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原　　著

Effects of *In vitro* and *In vivo* Ethanol and Acetaldehyde on Exocrine Pancreatic Secretion

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Introduction

It has been suggested that ethanol contributes to the development of acute pancreatitis in man. Although many experimental studies have been reported, the exact mechanism by which ethanol induces pancreatitis remains unknown. *In vivo* studies have suggested that intravenous injections of ethanol inhibit[1,12,14,16,17] while oral intake stimulates pancreatic enzyme secretion[5,8,13]. *In vitro* studies have shown that ethanol stimulates pancreatic enzyme secretion and inhibits trypsin inhibitory capacity in the rabbit pancreas[15].

Although no investigator has succeeded in producing acute pancreatitis in experimental animals by ethanol, these reports suggest that the acute administration of ethanol may sensitize the pancreas to acute pancreatitis.

In this study the effects of *in vitro* and *in vivo* ethanol on pancreatic secretion were evaluated by an incubation system[10]. The effect of acetaldehyde on pancreatic secretion was also investigated.

Materials and Methods

All studies were performed on CD-1 female mice weighing 20–25 gm. Two series of experiments the effect of *in vitro* administration and the effect of *in vivo* administration of ethanol or acetaldehyde on pancreatic enzyme secretion were studied by an incubation system.

Effects of in vitro ethanol and acetaldehyde

Following an 18-hour fast, mice were sacrificed by decapitation, the pancreas was removed and *in vitro* secretory studies were performed in the presence of various doses of ethanol (n=48), ethanol plus sincalide, the C-terminal octapeptide of CCK-PZ, (n=24) or acetaldehyde (n=32).

Effects of in vivo ethanol and acetaldehyde

Key words: Acute pancreatitis, Ethanol, Acetaldehyde, Exocrine pancreatic secretion, Incubation study.
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Mice were divided into three groups: control (n=8), ethanol (n=8) and acetaldehyde (n=8) groups. The ethanol and acetaldehyde groups received 0.01 g of ethanol/g of body weight in a 20% solution or 2 μg of acetaldehyde/g of body weight every seven hours for 42 hours, and the control animals received an identical volume of 0.9% saline. For the first 24 hours, normal commercial chow was given; then the animals were fasted for 18 hours. The animals were sacrificed by decapitation, the pancreas was removed and secretory studies were performed by an incubation system.

Incubation studies[10]

The pancreas was placed in Krebs-Henseleit Bicarbonate buffer containing 5.6 mM glucose and gassed with 95% O_2–5% CO_2 at 37°C. The pancreas was cut into 5–6 fragments, washed and placed in incubation vessels containing 3 ml of buffer. The vessels were gassed with 95% O_2 5% CO_2, and pre-incubated at 37°C for 30 minutes with shaking. The buffer was removed and replaced with fresh medium containing test substances and incubated for 30 minutes. For the experiments on the *in vivo* effect of ethanol or acetaldehyde, sincalide was added at a concentration of 3×10^{-4} μg/ml. This had previously been shown to produce submaximal rates of protein and enzyme secretion. After incubation, pancreatic fragments were removed, blotted, weighed and homogenized in phosphate buffer, pH 6.9. The incubation media and homogenate samples were centrifuged, and the supernatants were assayed for digestive enzymes. Protein was measured by the method of Lowry et al[9]. Amylase was assayed by the method of Bernfeld[2]. Trypsinogen and chymotrypsinogen were assayed by the method of Glazer and Steer[7].

Analysis of data

The statistical significance of differences was evaluated by Student's t-test, and a p value of <0.05 was considered significant.

Results

Effects of in vitro ethanol and acetaldehyde

The presence of 0.03% to 1.0% of ethanol did not result in any significant change in the

Table 1. Effect of in-vitro administration of ethanol on exocrine pancreatic secretion (n=8 in each group).

Ethanol	0%	0.03%	0.3%	1.0%	3.0%	10.0%
Protein mg/g	2.7±0.1	2.5±0.2 NS	2.6±0.1 NS	2.7±0.2 NS	3.2±0.2 $p<0.05$	3.6±0.4 $p<0.05$
Amylase U/g	1016±58	1021±61 NS	1027±59 NS	1128±74 NS	1379±67 $p<0.01$	1522±167 $p<0.02$
Trypsinogen U/g	17.0±0.9	17.2±1.2 NS	17.3±1.7 NS	19.5±2.7 NS	24.0±1.8 $p<0.01$	28.8±4.0 $p<0.02$
Chymotrypsinogen U/g	31.7±2.2	29.5±1.2 NS	32.3±1.7 NS	37.8±4.5 NS	42.9±3.3 $p<0.02$	44.4±5.5 $p<0.01$

mean±SE

Table 2. Effect of in-vitro administration of ethanol plus 3×10^{-4} μg/ml CCK-OP, sincalide, on protein and amylase secretions (n=6 in each group).

Ethanol	0%	0.03%	0.3%	3.0%
Protein mg/g	6.0±0.2	6.5±0.6 NS	6.4±0.3 NS	5.4±0.4 NS
Amylase U/g	1498±44	1601±98 NS	1508±76 NS	1469±91 NS

mean±SE

Table 3. Effect of in-vitro administration of acetaldehyde on protein and amylase secretions (n=8 in each group).

Acetaldehyde	0 mM	0.1 mM	1 mM	10 mM
Protein mg/g	3.0±0.3	2.8±0.2 NS	3.0±0.1 NS	2.8±0.2 NS
Amylase U/g	897±49	868±113 NS	923±85 NS	819±81 NS

mean±SE

output of amylase, protein, trypsinogen or chymotrypsinogen. The administration of 3.0% and 10.0% ethanol significantly increased the output of amylase, protein, trypsinogen and chymotrypsinogen (Table 1). However, the addition of 3×10^{-4} μg/ml of sincalide abolished the stimulant effect of 3.0% ethanol on amylase and protein output (Table 2, Fig. 1). On the other hand, the presence of 0.1 mM to 10.0 mM of acetaldehyde did not cause significant changes in amylase or protein output (Table 3).

Effects of in vivo ethanol and acetaldehyde

Forty-two hours after the intraperitoneal injection of 0.01 g of ethanol/g body weight, various degrees of pancreatic edema and hemorrhage were observed. Inflammatory edema of the paren-

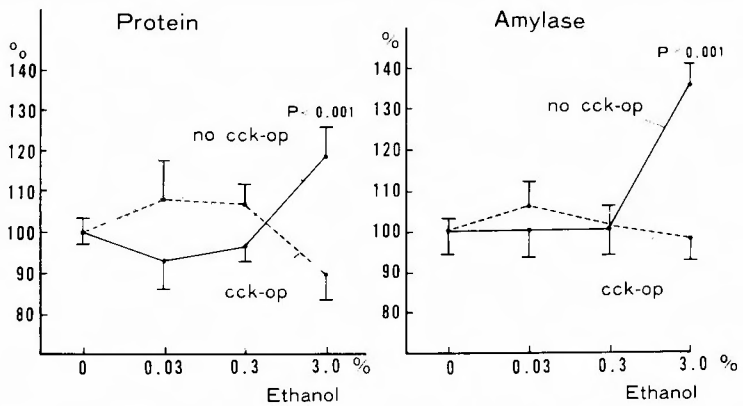

Fig. 1. Effect of 3×10^{-4} μg/ml CCK-OP, sincalide, on protein and amylase secretion in the presence of ethanol. Results are expressed as percent of level in the no ethanol group.

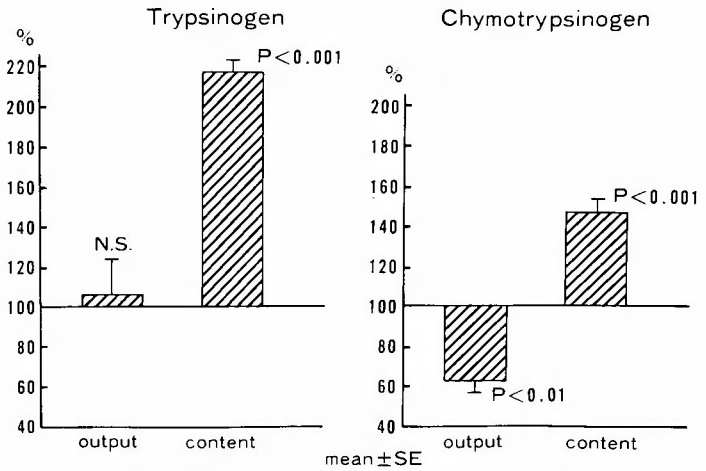

Fig. 2. Effect of intraperitoneal administration of ethanol on output and content of trypsinogen and chymotrypsinogen. Results are expressed as percent of the level in the no ethanol group.

chyma with congestion of zymogen granules was observed microscopically. A significant decrease of amylase and chymotrypsinogen output and a significant increase of trypsinogen and chymotrypsinogen content were noted in the ethanol group (Fig. 2). After acetaldehyde injection, there were no remarkable changes in the histology of the pancreas. In the secretion study, the doses used in this experiment did not significantly affect the protein output or the output or content of the enzymes (Table 4).

Discussion

These *in vitro* studies demonstrate that high concentrations of ethanol stimulate the secretion of pancreatic protein, amylase, trypsinogen and chymotrypsinogen. This result is in accord with those of our previous study in rabbits[15] and other[19]. In the *in vitro* study using rabbit pancreas,

Table 4. Effect of intraperitoneal administration of ethanol and acetaldehyde on output and content of amylase, trypsinogen and chymotrypsinogen.

	Amylase		Trypsinogen		Chymotrypsinogen	
	output U/g/30′	content U/g	output U/g/30′	content U/g	output U/g/30′	content U/g
Control n=8	1737±108	11527±418	31±3.1	240±13	68±6.0	393±15
Ethanol 0.01 g/g n=8	1192±101 p<0.01	10753±1146 NS	33±3.5 NS	519±14 p<0.001	43±4.5 p<0.01	563±27 p<0.001
Acetaldehyde 2 μg/g n=8	1652±136 NS	9544±430 NS	37±3.5 NS	220±0.7 NS	79±6.4 NS	350±20 NS

mean SE

significant increases of volume and output of protein, amylase, trypsinogen and chymotrypsinogen were observed with 3.0% ethanol, and the inhibitory capacity of trypsin was decreased[15]. However, no effect on secretion was noted when pancreatic slices were incubated in 0.3 and 1.0% ethanol, concentrations which are similar to the blood levels found during moderate to severe ethanol intoxication.

In the present *in vivo* study, mice receiving intraperitoneal injections of 0.01 g of ethanol/g body weight, showed no increase in the output of amylase, trypsinogen and chymotrypsinogen, although the content of trypsinogen and chymotrypsinogen was increased significantly.

These *in vitro* and *in vivo* experiments suggest that ethanol has no direct stimulant effect on pancreatic enzyme secretion under physiological conditions. In animal and man, the intravenous administration of ethanol inhibits secretin-, secretin plus CCK-PZ- or secretin plus gastrin-induced pancreatic secretion[3,4,11,16,17]. In this experiment, the increment of enzyme output in reaction to 3.0 to 10.0% ethanol in the incubation media was abolished by sincalide. In studies utilizing isolated guinea pig pancreas, Uhlemann et al[18] also observed that ethanol has an inhibitory effect on acinar cells that had been stimulated with various secretagogues. These results indicate that ethanol inhibits the pancreatic receptor response to secretagogues.

In contrast to the inhibitory effect of ethanol on pancreatic secretion, the content of pancreatic enzyme was markedly increased in the *in vivo* study. In other words, congestion of pancreatic enzyme caused by blockage of the secretory tract by ethanol was considered to be responsible for the development of ethanol-induced pancreatitis.

The metabolite of ethanol, acetaldehyde, did not have any effect on pancreatic enzyme secretion in the present *in vitro* and *in vivo* studies. Although a high concentration of acetaldehyde was found to prevent the free flow of exocrine secretion by constricting the outflow tract[6], no effect on the pancreatic acinar cells was seen in this experiment.

This experimental study suggests that congestion of proteolytic enzymes in ethanol intoxication plays an important role in the development of acute pancreatitis.

Summary

Effects of *in vitro* and *in vivo* ethanol and acetaldehyde on exocrine pancreatic function were studied in mice.

Significant increases in protein and enzyme output were noted with 3.0 and 10.0% ethanol, but not with 0 to 1.0% ethanol. In the *in vivo* study with intraperitoneal injections of 0.01 g of ethanol/g body weight, the content of trypsinogen and chymotrypsinogen was increased significantly, although the output of the enzymes was not increased. Acetaldehyde had no effect on pancreatic enzyme secretion in either *in vitro* or *in vivo* studies. Congestion of proteolytic enzyme may play an important role in the development of acute pancreatitis.

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和文抄録

In vitro, in vivo エタノール，アセトアルデヒド投与の膵外分泌に対する影響

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アルコールによる急性膵炎発症機序を解明する目的でマウスを用いエタノール，アセトアルデヒド投与時の膵酵素分泌動態を incubation system を用いて観察し以下の結果を得た．

1) In Vitro 実験：膵細胞を KHB 緩衝液で incubate し，種々の濃度のエタノールを投与すると，0～1％エタノール濃度では膵蛋白，酵素放出量に有意な変化がみられなかったが，エタノール濃度3～10％では膵蛋白，アミラーゼ，トリプシノーゲン，キモトリプシノーゲンの著明な上昇がみられた．しかし，CCK-OP (sincalide) を加えると3％エタノールによる膵蛋白，アミラーゼ分泌反応は有意に抑制された．

2) In vivo 実験：20％エタノール腹腔内投与ではトリプシノーゲン，キモトリプシノーゲン含有量の著明な増加を示した．

3) アセトアルデヒドは in-vitro, in-vivo 実験とも有意な膵酵素分泌反応をもたらさなかった．

以上よりアルコールは薬理学的濃度では膵に対し強力な酵素分泌刺激作用を有するが，生理学的濃度では acinar 細胞のレベルで膵酵素分泌を抑制し，zymogen 顆粒の鬱滞をもたらし，その結果膵炎が発症する機序が想定された．