EFFECT OF PROSTAGLANDINS E_2 AND F_2 ON THE SEROTONIN CONTENT IN MAST CELLS IN EXPERIMENTAL PANCREATITIS

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Acute pancreatitis is accompanied by disturbances of the hemodynamics [4] and by an increase in vascular permeability. There is evidence in the literature that prostaglandins (PG) are modulators of inflammation, and in particular, that they change vascular permeability [7, 11]. In experimental pancreatitis a considerable increase in the concentration of prostaglandin-like substances also is found in the pancreatic venous blood and in the peritoneal fluid [9].

Since the mast cells participate in the regulation of microvascular permeability [3], it was decided to study the effect of exogenous PG E_2 and $F_{2\alpha}$ on the serotonin content in the mast cells in experimental pancreatitis.

EXPERIMENTAL METHOD

Experiments were carried out on 45 male albino rats weighing 180-200 g, divided into three groups. In the animals of group 1 the splenic segment of the pancreas was cooled with ethyl chloride [5]. The results of the morphological control confirmed the presence of hemorrhagic pancreatitis. The rats of groups 2 and 3, after cooling of the pancreas, were given 1 ml of PG E_2 (from the Upjohn Company) perorally daily in a dose of 25×10^{-8} g/ml or PG $F_{2\alpha}$ intraperitoneally in a dose of 12.5×10^{-8} g/ml. To study the effect of the operation on the reaction of the mast cells in the early periods of the investigation, laparotomy was performed under ether anesthesia. The rats were decapitated on the 1st, 3rd, 7th, and 14th days of the experiment. The serotonin content per mast cell was determined fluormetrically [1] by means of the FMÉL-1A photometric luminescent attachment after treatment of preparations of the mesentery and subcutaneous loose connective tissue with paraform [8], and were expressed in relative fluorescence units (FU). In each group 300 cells were examined photometrically. The total number of measurements was 9000. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Values of the mean content of serotonin per mast cell at different times of experimental pancreatitis and under the influence of PG are given in Table 1. They show that one day after induction of pancreatitis in the animals of group 1 there was a sharp decrease in the intensity of fluorescence in the mast cells of the mesentery and loose connective tissue. The serotonin content in the mesentery fell by 63.5%, and in films of loose connective tissue it fell by 67.3% compared with the control animals. In rats undergoing mock operations the reaction of the mast cells was much weaker. Administration of PG E_2 had no significant effect on the intensity of fluorescence in the mast cells of the mesentery but in the loose connective tissue it led to normalization of the mean serotonin content in the mast cells. PG $F_{2\alpha}$ at this time of the experiment did not affect fluorescence of the mast cells of the mesentery or loose connective tissue. Later, on the third day of development of hemorrhagic pancreatitis the mean serotonin content per mast cell continued at a low level in the rats, namely 62.3% in the mesentery and 59.9% in films of loose connective tissue compared with the corresponding values in control animals. In rats undergoing mock operations the intensity of fluorescence in the mast cells of the mesentery and loose connective tissues was fully restored to normal. One week after induction of pancreatitis, when

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TABLE 1. Mean Serotonin Content (in FU) per Mast Cell in Mesentery and Loose Connective Tissue of Rats with Experimental Pancreatitis and under the Influence of PG E_2 and $F_{2\alpha}$ (M ± m)

Duration of experiment, days	Experimental conditions	Mesentery	P_1	P_2	Loose connective tissue	P_1	P_2
	Control	54,01±2,59			36,13±4,42		
	Laparotomy	43.7±5,17	>0,1	<0,001	22,4±3.92	<0,05	<0,05
1	1	19,7±4,38	<0,001	—	12,41±1.20	<0,001	—
	2	20,08±2,64	<0,001	>0,5	32,89±4,50	>0,5	<0,01
	3	14,80±1,01	<0,001	>0,2	15,29±1,68	<0,01	>0,2
	Laparotomy	51,50±3,04	>0,5	<0,01	32,98±4,05	>0,5	<0,05
3	1	33,76±3,06	<0,001	—	21,65±1,67	<0,02	—
	2	29,53±3,46	<0,001	>0,2	17,35±1,27	<0,01	>0,05
	3	16,80±3,39	<0,001	<0,02	15,56±2,92	<0,01	>0,1
	1	38,80±2,15	<0,01	—	25,60±2,04	>0,05	—
7	2	22,57±1,07	<0,001	<0,001	18,74±1,22	<0,01	<0,05
	3	13.29±1,67	<0,001	<0,001	15,80±0,83	<0,01	<0,01
	1	44,12±1,15	<0,02	—	28,16±1,93	>0,1	—
14	2	56,15±1,38	>0,2	<0,001	41,33±3,56	>0,2	<0,02
	3	8,55±0,64	<0,001	<0,001	16,19±1,16	>0,01	<0,01

Legend. P_1) Relative to control, P_2) relative to pancreatitis; 1) pancreatitis, 2) pancreatitis + PG E_2, 3) pancreatitis + PG $F_{2\alpha}$. Three rats used in each experiment and 300 cells in mesentery and loose connective tissue respectively were examined photometrically.

the serotonin content in the mast cells of the mesentery and loose connective tissue of the rat was low, administration of PG E_2 and $F_{2\alpha}$ caused a further decrease in the intensity of fluorescence of the mast cells. By the end of the second week of the experiment the serotonin content in the animals was increased in both populations of mast cells, but it was still lower than in the control rats. Under the influence of PG E_2 an increase in the intensity of fluorescence was observed in the mast cells of the mesentery and loose connective tissue and it did not differ significantly from the results obtained in the control animals. After injection of PG $F_{2\alpha}$, however, the serotonin content still remained low, namely 15.8% in the mesentery and 44.8% in the loose connective tissue compared with the controls.

In experimental hemorrhagic pancreatitis a decrease in the intensity of fluorescence of the mast cells of the mesentery and loose connective tissues was thus observed. Administration of PG E_2 caused an initial fall and subsequent rise in the serotonin content in the mast cells, whereas PG $F_{2\alpha}$ caused a stable decline in fluorescence of the mast cells. PG E_2 is known to activate adenylate cyclase and thus to promote accumulation of cyclic AMP in the cells [8]. Cyclic AMP, in turn, prevents liberation of biogenic amines from the mast cells [2, 6]. In the early period of the present experiments PG E_2, however, had no effect on the serotonin content in the mast cells. The reason evidently was that the stress effect of the mast cells was so great that even accumulation of cyclic AMP did not affect the liberation of biogenic amines. Other workers observed the same picture when, after treating mast cells with phosphodiesterase inhibitors, they obtained a tenfold rise in the cyclic AMP level and did not observe inhibition of liberation of biogenic amines under the influence of compound 48/80. The fall in the intensity of fluorescence of the mast cells at all times of the experiment in which PG $F_{2\alpha}$ was injected can be explained in all probability by the stimulating effect of this PG on guanylate cyclase and, correspondingly, on cyclic GMP synthesis. Cyclic GMP is known to stimulate the liberation of biogenic amines from the mast cells [10].

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EVALUATION OF THE PROTECTIVE EFFECT OF GAMMA-HYDROXYBUTYRIC ACID IN STRESS

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Stress is known to induce disturbances of the metabolism and function of the heart and also to cause ulcers in the gastrointestinal tract [9, 10]. Meanwhile activation of the inhibitory GABA-ergic system of the brain, protective in character, is observed during stress, for preliminary administration of the GABA metabolite sodium hydroxybutyrate (GHBA) prevents disturbances in the heart and stomach [8-10]. The writers showed previously that stress causes phasic injuries to the structure of the heart muscle which correspond to definite periods with different blood eosinophil counts [2, 5]. It has been found that changes in the blood eosinophil level, reflecting functional activity of the pituitary-adrenal system (PAS) in stress [3, 4], can serve as the criterion for quantitative evaluation of the effectiveness of drugs [6].

Accordingly the aim of the investigation described below was to study the effect of GHBA on the eosinophil, corticosterone, and noradrenal levels and the severity of injuries to the structure of the heart as a result of exposure to stress.

EXPERIMENTAL METHOD

Experiments were carried out on 78 male albino rats weighing 180-200 g with an initial eosinophil count of 220-340/μl peripheral blood at 9 a.m. Stress was produced by Desiderato's method [11]. GHBA in a dose of 100 mg/kg was injected subcutaneously 30 min before exposure to stress and again 2 and 4 h after the beginning of exposure. Control animals received physiological saline at the same time. After the end of exposure to stress at intervals of 3 h the eosinophils in 1 μl peripheral blood were counted in the animals of all groups (in a Goryaev's chamber, using Hinkleman's stain). The plasma corticosterone concentration was determined by chromatography on columns with silica-gel [1] 2 h after the end of stress and the noradrenalin concentration in the heart was determined fluorometrically [7]. To assess the protective effect of GHBA on the animal quantitatively, the method developed previously [6] was used; this involved calculating the ratio between the time required for eosinophilia to appear after the eosinopenia in animals of the control group (without GHBA) and the time taken for eosinophilia to appear in the animals of the experimental group (receiving GHBA). To detect structural injuries to the heart, Perls's reaction was carried out on serial topographic sections, followed by counterstaining with hematoxylin and eosin; the method of polarization microscopy also was used. Animals in all groups were killed for morphological investigation during the period of marked eosinophilia, for it was shown previously that the severest structural changes in the heart are observed at that time [2, 5].

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