MECHANISM OF ACTION OF ACTIVE ELASTASE ON PANCREATIC TISSUE

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Acute pancreatitis developed 15 min after injection of 0.65 mg active elastase into the pancreatic duct. The histological changes in the gland tissue increased from the edematous form to necrotic pancreatitis with a hemorrhagic component, and with an increase in the dose of elastase injected up to 2.6 mg the elastic structures of the blood vessels were destroyed. The changes described above were accompanied by a decrease in the proelastase content in the pancreatic tissue and a decrease in the inhibitory power of the blood serum. The results are evidence of the important role of elastase in the pathogenesis of acute pancreatitis.

KEY WORDS: pancreas; pancreatitis; active elastase.

The histological picture of acute pancreatitis in man and experimental animals as a rule is characterized by definite changes in the vascular system of the pancreas. Rich and Duff [5] postulated that in the course of development of acute pancreatitis certain "necrotic substances" with a necrotic action on the wall of the arteries and veins of the pancreas are liberated into the tissues of the gland. It subsequently was discovered that the enzyme elastase is produced in the pancreas: unlike other known pancreatic enzymes, elastase can destroy elastic structures and thereby induce characteristic changes in the organ [1, 2, 4].

The object of this investigation was to study changes in the various structures of the pancreas under the influence of active elastase, changes in the concentrations of proelastase and active elastase in the pancreatic tissue, and changes in the inhibitory power of the blood serum.

EXPERIMENTAL METHOD

Female rats weighing 160-180 g were used. Under ether–hexobarbital anesthesia, 0.65 or 2.6 mg elastase (1.65 and 6.6 units respectively) in 0.2 ml distilled water was injected into the pancreatic duct, after which the duct was ligated.

The animals were decapitated after various times and histological, histochemical, and electron-microscopic investigations carried out on the pancreatic tissue. Active elastase and proelastase in the pancreatic tissue and elastase inhibitor in the blood serum were determined by a modified method of Geokas [3]. To determine the total elastase activity, the tissue homogenate was pre-incubated with crystalline trypsin.

To determine the inhibitory power of the blood serum, the serum was first incubated with active elastase for 1 h at 37°C, and the residual elastase activity was then determined in a mixture consisting of 1 ml blood serum in a dilution of 1 : 25, 1 ml elastase, and 1 ml Tris-buffer. The modification of the technique was that at the end of the reaction the proteins were not precipitated with TCA and the color was not extracted from the supernatant with butanol.

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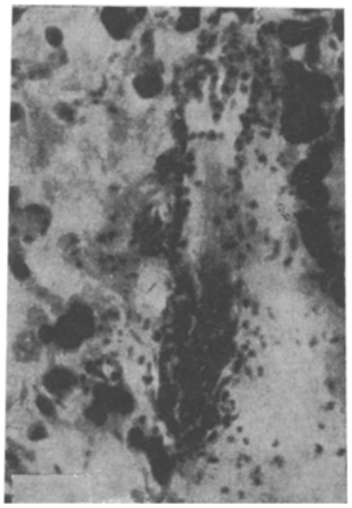

Fig. 1. Necrosis of acinar lobules. Venous congestion. Perivascular hemorrhages in the stroma. Hematoxylin–eosin, 300×.

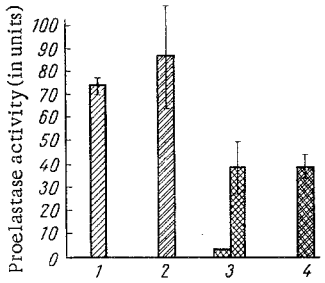

Fig. 2. Changes in proelastase concentration in pancreatic tissue in "elastase" pancreatitis: 1) intact rats; 2) injection of 0.3 ml distilled water; 3, 4) 15 and 60 min respectively after injection of 0.65 mg elastase.

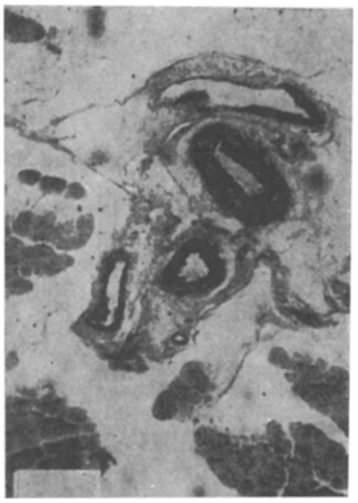

Fig. 3. Ill-defined outlines of elastic membrane of veins. Orcein, 300×.

EXPERIMENTAL RESULTS

Injection of active elastase into the pancreatic duct was found to produce acute pancreatitis accompanied by a decrease in the proelastase content in the pancreatic tissue and a decrease in the inhibitory power of the blood serum.

The microscopic picture under these circumstances was characterized by the various changes in the histological structure and ultrastructure of the acini that depended on the time after injection of the active elastase. The morphological changes in the pancreas 15 min after the injection of 0.65 mg active elastase were those of the edematous form of acute pancreatitis: edema, congestion of the blood vessels, recent focal hemorrhages, and inflammatory foci of infiltration of lymphocytes and polynuclear cells in the stroma of the pancrease, loss of the normal complex structure in the acinar lobules of the gland, accompanied by necrobiosis and necrosis of the acinar cells with a marked decrease in size of the zymogen granules in their cytoplasm. Some of the zymogen granules lay freely in the pericellular space. Side by side with acini showing the changes described above there were exocrine lobules of normal structure. The interlobular and intralobular ducts and the islets of Langerhans were unchanged. The epithelium lining the ducts was intact throughout their extent. Severe congestion and dilatation of the intralobular veins were conspicuous.

The morphological changes in the pancreas 60 min after the injection of elastase were progressive: a picture of acute necrotic pancreatitis with a hemorrhagic component developed. Most of the lobules were disorganized, with marked necrotic changes in the parenchymatous cells, with poorly distinguishable intralobular ducts, sharply dilated and congested veins, and diapedetic hemorrhages in the perivascular spaces (Fig. 1).

The integrity of the cell membrane of the remaining acinar cells was disturbed. The pericellular space contained numerous zymogen granules. The inflammatory infiltration of the gland was slight in degree.

The RNA content in the cytoplasm of the acinar cells was sharply reduced.

It will be clear from Fig. 2 that the histological changes described above were accompanied by changes in the proelastase concentration in the pancreatic tissue. Several causes, varying in importance, could be responsible for this fact. To a lesser degree, especially in the early stages after injection of active elastase, this could evidently be due to a decrease in the synthesis of the enzyme. The zymogen activity in the unchanged acinar cells was normal, and in some it was actually increased. The most likely explanation

is that the decrease in the concentration of proelastase was due to its activation and conversion into the active form. The process of activation of proelastase by active elastase in vitro takes place very rapidly. Under certain conditions in vivo a similar process can also take place (for example, after regurgitation of the intestinal contents), and, consequently, changes in the gland tissue are connected not only with the action of the injected elastase, but also with the effect of the newly activated elastase. However, the content of active elastase in the pancrease did not rise parallel with the decrease in the proelastase level. The level of active elastase in the pancreatic tissue evidently remained almost unchanged during the development of acute pancreatitis for at least two reasons.

1. Activated proelastase, like active elastase injected from an outside source, is partly inhibited. The blood serum inhibitor also participates in this process. A sharp decrease in its concentration was found after 15 min (100% in the control, 46.8% after 15 min and 42.7% after 60 min in the experiment).

2. The changes in elastase activity were evidently largely explained by its action on the pancreatic tissue. Electron-microscopic investigation revealed destruction of the acinar cells, characterized by marked dilatation and degranulation, followed by fragmentation and disintegration of the cisterns of the rough endoplasmic reticulum, was observed. The cell membrane of the acinar cells was destroyed. Large areas of ultrastructural components of exocrine cells not bounded by a cell membrane were formed in the acini.

In some cells defects were observed in the apical cell membrane forming intercellular ducts.

The most important factor in the action of elastase on the pancreatic tissue was fixation of the elastase on the elastic fibers. The severity of the vascular changes under these circumstances depended on the dose of elastase injected. If the dose of elastase was increased to 2.6 mg, the elastic membrane of the vein wall in some areas stained poorly with the orcein dye, evidence of disturbances of its structure (Fig. 3).

Acitve elastase, as a proteolytic enzyme with specific elastolytic properties, thus acts both on acinar cells, causing destruction of their structural elements, and on the elastic fibers of the blood vessel wall of the organ. The sequence of the processes under these circumstances can evidently be represented as follows: active elastase, breaking down desmosomal compounds, passes through defects in the large and small pancreatic ducts into the periacinar spaces and induces destruction of the acinar cells, as a result of which active elastase accumulates in the tissue of the gland (on account of the activation of proelastase), and direct contact between active elastase and the blood vessel wall also becomes possible.

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