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Effect of Hypothermia on Pancreatic Acinar Cells in Rats

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Abstract

This study was designed to evaluate the effects of direct pancreatic surface cooling on the exocrine pancreas. We measured the changes in serum amylase levels, pancreatic water, amylase and cathepsin B as a lysosomal enzyme, content, histological changes of acinar cells, and the subcellular distribution of cathepsin B after 1- 2- and 3-hours of direct pancreatic cooling in rats. In addition, we evaluated the in-vivo amylase and cathepsin B output stimulated by caerulein, in-vitro lysosomal and mitochondrial fragility as well as the pancreatic adenylate energy metabolism.

2-hours cooling showed slight yet significant changes, but 3-hours cooling caused most significant changes including hyperamylasemia, increased pancreatic amylase content and very mild histological changes. Furthermore, 3-hours cooling caused a remarkable redistribution of cathepsin B activity from the lysosomal fraction to the heavier zymogen fraction, and colocalization of the lysosomal enzyme with the digestive enzyme, the impaired amylase and cathepsin B output into pancreatic juice stimulated by caerulein as well as the accelerated fragility of lysosomes and mitochondria, and impaired pancreatic adenylate energy metabolism.

These results indicate the impaired exocrine pancreatic functions induced by direct pancreatic cooling injury induced by cooling as shown in the other models of experimental pancreatitis. Moreover, this cooling model of pancreatitis seems to be useful in understanding the early events in the pathogenesis of acute pancreatitis, and we must take these "cold" injuries of exocrine pancreas into considerations, particularly in the pancreas transplantation and in other major abdominal surgeries where the pancreas is exposed to cooling.

Introduction

Acute pancreatitis is a common finding in patients with accidental hypothermia and almost half of the hypothermic patients have been reported to have elevated serum amylase levels[6,21]. Acute pancreatitis has been reported to be found at necropsy of these patients at high incidence[8]. Moreover, there have been several reports about the elevated serum amylase levels and suggestive pancreatic injury after major abdominal operation[3,4,4], and in these major abdominal operations, we

Key words: Hypothermia, Exocrine pancreas, Lysosome, Mitochondria, Pancreatic adenylate energy charge level.
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cannot rule out the effects of hypothermia on the pancreas, either systemically or locally. In the hypothermic accident, ischemia resulting from the microcirculatory failure of hypothermia is considered to be a likely operative factor in the causation of the pancreatic damage. But it is not clear whether local pancreatic hypothermia has some effects on the exocrine pancreas. If this does indeed have some effects, then we must take the hypothermic effects on the exocrine pancreas into consideration, because during pancreatic transplantation, the preservative solution is always very cold and the donor pancreas is always kept cold before transplantation.

In this communication, we evaluated the effects of local pancreatic cooling on the exocrine pancreas, as possible as ruling out its systematic effects, from the various parameter such as serum amylase levels, pancreatic water content, pancreatic amylase and cathepsin B content, lysosomal and mitochondrial fragility as well as the distribution of lysosomal enzyme in acinar cells.

Materials and Methods

Animal Preparation 80 male Wistar rat, weighing about 350 g (Shizuoka Experimental Animals, Shizuoka, Japan) were used in this experiment. They were kept in light-dark cycle regulated (light: 5:00–7:00) and air-conditioned (23 ± 3°C) animal quarters in our university before the experiments, and were allowed to become acclimatized to standard laboratory conditions for at least 4 days. The rats were maintained throughout the study in accordance with the guidelines of the Animal Care Committee of Kyoto University. Experiments were begun after a 16-hour fast, starting at between 8:00–10:00 AM to rule out the effects of the rat's circadian rhythm on the exocrine pancreas. Anesthesia was induced by intraperitoneal pentobarbital injection (25 mg/kg). Catheterization (V-3 catheter, Insul-Tab, Woburn, MA, USA) to the superior vena cava (S.V.C.) via right external jugular vein as a venous line was performed. Another catheterization (PE50, Clay Adams, Parsippany, NJ, USA) to the right carotid artery for blood pressure monitoring was also performed, and the mean arterial blood pressure (MABP) was measured by a mercurial manometer (Stentor Engineering Corp., Kansas City, MO, USA). A temperature probe was also inserted into the rectum for body temperature monitoring. All the animals were kept on a heating pad at 40°C, and overhead lamps were arranged to maintain their core body temperature. Anesthesia was maintained by intravenous administration of pentobarbital (10 mg/kg) when necessary. All animals were divided into the following 2 groups:

Pancreas cooling group (C group) (40 rats)—After making a venous line, the abdomen was opened by an upper midline incision. Thirty ml of ice-cold (0°C) saline packed in a thin plastic bag was placed on the portion of pancreas from the ducdenal loop to the gastric, and splenic lobe, keeping it as far way as possible from other organs such as the liver, the spleen, and the intestines. This saline bag was exchanged for a new one every 30 minutes to keep pancreas cold. During this cooling, the abdomen was closed by small hemostatic clamps.

Sham cooling group (S group) (30 rats)—The abdomen was opened as in the C group, but a warm saline (38°C) containing bag was placed in the same position as in C group. The abdomen was also closed as in C group. During the experiments, all the animals were infused with heparinized (30 U/ml) saline at the speed of 0.58 ml/hr by using an infusion pump (Harvard Appratus, South Natick, MA, USA). In addition to the above two groups, normal fasted rats were used as a pure control group (25 rats).

Serum amylase levels At 1, 2, and 3 hours after cooling or sham-cooling, rats were killed by a

large dose of pentobarbital, and the abdomen was re-opened. Blood samples were taken from the inferior vena cava (I.V.C.) and serum amylase levels were measured.

Pancreatic water content Portions of the pancreas were removed quickly, and one small portions of the pancreas (ca. 300 mg) was used for the determination of pancreatic edema by comparing the weight obtained immediately after sacrifice (wet weight) to that of the same sample after incubation at 150°C for 48 hours in a desiccator (Isotemp Oven®, Fisher Scientific, Fair Lawn, NJ, USA) (dry weight). Pancreatic water content was expressed as a percent of the wet weight as an index of pancreatic edema.

Pancreatic amylase and cathepsin B content Another small portions of the pancreas (ca. 300 mg) were homogenized in 4 ml of cold phosphate-buffered saline (pH 7.4) containing 0.5% Triton X-100 (Fisher Scientific) by a Brinkmann Polytron (Brinkmann Instruments, Inc., Westbury, NY, USA) for the measurement of pancreatic amylase and cathepsin B content, as a lysosomal enzyme. Amylase and cathepsin B activities as well as deoxyribonucleic acid (DNA) concentration were measured in the resulting supernatant after low speed centrifugation (150 × g, 15 min, 4°C). Both pancreatic amylase and cathepsin B contents were expressed as U/mg DNA.

Histological examinations Other very small portions of the pancreas from each group at an each stage were fixed overnight in phosphate-buffered (pH 7.4) 10% neutral formaldehyde solution. After paraffin embedding, the portions were sectioned and stained with hemaloxylin-eosin. The sections were then examined by an independent observer and interstitial edema, acinar cell vacuolization, and inflammatory cell infiltration were graded on a 0–4+scale (0; no changed, 4+; maximum changed).

Subcellular distribution of amylase and cathepsin B activities The rest of the pancreas (ca. 700 mg) was homogenized in 6 ml of ice-cold 5 mM MOPS (3-(N-morpholino)propanesulfonic acid) (Sigma Chemical, St. Louis, MO, USA) buffer (pH 6.5) containing 1 mM $MgSO_4$ and 250 mM sucrose by 3 up-and-down strokes of a Dounce homogenizer (Wheaton, Millville, NJ, USA) in a cold room. Unbroken cells and debris were removed by low speed centrifugation (150 × g, 15 min, 4°C). The resulting supernatant was considered to be the entire sample for later calculations and to contain 100% of all measured components. Subcellular fractionations were performed by differential centrifugations as described by TARTAKOFF and JAMIESON[32], with minor modifications for the study of rat tissue[5]. Briefly, the above supernatant was centrifuged (1300 × g, 15 min, 4°C) to yield a "zymogen granule" pellet (1.3 kp) and another supernatant. This second supernatant was then centrifuged (12000 × g, 12 min, 4°C) to yield a "lysosomal and mitochondrial" pellet (12 kp) and the supernatant, which is considered to be "microsomal and soluble" fraction (12 ks). The various pellets obtained during the fractionations were resuspended individually in 2 ml of ice-cold 5 mM MOPS buffer. The amylase and cathepsin B activities in each fraction were measured and expressed as a percentage of the total activity as an index of the distribution of both digestive and lysosomal enzyme in the pancreatic acinar cells.

In-vivo secretion of amylase and cathepsin B into stimulated by pancreatic secretagogue For this in-vivo experiment, other new rats were used. After 3-hours of cooling in C group, or sham-cooling in S group, the abdomen was opened. After making an external biliary fistula at the hepatic hilum by catheterization (PE10) to the hepatic duct, another catheterization (V-1 catheter, Insul-Tabl) was performed to the pancreatico-biliary duct just distal to the duodenum to collect the pancreatic juice. After about 30 minutes stabilization, caerulein was infused at a dose of 0.2 μg/kg・hr for 1 hour to stimulate the pancreatic secretion for 1 hour. Pancreatic juice was collected in the pre-

weighed eppendorf tubes. The pancreatic juice volume was calculated by direct weighing, and both amylase and cathepsin B activities in the pancreatic juice were measured. Both amylase and cathepsin B outputs were expressed as U/kg · hr.

In-vitro incubation of lysosome and mitochondria For this experiment, too, other new rats in each group were used. After 3-hours of cooling or sham-cooling, the animals were sacrificed and portions of the pancreas were removed. About half of the pancreas was used in this in-vitro incubation experiment. Combined lysosomal and mitochondrial fractions were obtained as described above. This lysosomal and mitochondrial fraction, arbitarily considered to contain 100% of both lysosomal and mitochondrial enzymes activities. These fractions were resuspended in the same 5 mM MOPS buffer and incubated for varying intervals (30, 60, and 90 min) at 25°C in a shaking water bath under the room air. The samples were then recentrifuged (12000 × g, 12 min, 4°C) to separate the particulate from the soluble lysosomal and mitochondrial enzyme activity, each of which was individually measured after separation of the pellet and supernatant. As a lysosomal enzyme, cathepsin B activity was measured in both the pelleted and soluble fractions. Centrifugation and subsequent measurement of particulate and soluble lysosomal enzyme activity identified the rate and extent of in-vitro rupture of lysosomal enzyme containing organelles. Soluble cathepsin B activity, expressed as a percentage of the total cathepsin B activity was used as an index of lysosomal fragility. In the same samples, malate dehydrogenase (MDH) was measured as a mitochondrial enzyme, and MDH leakage from mitochondria was expressed in the same way as cathepsin B leakage as an index of mitochondrial fragility.

Pancreatic adenylate energy charge levels The other half of the pancreas was used for the determination of pancreatic adenylate energy charge levels. The pancreas was frozen in liquid nitrogen. The frozen tissues were powdered with a mortar and pestle in liquid nitrogen, and were then homogenized in 4 times their volume of chilled 6% (w/v) perchloric acid containing 1 mM edetic acid. The extract was centrifuged at 10000 × g at 0°C for 15 min. The supernatant was adjusted to pH 6.0 with cold 69% (w/v) K_2CO_3 and was recentrifuged at 10000 × g at 0°C for 5 min. The final supernatant was used to determine the concentration of adenine nucleotides (ATP, ADP and AMP) in the pancreatic tissue. The enrgy charge was calculated by the formula proposed by ATKINSON[1]:

energy charge (E.C.)=(ATP+1/2ADP)/(ATP+ADP+AMP)

Assays Amylase activity was measured with soluble starch (Sigma Chemical) as the substrate by the method of BERNFELD[23]. Cathepsin B activity was measured fluorometrically by the method of McDONALD and ELLIS[22] with CBZ-arginyl-arginine-β-nephthylamide (Bachem Bioscience, Philadelphia, PA, USA) as the substrate. DNA concentration was measured fluorometrically by the method of LABARCA and PAIGEN[19]. Malate dehydrogenase (MDH) was measured spectrophotometrically by the method of BERGMEYER[2]. ATP was measured enzymatically by the method of LAMPRECHT and TRAUTSCHOLD[20], and ADP and AMP by the method of JAWOREK and Coworkers[16].

Data Presentation The results reported here represent the means±SEM for n determinations. Differences between groups were evaluated by ANOVA with TUKEY method, except for the histological changes. For evaluating the histological changes, the WILCOXON rank-sum test was used. Significant differences were defined as those associated with a probability value (p) of less than 0.05 ($p<0.05$).

Results

Rectal temperatures in the pancreas cooling group (C group) tended to be lower compared with those in the sham-cooling group (S group), but there were no significant differences between these two groups at any stage (Table 1). The mean arterial blood pressure (MABP) showed the same tendency, but there were no significant differences between the two groups, showing no systemic bypotension (Table 2).

Serum amylase levels Serum amylase levels in the pancreas cooling group (C group) showed a gradual increae. At 3 hours after cooling, significantly higher levels (12±2 U/ml) were seen in C group compared with the sham cooling group (S group) (7±1 U/ml) and normal rats (6±1 U/ml) (Figure 1).

Pancreatic water content Pancreatic water content tended to increase gradually as cooling time prolonged, but there were no significant differences between these two groups and normal rats (Table 3).

Pancreatic amylase and cathepsin B content Pancreatic amylase content showed a gradual significant increase compared with the sham-cooling group, particularly at 2 and 3 hours after cooling. This seems to show the congestion of digestive enzymes induced by direct cooling. Cathepsin B content in the cooling group tended to increase compared with those in the sham-cooling group, but there were no significant differences between these two groups and normal rats (Table 4).

Histological changes Direct pancreatic cooling induced very mild interstitial edema and acinar cell vacuolization compared with the sham-cooling group, but there were no significant differences between these two groups (Table 5).

Subcellular distribution of amylase and cathepsin B Three hours direct cooling of the pancreas caused a significant decrease of amylase activity in the zymogen fraction (1.3 kp) and a significant increase in the microsomal and soluble fraction (12 ks). This means that increased fragility of amylase containing organella in the zymogen granule fraction during the process of subcellular fractionation was induced by prolonged pancreatic cooling. However, shorter cooling duration (1 and 2 hours) induced no significant changes (Figure 2). Two and 3 hours cooling of the pancreas caused a significant increase cathepsin B activity in the zymogen fraction (1.3 kp) and a significant decrease in the lysosomal fraction (1.2 kp) compared with the sham-cooling group. This indicates a redistribution of the lysosomal enzyme from the lysosomal fraction to the heavier zymogen fraction (Figure 3). This redistribution also indicates the colocalization of the lysosomal enzyme with the digestive enzyme in the same subcellular fractions.

In-vivo amylase and cathepsin B output stimulated by caerulein (0.2 μg/kg・hr) Three hours cooling of the pancreas caused a significant decrease in the pancreatic juice volume (0.85±0.12 ml/kg・hr) stimulated by caerulein (0.2 μg/kg・hr), compared with the sham-cooling (1.64±0.18 ml/kg・hr) and normal rats (1.72±0.16 ml/kg・hr) (Figure 4a). Three hours cooling of the pancreas also caused a significant decrease in both amylase output (3367±528 U/kg・hr) and cathepsin B output (9±3 U/kg・hr) stimulated by caerulein compared with the sham-cooling group (amylase output; 8251±746 U/kg・hr, cathepsin B output; 22±2 U/kg・hr) and normal rats (amylase output; 8442±821 U/kg・hr, cathepsin B output; 21±2 U/kg・hr) (Figure 4b, c).

In-vitro lysosomal and mitochondrial fragility Three hours cooling of the pancreas caused a significant increase in cathepsin B leakage from lysosomes particularly for prolonged incubation time (≧60 min), compared with the sham-cooling and normal rats. This means that the accelerated

fragility of lysosomes was induced by direct cooling of the pancreas. Three hours cooling of the pancreas also caused a significantly accelerated fragility of the mitochondria in-vitro incubation compared with the sham-cooling and normal rats, thus indicating that the increased mitochondrial fragili-

Table 1 Effect of direct surface cooling on the changes in rectal temperature in rats

Group	n	Rectal temperature (°C) Hours after pancreatic cooling		
		1	2	3
C	8	35.3±0.3	34.9±0.4	34.8±0.4
S	6	36.1±0.4	36.0±0.6	35.7±0.3

C; direct surface cooling group, S; sham-cooling group.

Table 2 Effect of direct surface cooling of the pancreas on the changes in mean arterial blood pressure (MABP) in rats

Group	n	Mean arterial blood pressure (MABP) (mmHg) Hours after induction of cooling		
		1	2	3
C	8	94±7	92±6	89±8
S	6	98±6	96±9	93±7

C; direct surface cooling of pancreas group.
S; sham-cooling group.

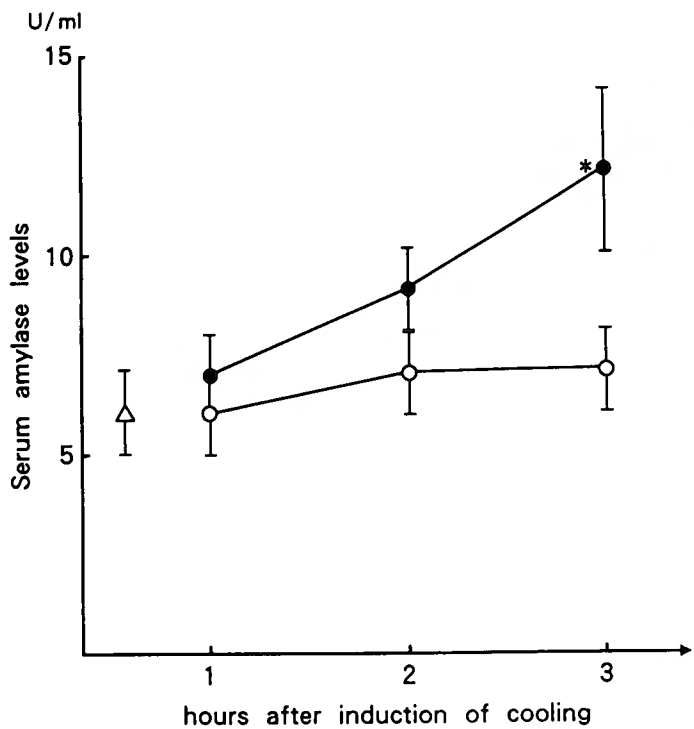

Fig. 1 Effect of direct pancreatic surface cooling on the changes in serum amylase levels in rats (●; direct surface cooling group (C group) (n=8), ○; sham-cooling group (S group) (n=6) △; normal rats (n=5)) (*; $p<0.05$ compared with S group and normal rats)

ty was induced by pancreatic cooling (Table 6).

Pancreatic adenylate energy metabolism Three hours cooling of pancreas caused a significant decrease of ATP in the pancreatic tissue and an increase in AMP in the pancreatic tissue compared with the sham-cooling and normal rats. Moreover, pancreatic energy charge levels in the cooling group were significantly lower compared with the sham-cooling and normal rats, which suggests that impaired pancreatic energy metabolism can be induced by prolonged cooling (Table 7).

Table 3 Effect of direct surface cooling of pancreas on the changes in pancreatic water content in rats

Group	n	Pancreatic water content (% of wet weight) Hours after induction of pancreatic cooling		
		1	2	3
C	8	75±2	76±2	79±2
S	6	74±2	75±2	76±2
Normal rats	5	74±1		

C; direct surface cooling of pancreas group,
S; sham-cooling group

Table 4 Effect of direct pancreatic surface cooling on the changes in pancreatic amylase and cathepsin B content in rats

Group	n	Pancreatic amylase content (U/mgDNA) Hours after induction of cooling			Pancreatic cathepsin B content (U/mgDNA) Hours after induction of cooling		
		1	2	3	1	2	3
C	8	482±56	579±46*	632±53**	1278±193	1377±214	1392±246
S	6	443±37	452±39	471±42	1214±153	1299±172	1326±204
Normal rats	5	423±48			1094±128		

Both pancreatic amylase and cathepsin B content were expressed as U/mgDNA.
(C; pancreatic direct surface cooling group, S; sham-cooling group, *; $p<0.05$, **; $p<0.02$ compared with S group and normal rats)

Table 5 Effect of direct pancreas surface cooling on the pancreatic histological changes in rats

Group	n	Histological changes of acinar cells								
		Interstitial edema Hours after induction of cooling			Acinar cell vacuolization Hours after induction of cooling			Inflammatory cell infiltration Hours after induction of cooling		
		1	2	3	1	2	3	1	2	3
C	8	0 [0] (0)	0 [0.3±0.2] (0～1)	1+ [0.8±0.2] (0～1)	0 [0] (0)	0 [0.3±0.2] (0～1)	1+ [0.6±0.2] (0～1)	0 [0] (0)	0 [0] (0)	0 [0.1±0.1] (0～1)
S	6	0 [0] (0)	0 [0] (0)	0 [0.2±0.2] (0～1)	0 [0] (0)	0 [0] (0)	0 [0] (0)	0 [0] (0)	0 [0] (0)	0 [0] (0)

Histological changes were independently graded on a scale from 0 (no change) to 4+ (maximum change) and the values were expressed as means rounded to the nearest whole numbers
(C; pancreas direct surface cooling group, S; sham-cooling group, ( ); the range of values, [ ]; mean±SEM of values)

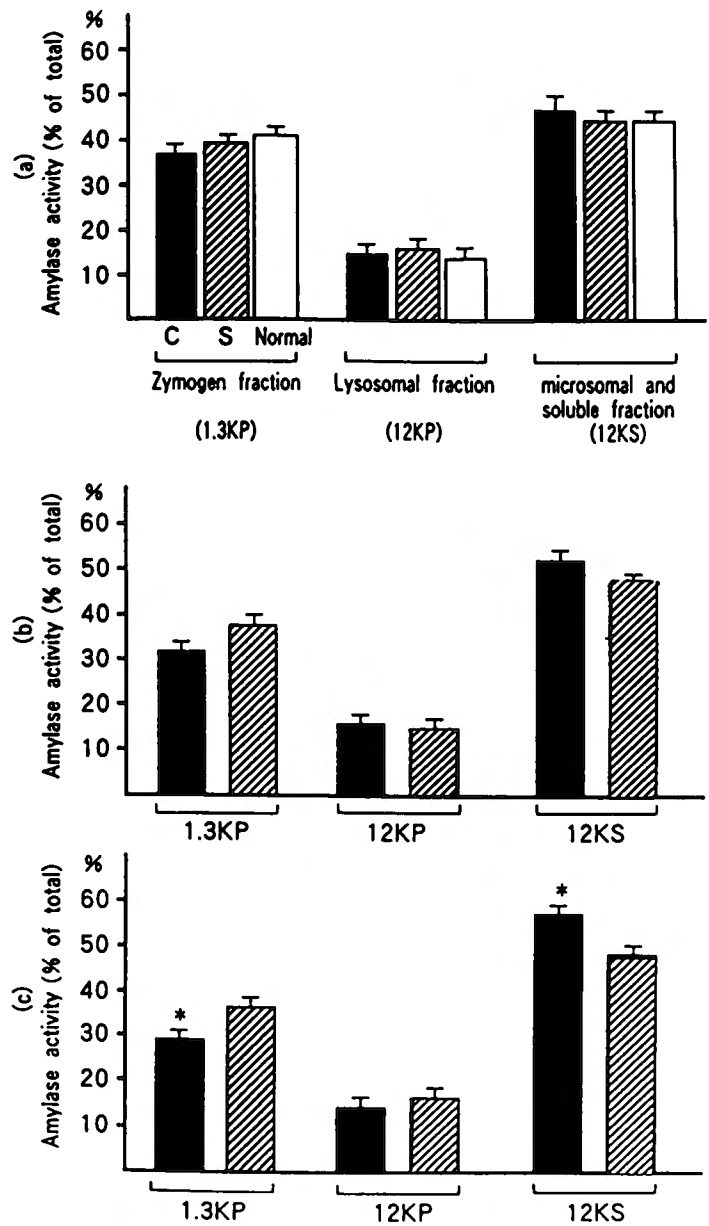

Fig. 2 Effect of direct pancreatic surface cooling on the changes in subcellular amylase distribution in acinar cells in rats at 1 (a), 2 (b), and 3 hours (c) after the induction of surface cooling
Amylase activity was expressed as a percentage of total activity. (■, direct surface cooling group (C group) (n=8), □; sham-cooling group (S group) (n=6), □; normal rats (n=5)) (*; $p<0.05$ compared with S group and normal rats)

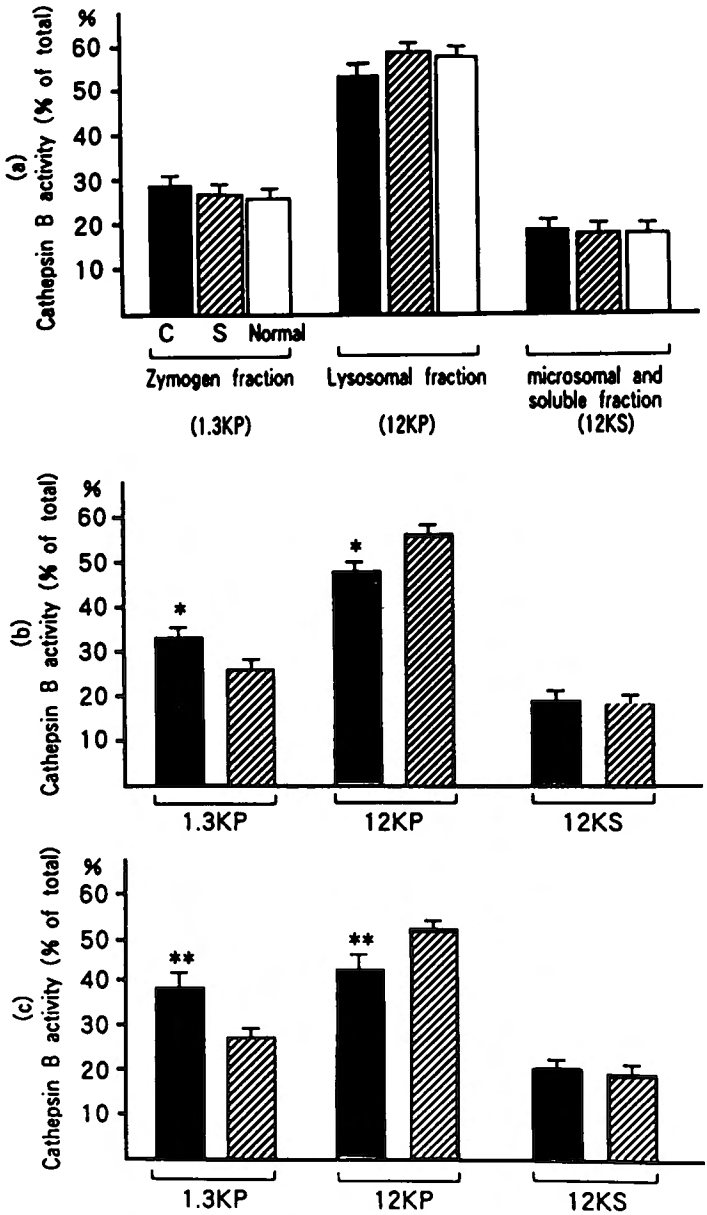

Fig. 3 Effect of direct pancreatic surface cooling on the changes in subcellular cathepsin B distribution in acinar cells in rats at 1 (a), 2 (b), and 3 hours (c) after the induction of cooling
Cathepsin B activity in each fraction was expressed as a percentage of total activity. (■; C group (n=8), □; S group (n=6), □; normal rats (n=5)) (*; $p<0.05$, **; $p<0.02$ compared with S group and normal rats)

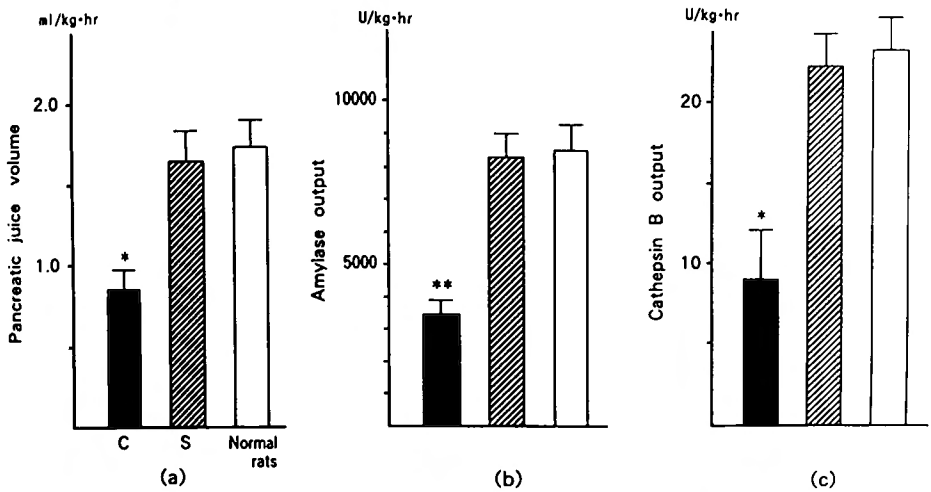

Fig. 4 Effect of 3-hours of direct pancreatic surface cooling on the changes in pancreatic juice volume (a), amylase output (b), and cathepsin B output (c), stimulated by caerulein (0.2 μg/kg · hr) in rats
Pancreatic juice volume was expressed in ml/kg · hr and both amylase and cathepsin B outputs were expressed in U/kg · hr. (■, C group (n=8), □; S group (n=6), □; normal rats (n=5)) (*; $p<0.02$, **; $p<0.01$ compared with S group and normal rats)

Table 6 Effect of 3-hours of direct pancreatic surface cooling on the changes in lysosomal and mitochondrial fragility in rats

Group	n	Cathepsin B leakage from lysowomal (% of total) Incubation time (min)			MDH leakage from mitochondria (% of total) Incubation time (min)		
		30	60	90	30	60	90
C	8	7±1	22±2	38±3	8±1	25±2	43±3
S	6	7±1	16±2*	26±2**	7±1	19±2*	32±2**
Normal rats	5	61±	14±2*	23±2**	7±1	18±2*	29±2**

Cathepsin B leakage from lysosomes and malate dehydrogenase (MDH) leakage from mitochondria were expressed as a percentage of the total activity.
(C; pancreas direct surface cooling, S; sham-cooling group, *; $p<0.05$, **; $p<0.02$ compared with C group)

Table 7 Effect of 3-hours of direct pancreatic surface cooling on the changes in pancreatic adenylate concentration and adenylate energy metabolism in rats

Group	n	Pancreatic adenylate concentration and energy charge level (E.C.)			
		ATP	ADP	AMP	E.C.
C	8	0.204±0.021	0.056±0.008	0.028±0.005	0.80±0.02
S	6	0.346±0.034*	0.063±0.012	0.012±0.003**	0.87±0.03*
Normal rats	5	0.282±0.023**	0.061±0.006	0.010±0.002**	0.89±0.02**

The values are expressed as micromoles per 100 mg wet pancreas, except in energy charge (energy charge=(ATP+1/2ADP)/(ATP+ADP+AMP))
(C; direct surface cooling group, S; sham-cooling group, *; $p<0.05$, **; $p<0.02$ compared with C group)

Discussion

There have been several reports about the putative relationship between hypothermia and acute pancreatic injury[6,8,21], but the extact mechanism by which the pancreatic acinar cells are injured is unclear. In this study, using the direct pancreatic surface cooling method, we evaluated the effect of local pancreatic hypothermia on the exocrine pancreas. This direct cooling method seems to have less systemic effects compared with accidental hypothermia, because the systemic blood pressure and rectal temperature remain within their acceptable ranges. Direct surface cooling caused moderate hyperamylasemia, moderate increases in pancreatic water content, moderate congestion of amylase in the acinar cells, and very mild histological changes. Moreover, this direct cooling caused the redistribution of the lysosomal enzyme from the lysosomal fraction to the hearvier zymogen fraction. This indicates the colocalization of the lysosomal enzyme with the digestive enzyme, in addition to the accelerated lysosomal and mitochondrial fragility in the in-vitro incubation. The cooling impaired amylase and cathepsin B output stimulated by caerulein in-vivo and also impaired pancreatic adenylate energy metabolism.

Generally, pancreatic digestive enzymes and lysosomal hydrolases are transported separately from the Goligi apparatus to their own subcellular compartments, condensing vacuoles and lysosomes[18,25,27,29]. Theoretically there is no colocalization of these two types of enzymes[27,28,18,15] in the same subcellular compartment of acinar cells. However, colocalization of pancreatic digestive enzymes with lysosomal enzymes has been reported in the normal physiological state[12,13] as well as in three experimental models of pancreatitis: caerulein-induced pancreatitis[28,33], diet-induced pancreatitis[17,23], and duct-obstructed pancreatitis[13,24]. Although the ultimate degree of pancreatic injury differs considerably in these models, this colocalization of the digestive enzyme with the lysosomal hydrolases seems to be an important common triggering event in the development of acute pancreatitis[30,31]. Since cathepsin B as a lysosomal hydrolase, can activate trypsinogen[7,9,10,26] and trypsin can activate many other key enzymes responsible for acute pancreatitis[7,9,10,26].

In this study, redistribution and colocalization of lysosomal enzymes with the digestive enzyme was observed, and this colocalization phenomenon seems to be a universal etiology for pancreatic injury.

On the other hand, in our recent studies, lysosomal enzymes can be secreted into the pancreatic juice both in the basal state and by the stimulation of pancreatic secretagogues such as secretin or caerulein, and these lysosomal enzyme secretions seem to be important for the maintenance of the normal cellular organization of acinar cells[12–14]. This direct cooling of the pancreas caused an impaired cathepsin B output into pancreatic juice in addition to impaired amylase secretion, which indicates the possible congestion of lysosomal enzymes in the acinar cells. In our other recent studies, we have reported the accelerated fragility of lysosomes and mitochondrias in the caerulein-induced pancreatitis model[11,15]. This pancreatic cooling model also induced increased fragility of these subcellular organelles. Furthermore, we can expect impaired pancreatic energy metabolism in this pancreatic cooling model, because of the increased subcellular organellar fragility such as mitochondria.

Since we did not evaluate the microcirculation of the pancreas, or the core temperature of the pancreas, we cannot explain clearly how the direct surface cooling of pancreas injures the exocrine pancreas. Although direct cooling of the pancreas may initially impair the microcirculation of the pancreas, or impair the exocrine pancreatic function, direct cooling also impaired lysosomal and

digestive enzyme secretion and caused the redistribution and colocalization of lysosomal enzymes with digestive enzymes. This missorting of lysosomal enzymes seems to be closely related to the increased and accelerated lysosomal and mitochondrial fragility, as well as the impaired pancreatic adenylate energy metabolism.

Finally, these results call our attention to organ preservation during pancreas transplantation. Since the pancreas is usually preserved at very low temperatures, and this cooling preservation may impose some exocrine pancreatic malfunctions before organ transplantation, we must take into consideration that these cool preserved pancreas are very vulnerable to acute pancreatitis.

At present our direct surface cooling model of pancreatitis seems to be a useful tool in understanding the pathogenesis of acute pancreatitis, because no special tools or agents or drugs are needed in this model.

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和文抄録

ラットにおける膵表面冷却の膵外分泌系におよぼす影響について

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膵表面冷却が膵外分泌系におよぼす影響を解明する目的にて，ラットにおいて1，2，3時間の冷却後の血清 amylase 値，膵水分量，膵組織学的変化およびライソゾーム酵素である cathepsin B の膵腺房内分布を検討した．さらに，冷却後の caerulein 刺激下での amylase と cathepsin B の分泌量，in-vitro でのライソゾームとミトコンドリアの脆弱性とともに，膵エネルギー代謝も検討した．

3時間の冷却により高 amylase 血症とともに，cathepsin B 活性のライソゾーム分画よりチモーゲン分画への移動・再分布が観察された．さらに，amylase と cathepsin B 分泌量の低下と膵エネルギー代謝の障害が観察された．これらの結果は，膵表面冷却が誘起する膵外分泌系障害を示すとともに，特に膵外分泌系が冷却に暴露される膵移植や腹部外科手術時には，これらの膵における“cold”injury を考慮に入れる必要があるものと思われた．