MORPHOLOGICAL AND FUNCTIONAL CHANGES INDUCED BY DALARGIN IN THE PANCREAS IN THE NORMAL STATE AND IN EXPERIMENTAL PANCREATITIS

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Modern views on the etiology and pathogenesis of acute pancreatitis contain many doubtful propositions and concepts. One view which has been held for a long time postulates an important role for pancreatic enzymes in the mechanisms of formation of the pathological changes in the gland tissue, which are regarded as the morphological basis of the combination of functional disturbances and the clinical picture accompanying pancreatitis. There have been numerous attempts to use various ways of inhibiting pancreatic function or the activity of pancreatic enzymes for therapeutic purposes [3, 6, 8]. Existing views on inhibition of the external secretory function of the pancreas by enkephalins [9, 10] have led to the suggestion that the hexapeptide dalargin, synthesized in the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, which is an anlalog of Leu-enkephalin, may have an inhibitory influence of some kind on pancreatic secretion, and, consequently, it would possess pharmacotherapeutic activity in pancreatitis.

This assumption was tested by using different species of animals and models of experimental pancreatitis [1]. Considering that the effects of enkephalins and their analogs, including dalargin, which are ligands of opiate receptors, may be significantly distorted under general anesthesia [5], the experiments were carried out on unanesthetized animals.

EXPERIMENTAL METHODS

In series I of chronic experiments on eight dogs with Basov gastric fistulas and with Solov'ev pancreatic fistulas, the dose-dependent effect of parenteral injection of dalargin on pancreatic secretion stimulated by different agents was investigated: by a standard meat diet, by intraintestinal instillation of hydrochloric acid or a solution of hydrolysin acidified to pH 1.8, and also by intravenous infusion of secretin (Fluka, Switzerland) and of the octapeptide cholecystokinin. Dalargin was injected in doses of 50 μg/kg per hour intravenously, intramuscularly, and subcutaneously, both by the bolus method and continuously by means of a peristaltic pump (Zalimp, Poland). Experiments with injection of dalargin alternated with experiments according to a similar scheme, in which the peptide was replaced by physiological saline. The proteolytic activity of the pancreatic juice was determined spectrophotometrically [7].

In the experiments of series II on 89 male rats weighing 180-230 g the action of dalargin was studied on the morphological features of experimental pancreatitis (EP), induced by deep cooling of both surfaces of the splenic portion of the pancreas (SPP) [4]. Animals with EP of the first group were given an intraperitoneal injection of dalargin in a dose of 50 μg/kg body weight before the operation and again every 8 h during the source of the experiment.

Animals with EP of group 2 were given injections of sterile physiological saline by the same schedule. Rats undergoing a mock operation (MR) and intact rats (IR) took part in the experiments simultaneously. The MR were subjected to all manipulations involved in production of EP except cooling of the pancreatic tissue with ethyl chloride. The MR received dalargin by the same schedule as rats with EP. IR were kept throughout the period of the experiments under the same conditions as the animals of the first three groups. The

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TABLE 1. Effect of Dalargin on Secretion of Pancreatic Juice (numerator, in ml/30 min) and of Proteases in its Composition (denominator, in units/30 min) in Dogs (M ± m)

30-min periods	Stimulator of secretion			
	100 g meat	secretion + CCK	hydrochloric acid	hydrolysin
1	1,4±0,1 / 4,1±0,8	1,6±0,2 / 6,2±1,4	1,2±0,2 / 2,4±0,6	0,9±0,2 / 1,8±0,4
2	1,1±0,1 / 2,9±0,3	1,5±0,2 / 5,2±1,1	1,1±0,2 / 1,8±0,5	0,9±0,2 / 1,9±0,4
3	12,4±1,7 / 24,8±3,2	17,8±2,5 / 31,6±3,3	7,8±1,9 / 13,3±6,8	12,8±3,7 / 14,6±3,5
4	16,9±2,1 / 27,8±3,9	18,1±2,5 / 27,6±4,6	12,3±2,7 / 17,5±8,3	15,4±4,1 / 12,9±3,2
5	3,8±0,9 / 6,2±1,5	9,4±1,6 / 11,9±2,4	5,5±1,2 / 5,9±1,8	5,5±1,5 / 4,0±1,7
6	1,5±0,4 / 3,2±1,2	10,7±2,4 / 14,4±3,9	4,3±0,8 / 3,0±1,5	3,4±0,6 / 1,9±0,8
7	8,4±2,0 / 21,6±4,5	11,9±1,7 / 17,7±3,7	6,6±1,0 / 9,4±5,2	9,0±2,8 / 6,8±2,0
8	8,9±1,3 / 22,1±3,1	13,2±2,0 / 26,2±5,2	8,9±1,6 / 13,5±5,2	12,9±4,0 / 14,1±4,3

Legend. 1,2) Before stimulation of secretion, 3-8) stimulated secretion. Peptide injection in periods 5 and 6.

rats were decapitated after 1, 3, and 7 days. The pancreas was fixed in 10% formalin, buffered by Lillie's method (pH 7.4) and cooled to 4°C, and embedded in paraffin wax. Sections from SPP and the duodenal part of the pancreas (DPP), 5-7 μ thick, were stained with hexatoxylin and eosin and by Van Gieson's method.

EXPERIMENTAL RESULTS

The results (Table 1) indicate that dalargin inhibited stimulation of PS as regards both the volume of juice secreted and its content of protein and bicarbonates. Infusion of dalargin led to a sharp decrease in secretion of proteases, lipase, and amylase in the composition of the juice. Analysis of the data in order to elucidate the possible mechanism of dalargin-induced inhibition of PS suggested that the peptide inhibits release of endogenous secretin and cholecystokinin–pancreozymin, and that the Leu-enkephalin analog exerts a direct inhibitory action on the pancreas.

The dynamics of pathomorphological changes in rats of the second group with EP which were reproduced according to the mentioned model, macroscopically and histographically corresponded to the description in [2]. It follows to note that cysts in the DPP were formed in the majority of rats with EP, and in animals which did not receive treatment, their sizes were from 1 to 2.2 cm and in those receiving dalargin, they decreased to 0.3-1 cm in diameter. On one rat the cyst was absent.

In rats receiving dalargin, large zones of necrobiosis of the acini were seen in the tissue of SPP after 24 h, together with foci of colliequative necrosis in the central zone and with small foci of hemorrhagic infiltration. Particular features of these zones were preservation of the viability of the connective-tissue cells of the interacinar spaces and interacinar vessels, slight leukocytic infiltration with the absence of any dystrophic or necrotic changes in the leukocytes (Fig. 1a), and acute vascular changes. In DPP inter- and intralobular edema was present, the acini within the lobules were unchanged, and the acinar cells (AC) had a widened zone of basophilia and reduced eosinophilia of the apical zones.

On the 3rd day the leukocytic barrier around the focus of necrosis in SPP was reduced in size, and destruction of polymorphs was not observed. In the adjacent zone individual acini were atrophied, and the interlobular spaces were widened, moderately sclerosed, and infiltrated by mononuclear cells. In some cases there was a sharp boundary between areas

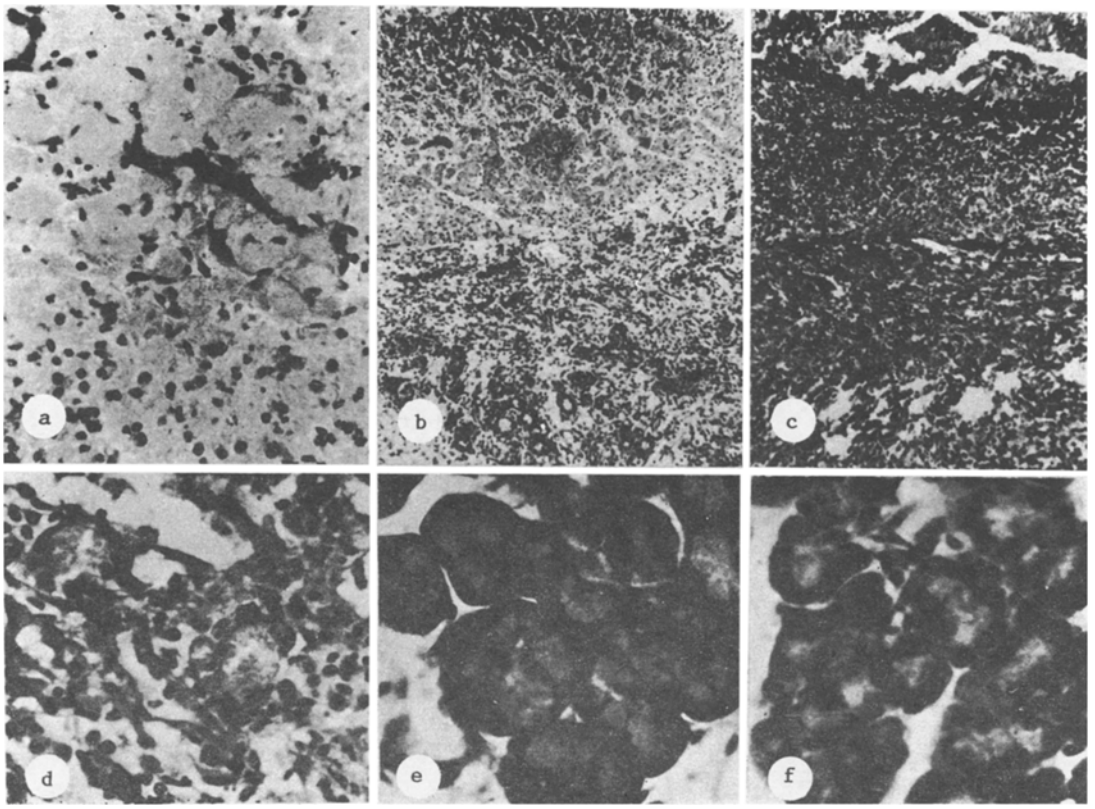

Fig. 1. Structural changes in pancreas during EP (a-d) under normal conditions (e), and after administration of dalargin (f). a) Interacinar capillaries in zone of necrosis of pancreas; b) narrow inflammatory demarcation barrier around necrotic tissues, atrophy of pancreatic lobules; c, d) wall of pseudocyst of pancreas with numerous blood vessels in connective tissue and diffuse leukocytic infiltration of its inner surface; e) reduction of basophilia and narrowing of zones of eosinophilia of cytoplasm of AC. Magnification: a, d, e, f) 400×; b, c) 100×.

of tubulo-epithelial regeneration and the intact lobules, in which there were signs of reduced secretory activity of AC (Fig. 1b). Inflammatory changes were absent in the intact areas of the pancreas in the animals of this group, and the pattern of the lobules and acini in them was unchanged. The nuclei of AC were hypertrophied and the zone of basophilia of their cytoplasm was enlarged. Accumulation of secretion was sharply reduced in individual acini.

A cavity filled with necrotic debris and bounded by a narrow inflammatory leukocytic barrier of demarcation was still present on the 7th day in SPP of rats receiving dalargin. Polymorphs penetrated into the necrotically changed tissues and broke them up into fragments. Outside the demarcation barrier there was a zone of loose granulation tissue containing a very few fibroblasts but numerous congested blood vessels. In the outer part of the capsule the pseudocysts contained single thin-walled tubular structures, lined with flattened epithelium (Fig. 1c, d). Small lobules of the pancreas with signs of perivascular and periductal sclerosis and of moderately chronic inflammation appeared in the peripheral zone of scar tissue. The lobules in DPP had their usual structure. No signs of inflammation or sclerosis were present in the interlobular spaces, but single large lymph nodes were seen.

Histological study of the pancreatic tissue of MR 24 h after the mock operation revealed evidence of moderate congestion of the vessels and of slight interlobular edema. Clear division of the cytoplasm of AC into wide, basophilically stained areas and eosinophilic apical areas was observed. On the 3rd day the zone of basophilia of AC was enlarged in the rats of this group by comparison with IR, and eosinophilia of the cytoplasm was reduced, evidence of decreased accumulation of secretion in AC (Fig. 1e, f). On the 7th day the boundaries between the areas of basophilia and eosinophilia were indistinct, and small nuclei of AC, poor in chromatin, appeared, indirect evidence of lowering of the intensity of synthesis in AC. Dystrophic changes were not found in AC. After 24 h, under the influence

of dalargin, the pathomorphological picture of acute EP was characterized by predominance of zones of incomplete necrosis of the lobules with preservation of their blood supply, accompanied by restoration of the microcirculation in the zones of damage and the boundary areas of the gland. On the 3rd day progression of necrosis had ceased, complete resorption of the necrotic tissues had occurred, while secretory activity of AC adjacent to the focus of damage and the intact zones was depressed. On the 7th day the zone of necrosis was demarcated and reduced in size, ability to undergo resorption due to polymorphs was preserved, atrophic changes in the acinar tissue of the transitional zone were reduced, and they were separated from intact parenchyma by a clear line of demarcation.

Comparison of the pathomorphological chagnes described above with the data on inhibition of PS by dalargin suggests that the beneficial effect of dalargin on the source of acute EP observed is due mainly to its action on the functional state of the body as a whole, including on the parameters of the microcirculation.

LITERATURE CITED

1. A. K. Georgadze, N. K. Permyakov, V. A. Vinogradov, et al., Farmakol. Toksikol., No. 5, 101 (1985).
2. A. S. Kanayan, N. K. Permaykov, G. P. Titova, and K. P. Arabadzhyan, Arkh. Patol., No. 4, 64 (1984).
3. A. G. Kryuchok, G. L. Gurevich, P. P. Murzenok, and A. D. Taganovich, Patol. Fiziol., No. 2, 74 (1976).
4. V. A. Malkhasyan and P. R. Simovoryan, Éksp. Khir., No. 3, 30 (1972).
5. D. Northrop, M. Kunitz, and R. Herriott, in: Crystalline Enzymes, D. Northrop (ed.), Columbia Univ. Press, New York (1948).
6. V. G. Smagin, V. A. Vinogradov, and S. A. Bulgakov, Ligands of Opiate Receptors [in Russian], Moscow (1983).
7. L. N. Starodubtseva, Vestn. Khir., No. 6, 58 (1978).
8. P. Fric, E. Kasafirek, I. Slaby, and I. Marek, Čas. Lek. Česk., 124, 41 (1985).
9. S. J. Konturek, J. Tasler, M. Cieszkowski, et al., Gastroenterology, 74, 851 (1978).
10. S. J. Konturek, J. Tasler, A. V. Schally, and W. Y. Chey, Gastroenterology, 76, 1174 (1979).

EFFECT OF SYNTHETIC L-ENKEPHALIN ANALOGS ON VIRAL AREAS OF THE PANCREAS IN EXPERIMENTAL PANCREATITIS

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Elucidation of the role of peptide neurohormones in the regulation of widely different functions of the body in health and disease is an urgent problem that has been actively researched in the last decade. Many investigations have been devoted to the effect of neuropeptides on organs of the digestive system and, in particular, on the secretory activity of the pancreas [1, 2, 7-11]. Dalargin, a synthetic Leu-enkephalin analog, depresses the secretory activity of exocrine pancreocytes (EP) in intact dogs, which is accompanied by reduction of the protein component of the zymogen granules (ZG) [4]. However, the action of enkephalins on repair processes in the pancreas arising in pancreatitis has received little study.

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