and trypsinogen content due to congestion of digestive enzymes during PDO. There was also a redistribution of cathepsin B from the lysosomal fraction to the zymogen fraction. In normal rabbits and in those with only secretin infusion, caerulein stimulated the secretion of cathepsin B, into pancreatic juice. Just after PDO, the secretion of cathepsin B, amylase and trypsinogen significantly decreased. By 24 hours after PDO, the output of cathepsin B stimulated by caerulein and secretin had increased significantly. Amylase and trypsinogen output were also significantly increased at this stage, in both the secretin and caerulein fractions.

These results indicate that the secretion of lysosomal enzymes into pancreatic juice is stimulated by gut hormones, such as caerulein, in the normal physiological state and in pathological states, such as PDO.

These results also show augmented secretion of both lysosomal enzymes and pancreatic digestive enzymes in the recovery stage after PDO and their important roles at this stage. Lysosomal enzymes also seem to play some physiological roles in the pancreatic ductal system in normal physiological states as well as their roles in pathological states, because cathepsin B can activate trypsinogen, and trypsin can activate many other enzymes.

Key words: Pancreatic duct obstruction, Amylase, Trypsinogen, Cathepsin B,
索引語 膵管閉塞, アミラーゼ, トリプシンノーゲン, カテプシンB

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Introduction

Both pancreatic digestive enzymes and lysosomal hydrolases are separated in the normal physiological state in pancreatic acinar cells for the maintenance of normal cellular organization, because the mixture of these two enzymes is potentially dangerous to the acinar cells. However, there have been several descriptions of the secretory profiles of lysosomal enzymes in many cell lines^{8,9,18,20}) and their presence in pancreatic juice has been noted, as well as the possible secretion of lysosomal enzymes into pancreatic juice in normal subjects¹⁶) and in patients with chronic calcifying pancreatitis⁷). These reports suggest that lysosomal enzymes play some physiological and pathological roles in biological fluids¹³).

On the other hand, gallstone pancreatitis, which is the most common form of acute pancreatitis in humans¹), seems to be triggered by the passage 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 which pancreatic duct obstruction could induce pancreatitis has not been clarified.

In this study, we evaluated the secretion of lysosomal enzymes into pancreatic juice during stimulation by the pancreatic secretagogue secretin and careulein and the effects of short-term pancreatic duct obstruction on exocrine pancreatic function and on the cellular and organelle fragility of acinar cells.

Materials and Methods

Male White rabbits weighing 2.4–3.0 kg were used in this experiment. The animals were given free access to tap water and diet before the experiment. After a 16-hour fast, the animals were divided randomly into the following two groups; 1 Pancreatic duct obstruction and secretin infusion group (PDO group). Under intravenous sodium pentobarbital anesthesia (35 mg/kg) laparotomy was performed, and the pancreatic duct was closed with a metal clip just adjacent to the duodenum. Secretin (2 CU/kg. hr) in heparinized saline (30 U/ml) was then infused for 3 hours with an infusion pump at a speed of 1.58 ml/hr. Then the metal clip was removed gently, and the abdomen was closed. After operation, the rabbits were infused continuously with lactate-Ringer solution containing 10% glucose at a speed of 1.58 ml/hr, and they were given tap water ad lib but no food. 2 Secretin infusion group without PDO (S group). After gentle manipulation of the pancreatic duct near the duodenum, secretin (2 CU/kg. hr) was infused for 3 hours.

The animals were killed with a large dose of pentobarbital, and the pancreas was removed.

Normal fasted rabbits formed a control group.

In the PDO group, before and 1, 2, 3, 9, 15, 21, and 27 hours after secretin infusion, blood samples of 0.5 ml were obtained through the venous catheter, and the serum amylase levels were measured. In each group, just after and 12, and 24 hours after the secretin infusion, the animals were killed with a large dose of pentobarbital, and the pancreas was removed.

One part of the pancreas was used for the estimation of pancreatic water content by a comparison of the wet weight immediately after removal with the dry weight after dessication at 150°C for 48 hours in a dessicator.

About one third of the pancreas was homogenized in a Brinkmann Polytron in 3 ml of cold phosphate-buffered (pH 7.4) saline containing 0.5% Triton X-100 (Fisher Scientific, Fair Lawn, NJ, U. S. A.) and after low speed centrifugation (150 × g, at 4°C for 15 min), the resulting supernatant

was tested for amylase activity, trypsinogen activity, cathepsin B activity and deoxyribonucleic acid (DNA) content. The amylase, trypsinogen, and cathepsin B activities were expressed as U/mg DNA as an index of pancreatic tissue content.

The other two thirds of the pancreas was homogenized in 6 ml of cold 300 mM sucrose solution with up and down strokes of a Dounce homogenizer and separated into various subcellular fractions by differential centrifugation. The protocol originally developed by Tartakoff and Jemieson¹⁹⁾ and modified for studies in rat tissue⁵⁾ was again modified to permit optimum separation for rabbit pancreatic cell fractions¹⁷⁾. Briefly, the homogenate was centrifuged ($150 \times g$ at 4°C for 10 min) and the supernatant was centrifuged ($1000 \times g$ at 4°C for 15 min) to obtain a zymogen granule-rich pellet and supernatant. That supernatant was centrifuged ($12,000 \times g$ at 4°C for 12 min), yielding a lysosome and mitochondria-rich pellet and a supernatant that contained microsomal and soluble fractions. The various pellets obtained during fractionation were resuspended individually in 2 ml of cold 300 mM sucrose solution, and the amylase and cathepsin B activities in each fraction were measured and expressed as percentages of the total activity.

In other PDO animals infused with secretin for 3 hours the pancreatic duct just adjacent to the duodenum was catheterized under pentobarbital anesthesia, the pylorus was ligated, and tube gastrostomy was performed. After placement and exteriorization of the various cannulas, the abdominal wound was closed. To stimulate and maintain pancreatic fluid secretion, secretin was infused through the venous catheter at a rate calculated to deliver 2.0 CU/kg. hr for 1 hour at a speed of 1.58 ml/hr of heparinized saline after 30 min for stabilization. After this hour, to stimulate the digestive enzyme secretion caerulein ($0.2 \mu\text{g}/\text{kg. hr}$) was added to the perfusate for 2 hours. Pancreatic juice was collected in ice-chilled Eppendorf preweighed tubes hourly (S: secretin fraction; CS₁: first hour caerulein fraction; CS₂: second 1 hour caerulein fraction). Amylase, trypsinogen and cathepsin B activities in each fraction were measured and expressed as U/kg. hr.

Amylase was measured by the method of Bernfeld³⁾. Trypsinogen was measured by the method of Hummel¹⁰⁾. Cathepsin B activity was measured by the method of McDonald and Ellis¹⁴⁾. Deoxyribonucleic acid (DNA) was measured by the method of LaBarca and Paigen¹²⁾.

The results reported represent mean \pm SEM values for *n* determinations, each from a different animal. The significance of difference was evaluated by ANOVA, and a *p* value of 0.05 was considered to significant.

Results

In the PDO group, serum amylase levels were significantly higher at 1 hours (11 ± 2 U/ml), 2 hours (15 ± 2 U/ml) and 3 hours (22 ± 3 U/ml) than in the group with infusion only (pre infusion: 7 ± 1 U/ml; 1 hour: 6 ± 1 U/ml; 3 hours: 7 ± 1 U/ml) and in normal rabbits (6 ± 1 U/ml). Serum amylase levels were still significantly high in the PDO groups at 6 hours (14 ± 3 U/ml vs pre-obstruction value of 6 ± 1 U/ml), but were almost back to normal at 12 hours (9 ± 2 U/ml), 18 hours (8 ± 2 U/ml), and 24 hours (7 ± 1 U/ml) (Fig 1).

Pancreatic duct obstruction for 3 hour and secretin infusion caused a significantly greater pancreatic water content than in the secretin only and normal control animals, but 24 hours after PDO, the pancreatic water content was almost normal.

After pancreatic duct obstruction for 3 hours and secretin infusion, both the pancreatic amylase and the trypsinogen content were significantly greater than in the secretin only group and in normal

controls, and 12 hours after obstruction, these values were still significantly high, but by 24 hours after obstruction, they had returned almost to normal. Recovery from this enzyme congestion seems to require almost 24 hours. The pancreatic cathepsin B content was somewhat greater in the PDO group than in the control groups, but not significantly so, and there were no significant differences among all the groups (Table 1).

Pancreatic duct obstruction (PDO) for 3 hours plus secretin infusion caused a marked shift of cathepsin B activity from the lysosomal fraction ($24 \pm 2\%$) to the zymogen-rich fraction ($54 \pm 3\%$). The percentages in the secretin only group were: $52 \pm 3\%$ and $25 \pm 2\%$. In the normal

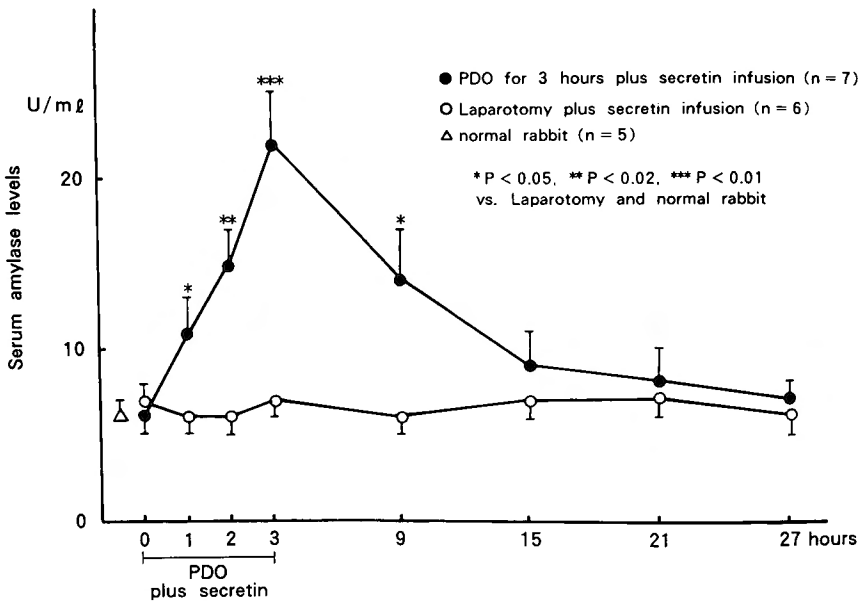


Fig 1 Effects of short-term pancreatic duct obstruction (PDO) plus secretin infusion (2.0 CU/kg. hr) on serum amylase levels. The values are expressed as mean \pm SEM.

Table 1 Effects of pancreatic duct obstruction and secretin infusion on pancreatic water, amylase, trypsinogen and cathepsin B content.

Group	n	Pancreatic water content (%)	Pancreatic amylase content (U/mg DNA)	Pancreatic trypsinogen content (U/mg DNA)	Pancreatic cathepsin B content (U/mg DNA)
PDO + secretin	7	85 \pm 3	562 \pm 31	38 \pm 4	1715 \pm 273
Secretin	6	75 \pm 2*	354 \pm 27**	19 \pm 3**	1493 \pm 189
12 hours after PDO + secretin	7	81 \pm 2	516 \pm 39†	32 \pm 4†	1656 \pm 252
24 hours after PDO + secretin	7	78 \pm 2*	401 \pm 36*	23 \pm 2*	1557 \pm 248
Normal rabbit	5	76 \pm 2*	378 \pm 23**	18 \pm 2**	1528 \pm 216

The values are expressed as mean \pm SEM for n determinations. PDO + secretin: pancreatic duct obstruction and secretin infusion; secretin: laparotomy and secretin infusion; *: $p < 0.05$; **: $p < 0.01$ compared with PDO + secretin; †: $p < 0.05$ compared with 24 hours after PDO + secretin; and $p < 0.02$ compared with secretin group and normal group.

rabbits the lysosomal fraction was $55 \pm 3\%$ and zymogen fraction, $23 \pm 2\%$. These findings indicate a redistribution of lysosomal enzyme induced by PDO plus secretin infusion. At 12 hours after PDO, the redistribution was less, but still significant (lysosomal fraction, $37 \pm 3\%$; zymogen, $42 \pm 3\%$) and 24 hours the redistribution had almost disappeared (lysosomal fraction, $49 \pm 2\%$; zymogen fraction, $29 \pm 2\%$). The microsomal and soluble fraction, was approximately the same in all the groups.

Approximately 40% of the amylase in the pancreas of normal animals and those receiving only secretin was located in the fraction considered to be the zymogen, and about 40% remained in the microsomal and soluble fraction. PDO for 3 hours plus secretin infusion increased significantly the amylase activity in this microsomal and soluble fraction ($57 \pm 4\%$ vs $39 \pm 3\%$ in normal animals and $43 \pm 3\%$ in the secretin only group) and decreased significantly the amylase activity in the zymogen fraction ($24 \pm 2\%$ vs $36 \pm 3\%$ in normal rabbit and $34 \pm 2\%$ in the secretin only group). This fragility of amylase-containing organelles was still apparent 12 hours after PDO but by 24 hours after PDO, this fragility had almost disappeared (Fig 2).

Just after PDO and secretin infusion, the volume of pancreatic juice decreased to 30–40% of that of the control groups, but 12–24 hours after PDO, it returned to almost the normal value (Table 2). There was only a little cathepsin B activity in the secretin only fraction (S), but there was con-

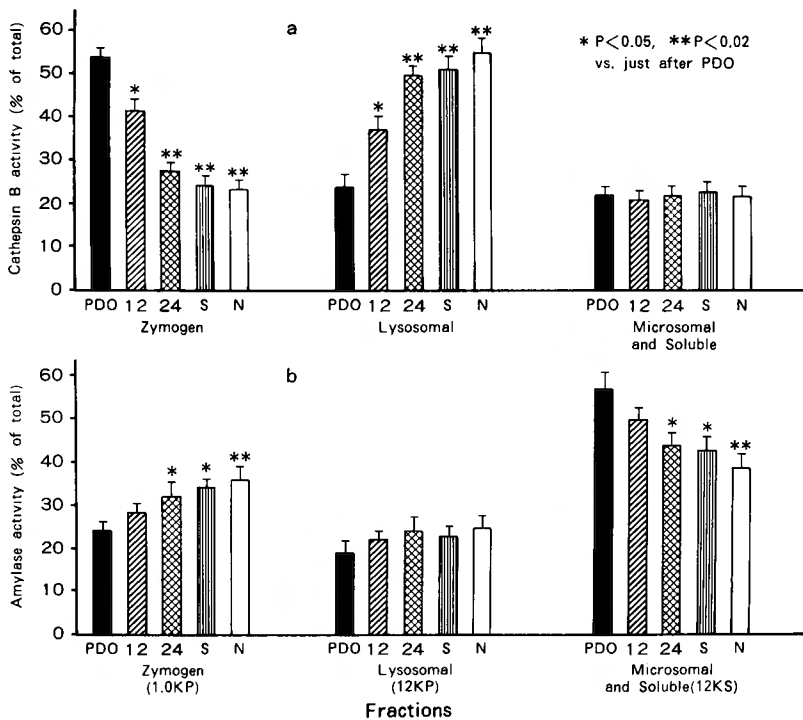


Fig 2 Effects of short-term pancreatic duct obstruction plus secretin infusion on distribution of cathepsin B (a) and amylase activity (b) in subcellular fractions.

The values are expressed as mean \pm SEM. (PDO: just after PDO and secretin infusion (n=7); 12: 12 hours after PDO plus secretin infusion (n=7); 24: 24 hours after PDO plus secretin infusion (n=7); S: just after secretin infusion only (n=6); N: normal fasted animals (n=5)).

Table 2 Effects of pancreatic duct obstruction and secretin infusion on volume of pancreatic juice under stimulation by secretin (2.0 CU/kg. hr) and secretin plus caerulein (0.2 μ g/kg. hr).

Group	n	Pancreatic juice volume (ml/kg. hr)		
		Pancreatic juice fraction		CS ₂
		S	CS ₁	
PDO + secretin	7	0.23 \pm 0.08	0.35 \pm 0.07	0.42 \pm 0.09
Secretin	6	0.68 \pm 0.09*	0.85 \pm 0.06*	0.89 \pm 0.09*
12 hours after PDO + secretin	7	0.73 \pm 0.06*	0.98 \pm 0.07*	1.03 \pm 0.05*
24 hours after PDO + secretin	7	0.83 \pm 0.05*	1.01 \pm 0.06*	0.96 \pm 0.08*
Normal rabbit	5	0.76 \pm 0.04*	1.04 \pm 0.09*	1.08 \pm 0.07*

The values are expressed as mean \pm SEM for n determinations. *: $p < 0.01$ compared with PDO + secretin group.

siderable activity in the caerulein plus secretin fraction (CS₁ and CS₂). These findings show that caerulein stimulates cathepsin B output into pancreatic juice. However, just after PDO, in accordance with the changes in pancreatic juice, the cathepsin B output decreased in both the secretin fraction (1 \pm 1 U/kg. hr) and the caerulein plus secretin fractions (CS₁, 4 \pm 1 U/kg. hr; CS₂, 5 \pm 1 U/kg. hr vs values in normal rabbits: S, 2 \pm 1 U/kg. hr; CS₁, 12 \pm 2 U/kg. hr, and CS₂, 10 \pm 2 U/kg. hr). In the secretin only group the values were: S, 3 \pm 1 U/kg. hr; CS₁, 11 \pm 1 U/kg. hr; and CS₂, 12 \pm 2 U/kg. hr). At 12 hours after PDO, the cathepsin B output was slightly less than in the controls (S, 2 \pm 1 U/kg. hr; CS₁, 8 \pm 2 U/kg. hr; and CS₂, 9 \pm 2 U/kg. hr) but by 24 hours after PDO the cathepsin B output was significantly higher than normal in both the secretin fraction (S, 7 \pm 2 U/kg. hr) and the caerulein plus secretin fraction (CS₁, 19 \pm 3 U/kg. hr; CS₂, 22 \pm 2 U/kg. hr). The trypsinogen output in the secretin fraction (S) was slight, and considerably greater in the caerulein plus secretin fractions (CS₁, CS₂). It was low just after PDO in both the secretin fraction (S, 2 \pm 1) and the caerulein plus secretin fraction (CS₁, 12 \pm 2 U/kg. hr; CS₂, 14 \pm 2 U/kg. hr vs the controls: S, 3 \pm 1 U/kg. hr; CS₁, 33 \pm 3 U/kg. hr; CS₂, 35 \pm 2 U/kg. hr) and the secretin only group; S, 4 \pm 1 U/kg. hr; CS₁, 30 \pm 3 U/kg. hr; CS₂, 32 \pm 3 U/kg. hr). By 12 hours after PDO, the trypsinogen output had almost recovered but was still below that in the controls (S, 3 \pm 1 U/kg. hr; CS₁, 18 \pm 3 U/kg. hr; CS₂, 20 \pm 2 U/kg. hr), but by 24 hours after PDO the trypsinogen output had increased significantly in both the secretin fraction (S, 8 \pm 2 U/kg. hr) and the caerulein plus secretin fraction (CS₁, 45 \pm 4; CS₂, 48 \pm 3 U/kg. hr). There was little amylase activity in the secretin fraction (S) and considerably more in the caerulein plus secretin fractions (CS₁ and CS₂). Just after PDO, the amylase output decreased significantly (S, 52 \pm 1 U/kg. hr; CS₁, 316 \pm 35 U/kg. hr; CS₂, 352 \pm 41 U/kg. hr vs the normal values: S, 74 \pm 18; CS₁, 842 \pm 46 U/kg. hr; CS₂, 913 \pm 88 U/kg. hr); in the secretin only group: S, 82 \pm 9 U/kg. hr; CS₁, 816 \pm 51 U/kg. hr; CS₂, 852 \pm 84 U/kg. hr. By 12 hours after PDO, the amylase output had recovered somewhat (S, 80 \pm 15 U/kg. hr; CS₁, 624 \pm 64 U/kg. hr; CS₂, 653 \pm 78 U/kg. hr), but it was still slightly lower than in the control groups.

By 24 hours after PDO, the amylase output had increased significantly in both the secretin fraction (S, 151 \pm 13 U/kg. hr) and the caerulein plus secretin fractions (CS₁, 1218 \pm 102 U/kg. hr; and CS₂, 1314 \pm 132 U/kg. hr) in parallel with the changes in trypsinogen output (Fig 3).

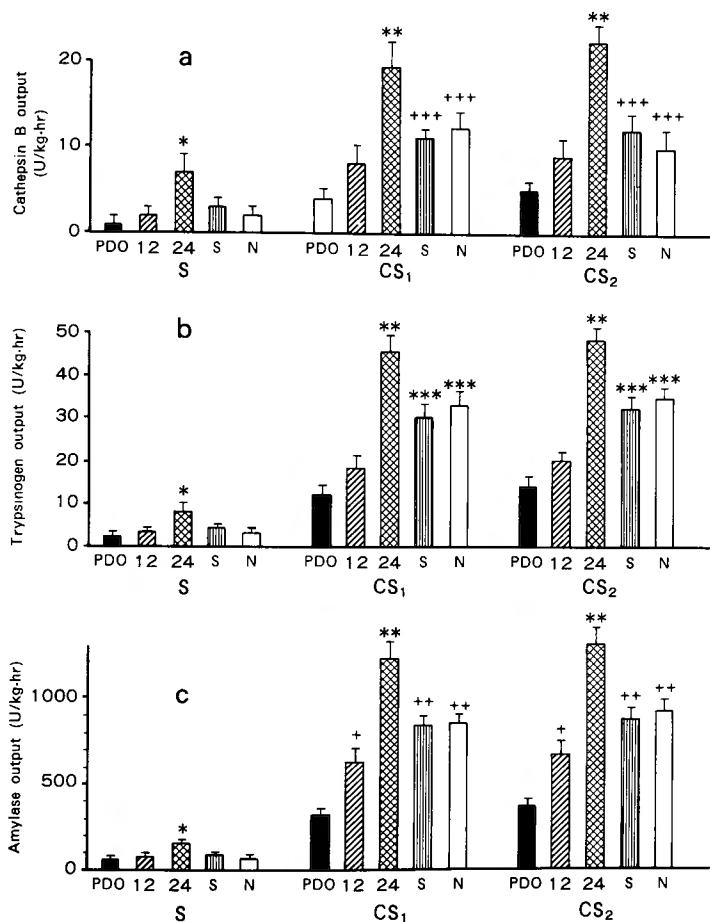


Fig 3 Effects of short-term pancreatic duct obstruction plus secretin infusion on cathepsin B (a), trypsinogen (b), and amylase output (c) under stimulation by secretin (S) or secretin plus caerulein (CS₁, CS₂). The values are expressed as mean ± SEM. *: p < 0.05 compared with other groups; **: p < 0.01 compared with just after PDO group and p < 0.05 compared with other groups; ***: p < 0.05 compared with just after PDO group, and 12 hours after PDO group; +: p < 0.05 compared with just after PDO group; ++: p < 0.02 compared with just after PDO group; +++: p < 0.01 compared with just after PDO group.

Discussion

In our present rabbit model, the pancreatic amylase and trypsinogen content increased after short-term PDO showing that although protein synthesis continues, discharge of newly synthesized digestive enzymes does not occur and the concentration of digestive enzymes within the acinar cells increases. In addition, our subcellular fractionation studies indicate that short-term PDO leads to a redistribution of cathepsin B activity and that, as a result, lysosomal hydrolase becomes localized within a fraction that is rich in digestive enzymes. This phenomenon of colocalization of these two enzymes is likely to be the result of crinophagy⁶. Colocalization could, under appropriate conditions, lead to the intra-acinar cell activation of potentially dangerous digestive enzymes.

Another important finding in this study was that during recovery from short-term PDO the output of both pancreatic digestive enzymes and lysosomal enzymes stimulated by a pancreatic secretagogue was significantly greater than in the controls. This augmented secretion of small amounts of colocalized lysosomal hydrolases and digestive enzymes into pancreatic juice and into the pancreatic duct system, as well as the redistribution of lysosomal enzymes in pancreatic acinar cells, may be important in relation to the etiology of gallstone pancreatitis in humans. Attacks of gallstones are often repeated, and after the first obstruction, if the secretion of pancreatic secretagogues such as cholecystokinin and secretin is stimulated by food intake, increased amounts of colocalized digestive enzymes and lysosomal hydrolases might be secreted into the pancreatic juice. When obstruction is caused again by another stone or attack, or when edema of the sphincter of Oddi persists, drainage of these digestive enzymes and lysosomal hydrolases into the pancreatic juice is blocked; in addition, within the acinar cells there will be another redistribution of lysosomal enzyme. 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 make this space wider and to facilitate the entry of digestive enzymes into the systemic circulation or the interstitium of the pancreas. Under these conditions, the exocrine pancreas is exposed to activation of digestive enzymes by lysosomal hydrolase both within the acinar cells where lysosomal enzyme is colocalized in zymogen granules and outside the acinar cells where colocalization of lysosomal and digestive enzymes takes place in the pancreatic duct system and pancreatic interstitium. These events can lead to ductal hypertension and can damage the protective barrier of the ductal epithelium¹¹⁾ by simple mechanical pressure or by the reflux of infected biliary juice, which sometimes occurs in cholelithiasis, and the pancreas will be more susceptible to autodigestion from both within and outside the acinar cells.

Although the factors responsible for triggering and worsening pancreatitis are not clearly understood, this secretion of lysosomal enzymes, which can potentially activate the pancreatic digestive enzymes, into the pancreatic juice in large amounts when stimulated by pancreatic secretagogues would seem to convert mild and edematous pancreatitis into severe hemorrhagic and necrotic pancreatitis; it may be one of the factors which trigger pancreatitis in the common channel theory¹⁵⁾.

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和文抄録

膵管閉塞（3時間）の膵ライソゾームおよび 消化酵素動態に及ぼす影響

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京極 高久, 四元 文明, 今村 卓司, 大塩 学而

短時間（3時間）の膵管閉塞の膵ライソゾーム酵素、膵消化酵素動態に及ぼす影響を検討するために家兎を用い3時間の膵管閉塞および開放後の血中アミラーゼ、膵水分、アミラーゼ、トリプシノーゲン、カテプシンB含量、カテプシンBの分布、セクレチンおよびセルレイン刺激下のアミラーゼ、トリプシノーゲン、カテプシンB分泌を経時的に観察した。その結果、3時間の膵管閉塞後には著明な膵液の鬱滞によると考えられる有意な血中アミラーゼ、膵水分、アミラーゼ、トリプシノーゲン含量の増加がみられ、細胞内カテプ

シンBのライソゾーム分画からチモーゲン分画への移動がみとめられた。正常ラットにおいてはセルレイン刺激に反応して膵消化酵素、カテプシンBの膵液中への分泌がみられたが、膵管結紮開放直後はこれらの分泌は低下した。しかし、開放24時間後には著明な分泌の増加がみられた。このような結果は膵管結紮によって膵内においてはライソゾーム酵素と消化酵素の鬱滞が起り、何らかの条件下では消化酵素の活性化が惹起される可能性を示唆した。