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Alcohols enhance caerulein-induced zymogen activation in pancreatic acinar cells

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Abstract

Activation of zymogens within the pancreatic acinar cell is an early feature of acute pancreatitis. Supraphysiological concentrations of cholecystokinin (CCK) cause zymogen activation and pancreatitis. The effects of the CCK analog, caerulein, and alcohol on trypsin and chymotrypsin activation in isolated pancreatic acini were examined. Caerulein increased markers of zymogen activation in a time- and concentration-dependent manner. Notably, trypsin activity reached a peak value within 30 min, then diminished with time, whereas chymotrypsin activity increased with time. Ethanol (35 mM) sensitized the acinar cells to the effects of caerulein (10^{-10} to 10^{-7} M) on zymogen activation but had no effect alone. The effects of ethanol were concentration dependent. Alcohols with a chain length of ≥ 2 also sensitized the acinar cell to caerulein; the most potent was butanol. Branched alcohols (2-propanol and 2-butanol) were less potent than aliphatic alcohols (1-propanol and 1-butanol). The structure of an alcohol is related to its ability to sensitize acinar cells to the effects of caerulein on zymogen activation.

Keywords

trypsinogen activation peptide; trypsin; chymotrypsin; ethanol; pancreatitis

ABERRANT ACTIVATION OF ZYMOGENS within the pancreas has long been proposed as a critical step in the pathogenesis of pancreatitis. A close link between the intracellular activation of zymogens and the initiation of pancreatitis has recently been established; both pancreatic trypsin and elastase activities increase within the first hour after the initiation of experimental pancreatitis. A surrogate marker of trypsinogen activation, trypsinogen activation peptide (TAP), is generated in pancreatic acinar cells within 30 min of initiating experimental pancreatitis and is elevated in the serum and urine of patients with acute pancreatitis (6). The key role of protease activation in disease is further supported by studies that demonstrate that protease inhibitors can ameliorate or inhibit experimental and endoscopic retrograde cholangiopancreatography-induced pancreatitis (2,10,28). An additional link between abnormal zymogen activation and acute pancreatitis comes from the finding that mutations in the trypsinogen gene cause hereditary pancreatitis (5,29). Thus experimental and clinical evidence support a role for zymogen activation in acute pancreatitis.

One of the best-described and -studied models of acute pancreatitis is the caerulein-induced model (4). Supraphysiological or hyperstimulating doses (10- to 100-fold greater than those that elicit maximum secretion) of caerulein, a cholecystokinin (CCK) analog, induce

pancreatitis. Isolated pancreatic acini have been used to examine the earliest cellular events in pancreatitis. Similar to the *in vivo* response, caerulein hyperstimulation of acini is associated with zymogen activation as reflected by the generation of TAP, enhanced tryptic activity, and the proteolytic conversion of procarboxypeptidase A₁ (PCA₁) to its mature form carboxypeptidase A₁ (CA₁) (7,11,24). These CCK-hyperstimulation models may be clinically relevant, because administration of CCK antagonists has been shown to ameliorate pancreatic injury caused by diet, bile salts, and ischemia, suggesting a more general role for CCK receptors in the development of pancreatitis (13,25a). In these and other forms of pancreatitis, sensitization to the harmful effects of CCK may play a role in disease. Ethanol has been shown to sensitize acinar cells to CCK-induced PCA₁ processing *in vitro* and to sensitize to various forms of pancreatitis *in vivo* (17-19,27). Thus ethanol enhancement of the CCK-dependent induction of pancreatitis may play a role in ethanol toxicity.

The present study addresses several issues relevant to zymogen activation and the effects of ethanol in isolated pancreatic acini. First, the effects of caerulein-dependent processing of several zymogens are examined. We observed that trypsinogen and chymotrypsinogen exhibit distinct patterns of activation in response to caerulein. Second, the effects of ethanol and other alcohols on caerulein-induced zymogen activation are studied. Ethanol sensitized the acinar cell to caerulein-induced trypsin and chymotrypsin activation. Other short-chain *N*-aliphatic alcohols enhanced the effects of caerulein on the acinar cell. These studies suggest that CCK receptor activation can initiate different patterns of zymogen activation in pancreatic acinar cells and that the extent of activation can be enhanced by a distinct set of short-chain alcohols.

MATERIALS AND METHODS

Acinar preparation

Acini were isolated as described, with minor modifications (26). Briefly, fasted male Sprague-Dawley rats weighing 80–120 g (Charles River Labs, Wilmington, MA) were killed by CO₂ using a protocol approved by the Yale University Animal Care and Use committee. The pancreas was removed and placed into 6 ml of acinar media consisting of (in mM): 40 Tris (pH 7.4), 95 NaCl, 4.7 KCl, 0.6 MgCl₂, 1.3 CaCl₂, 1 NaH₂PO₄, 10 glucose, and 2 glutamine, plus 0.1% BSA, and 1× MEM-amino acids (GIBCO-BRL, San Jose, CA) containing 50 units/ml of collagenase Type-4 (Worthington, Freehold, NJ). The pancreas was insufflated for 5 min with the collagenase media. After being minced, the pancreas was placed into a 50-ml flask in a total of 12 ml of collagenase media and incubated for 60 min at 37°C with shaking (120 rpm). At the end of incubation, the digest was filtered through a 300- to 400-μm mesh (Sefar American, Depew, NY). Isolated acini were distributed evenly among the 24 wells (0.5 ml suspension/well) of a 24-well Falcon tissue culture plate (Becton Dickinson, Franklin Lakes, NJ).

Acinar experimental protocol

Tissue culture plates containing acini were incubated for 60 min at 37°C under constant O₂ with shaking (80 rpm). The media were exchanged for 0.5 ml of fresh media and incubated for an additional 60 min. At this time, treatments were added to cells without changing media. Samples (medium and cells) were collected and placed in 1.5 ml Eppendorf tubes and stored at –80°C.

Enzymatic activity assays

Frozen homogenates containing medium and cells were thawed on ice and homogenized, and 100 μl was added to wells of a 24-well tissue culture plate with 350 μl of trypsin assay buffer (50 mM Tris, pH 8.1, 150 mM NaCl, 1 mM CaCl₂, and 0.01% BSA). Finally, 50 μl of 400 μM enzyme substrate (trypsin 3135-v, chymotrypsin 3114-v; Peptides International,

Louisville, KY) diluted in trypsin assay buffer (40 μ M final) was added to each well. The plate was immediately read using a fluorometric microtiter plate reader (model HTS 7000; Perkin-Elmer Analytical Instruments, Shelton, CT) using an excitation wavelength of 380 nm and emission of 440 nm. Twenty data points were collected over an 11-min period. The slope of the line represented enzyme activity and was normalized to total amylase activity and expressed as relative fluorescence units per second per microgram total amylase.

Amylase assay

Amylase activity in acinar homogenates was determined using the Phaebedas test kit (Pharmacia diagnostic, Rochester, NY).

TAP assay

Affinity-purified rabbit antibody to the five amino acids [(Asp)4-Lys] adjacent to the activation site in trypsinogen (TAP-5 Ab) was prepared as described (16). Competitive enzyme-linked immunosorbent assay was used to measure the amount of TAP. Ninety-six-well plates were coated overnight at 4°C with rabbit albumin-(Asp)4-Lys conjugate (50 ng peptide/well) in 50 mM NaHCO₃ at pH 9.5. The next day plates were washed three times with wash buffer composed of 50 mM Tris, 150 mM NaCl, 0.05% Tween-20, pH 8.2, and then were blocked for 1 h with 200 μ l of blocking buffer (50 mM Tris, 5 mM EDTA, 0.05% Tween-20, 0.1% BSA, pH 8.2). Samples containing medium and cells were solubilized by adding Triton X-100 to a final concentration of 0.1%. Postcollection activation of trypsin in samples was prevented by the addition of benzamidine to a final concentration of 10 mM. Before use, all samples were vortexed vigorously for TAP determination. Samples (75 μ l) or standards in blocking buffer were placed into each well followed by 25 μ l of TAP-5 antibody in blocking buffer at a final ratio of 1:10,000. The plate was incubated 90 min at room temperature. The plate was then washed three times with wash buffer, incubated with 50 liters of alkaline phosphatase-conjugated goat anti-rabbit IgG (ICN Biomedicals, Costa Mesa, CA), and diluted 1:2,500 in blocking buffer for 60 min at room temperature. The plate was then washed three times with wash buffer followed by the addition of 200 μ l of Sigma Fast *p*-nitrophenyl phosphate substrate (Sigma, St. Louis, MO) and incubation at 37°C for 60–90 min. Absorbance was measured at 405 nm. TAP concentrations were extrapolated from a standard curve with a linear range of 1.5–5,000 pM/well.

Statistical analysis

Data represent the means $\pm$ SE of at least three individual experiments, with each experiment being performed in triplicate. A paired Student's *t*-test analysis was used to determine statistical significance.

RESULTS

To examine the time-dependent zymogen activation by caerulein hyperstimulation, isolated pancreatic acinar cells were treated with 100 nM caerulein. The tryptic and chymotryptic activities, as well as the generation of TAP, were determined (Fig. 1A). Caerulein hyperstimulation induced trypsin activity in a time-dependent manner. Tryptic activity had increased over basal levels by 15 min and peaked at 30 min. Tryptic activity decreased by 25% over the next 60 min but remained greater than basal levels.

Caerulein hyperstimulation also induced an increase in chymotrypsin activity and TAP accumulation. In contrast to tryptic activity, these values continued to increase with time. Moreover, the time needed for chymotrypsinogen activation and TAP accumulation to show a significant increase over basal values was longer (30 min for chymotrypsin and >30 min for TAP). The maximum level of chymotrypsin activity and TAP accumulation was observed at

90 min. The relative increases over basal for each marker of zymogen activation were calculated (Fig. 1B). The maximal increase of tryptic activity over basal state was threefold, whereas there was an 11-fold increase for chymotryptic activity and a >30-fold increase for TAP levels. These findings demonstrate that zymogens can exhibit distinct patterns and degrees of activation.

To examine the effects of ethanol on zymogen processing, isolated pancreatic acinar cells were treated simultaneously with 100 nM caerulein and increasing concentrations of ethanol for 60 min. Untreated cells or cells treated with 200 mM ethanol generated similar amounts of TAP. Treatment with 100 nM caerulein caused a TAP accumulation of 10-fold over untreated cells. Ethanol at 5 mM did not affect the amount of TAP generated by 100 nM caerulein; however, a dose-dependent enhancement of caerulein-induced TAP generation was observed with higher concentrations of ethanol (35, 50, 100, and 200 mM) (Fig. 2). An ethanol concentration of 35 mM corresponds to a blood alcohol value of ~0.15%; a concentration both physiologically and disease relevant was used for the subsequent experiments unless otherwise noted.

After 30 min incubation with ethanol, trypsin activity and TAP generation were significantly enhanced; chymotrypsin activity was enhanced after 60 min of incubation. (Fig. 3). Ethanol did not alter the biphasic pattern of trypsin activation or the monophasic patterns of chymotrypsin activation and TAP generation induced by caerulein.

Previous reports have shown that serine protease inhibitors reduce caerulein-induced zymogen activation (7,12,15). The effect of two serine protease inhibitors on TAP generation was measured. The serine protease inhibitors benzamidine (1 mM) and FUT-175 (10 μ M) completely abolished the generation of TAP induced by 100 nM caerulein with or without ethanol (Fig. 4).

The effect of ethanol on the concentration-dependent increase in zymogen activation by caerulein was examined (Fig. 5). There was a tendency for 35 mM ethanol to enhance activation at all caerulein concentrations. When background levels are subtracted, the remaining values even at the lowest concentrations of caerulein revealed average increases of 2.1- and 3-fold in trypsin and chymotrypsin activities, respectively.

The effect of *N*-butanol, a four-carbon alcohol, on chymotryptic activity induced by 100 mM caerulein was examined. As shown in Fig. 6, butanol caused a concentration-dependent increase in chymotrypsin activity when added with caerulein. Activation with 1 mM butanol and caerulein was significantly greater than caerulein alone, and a half-maximal effect was observed with 5 mM butanol. Its sensitization to caerulein reached a plateau at 25 mM. Butanol had much less of an effect on trypsin activity. Butanol also sensitized the acinar cell to lower concentrations of caerulein (Fig. 7). Although both butanol and ethanol sensitize the acinar cell to the effects of caerulein, butanol was much more potent.

To determine the relationship between the structure of short-chain alcohols and sensitization to caerulein, alcohols of 1–4 carbon chain lengths were tested for their affect on chymotrypsinogen activation (Fig. 8). The alcohols alone had little to no effect on chymotrypsin activities (data not shown). All 1–4 carbon *N*-linked alcohols significantly enhanced chymotrypsin activity induced by 100 nM caerulein. The amount of sensitization related directly to chain length; methanol was the least potent and 1-butanol the most potent. The branched alcohols 2-propanol and 2-butanol had significantly less effect than their unbranched counterparts.

To examine the effect of alcohols on another caerulein response, the effect of 10 mM butanol and 35 mM ethanol on acinar cell amylase secretion was measured (Fig. 9). The secretory response was maximal at 1 nM caerulein and then decreased at higher concentrations. The

addition of either 35 mM ethanol or 10 mM butanol with caerulein did not change secretion. Additionally, ethanol demonstrated neither time- nor concentration-dependent effects on amylase secretion (Fig. 10). These findings suggest that the sensitizing actions of ethanol and butanol affect pathways linked to zymogen activation but not to amylase secretion.

DISCUSSION

The intracellular activation of pancreatic digestive zymogens is an early feature of acute pancreatitis. Supraphysiological concentrations of CCK cause zymogen activation and pancreatitis. Ethanol, a common cause of acute pancreatitis, may promote this damaging effect of CCK through two mechanisms. First, ethanol may directly sensitize the acinar cell to the effects of CCK (8). Second, ethanol may stimulate CCK release from duodenal I-cells (23). The present study uses isolated pancreatic acini to confirm that supramaximal concentrations of caerulein cause zymogen activation in isolated pancreatic acini. The observed increase in trypsin activity before an increase in chymotrypsin activity is consistent with the requirement for trypsin to convert chymotrypsinogen to chymotrypsin. After hyperstimulation, trypsin activity was found to reach a peak value and then decrease, whereas TAP, a marker for trypsinogen activation, steadily increased. The continued generation of TAP suggests that trypsinogen activation persists throughout the treatment period. The decrease in trypsin activity with time is likely due to both its inhibition by the pancreatic trypsin inhibitor and trypsin degradation. Chymotrypsin is not subject to these downregulatory mechanisms and was a much more sensitive marker than trypsin of enzyme activation after 60 min of treatment.

Agents that enhance the effects of CCK on zymogen activation might be relevant to human disease. Ethanol by itself has not been shown to induce pancreatitis or zymogen activation in experimental models of pancreatitis. However, it has been reported to sensitize the pancreas to the effects of CCK in vivo, suggesting that the two agents may act synergistically (17). Utilizing the proteolytic conversion of PCA₁ to CA₁ as a marker for zymogen proteolysis, we demonstrated that ethanol could enhance the zymogen processing induced by caerulein (8). However, these assays did not directly demonstrate the generation of active zymogens. We now show that ethanol at a physiologically relevant dose enhances zymogen activation induced by both physiological and hyperstimulation doses of caerulein.

To explore the mechanisms for ethanol's actions, we used the observation that other short-chain aliphatic alcohols often have actions similar to ethanol (1). These alcohols (methanol, propanol, and butanol) have a wide range of effects that include inducing abnormal phospholipid and fatty acid metabolism, changing the cellular redox state, disruption of energy generation, stimulating reactive oxygen production, changing membrane fluidity, and affecting intracellular signaling. We show that alcohols ranging from 1 to 4 carbons in length enhance zymogen activation induced by caerulein; the order of alcohols' sensitizing potencies is 1-butanol > 2-butanol > 1-ethanol = 1-propanol > methanol with 2-propanol having no effect. Alcohols with *N*-linked OH groups are more potent in their sensitizing effects than branched-chain alcohols. This ranking is in contrast to the effect of these alcohols on membrane fluidity. For example, in *Torpedo californica* cell membranes, alcohols increased the membrane fluidity in the order of propanol > *N*-butanol > *n*-butanol > ethanol (9). This suggests that the effect of alcohols in the acinar cell might be related to factors other than membrane fluidity.

Ethanol has been shown to affect many cellular second messenger pathways. The one common feature of all short-chain alcohols is their metabolism by phospholipase D (PLD). The enzyme catalyzes the conversion of phosphatidylcholine (PC) to phosphatidic acid (PA). Two isoforms of PLD are expressed in distinct regions of the cell: PLD₁ is found in the perinuclear region, on the Golgi, ER, and late endosomes, whereas PLD₂ is found in the plasma membrane (3). Among the cellular targets of PA are processes that regulate transport of proteins from the ER

to the Golgi complex and from the trans-Golgi network to the plasma membrane. Moreover, PA serves as a source of diacylglycerol, a potent activator of protein kinase C. In the presence of short-chain alcohols, PLD preferentially converts PC to phosphoalcohols, thus reducing PA and diacylglycerol levels (1). The metabolism of alcohols by PLD reduces the generation of PA and PC and generates phosphatidylalcohols (20). It is interesting to note that the short-chain alcohols' ability to affect PLD is in the order of butanol > ethanol = propanol, the same as the rank order effectiveness of these short-chain alcohols on zymogen activation.

The role of PLD in acinar cells is unclear. Acinar cells have been shown to express PLD; moreover, PLD₁ has been localized to secretory granules, where it is thought to play a role in zymogen secretion. Activation of CCK receptors has been reported to affect PLD activity by some authors but not by others (22,25). Interestingly, acute ethanol ingestion by rats has been shown to affect PLD activity in pancreatic acinar cells (21). If alcohol is affecting CCK-induced activation through PLD, it may act by diminishing either PA or diacylglycerol generation or by producing phosphatidylalcohols. Studies of the metabolic effects of phosphatidylalcohols are limited. Phosphatidylethanol and phosphatidylbutanol have been shown to increase the activity of calcium ATPases and to diminish PA-dependent tyrosine phosphorylation (1). Further studies examining the role of PLD in zymogen activation are needed.

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REFERENCES

1. Baker RC, Kramer RE. Cytotoxicity of short-chain alcohols. *Annu Rev Pharmacol Toxicol* 1999;39:127–150. [PubMed: 10331079]
2. Cavallini G, Tittobello A, Frulloni L, Masci E, Mariani A, DiFrancesco V. Gabexate for the prevention of pancreatic damage related to endoscopic retrograde cholaniopancreatography. *N Engl J Med* 1996;335:919–923. [PubMed: 8786777]
3. Exton JH. Regulation of phospholipase D. *Biochim Biophys Acta* 1999;1439:121–133. [PubMed: 10425390]
4. Gorelick, FS.; Adler, G.; Kern, HF. Cerulein-induced pancreatitis. In: Go, VLW.; DiMagno, EP.; Gardner, JD.; Lebenthal, E.; Reber, HA.; Scheele, GA., editors. *The Pancreas: Biology, Pathobiology, and Disease*. Raven; New York: 1993. p. 501-526.
5. Gorry MC, Gabbazadeh D, Furey W, Gates LK Jr, Preston RA, Aston CE, Zhang Y, Ulrich C, Ehrlich GD, Whitcomb DC. Mutations in the cationic trypsinogen gene are associated with recurrent acute and chronic pancreatitis. *Gastroenterology* 1997;113:1063–1068. [PubMed: 9322498]
6. Heath DI, Wilson C, Gudgeon AM, Jehanli A, Shenkin A, Imrie CW. Trypsinogen activation peptides (TAP) concentrations in the peritoneal fluid of patients with acute pancreatitis and their relation to the presence of histologically confirmed pancreatic necrosis. *Gut* 1994;35:1311–1315. [PubMed: 7525422]
7. Hofbauer B, Saluja AK, Lerch MM, Bhagat L, Bhatia M, Lee HS, Frossard JL, Adler G, Steer ML. Intra-acinar cell activation of trypsinogen during caerulein-induced pancreatitis in rats. *Am J Physiol Gastrointest Liver Physiol* 1998;275:G352–G362.
8. Katz M, Carangelo R, Miller LJ, Gorelick F. Effect of ethanol on cholecystokinin-stimulated zymogen conversion in pancreatic acinar cells. *Am J Physiol Gastrointest Liver Physiol* 1996;270:G171–G175.
9. Lasner M, Roth LG, Chen CH. Structure-functional effects of a series of alcohols on acetylcholinesterase-associated membrane vesicles: elucidation of factors contributing to the alcohol action. *Arch Biochem Biophys* 1995;317:391–396. [PubMed: 7893154]
10. Lasson A, Ohlsson K. Protease inhibitors in acute pancreatitis: correlation between biochemical changes and clinical course. *Scand J Gastroenterol* 1984;19:779–786. [PubMed: 6083596]

11. Leach SD, Modlin IM, Scheele GA, Gorelick FS. Intracellular activation of digestive zymogens in rat pancreatic acini. Stimulation by high doses of cholecystokinin. *J Clin Invest* 1991;87:362–366. [PubMed: 1985109]
12. Mithofer K, Fernandez-del Castillo C, Rattner D, Warshaw AL. Subcellular kinetic of early trypsinogen activation in acute rodent pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 1998;274:G71–G79.
13. Niederau C, Liddle RA, Ferrell LD, Grendell JH. Beneficial effects of cholecystokinin-receptor blockade and inhibition of proteolytic enzyme activity in experimental acute hemorrhagic pancreatitis in mice. Evidence for cholecystokinin as a major factor in the development of acute pancreatitis. *J Clin Invest* 1986;78:1056–1063. [PubMed: 2428840]
15. Otani T, Atomi Y, Kuroda A, Muto T, Tamura M, Fukuda S, Akao S, Gorelick FS. Distribution of a synthetic protease inhibitor in rat pancreatic acini after supramaximal secretagogue stimulation. *Pancreas* 1997;14:142–149. [PubMed: 9057186]
16. Otani T, Chepilko SM, Grendell JH, Gorelick FS. Co-distribution of trypsinogen activation peptide and the granule membrane protein, GRAMP-92, in rat cerulein-induced pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 1998;275:G999–G1009.
17. Pandolfi SJ, Periskic S, Gukovsky I, Zaninovic V, Jung Y, Zong Y, Solomon TE, Gukovskaya AS, Tsukamoto H. Ethanol diet increases the sensitivity of rats to pancreatitis induced by cholecystokinin octapeptide. *Gastroenterology* 1999;117:706–716. [PubMed: 10464148]
18. Ponnappa BC, Marciniak R, Schneider T, Hoek JB, Rubin E. Ethanol consumption and susceptibility of the pancreas to cerulein-induced pancreatitis. *Pancreas* 1997;14:150–157. [PubMed: 9057187]
19. Ramo O. Antecedent long term ethanol consumption in combination with different diets alters the severity of experimental acute pancreatitis in rats. *Gut* 1987;28:64–69. [PubMed: 3817587]
20. Rydzewska G, Jurkowska G, Gabryelewicz A. Phospholipase D mediated transphosphatidylolation as a possible new pathway of ethanol metabolism in isolated rat pancreatic acini. *J Physiol Pharmacol* 1996;47:385–95. [PubMed: 8807565]
21. Rydzewska G, Jurkowska G, Gabryelewicz A. The influence of acute ethanol ingestion on phospholipase D activity in rat pancreas. An in vitro and in vivo study. *Int J Pancreatol* 1996;20:59–68. [PubMed: 8872525]
22. Rydzewska G, Morisset J. Specificity of phospholipase D activation by cholecystokinin and phorbol myristate acetate but not by carbamylcholine and A23187 in rat pancreatic acini. *Digestion* 1995;56:127–136. [PubMed: 7750666]
23. Saluja A, Maitre N, Runzi M, Dawra R, Nishino H, Steer ML. Ethanol induced increase in plasma cholecystokinin (CCK) level is mediated by the release of a CCK-releasing peptide into the duodenum. *J Clin Invest* 1997;99:506–512. [PubMed: 9022085]
24. Saluja AK, Donovan EA, Yamanaka K, Yamaguchi Y, Hofbauer B, Steer ML. Cerulein-induced in vivo activation of trypsinogen in rat pancreatic acini is mediated by cathepsin B. *Gastroenterology* 1997;113:304–311. [PubMed: 9207291]
25. Sarri E, Ramos B, Salido G, Claro E. Cholecystokinin octapeptide CCK-8 and carbachol reduce [³²P]orthophosphate labeling of phosphatidylcholine without modifying phospholipase D activity in rat pancreatic acini. *FEBS Lett* 2000;486:63–67. [PubMed: 11108844]
- 25a. Satake K, Kimura K, Saito T. Therapeutic effects of loxiglumide on experimental acute pancreatitis using various models. *Digestion* 1999;60(Suppl):164–168.
26. Schultz GS, Sarras MP, Gunther GR, Hull BE, Alicea HA, Gorelick FS, Jamieson JD. Guinea pig pancreatic acini prepared with purified collagenase. *Exp Cell Res* 1980;130:49–62. [PubMed: 6256185]
27. Siech M, Weber H, Letko G, Dummmler W, Schoenberg MH, Beger HG. Similar morphological and intracellular biochemical changes in alcoholic acute pancreatitis and ischemic acute pancreatitis in rats. *Pancreas* 1999;14:32–38. [PubMed: 8981505]
28. Suzuki M, Isaji S, Stanten R, Frey CF, Ruebner B. Effect of protease inhibitor FUT-175 on acute hemorrhagic pancreatitis in mice. *Int J Pancreatol* 1992;11:59–65. [PubMed: 1374785]
29. Whitcomb DC, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, Martin SP, Gates LK, Amann ST, Toskes PP, Liddle R, McGrath K, Uomo G, Post JC, Ehrlich GD. Hereditary

pancreatitis is caused by a mutation on the cationic trypsinogen gene. Nat Genet 1996;14:141–145. [PubMed: 8841182]

Fig. 1.

Caerulein-increases markers of zymogen activation in isolated pancreatic acini. *A*: time dependence. *B*: relative changes. Isolated acini were treated with 100 nM caerulein. Markers of zymogen activation in cells combined with medium were measured as described in MATERIALS AND METHODS. Data are plotted as a ratio to the maximum value for each marker in *A* and relative to basal value in *B* (after 90 min). Values are means $\pm$ SE of at least 4 experiments.

Fig. 2.

Ethanol, in a dose-dependent manner, enhances caerulein-induced generation of trypsin, chymotrypsin, and TAP. Isolated acini were treated with 100 nM caerulein and ethanol for 60 min, and assays were performed on the cells combined with the medium. Data are plotted as a ratio to 100 nM caerulein. Values are means $\pm$ SE of 7 experiments. * $P < 0.05$ vs. caerulein 100 nM.

Fig. 3.

Ethanol enhances caerulein-induced activation of trypsin, chymotrypsin, and TAP generation in a time-dependent manner. Isolated acini were treated with 100 nM caerulein continuously with or without 35 mM ethanol. Data are plotted as a ratio to the maximum amount obtained for caerulein. Values are means $\pm$ SE of 5 experiments. * $P < 0.05$ vs. no ethanol.

Fig. 4.

Serine protease inhibitors block ethanol-enhanced TAP generation by caerulein. Isolated acini were pretreated for 30 min with media (no inhibitor), 1 mM benzamidine (Ben), or 10 μ M FUT-175. Then, 100 nM caerulein with or without 35 mM ethanol were added to the cells. TAP was measured after 90 additional min of treatment in the cells and medium. Data are plotted as a ratio to 100 nM caerulein. Values are means $\pm$ SE of 5 experiments. * $P < 0.05$ vs. no inhibitor group.

Fig. 5.

Ethanol enhancement of zymogen activation depends on the concentrations of caerulein. Isolated acini were treated simultaneously with caerulein with or without 35 mM ethanol for 60 min, and the total trypsin, chymotrypsin, and TAP were measured. Data are plotted as a ratio to 100 nM caerulein. Values are means $\pm$ SE of 6 experiments. * $P < 0.05$.

Fig. 6.

Butanol, in a dose-dependent manner, enhances caerulein-induced zymogen activation.

Isolated acini were treated simultaneously with 100 nM caerulein with or without butanol for 60 min, and total trypsin and chymotrypsin activities were measured. Data are plotted as a ratio to 100 nM caerulein. Values are means $\pm$ SE of 6 experiments. * $P < 0.05$.

Fig. 7.

Butanol enhancement of zymogen activation depends on the concentrations of caerulein. Isolated acini were treated simultaneously with caerulein with or without 10 mM *N*-butanol for 60 min, and the total trypsin and chymotrypsin activities were measured. Data are plotted as a ratio to 100 nM caerulein. Values are means $\pm$ SE of 6 experiments. * $P < 0.05$ comparing caerulein to caerulein with butanol.

Fig. 8.

Short-chain alcohols enhance caerulein-induced (100 nM) chymotrypsin activity. Acini were treated with each alcohol (50 mM) simultaneously with 100 nM caerulein, and total chymotrypsin activity was measured. Data are plotted as fold stimulation of activity vs. 100 nM caerulein. Values are means $\pm$ SE of 3 experiments. * $P < 0.05$ or less; # $P < 0.05$.

Fig. 9.

Effects of alcohols on secretion. Amylase secretion into the medium was measured with increasing concentrations of caerulein in the absence of alcohol or with 35 mM ethanol or 10 mM butanol after 60-min treatments.

Fig. 10.

Ethanol does not effect time-dependent effects of caerulein on amylase secretion. Isolated acini were treated simultaneously with caerulein 0 nM (A), 1 nM (B), 100 nM (C) in the presence of increasing concentrations of ethanol (0–200 mM). Media were collected at various time points between 15 and 90 min to determine secreted amylase, whereas cells were collected at the end of the experiment for the determination of total amylase. Data are plotted as the average %secretion of 2 experiments $\pm$ the variance.