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PANCREATIC TISSUE GLUTAMATE DEHYDROGENASE ACTIVITY IN ACUTE EXPERIMENTAL PANCREATITIS AND ITS RESPONSE TO SODIUM THIOSULFATE

P. S. Simavoryan, I. L. Saakyan, and D. A. Gevorkyan

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KEY WORDS: glutamate dehydrogenase; pancreas, acute pancreatitis, sodium thiosulfate.

The study of glutamate dehydrogenase (GDH; EC 1.4.1.3) is of great interest in connection with the central role of this enzyme in amino acid and nitrogen metabolism, and also with the high biosynthetic activity of the pancreas. The study of GDH activity under the influence of sodium thiosulfate, a substance whose high therapeutic efficacy is documented by morphological and functional studies, is likewise of great importance. The intracellular localization of GDH and the character of its changes in foci of reactive inflammation and tissue destruction is another interesting topic.

GDH synthesis is known to take place on microsomal membranes, connections with which are effected through phosphatidylserine; it subsequently undergoes intracellular migration and is located in the mitochondria; molecules of cardiolipin, moreover, facilitate its transport through the membrane into the mitochondrial matrix [5].

The aim of this investigation was to study GDH activity and its subcellular distribution in different segments of the pancreas under normal conditions, in acute experimental subtotal pancreatic necrosis, and after administration of sodium thiosulfate.

EXPERIMENTAL METHOD

Experiments were carried out on 220 rats with a body weight of 200-250 g. The animals were divided into the following groups: control, mock operation (laparotomy + general anesthesia), with acute pancreatitis, and receiving or not receiving sodium thiosulfate. Acute hemorrhagic (subtotal) pancreatic necrosis was induced by cooling the splenic segment of the pancreas with ethyl chloride to −30°C [2]. Sodium thiosulfate was injected into the peritoneal cavity during the operation in the form of a 30% aqueous solution, after which a 1.5% solution was given instead of drinking water at the rate of 25 mg/100 g body weight. Splenic (subjected to direct injury) and duodenal (affected by reactive inflammation) segments of the pancreas were studied separately 3, 24, and 72 h and 7, 14, and 30 days after establishment of the disease. Enzyme activity was determined in mitochondrial (MCh) and microsomal (MS) fractions of pancreatic tissue obtained by differential centrifugation of a 10% tissue homogenate in 0.1 M sodium-phosphate buffer (pH 7.8) at 12,000*g* and 105,000*g* respectively; the supramicrosomal (cytosolic, CS fraction) also was studied. Enzyme activity was determined [6] in two directions: in the reaction of oxidative deamination of

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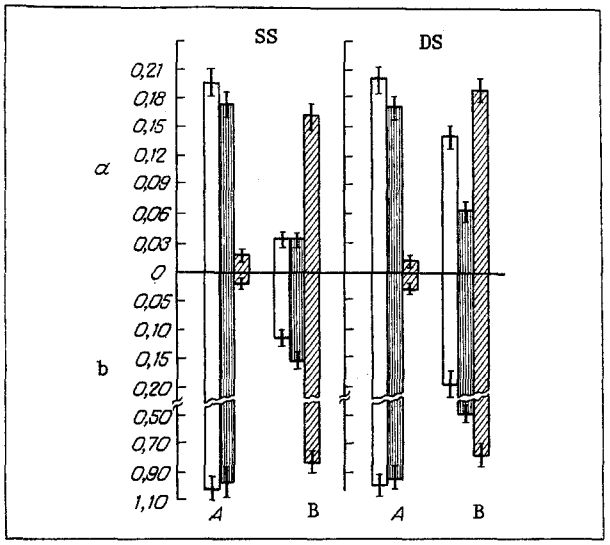

Fig. 1. NAD-GDH (a) and NADH-GDH (b) activity in MCh (unshaded column), MS (vertically shaded column) and CS (obliquely shaded column) fractions of pancreatic cells in control rats (A) and 24 h after induction of pancreatic necrosis (B) Ordinate, unit of activity.

glutamic acid (Glu), i.e., NAD-GDH, and reductive amination of α-ketoglutaric acid (α-KG), i.e, NADH-GDH. Activity was expressed as the number of micromoles of coenzyme oxidized (NADH) or reduced (NAD) per minute per gram dry weight of tissue.

EXPERIMENTAL RESULTS

Under normal conditions GDH activity is identical in value in the duodenal segment (DS) and splenic segment (SS) of pancreatic tissue, but the direction of the reaction is shifted strongly toward reductive amination of α-KG (Table 1). In pancreatic necrosis, a sharp reduction in activity of both NAD-GDH and NADH-GDH is observed in the focus of progressive tissue destruction (in SS). In DS, where a picture of interlobular and intracellular edema develops (3-24 h), by contrast with the focus of pancreatic necrosis, there is a comparatively small decrease in activity of NAD-GDH and NADH-GDH (by 1.5-2 times), and this continues later in the stages of formation of a pseudocyst (7th-14th days) and of exacerbation of chronic pancreatitis (30th day). Here there is a more significant decline in NADH-GDH activity in the stage of parenchymatous pancreatic necrosis (after 24 h), and the allosterically controlled reaction of oxidative deamination of Glu (NAD-GDH) is less severely affected. This emphasizes once again the role of this particular reaction, leading to the formation of two important compounds, namely α-KG (a substrate for the Krebs' cycle) and NADH (the principal electron generator and carrier) for the cells. In DS, at all the times of testing, a corrective effect of sodium thiosulfate could not be observed from GDH activity in the reaction in both directions. In the segment involved primarily (SS), however, in the acute phase (24-72 h) of the disease, the low background level of enzyme activity was distinctly raised under the influence of sodium thiosulfate, probably due, on the one hand, to the membrane-stabilizing effect of the compound on intact (viable) pancreatic cells, and on the other hand, to the stimulating action of thiosulfate on migration of inflammatory cells, rich in dehydrogenases, into the focus of injury [3].

In the next stage we studied the distribution of GDH activity in subcellular fractions of pancreatic cells under normal conditions and in the phase of parenchymatous pancreatic necrosis. It was shown for the first time that in the normal pancreas, the same GDH activity is found in MS, extracted in phosphate buffer, as in MCh, but at the same time, that its value in the CS fraction (Fig. 1) is low (close to zero). In acute subtotal pancreatitis, redislocation of the enzyme apparently takes place in a focus of pancreatic necrosis, and is manifested as a sharp fall in its activity both in MCh and in MS (five-sixfold), accompanied by a sharp rise in CS, possibly as a result of damage to membranes of the subcellular structures of the pancreocytes. In the segment with reactive inflammation (DS) the changes were different in character: in the MS-fraction of the cells NAD-GDH activity was reduced by two-thirds and NADH-GDH activity by half, whereas in MCh it was reduced by 25% and 80% respectively, with a simultaneous increase by many times in CS. In this case what takes place is evidently labilization of enzyme-phospha-

TABLE 1. NAD-GDH (μmoles NAD/g dry weight/min) and NADH-GDH (μmoles NADH/g tissue/min) Activity in MCh Fraction of Pancreatic Cells of Normal Rats, Rats Undergoing Mock Operation (A), during the Course of Acute Experimental Pancreatitis (B), and Treatment with Sodium Thiosulfate (C)

Reaction	Sample	Control	Times of observation (in h)								
			3 h			24 h			72 h		
			A	B	C	A	B	C	A	B	C
NAD-GDH	DS	0,150±0,007	0,100±0,011*	0,078±0,013*	0,090±0,011*	0,154±0,011*	0,097±0,08*+	0,087±0,010*	0,135±0,011	0,093±0,009*+	0,120±0,022
		(24)	(14)	(22)	(14)	(12)	(12)	(12)	(12)	(12)	(14)
	SS	0,140±0,010	0,090±0,017*	0,029±0,003*+	0,023±0,06*+	0,150±0,018	0,025±0,001*+	0,040±0,007*+○	0,177±0,010*	0,016±0,08*+	0,070±0,007*+○
NADH-GDH	DS	0,826±0,046	0,487±0,044*	0,500±0,034*	0,310±0,039*+○	0,747±0,124	0,172±0,038*+	0,255±0,026*+	0,733±0,026*+	0,484±0,031*+	0,466±0,067*+
		(24)	(12)	(16)	(12)	(12)	(12)	(16)	(14)	(12)	(12)
	SS	0,805±0,067	0,508±0,060*+	0,075±0,011*+	0,108±0,017*	0,767±0,149	0,120±0,019*+	0,220±0,027*+○	1,379±0,144*	0,147±0,015*+	0,442±0,015*+○
		(26)	(16)	(22)	(18)	(12)	(14)	(18)	(14)	(12)	(16)

Legend. Number of animals given in parentheses. Asterisk indicates significance of differences from control, + sign — from mock operation, circle — pancreatitis with treatment.

tidylserine bonds in the MS itself, and also in the transport system at the level of components of CS, rather than slowing of the rate of GDH biosynthesis in MS. It is admitted that a central place in the mechanism chymotrypsin of labilization of these bonds is occupied by proteases (trypsin, chymotrypsin), the content of which in the pancreatic tissues and blood is substantially increased (three-fourfold). Additional indirect proof is given by the increase in the phosphatidylserine content (about twofold) in the pancreatic tissue.

Thus because of damage to membranes of the subcellular organelles of the pancreocytes in pancreatic necrosis, their function is disturbed and a phenomenon of "enzyme leakage" takes place [1], including leakage of GDH, into the cytosol, and subsequently into the blood stream and peritoneal cavity; this is an essential link in the chain of the pathobiochemical mechanism of damage and formation of acute pancreatitis.

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DETECTION OF SEQUENCES IN THE STRUCTURE OF INFLUENZA VIRUS PROTEINS SIMILAR TO VASOACTIVE INTESTINAL PEPTIDE

I. N. Zhilinskaya, L. I. Brodskii, L. V. Alekseenko, and O. I. Kiselev

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In the course of their reproduction viruses utilize receptors of the host cell specific for certain neurotransmitters, including regulatory peptides. For instance, in order to infect B lymphocytes, Epstein–Barr virus utilizes the receptor [10], rubella virus uses the acetylcholine receptor [11], rheoviruses use the beta-adrenergic receptor [8], vaccinia virus the receptor of epidermal growth factor [9], and HIV virus uses the T4 receptor factor. These data indicate that viral proteins or their fragments can act successfully as agonists of many of the more important regulatory peptides.

This paper gives information on the discovery of amino acid sequences in the structure of influenza virus proteins similar to those of a regulatory peptide, namely vasoactive intestinal peptide (VIP). Comparison of these sequences is particularly interesting, first, because of the high concentration of receptors to this regulatory peptide in the upper respiratory tract, the lungs, and brain [14], i.e., of organs particularly intensively involved in influenzal infection, and second, because changes in the body observed following injection of VIP are similar to the pathological changes found in influenza.

All-Union Influenza Research Institute, Ministry of Health of the USSR, Leningrad. Faculty of Biology, M. V. Lomonosov State University, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR I. P. Ashmarin.)

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