7. I. D. Stal'naya and T. T. Garishvili, Modern Methods of Biochemistry, ed. V. N. Orekhovich [in Russian], Moscow (1977), pp. 68-68.
8. S. Boyden, Meth. Med. Res., 10, 152 (1964).
9. D. Hungerford, I. Donnal, and S. Beck, Am. J. Hum. Genet., 11, 215 (1959).
10. G. Middelbroch and P. Dubus, J. Exp. Med., 88, 66 (1948).

COMPARATIVE EFFICACY OF REGULATORY PEPTIDES IN EXPERIMENTAL ACUTE PANCREATITIS

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Traditional methods of etiotropic therapy of acute pancreatitis currently available do not enable the development of this disease to be prevented to an adequate degree. Protease inhibitors (gordox, contrykal, etc.), for instance, which do not depress pancreatic function, can only lower the circulating blood levels of pancreatic enzymes, whereas preparations acting on the synthesis of these enzymes (various cytostatics), in the optimal therapeutic concentrations used, also have a general toxic action on vital organs and systems of the body [5]. Reports have recently been published on the important role of regulatory peptides (RP) in the maintenance of homeostasis in various types of pathology, including pancreatitis [3, 6, 8]. However, in the few publications on this problem, the efficacy of peptide bioregulators was assessed mainly on the basis of biochemical tests, carried out in forms of acute pancreatitis which had already developed [1, 2]. Meanwhile there have been virtually no studies of pancreatic function during prophylactic administration of RP in the early stages of pancreatic toxemia, and equally no studies of protective factors promoting detoxication (phagocytic cells of the reticuloendothelial system – RES, fixed macrophages of the liver and spleen).

The aim of this investigation was to compare the efficacy of various RP for the prevention of experimental acute pancreatitis, using radionuclide methods to investigate functional activity of pancreatic cells and cells of RES in the liver.

EXPERIMENTAL METHOD

Experiments were carried out on 12 mongrel dogs weighing 15-20 kg, anesthetized with pentobarbital (2% solution, dose 25 mg/kg), after premedication with trimeperidine (2% solution, 0.5 ml/kg) and atropine (0.3 ml/10 kg body weight). Experimental acute pancreatitis (EAP) was induced by transduodenal injection of autologous bile into the chief pancreatic duct in a dose of 0.5 ml/kg, followed by stimulation of secretion by secretin and cerulein (2 units/kg respectively of each; from "Boots," England). A leg vein was cannulated at the same time for injection of the test preparations, including radiopharmaceuticals (RPH), and blood was taken at intervals to determine α-amylase and trypsin levels in the blood serum as in [9, 10]. EAP and hemorrhagic pancreatic necrosis developed in this model in the course of 1.5-2 h after initiation, as confirmed by morphologic and ultastructural investigations [4]. The animals were investigated by gamma-camera (Searle, the Netherlands), equipped with PDP 11/34 computer (USA). Recording began from the time of intravenous injection of RPH (a colloidal solution of ^{198}Au in a dose of 11.1 MBq and ^{75}Se-methionine in a dose of 14.8 MBq) and continued for 30 min with serial scans with an interval of 1 min, with the dog lying in the supine position. The quantitative analysis was conducted in two stages: the first consisted of identifying the "zone of interest" over the region

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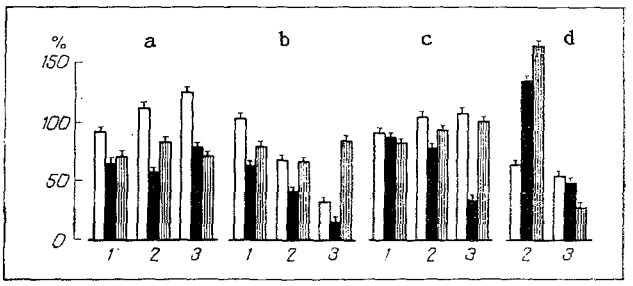

Fig. 1. Functional activity of RES and pancreatic cells after administration of RP to intact dogs (1) and animals with EAP 3 h (2) and 3 days (3) after initiation (M ± m). a) Dalargin; b) calcitonin; c) somatostatin; d) control – placebo (physiological saline); Ordinate, level of uptake by phagocytic cells of RES and pancreocytes (in % of values for intact dogs) relative to test indicator substances. Unshaded columns – rate of clearance of blood from intravenously injected inert ^{198}Au colloid; black columns – degree of accumulation of ^{198}Au colloid by hepatic macrophages; vertically shaded columns – degree of accumulation of ^{75}Se-methionine by pancreocytes.

of the heart, pancreas, liver, and thigh; the second consisted of radiochromograms in these "zones of interest," by means of which the functional state of the test systems and organs could be evaluated with the aid of specially devised computer programs. The criterion of this evaluation for the pancreas was the degree of accumulation of ^{75}Se-methionine by the pancreocytes, and for cells of the RES it was the level of their phagocytic activity, judged by the rate of clearance of the blood from intravenously injected ^{198}Au, and also the uptake of ^{198}Au by liver macrophages, for this particular indicator is taken up preferentially by stellate reticuloendotheliocytes [7]. The phagocytic index K, i.e., clearance of the test colloid by all cells of RES, was calculated by the equation:

$$K = \frac{\lg C_1 - \lg C_2}{T_2 - T_1},$$

where C_1 and C_2 denote activity of the radiochronograms above the region of the heart, recorded during time $T_2 - T_1$. The phagocytic index of the hepatic macrophages (K_h; the degree of accumulation of colloidal gold) was calculated by an analogous equation with modifications, in which $C_2 - C_1$ denotes activity of radiochronograms above the region of the liver, recorded during the same time $T_2 - T_1$.

There were four series of experiments. In series I the functional state of the pancreas and cells of the RES was studied in intact dogs. The results of this series served as the control for series II, in which the corresponding parameters were studied after administration of regulatory peptides, somatostatin, 4 units/kg/h ("Serono," West Germany), calcitonin, 2 units/kg/h ("Sandoz," Switzerland), and dalargin, 50 μg/kg/h. In series III the same preparations were given simultaneously with the beginning of the operation on the dogs subjected to EAP. The tests were carried out at intervals: 3 h and on the 3rd day after the operation. Animals with EAP and receiving a placebo (physiological saline) served as the control.

EXPERIMENTAL RESULTS

The action of the RP tested on the intact pancreas was consistently one of inhibiting accumulation of the label by the gland by 25% compared with the initial level of uptake of ^{75}Se-methionine by the cells (without infusion of RP), which was estimated 2 weeks before the present experiment began. The phagocytic activity of RES cells on intact animals was virtually unchanged after administration of RP, for despite a decrease in the phagocytic index of the hepatic macrophages (K_h), clearance of the test colloid ^{198}Au from the blood was not reduced (Fig. 1).

On induction of EAP a marked increase was observed in the uptake of labeled methionine by the pancreatic cells in the early stage (after 3 h), followed by a considerable decrease later during the experiment (3 days after initiation of EAP). A similar time course was found when the rate of accumulation of colloidal gold by hepatic macrophages was investigated, but the intensity of clearance of the blood from the injected indicator fell sharply even in the early period, and continued to fall until the end of the observation. Two of these animals died at the end of the 3rd day and one on the 4th day of the course of endogenous toxemia caused by EAP.

TABLE 1. Serum Pancreatic Enzyme Levels of Intact Dogs and Dogs with EAP after Administration of RP (M ± m)

Group of dogs	α-Amylase, U/liter	Trypsin, U/liter
Initial parameters of intact dogs (n = 12)	260,4±25,0	5,3±0,8
Administration of somatostatin (n = 3)	245,6±8,0	4,7±0,02
Administration of calcitonin (n = 3)	249,3±18,0	5,0±0,8
Administration of dalargin (n = 3)	252,1±25,0	5,0±0,72
With EAP (control): after 3 h (n = 3)	1041,0±62,0*	11,04±1,9*
after 3 days (n = 3)	804,0±74,0*	9,32±0,9*
With EAP, receiving somatostatin: after 3 h (n = 3)	337,0±12,6**	7,4±1,1**
after 3 days (n = 3)	303,7±13,5**	6,7±1,04**
With EAP, receiving calcitonin: after 3 h (n = 3)	406,6±15,4*,**	7,8±1,42**
after 3 days (n =3)	323,8±11,6**	7,14±1,09**
With EAP, receiving dalargin: after 3 h (n = 3)	516,6±31,4*,**	8,1±0,98*,**
after 3 days (n = 3)	360,6±37,0**	7,7±0,87*

Legend. Number of animals given in parentheses. *p < 0.05 compared with initial values for intact dogs; **p < 0.05 compared with parameters in control at same time of investigation.

The trend of the state of pancreatic function in dogs with EAP treated with RP differed essentially from that in the control (untreated) animals. In the first place, hyperactivation of the pancreocytes was limited in the early stage and their inhibition was prevented in the late stage of EAP. In animals receiving somatostatin, for instance, the degree of accumulation of methionine by pancreatic cells at all times of EAP was virtually identical with the initial values before its induction. Dalargin, like somatostatin, abolished the excessive functional activity of the pancreas characteristic of the early stages of endogenous pancreatic toxemia, but infusion of dalargin prevented the subsequent (toward the 3rd day) decrease in uptake of RPH by a lesser degree, although it was nevertheless 45.2% higher than in the untreated dogs. Injection of calcitonin caused an even greater (compared with somatostatin and dalargin) decrease in methionine accumulation by the pancreatic tissue 3 h after initiation of EAP, and prevented the decrease in the ability of the pancreocytes to accumulate RPH on the 3rd day of pancreatic toxemia quite effectively, as shown by the higher (by 56.4%) degree of accumulation of RPH compared with the control at the same time of investigation.

The level of the ingestive capacity of the phagocytic cells of RES in animals receiving RP was significantly higher in the control at all times of the investigation, except that calcitonin had virtually no effect on the rate of elimination of colloidal gold from the blood stream, combined with a low value of K_h. The greatest intensity of phagocytosis was observed after injection of dalargin, despite the relative fall in the degree of accumulation of the test colloid by stellate reticuloendotheliocytes of the liver compared with the initial value. This phenomenon of desynchronization of general clearance of foreign material from the blood and the fixed macrophages of the liver, which make the main contribution to realization of the above-mentioned blood clearance, may be due to a compensatory and adaptive distribution of the functional load to other cells of RES and, in particular, to alveolar and splenic macrophages.

In the final stage of the work activity of pancreatic enzymes was studied in intact animals and in dogs with induced EAP, receiving RP or a placebo. It will be clear from Table 1 that serum levels of pancreatic enzyme activity remained significantly unchanged

after administration of the test RP to intact animals and lay within the limits of their initial values. Meanwhile induction of EAP was accompanied by marked activity of the excretory function of the pancreatic exocrine tissue as early as in the first 3 h after the beginning of initiation of the disease (in this case the α-amylase level was increased fourfold and the trypsin level 2.5-fold). During development of destruction of the parenchyma of the pancreas (by the 3rd day of the postoperative period) a fall in the α-amylase level by 22.8% and the trypsin level by 15.6% compared with their values after 3 h was observed. Infusion of RP in dogs with EAP had a significant inhibitory action on the external excretory function of the gland. For instance, only 3 h after the beginning of development of EAP somatostatin depressed α-amylase activity by 77.7%, calcitonin activity by 61%, and dalargin by 50.6%. Inhibition of trypsin activity amounted to 33, 29.4, and 26.7%, respectively. A further progressive decline in the level of enzyme toxemia was observed toward the 3rd day.

Administration of RP for the etiotropic prophylaxis of EAP thus demonstrates that the preparations used were sufficiently effective, as shown by the relative prevention of development of acute inflammation of the pancreatic parenchyma. The strongest protective action on the animals with EAP was exhibited by somatostatin. Dalargin and calcitonin were pharmacologically less effective, although when dalargin was used, the most effective phagocytosis by cells of the RES was observed at all times after induction of EAP.

LITERATURE CITED

1. A. K. Georgadze, N. K. Permyakov, V. A. Vinogradov, et al., Farmakol. Toksikol., No. 5, 101 (1985).
2. A. K. Georgadze, N. K. Permyakov, V. A. Penin, et al., Neuropeptides: Their Role in Physiology and Pathology [in Russian], Tomsk (1985), pp. 167-168.
3. G. N. Kryzhanovskii, V. D. Slepushkin, G. K. Zoloev, et al., Patol. Fiziol., No. 6, 47 (1987).
4. N. K. Permyakov and G. P. Titova, Abstracts of Proceedings of the 5th All-Russian Congress of Surgeons [in Russian], Sverdlovsk (1978), pp. 73-76.
5. V. S. Savel'ev, V. M. Buyanov, and Yu. V. Ognev, Acute Pancreatitis [in Russian], Moscow (1983).
6. V. G. Smagin, V. A. Vinogradov, and S. A. Bulgakov, Ligands of Opiate Receptors [in Russian], Moscow (1983).
7. N. A. Fedorov, B. E. Movshev, R. V. Nedoshivina, and I. K. Koryakina, Burn Autointoxication: Its Immunologic Control [in Russian], Moscow (1985).
8. E. I. Chazov, M. I. Titov, V. A. Vinogradov, et al., Vopr. Med. Khimii, No. 3, 47 (1984).
9. W. T. Caraway, Am. J. Clin. Pathol., 32, 97 (1959).
10. F. Erlange, N. Kokowsky, and W. Cohen, Arch. Biochem., 95, 271 (1961).