EXPERIMENTAL STUDY OF THE HEPATOPROTECTIVE ACTION OF TRANSCRANIAL PRECUTANEOUS ELECTRICAL STIMULATION AND OF THE SYNTHETIC LEU-ENKEPHALIN ANALOG DALARGIN

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Transcranial percutaneous electrical stimulation (TPES), under conditions inducing electroanesthesia (EA), as was shown previously [5], reduces after 72 h the outflow of hepatospecific enzymes — histidase (HD, EC 4.3.1.3) and urocanase (UC; EC 4.2.1.49) — into the blood stream of albino rats with experimental acute cholestasis and pancreatitis. The results suggested that EA has a hepatoprotective effect. Considering the advantages which combined electrical and pharmacological anesthesia (EPA) has been shown to have over pharmacological anesthesia alone in clinical practice of patients with pathology of the hepatopancretico-duodenal region, it was decided to continue the study of the mechanisms of the hepatoprotective action of TPES as a component of EPA. There is information in the literature that the mechanism of action of EA involves stimulation of certain brain structures and the release of endogenous opioid peptides from them [4].

The aim of this investigation was to study the mechanisms of the effect of the synthetic hexapeptide dalargin (DG), a leu-enkephalin analog, and its long-acting conjugate, on the liver.

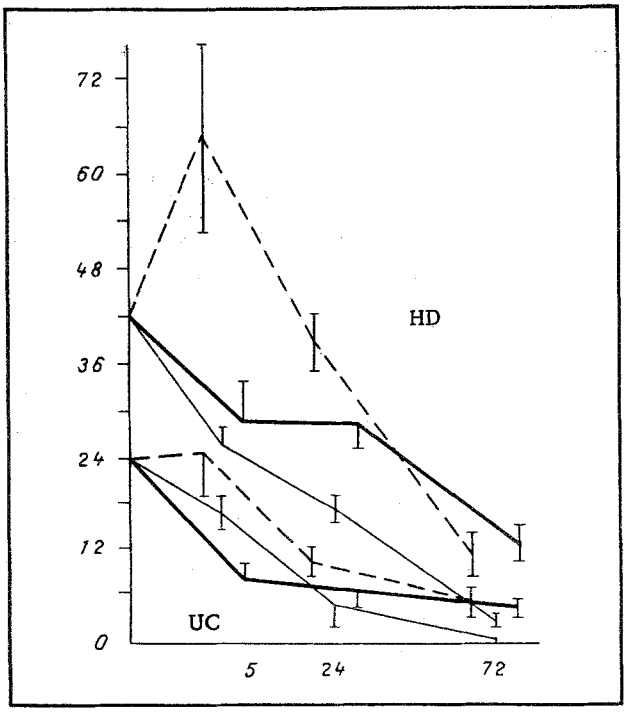

Fig. 1. Time course of hepatospecific enzyme activity in blood serum of experimental animals. Abscissa, time after procedure (in h); ordinate, enzyme activity (in pmoles/ml/sec). Broken line represents control (physiological saline); bold continuous line — DG; thin continuous line — long-acting DG.

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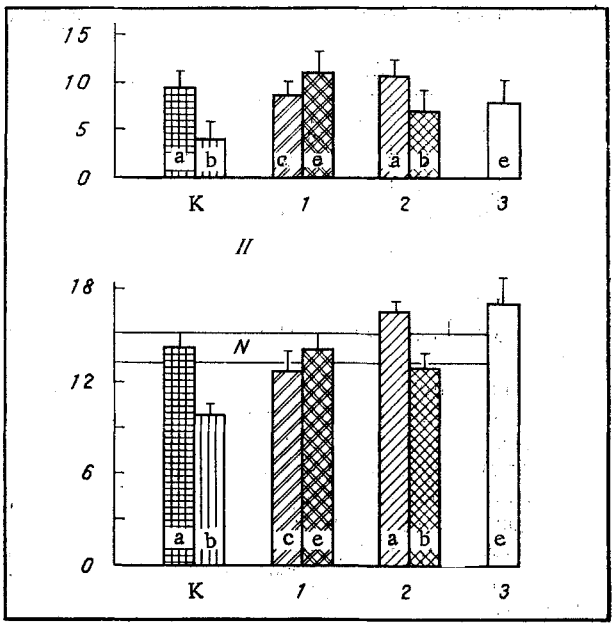

Fig. 2. HD (I) and UC (II) activity in liver tissue of experimental animals. Ordinate, enzyme activity (in pmoles/mg protein/sec). K) Control (physiological saline): a) after 24 h, b) after 72 h; 1: c) diazepam, e) diazepam + ES; 2: a) DG after 5 h; b) long-acting DG after 72 h.

EXPERIMENTAL METHOD

Experiments were carried out on 362 male albino rats weighing 180-200 g by a method developed by the writers on a model of acute cholestasis and pancreatitis [7]. The following experiments were carried out on rats undergoing the corresponding operations. In series I the animals were subjected to TPES after preliminary intraperitoneal injection of diazepam (Seduxen) in a dose of 10 mg/kg; a second dose of diazepam (5 mg/kg) was given 2.5 h later, and electrical stimulation (ES) was given for 5 h; rats receiving diazepam alone by intraperitoneal injection, at the same intervals and in the same doses, served as the control. In series II the animals were given in intraperitoneal injection of DG, diluted in 1 ml of isotonic NaCl solution, in a dose of 10^{-9} mole/100 g body weight; control animals received 1 ml of isotonic NaCl solution by the same method. In series III the rats were subjected to ES and received injections of diazepam and DG; the times of the procedures and the doses and intervals were the same as in series I and II. The investigations were carried out 5, 24, and 72 h after the procedures. The following parameters were studied: HD and UC activity in the blood serum and liver by the method in [1, 2], activity of the membrane-bound enzyme 5'-nucleotidase (5-ND) in the liver by the method in [8].

A special series of experiments also was carried out in vitro to study the possibility of a direct inhibitory action of DG on enzyme activity in the blood serum and liver. For this purpose samples of blood serum and 10% liver homogenate were incubated in the presence of DG in a concentration of 10^{-7}-10^{-9} M, after which HD and UC activity in the samples was determined.

EXPERIMENTAL RESULTS

In view of the role of endogenous opioid peptides which has been postulated in the realization of the hepatoprotective effect of TPES, it was decided to study the effect of DG and its long-acting conjugate on the state of the liver. In the animals undergoing experimental operations, DG was found to lower HD and UC activity in the blood after 5 h by 55.7 and 67.1% respectively compared with the control (isotonic NaCl solution), and to increase UK activity by 25% ($p < 0.01$) and 5-ND activity by 32.8% ($p < 0.001$; Figs. 1-3) in the liver tissue, which can be regarded as evidence of a marked hepatoprotective action, due to stabilization of the hepatocyte membranes. However, the action of this preparation was of short duration, and after only 24 h DG no longer had any marked effect on the test parameters compared with the control (Fig. 1). Injection of long-acting DG into the animals prolonged the effect of the preparation so that it lasted throughout all stages of the investigation.

TABLE 1. Serum HD and UC Activity (in pmoles/ml/sec) in Experimental Animals after 72 h (M ± σ; n = 10)

Enzyme	Experimental conditions			Diazepam + ES + DG
	diazepam	diazepam + ES	DG	
UD	33,6±2,2	23,8±0,9	16,3±2,6	11,17±1,8
UC	10,5±0,8	4,0±0,4	5,6±5,6	2,1±0,7

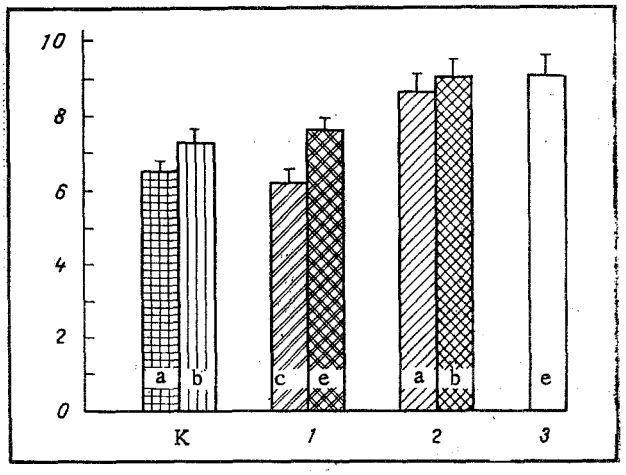

Fig. 3. 5'-ND activity (in μg P/mg protein) in liver tissue of experimental animals. Legend as to Fig. 2.

To discover whether the fall in HD and UC activity in the blood serum was due to a direct inhibitory action of the preparation on these two enzymes in the blood serum and liver, a special series of experiments was carried out in vitro; its results showed that DG, in the concentrations tested (10^{-9} M), does not inhibit HD or UC in blood serum or liver tissue.

To study possible summation of the effects of the exogenous opioid and activation of endogenous opioids as a result of TPES, enzyme activity against the background of their combined use was determined in the experiments of series III. The results of this series showed a marked decrease in blood HD and UC activity ($p < 0.01$), after combined action of TPES and DG (Table 1). Investigation of levels of HD and UC activity in the liver tissue of experimental rats subjected to ES revealed that after 72 h, i.e., when the most marked hepatoprotective effect was observed, HD activity in the liver was 61.7% higher ($p < 0.02$) than that in the control animals (without ES), and amounted to 12 pmoles/mg protein/sec (Fig. 2).

Determination of 5-ND activity in the liver tissue of the rats showed that in the experimental animals not subjected to ES (series I, control) activity of this enzyme was depressed by 38.8% compared with that in intact rats (Fig. 3). In experimental series I (ES + diazepam) this decrease was less marked (by 25.5%), evidence of some improvement in the metabolic status of the hepatocyte membranes. In series II and III, on animals undergoing the experimental operations, levels of 5-ND activity in the liver tissue after injection of DG alone and also after the combined use of TPES and DG did not differ statistically significantly from the normal values (Fig. 3).

It can be concluded from the results that the hepatoprotective properties of TPES, discovered by the writers previously, are evidently partly due to activation of endogenous opioid peptides, which creates optimal conditions for stabilization of the hepatocyte membrane in a model of acute cholestasis and pancreatitis.

At the same time, the possibility of a direct hepatoprotective action of the original DG preparation cannot be ruled out.

The experimental results suggest that a method of general anesthesia including ES and injection of DG may provide optimal anesthesiologic protection under clinical conditions during the surgical treatment of hepato-pancreatico-duodenal pathology.

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EFFECT OF DELTA-SLEEP PEPTIDE ON EPILEPTIC ACTIVITY DURING METRAZOL KINDLING

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Delta-sleep inducing peptide (DSIP) has an antiepileptic action when single foci of of epileptic activity and multifocal epileptic complexes are formed in the cerebral cortex of rats and cats [2]. It was considered interesting to study the antiepileptic efficacy of DSIP on other models of epileptic activity. One model of progressively increasing predisposition to epileptic activity is metrazol kindling, which arises as a result of repeated injections of subconvulsive doses of metrazol [5].

The effect of DSIP on epileptic activity in rats and mice was investigated in the study described below.

EXPERIMENTAL METHOD

Experiments were carried out on $(CBA \times C57Bl/6)F_1$ mice weighing 18-24 g and on Wistar rats weighing 180-250 g. Kindling was induced by daily (for 3 weeks) intraperitoneal injection of metrazol in a dose of 30 mg/kg. The intensity of the convulsions was estimated in points on the following scale: 0) absence of epileptic response; 1) paroxysmal twitchings; 2) clonic convulsions of the whole trunk; 3) clonic convulsions of the forelimbs, the animal raising itself on its bind limbs (kangaroo posture); 4) marked clonico-tonic convulsions with the animal falling on its side, and a phase of tonic extension; 5) repeated clonico-tonic convulsions with the animal falling on its side, terminating in death of some of the animals. The latent period of development of the first epileptic manifestations, and the mortality also were determined. DSIP in a dose of 100 μg/kg was injected intraperitoneally in physiological saline. The epileptic response of the animals was tested after 15-17 h. The action

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