JOURNAL OF SURGICAL RESEARCH 47, 360–364 (1989)

Effects of Chronic Alcohol Intake and Secretory Stimulation on Sodium Taurocholate-Induced Pancreatic Necrosis in the Rat

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Submitted for publication May 10, 1988

The effects of long-term alcohol intake and pancreatic secretory stimulation on pancreatic necrosis were studied in rats with acute pancreatitis induced by an intraductal injection of sodium taurocholate. Neither alcohol nor pancreozymin alone influenced the extent of pancreatic tissue damage in this experimental model. However, when animals were exposed to both alcohol and pancreozymin, the tissue lesions induced by the bile salt were wider than those in the presence of either alcohol or secretory stimulation alone. The results indicate that secretory stimulation with pancreozymin superimposed on chronic alcohol intake sensitizes the pancreas to the injury caused by intraductal bile salt administration.

INTRODUCTION

Hypersecretion has been proposed to participate in the development of tissue damage in acute pancreatitis, and inflammatory changes have been produced in certain experimental protocols by secretory stimulation [11, 14].

The effects of chronic alcohol intake on the secretion of pancreatic proteins have been studied extensively. Differences in the patterns of secretion were found between alcoholic and nonalcoholic rats before and after stimulation of pancreatic secretion [26]. However, it has not been possible to induce experimental pancreatitis by peroral alcohol alone [6, 22]. Recently, Tiscornia and Dreiling [24] emphasized the crucial role of two major factors in the pathogenesis of alcohol-induced pancreatitis: an elevated intrapancreatic acetylcholine level and an increased pancreatic exocrine response to pancreozymin. Both would lead to pancreatic damage by supranormal stimulation of pancreatic exocrine secretion.

The purpose of the present work was to study the effects of long-term alcohol intake and secretory stimulation with pancreozymin on the vulnerability of rat pancreas to intraductal bile salt injection.

METHODS

Animals

Forty male Wistar rats were used. At the age of 2 months the animals were randomly divided into two groups. Twenty animals were allowed to drink alcohol only as a 15% (w/v) solution of ethanol in water for 3 months. Twenty control animals were offered water. The animals were housed in individual cages and had free access to food (Ewos R3, Ewos Ab, Södertälje, Sweden; carbohydrate, 52%; protein, 40%; fat, 6%). The daily ethanol consumption was about 3 grams pure ethanol per rat. The alcoholic and control animals were further divided into four subgroups as described in Table 1.

Operation

Acute pancreatitis was induced by a standardized method [1]. The anesthetized animals were laparotomized, and a blunt injection needle was introduced into the common biliopancreatic duct. The hepatic duct was closed, and 5% sodium taurocholate (Sigma, 0.1 ml/100 g body wt) dissolved in saline was injected at the rate of 0.2 ml/60 sec into the pancreatic duct system by an infusion pump. Pancreatic secretion was stimulated with intraperitoneal pancreozymin (Boots, Nottingham, England; 0.75 U/100 g body wt) at three different time points before the induction of pancreatitis (Table 1). This dose of pancreozymin was found to increase pancreatic secretion up to sixfold in preliminary experiments (unpublished data).

Samples

Pancreatic tissue for histological examination was fixed in 10% buffered formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin. Tissues for electron microscopy were fixed by immersion in 3% glutaraldehyde in 0.1 *M* phosphate buffer at pH 7.4 for 24 hr and postfixed in 1% osmium tetroxide for 1 hr. The samples were washed in the buffer, dehydrated in acetone, and embedded in Epon 812. Thin sections were stained

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TABLE 1

Experimental Protocol

Animals exposed to alcohol	
Group A (5 rats)	Sodium taurocholate, the amount of necrosis measured after 9 hr
Group B (5 rats)	Pancreozymin 15 min before sodium taurocholate, the amount of necrosis measured after 9 hr
Group C (5 rats)	Pancreozymin 45 min before sodium taurocholate, the amount of necrosis measured after 9 hr
Group D (5 rats)	Pancreozymin 90 min before sodium taurocholate, the amount of necrosis measured after 9 hr
Animals not exposed to alcohol	
Group E (5 rats)	Controls for group A
Group F (5 rats)	Controls for group B
Group G (5 rats)	Controls for group C
Group H (5 rats)	Controls for group D

with uranyl acetate and lead citrate and studied in an electron microscope.

Experimental Protocol

The experimental protocol is presented in Table 1. Samples for light and electron microscopies were taken 9 hr after the intraductal bile salt injection and the extent of necrosis in the pancreas was measured.

Morphometric Analysis

The whole pancreas with associated duodenum and spleen was removed, attached with pins to a cork plate, and divided into four equally sized parts, and one section from each part was used for the quantitative histological analysis. The percentage of the necrotic parenchyma of the total pancreatic parenchyma was calculated by the systematic point counting method [13]. For this purpose a square grid, consisting of thin perpendicular lines 2 cm apart was drawn on paper. The line intersections formed the test point lattice. Then, the sections were projected

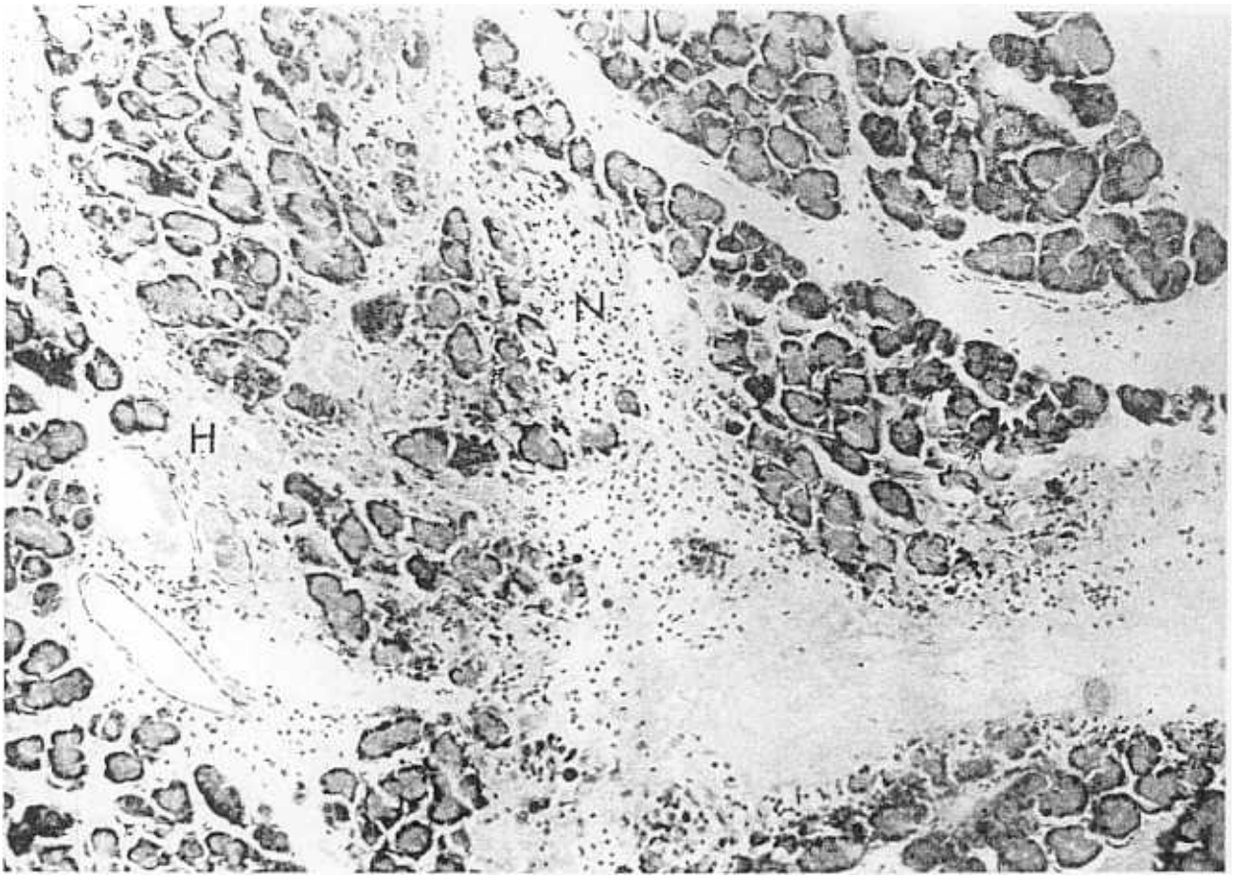

FIG. 1. Necrotic (N) and nonnecrotic pancreatic tissue in a rat after 3 months of alcohol intake, pancreozymin stimulation, and intraductal bile salt injection. H, hemorrhage. H-E, ×150.

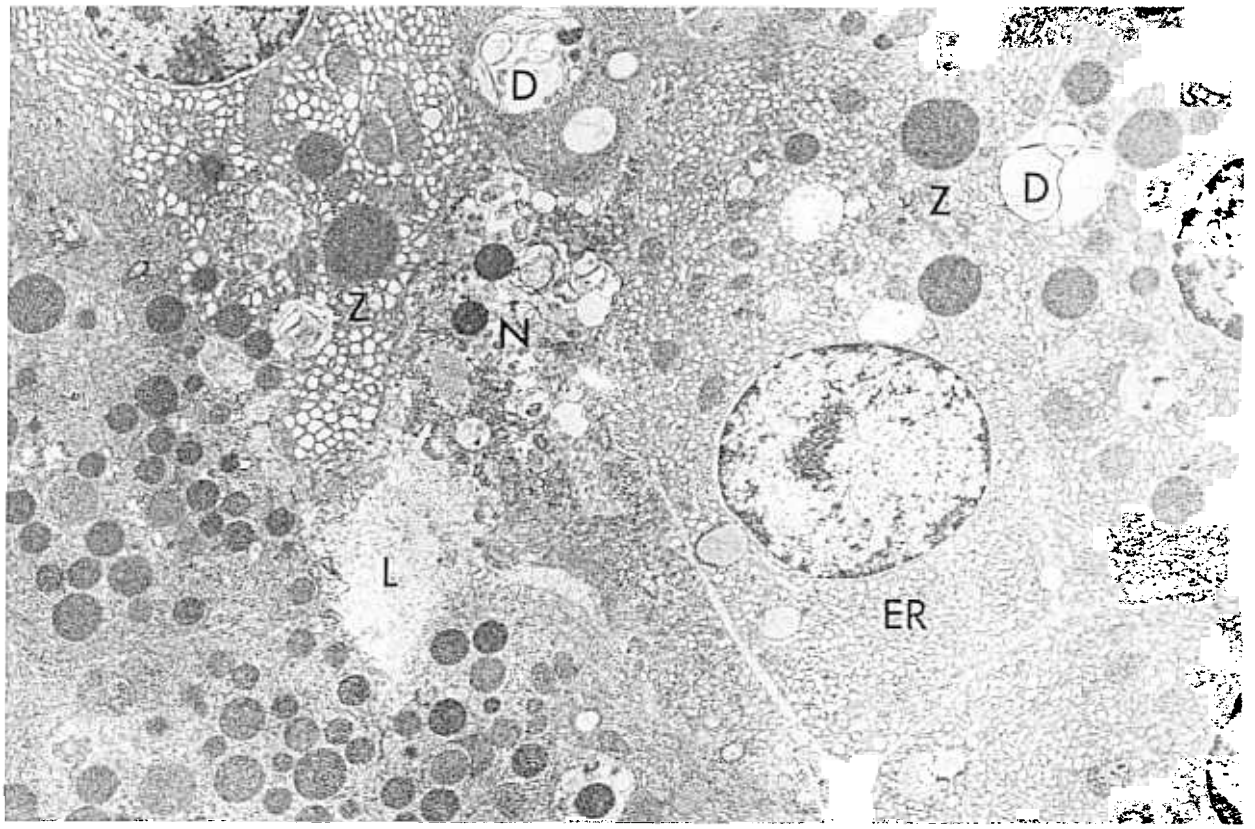

FIG. 2. Acinar cell changes in rat pancreas after 3 months of alcohol intake, pancreozymin stimulation, and intraductal bile acid injection. There are swollen profiles of the rough-surfaced endoplasmic reticulum (ER) and focal degenerative areas with myelin figures (D) in the cytoplasm. Z, zymogen granules; N, remnants of a necrotic acinar cell; L, acinar lumen. ×5200.

with a slide projector onto this paperboard and the percentage of the test points lying on the necrotic parenchyma of the total test points lying on pancreatic parenchyma was calculated. The total number of the test points lying on pancreatic parenchyma was about 500 per pancreas.

Statistical Analysis

The student *t* test was used for statistical analysis.

RESULTS

The weight of the alcoholic animals (434 ± 36 g) was significantly lower ($P < 0.05$ in two-sided test) than that of the control animals (467 ± 49 g) after the 3-month test period.

Morphology

The pancreas became edematous and red in color immediately after the bile salt injection. Wide areas of necrosis, hemorrhages, and interstitial edema were typical features in the histological sections (Fig. 1). Electron microscopy revealed myelin figures, swelling of endoplasmic reticulum and mitochondria, and dissolution of cellular membranes (Fig. 2). Zymogen granules were relatively well preserved. Neither alcohol intake nor pancreozymin stimulation added any histological or ultrastructural features to the pancreatic necrosis caused by the bile salt injection.

Morphometric Analysis

Long-term alcohol intake alone had no effect on the extent of the damage caused by an intraductal injection of sodium taurocholate (Fig. 3, groups A and E). However, there was significantly ($P < 0.01$ in two-sided test) more necrosis in the alcoholic rats (groups B and C) than in the control rats (groups F and G) when the pancreatic secretion was stimulated 15 or 45 min before the induction of pancreatitis (Fig. 3). When the time between the secretory stimulation and the induction of pancreatitis was 90 min (groups D and H), no statistically significant difference was found. There were no differences in the extent of pancreatic necrosis between groups F (nonalcoholic, pancreozymin 15 min before sodium taurocholate) and E (nonalcoholic, sodium taurocholate), or groups G (nonalcoholic, pancreozymin 45 min before sodium taurocho-

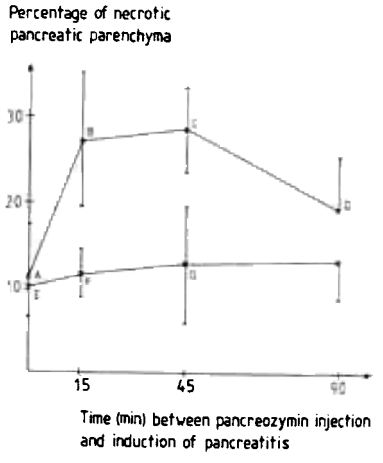

FIG. 3. The percentage of necrosis in pancreatic parenchyma 9 hr after intraductal injection of sodium taurocholate and secretory stimulation with pancreozymin. In groups A and E, the pancreatic secretion was not stimulated. The points on each curve represent the means ± SDs of five alcoholic (o) and five control (x) animals. The differences between groups B and F as well as between groups C and G are statistically significant ($P < 0.01$ in two-sided tests). A—alcoholic, sodium taurocholate; B—alcoholic, pancreozymin 15 min before sodium taurocholate; C—alcoholic, pancreozymin 45 min before sodium taurocholate; D—alcoholic, pancreozymin 90 min before sodium taurocholate; E—nonalcoholic, sodium taurocholate; F—nonalcoholic, pancreozymin 15 min before sodium taurocholate; G—nonalcoholic, pancreozymin 45 min before sodium taurocholate; H—nonalcoholic, pancreozymin 90 min before sodium taurocholate.

late) and E (nonalcoholic, sodium taurocholate). The differences between groups B (alcoholic, pancreozymin 15 min before sodium taurocholate) and A (alcoholic, sodium taurocholate) as well as between groups C (alcoholic, pancreozymin 45 min before sodium taurocholate) and A (alcoholic, sodium taurocholate) were statistically significant ($P < 0.05$ and $P < 0.01$, respectively, two-sided tests).

DISCUSSION

The present study demonstrated that long-term alcohol intake alone does not have any effect on the extent of pancreatic damage caused by intraductally injected sodium taurocholate. Neither had the stimulation of the pancreatic secretion alone any statistically significant effect on the extent of the sodium taurocholate-induced damage. However, when the animals were exposed to both long-term alcohol intake and pancreatic secretory stimulation preceding the induction of acute pancreatitis, the parenchymal lesions were considerably more extensive than those in the presence of only one of these two factors.

The diversity of the models used for induction of acute pancreatitis in experimental animals indicates that none of them is superior to the others in relevance to human acute pancreatitis. Although the ductal pancreatitis used in the present study most probably best resembles the human acute hemorrhagic necrotizing pancreatitis [12], there are, however, differences in both the course of the disease and the histology and ultrastructure [1–3]. Thus, extrapolations of the results of studies such as ours to human acute pancreatitis must be done critically. The necrosis in the present model of acute pancreatitis is induced by taurocholate, since intraductal saline injection causes minor nonspecific changes only, related to the injection pressure [1, 4].

Alcohol-exposed animals weighed less than the controls in the present experiment. Thus, the decrease in caloric intake may participate in mediating the effects of chronic alcohol intake and secretory stimulation on pancreatic necrosis. The present experimental model is consistent with malnutrition as one of the consequences of chronic alcoholism.

Pancreozymin stimulation reached its maximum effect on rat pancreatic secretion at a dose level of 0.4–0.8 U/100 g body wt and higher doses did not increase the secretory response [7, 8]. The rate of flow of pancreatic juice increased up to 10-fold after the administration of 0.8 U pancreozymin/100 g body wt. The dose of pancreozymin used in the present study (0.75 U/100 g) increased the flow up to 6-fold.

According to Tiscornia and Dreiling [24], an increased pancreatic exocrine response to pancreozymin, an elevated intrapancreatic acetylcholine level, and intact duodenopancreatic reflexes are major factors in the pathogenesis of alcohol-induced pancreatitis. Chronic alcohol intake caused a marked increase in the pancreatic exocrine response to pancreozymin stimulation in both humans [15] and experimental animals [20, 25]. Jalovaara and Huttunen [10] and Singh [21] suggested that the decreased pancreatic secretory trypsin inhibitor/proteolytic enzyme ratio caused by long-term alcohol ingestion might favor premature activation of proenzymes in pancreatic ducts. Stimulation of pancreatic secretion would further worsen the balance by increasing the enzyme secretion especially after chronic alcohol exposure [20, 25, 15].

The results of the present study are in accordance with the study of Jalovaara and Apaja [9], indicating that alcohol pretreatment alone has no effect on the severity of experimentally induced acute pancreatitis. However, Rämö *et al.* [17] found that long-term alcohol ingestion causes an increase in the serum phospholipase A_2 activity and mortality in acute experimental pancreatitis in rats.

According to Sarles [18], the earliest recognizable changes in the rat and the human pancreas caused by alcohol are the precipitation of proteinaceous plugs within pancreatic ductules and the subsequent obstruction of the ducts by these precipitates. Chemical analysis has revealed that these protein plugs contain pancreatic enzyme proteins [19]. The most widely held view is that these protein plugs lead to ductal obstruction and inflammation and destruction of pancreatic tissue during the development of alcoholic pancreatitis [18, 27]. The results of the present study further emphasize the importance of synergism be-

tween long-term alcohol intake and increased pancreatic secretion in the pathogenesis of alcoholic pancreatitis.

Steer *et al.* [23] questioned the previously accepted concept that the proteolytic enzymes are activated within the pancreatic duct or intercellular space. They suggested that the activation may occur within the pancreatic acinar cell, subsequent to the mixing of digestive enzymes and lysosomal hydrolases. Rinderknecht *et al.* [16] found that long-term alcohol intake and stimulation of secretion caused an increase in the secretion of lysosomal hydrolases by acinar cells. They further found that, in addition to an increased amount of lysosomal enzyme activities in the pancreatic juice of alcoholics at the resting state, their increase elicited by secretagogues was more pronounced in alcoholics than in nonalcoholic individuals. The results of the present study indirectly support these findings, viz., the potent synergistic and damage-expanding effects of long-term alcohol intake and pancreatic secretory stimulation. Moreover, the occurrence of human acute pancreatitis after a large meal [5] could be thought to result from a strong stimulatory effect of such a meal on pancreatic secretion.

ACKNOWLEDGMENTS

This work was supported by the Finnish Foundation for Alcohol Studies and the Paulo Foundation.

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