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QUARTERLY

Kinetic properties of AMP deaminase in acute experimental pancreatitis

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The inflammatory process in pancreas is accompanied by activation of proteolytic enzymes like trypsin and elastase which can be detected within the tissue [1, 2]. In the normal exocrine pancreas the majority of proteins consists of digestive enzymes synthesized as inactive proenzymes and targeted for export from acinar cells. Proenzymes are concentrated within secretion zymogen granules and further discharged by exocytosis. Cathepsin D is targeted into lysosomes thus avoiding intracellular trypsinogen activation.

Growing evidence points to free radical involvement in the pathogenesis of acute pancreatitis [3]. Hydroperoxides are activators of proteases specifically cleaving the proenzyme of phospholipase A_2 to the active enzyme form [4]. Lysophosphatidyl cholines are potent fusogens [5] and evidence exists for an extensive intracellular fusion process [6] and formation of large vacuoles during pancreatitis. In these vacuoles cathepsin D and trypsinogen are colocalized [7]. In the course of acute pancreatitis phospholipase A_2 activity has been found to be elevated.

AMP-deaminase (AMP aminohydrolase EC 3.5.4.6, AMP-D), widely distributed in animal and human tissues, catalyses irreversible deamination of AMP to IMP [8]. The activity of this enzyme seems to control the rate of adenylate catabolism [9]. The form of AMP-D present in the heart but not in the skeletal muscle was found to be activated by ATP, this effect being further enhanced by phosphatidylcholine (PC) as well as by natural membranes [10].

No effect of lipid activators on AMP deaminase from the heart of rat with experimental cardionecrosis has been observed [11]. A similar loss of PC sensitivity of AMP-D from the heart as a consequence of limited proteolysis was described [12]. The interaction of pancreatic AMP-D with PC species has not been investigated so far.

The purpose of this study was to evaluate the possible relationship between time dependent changes in the pancreatic tissue lipid hydroperoxide (LOOH) level, large vacuole formation and modification of AMP-D kinetics in the course of cerulein-induced pancreatitis.

It was expected that possible cerulein-mediated LOOH formation through phospholipase A_2 activation triggers intracellular fusion phenomena, trypsinogen activation and subsequent proteolytic AMP-D modification.

AMP-D from rat pancreas was purified by cellulose phosphate chromatography as described previously [10], with slight modifications, i.e. the extraction buffer contained 100 μM phenylmethylsulphonyl fluoride (Sigma Chemical Co., St. Louis, MO, U.S.A) and trypsin inhibitor from soybean (Sigma Chemical Co., St. Louis, MO, U.S.A.) 5 mg/ml. Enzyme

[1]Abbreviations: AMP-D, AMP deaminase; LOOH, lipid hydroperoxide; PC, phosphatidylcholine.

Table 1

Lipid hydroperoxide (LOOH) level in rat pancreas.

Group A, control rats; group B, cerulein induced pancreatitis (3 h); group C, cerulein induced pancreatitis (6 h).

Groups	LOOH nmol/mg of protein	LOOH nmol/mg of dry weight	Weight of pancreas (g)
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
A	51 ± 23	28 ± 5	0.63 ± 0.23
B	110 ± 13	52 ± 19	1.73 ± 0.46
C	60 ± 15	32 ± 5	1.98 ± 0.35

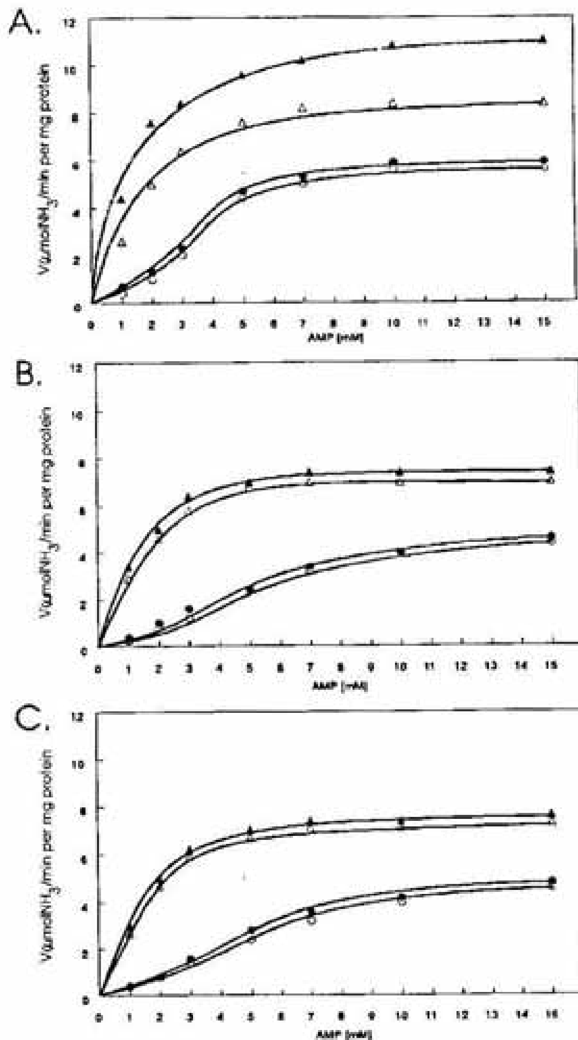

Fig. 1. Substrate saturation curves for AMP deaminase isolated from rat pancreas.

A, Without pretreatment; B, after the enzyme had been subjected to limited proteolysis by preincubation with trypsin; C, enzyme isolated from pancreas after 6 h infusion of cerulein. The incubations were carried out without effectors added (○), with addition of 1 mM ATP (Δ), in the presence of 375 nmol of phosphatidylcholine-containing liposomes (●), and the presence of 1 mM ATP plus 375 nmol phosphatidylcholine-containing liposomes (▲). See text for additional details.

assays were carried out as previously described [10].

Limited proteolysis of pancreatic AMP-D was performed by incubating the isolated enzyme with trypsin at the 125:1 (w/w) ratio at 4°C in potassium succinate buffer, pH 6.4, for 20 min.

Eighteen rats were divided into three groups: in group 1, six rats served as control; in six rats of group 2 infusion of cerulein (10 μg $kg^{-1}h^{-1}$, Carlo Erba, Farmitalia) was performed for 3 h; in group 3, six rats received cerulein infusion for 6 h. Rats were cannulated in jugular veins with silastic tubes for cerulein administration.

Autopsy of pancreas after 3 h of cerulein infusion showed, under a light microscope, interstitial edema, acinar cell vacuolization and leukocyte margination in pancreatic capillaries (not shown).

Table 1 presents the mean LOOH levels in pancreatic tissue estimated as described by Thomas & Poznansky [13]. In the control group of rats receiving physiological saline, LOOH amounted only to 51 or 28 nmoles per mg protein or mg dry weight, respectively. After 3 h of cerulein infusion about 115% increase of LOOH concentration was observed.

Our electronmicroscopic inspection of pancreatic cells revealed several cytoplasmic vacuoles after 3 h of cerulein infusion. The observations point to a close contact of some zymogen granules with vacuoles. After 6 h numerous large vacuoles were visible. Degenerating zymogen granules lost their surrounding membrane coat and there appeared flocculent debris of variable density as a sign of degradation processes (not shown).

It has been demonstrated previously that AMP-D isolated from cardionecrotic rat hearts was no more sensitive to phospholipids [11]. As may be seen from Fig. 1, the same change of the

regulatory properties was achieved by subjecting pancreatic AMP-D to limited proteolysis. The enzyme preincubated with trypsin lost its sensitivity to phospholipid bilayers, being still activated by ATP.

The enzyme isolated from pancreatic tissue after 3 h of cerulein infusion retained the ability to interact with phospholipids (not shown), whereas the enzyme isolated from pancreas after 6 h of cerulein treatment mimics perfectly AMP-D modified by proteolysis (Fig. 1).

Our results indicate that estimation of pancreatic AMP-D activity could be a useful tool for detecting intracellular proteolysis.

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