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.

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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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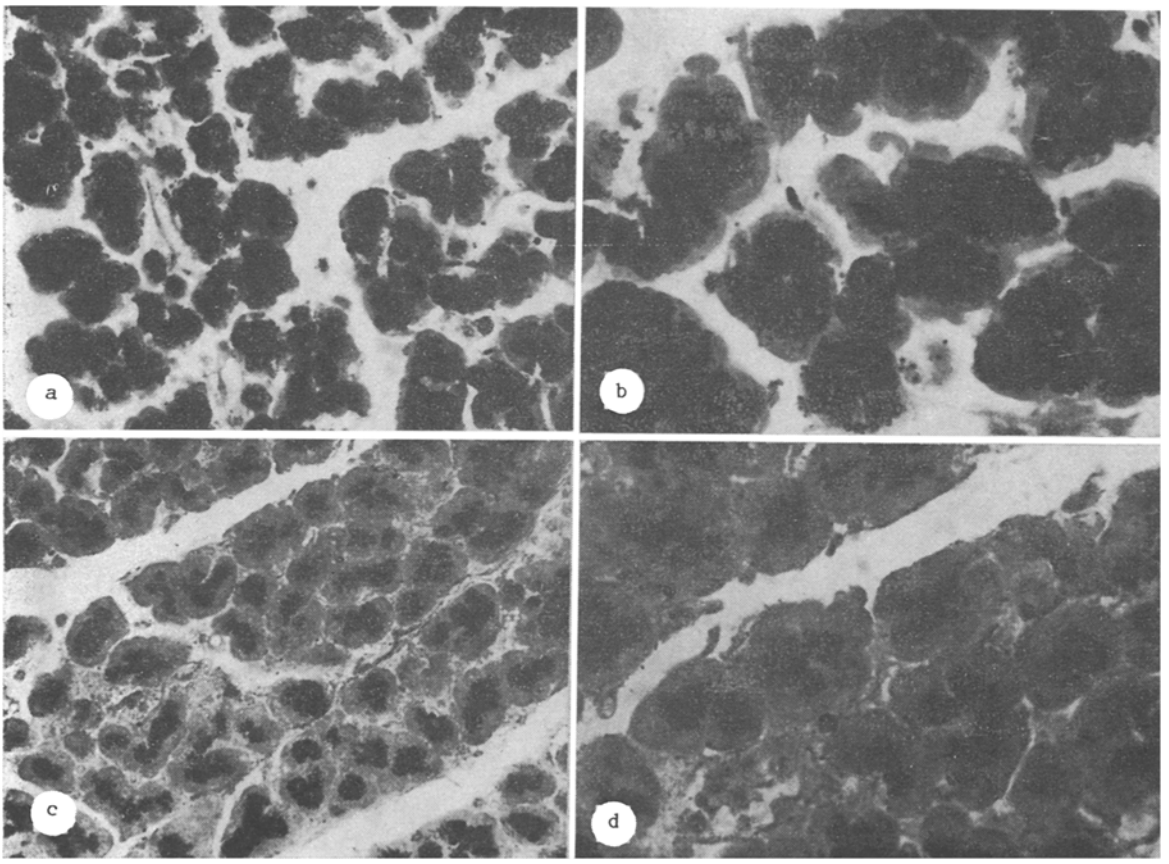

Fig. 1. Fig. 1. Content and distribution of ZG in pancreocytes of intact segment of pancreas in rats treated with tageflar (a, b) and dalargin (c, d) 24 h after induction of pancreatitis. Gram–Weigert stain. Magnification: a, c) 200, b, d) 400×.

The aim of this investigation was to study the effect of tageflar and dalargin, synthetic L-enkephalin analogs, on repair and secretory processes in viable areas of the pancreas in experimental pancreatitis. The enkephalins used were synthesized in the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR.

EXPERIMENTAL METHOD

Experiments were carried out on 181 albino rats weighing 180-200 g, divided into four groups: 1) intact rats (n = 16), 2) control rats with experimental pancreatitis (n = 55), 3) pancreatitis + tageflar (n = 55), 4) pancreatitis + dalargin (n = 55). Pancreatitis was induced by cooling the splenic segments of the pancreas (two-thirds of the weight of the organ) with ethyl chloride. Tageflar and dalargin, diluted with sterile physiological saline, were injected intraperitoneally in a dose of 0.1 mg/kg immediately after injury to the pancreas, and 2 and 24 h later. Rats of all groups were decapitated after starvation for 18 h on the 1st, 3rd, 7th, 14th, and 30th days after induction of pancreatitis. The undamaged (duodenal) segment of the pancreas was fixed in a mixture of alcohol–acetic acid–formalin (AAF) and also in Lillie's buffered formalin and embedded in paraffin wax. The percentage of binuclear EP was determined in paraffin sections 4 μ thick, stained with hexatoxylin and eosin (material fixed in AAF), and the area of the nucleus and cytoplams of the EP was determined by a dot counting method. To detect ZG, sections through the pancreas fixed with formalin were stained by the Gram–Weigert method. The intensity of incorporation of ^{14}C-leucine into proteins in the duodenal segment of the pancreas was determined in vitro. Radioactivity was measured in an SL-4221 liquid scintillation spectrophotometer (Intertechnique, France) in Bray's scintillator, after preliminary solubilization of the proteins in 0.5 M Protosol.

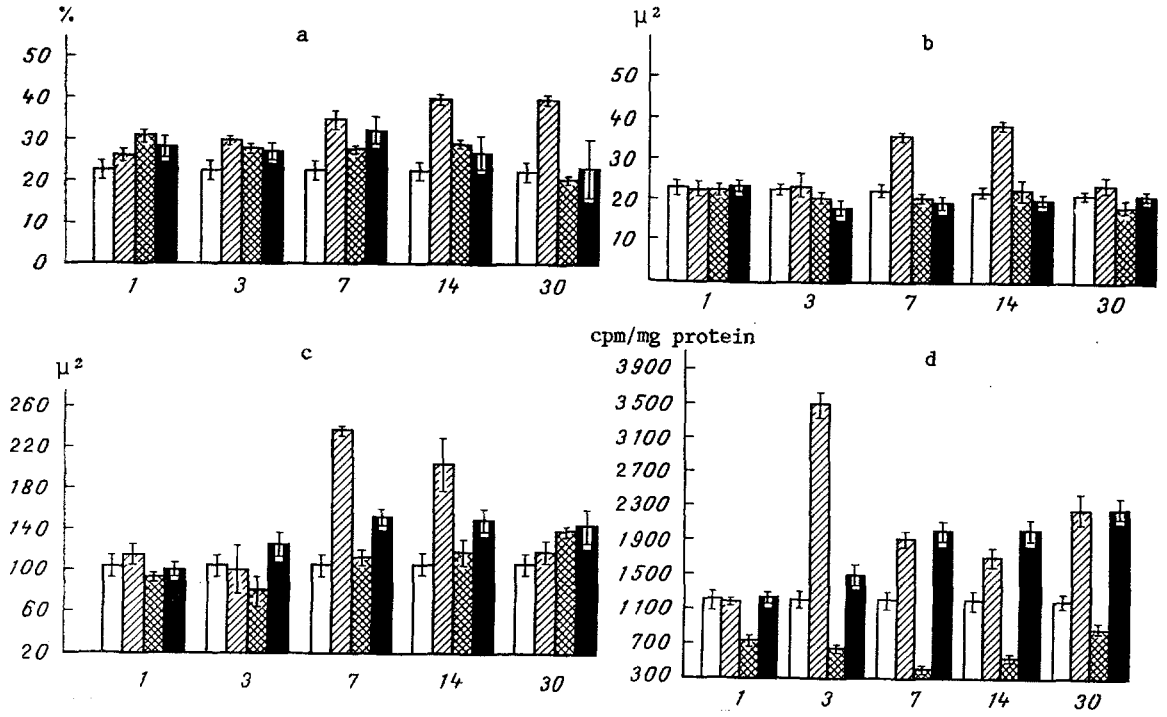

Fig. 2. Morphometric parameters of EP and ^{14}C-leucine incorporation into protein in intact segments of pancreas. a) Percentage of binuclear EP; b) area of nucleus of EP (in μ^2); c) area of cytoplasm of EP (in μ^2); d) intensity of incorporation of ^{14}-C leucine into protein (in cpm/mg protein). Unshaded columns) intact rats; obliquiely shaded) pancreatitis; cross-hatch) pancreatitis + tageflar; black) pancreatitis + dalargin. Abscissa, times during experiment (in days).

EXPERIMENTAL RESULTS

Interlobular, interacinar, and a weak degree of intracellular edema with translucency and swelling of the cytoplasm of EP developed in the duodenal segment, against the background of parenchymatous necrosis of the splenic segment in the control group 24 h after injury to the pancreas. Under the influence of tageflar and dalargin, only slight interlobular edema of the duodenal segment was observed. The action of the peptides on the content and distribution of ZG varied. For instance, tageflar led to thinning of the basal portion of the cytoplasm of EP in most acini, together with accumulation of ZG in it (Fig. 1a, b), whereas in the case of dalargin the basal portion was quite wide, and ZG had a predominantly apical orientation (Fig. 1c, d). This relationship between the basal and apical portions of the cytoplasm of EP under the influence of the peptide persisted throughout the experiment. The number of binuclear EP in the control group was almost doubled after 14-30 sec of observation, evidence of intensive functioning of the residual exocrine parenchyma of the pancreas [3, 5, 6]. In the series in which neuropeptides were used the number of binuclear EP was increased only at the 7th and 14th seconds (tageflar) or at the 7th second (dalargin) (Fig. 2a).

In the control group hypertrophy of the nucleus and cytoplasm of EP developed at the 7th and 14th seconds (Fig. 2b, c). Under the influence of tageflar the area of the nucleus was everywhere within normal limits (Fig. 2b), whereas the area of the cytoplasm was increased a little ($p < 0.01$) only at the 30th second of observation (Fig. 2c). Dalargin caused a decrease in the area of the nucleus of EP at the 3rd second, but at all other times of the experiment it was within normal limits (Fig. 2b); the area of the cytoplasm was gradually increased until the 7th second and remained at that level until the 30th second (Fig. 2c). Tageflar and dalargin had opposite effects on the nucleo-cytoplasmic ratio (Table 1). When tageflar was given this parameter was changed only at the 30th second, when the area of the cytoplasm was increased but that of the nucleus was unchanged. In the series with dalargin, the nucleo-cytoplasmic ratio was below normal from the 3rd second until the end of the period of observation, due to an increase in area of the cytoplasm whereas the area of the nucleus of EP was unchanged (3 sec) (Table 1).

It was interesting to study the effect of enkephalins on the intensity of protein synthesis, estimated as incorporation of ^{14}C-leucine (Fig. 2d). For instance, in the control

TABLE 1. Nucleo-Cytoplasmic Ratios in Exocrine Pancreocytes of Intact Segment of Pancreas in Experimental Pancreatitis uner the Influence of Neuropeptides

Experimental conditions	Times of experiments, days				
	1	3	7	14	30
Pancreatitis	0,19±0,01	0,25±0,03	0,16±0,03	0,20±0,02	0,22±0.02
p	>0,1	>0,25	>0,1	>0,5	
Pancreatitis + tageflar	0,24±0,004	0,26±0,003	0,19±0,01	0,20±0,02	0,14±0.01
p	>0,25	>0,05	>0,1	>0,25	<0,01
p_1	<0,001	>0,25	>0,25		<0,02
Pancreatitis + dalargin	0,23±0,01	0,15±0,01	0,14±0,01	0,15±0,01	0,17±0,02
p	>0,5	<0,01	<0,01	<0,01	>0,05
p_1	<0,05	<0,02	>0,5	>0,05	>0.1

Legend. This parameter for intact rats was 0.22 ± 0.02; p) relative to intact rats; p_1) relative to pancreatitis. In all groups five animals were tested (there were 11 intact rats).

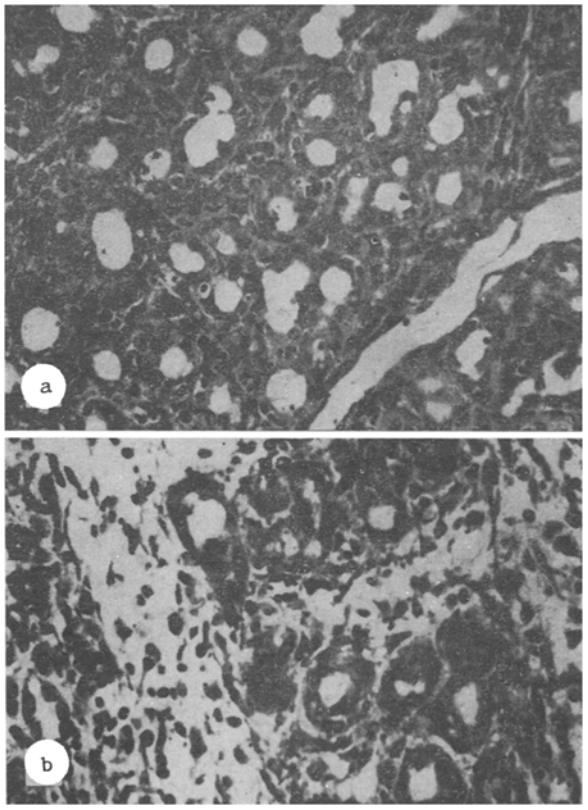

Fig. 3. Boundary zone of pancreas at 3rd second of experiment. a) Tubuloepithelial reconstruction of acini in control; b) tubulo-epithelial structures are few in number, lined with dystrophic epithelium, and surrounded by loose connective tissue (pancreatitis + tageflar). Hematoxylin and eosin, 200×.

group hypertrophy of EP was preceded by a sharp increase (almost threefold) in the intensity of protein synthesis. The action of tageflar was characterized by depression of protein synthesis throughout the experiment, but more especially at the 7th second (by 66%). Under the influence of dalargin the intensity of protein synthesis rose progressively starting from the 3rd second; from the 7th day onward this parameter was the same as in the control animals. Inhibition of protein synthesis by tageflar probably takes place by the feedback principle and is due to accumulation of ZG in the cytoplasm of EP, for we know that up to 90% of leucine is utilized for synthesis of zymogen proteins [12]. We also know that dalargin does not affect the ability of EP to release the secretory product into the lumen of the

ducts [4], as our own data also indicate (Fig. 1c, d). The increase in the intensity of protein synthesis in the late stages of the experiment against the background of dalargin administration, just as in the control, evidently reflects a compensatory reaction aimed at restoring the lost pancreatic function. Both neuropeptides acted similarly on reconstruction of acini into tubular complexes, which developed in the boundary zone. In the control this process was most marked at the 3rd second, when whole lobules underwent reconstruction (Fig. 3a). In animals receiving tageflar and dalargin, reconstruction of the acini was significantly delayed and the tubular structures were lined mainly with dystrophic epithelium (Fig. 3b), which was later replaced by connective tissue.

In the control group repair processes in viable areas of the pancreas thus follow the course mainly of intracellular regeneration, in agreement with the generally accepted view [5]. Administration of tageflar and dalargin (synthetic L-enkepahlin analogs) in experimental pancreatitis prevents the development of interacinar and intracellular edema in the damaged segment of the pancreas. Tageflar has no significant effect on the morphometric parameters of EP, but in all probability the process of release of secretion from EP is disturbed under the influence of this peptide, leading to accumulation of secretion in the cytoplasm and inhibition of protein synthesis. Under the influence of dalargin moderate hypertrophy of EP is observed with intensification of protein synthesis combined with preservation of the external secretory ability of EP, which in our view is a sign of a compensatory reaction in the viable areas of the pancreas. The peptides have no significant effect on the formation of binuclear EP and they inhibit tubular reconstruction of the acini in the boundary zone.

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