EFFECT OF OPIOID PEPTIDES ON THE LYMPHATIC DRAINAGE OF THE PANCREAS IN RATS AND DOGS

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During the last few years many investigations have demonstrated the effect of opiates and of endogenous opioids on the pancreas. In particular, they have been shown to inhibit the exocrine function of this organ [8, 9]. It has been suggested that ligands of opiate receptors can be used in the treatment of acute and chronic pancreatitis [5]. The enkephalin analog Tyr-D-Ala-Gly-Phe-Leu-Arg, known as Dalargin, has been synthesized at the All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, and it is currently being used therapeutically in clinical gastroenterology [1, 6].

In dogs with experimental pancreatic necrosis Dalargin has been shown to inhibit the synthesis and activity of proteolytic enzymes in the pancreas and to depress the kininogenase and lipolytic activity of the gland. By preventing the development of erythrostasis and microthrombosis, Dalargin improved the microcirculation in the pancreas and restricted the size of the foci of necrosis, thereby preventing progressive destruction of the organ [2]. Dalargin was found to increase the regional lymph flow in the mesentery of the small intestine [3]. The lymph-stimulating action of enkephalins also has been demonstrated by other workers [4, 7].

Changes in the lymph flow in the pancreas may be of essential importance for the development of pathological processes in the gland, and accordingly the aim of the present investigation was to study the effect of Dalargin and of certain other ligands of opiate receptors on the drainage function of the pancreatic lymphatic system in rats and dogs.

EXPERIMENTAL METHOD

Experiments were carried out on 75 male and female Wistar rats weighing 200-250 g and on three dogs weighing 12-15 kg.

The rats were anesthetized by intraperitoneal injection of pentobarbital (60 mg/kg) and placed on a thermostatically controlled table to maintain their body temperature at 37°C. A midline laparotomy was performed on the animals and 1 µl of a 1% solution of Evan's blue in physiological saline was injected beneath the capsule of the pancreas at each of 3 points, to form a depot of the dye. Immediately after injection of the Evans' blue, the rats were given a subcutaneous injection of 0.2 ml of physiological saline and the mean time for disappearance of the dye from its three depots was measured. After 30 min a second injection of Evans' blue was given at three points beneath the capsule of the pancreas, and the effect of subcutaneous injection of Dalargin (10-1000 µg/kg), naloxone (1 mg/kg), or a combination of Dalargin (100 µg/kg) and naloxone on the rate of elimination of the dye was studied. The effect of Dalargin, of the synthetic opioid peptides Tyr-D-Ala-Gly-Phe-D-Leu(DADLE) and Tyr D-Ala(Me)Phe-Gly-ol(DAGO), and also of N-allylnormetazocine (SKF 10047), injected subcutaneously in a dose of 100 µg/kg, on the rate of elimination of the dye from beneath the capsule of the pancreas, was studied in a separate randomized blind test. The control group of animals received a subcutaneous injection of 0.2 ml of physiological saline. As a first step the basal rate of elimination of the dye was determined in all animals.

In chronic experiments on dogs, the animals were anesthetized with hexobarbital (1 g, intravenously), the thoracic lymph duct and jugular vein were catheterized, laparotomy was

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TABLE 1. Effect of Dalargin on Rate of Elimination of Evans' Blue from beneath Capsule of Rat Pancreas (M ± m)

Elimination time of dye, sec	Dose of Dalargin, μg/kg			
	10	30	100	1000
Basal	20,63±1,51	16,67±1,33	18,83±0,99	17,07±1,29
After injection of Dalargin	20,65±1,32	11,33±0,57*	12,70±1,06*	9,30±1,13*

Legend. Here and in Table 2, *p < 0.05.

TABLE 2. Effect of Dalargin and Specific Agonists of Different Subpopulations of Opioid Receptors on Elimination Time of Evans' Blue from beneath Pancreatic Capsule of Rats (M ± m)

Substance injected	Elimination time, %
Control	100
Physiological saline	77,48±2,31
Dalargin (100 μg/kg)	47,39±2,27*
DAGO (100 μg/kg)	74,90±1,43
DADLE (100 μg/kg)	93,03±2,27*
SKF 10047 (100 μg/kg)	76,93±5,90

performed, and a catheter was introduced beneath the capsule of the pancreas and fixed in the parapancreatic cellular tissue. The lymph flowing from the thoracic duct began to be collected 24 h after the operation, and collection continued for 3.5 h. Meanwhile an infusion of physiological saline was given via the jugular vein in a volume corresponding to the volume of lymph collected. After removal of two 30-min samples of lymph, the dogs were given an injection of 0.5 ml of a 1% solution of Evans' blue through a catheter into the parapancreatic cellular tissue and, at the same time, Dalargin was injected subcutaneously in a dose of 60-80 μg/kg. Collection of 30-min portions of the lymph continued thereafter. Every 30 min after the beginning of the experiment, blood samples in a volume of 5 ml were taken from the jugular vein. In control experiments, instead of Dalargin the dogs were given a subcutaneous injection of 1 ml of physiological saline. Altogether 11 experiments were performed, in which either Dalargin or physiological saline was injected.

Entry of Evans' blue into the lymph and blood was identified by a change in the optical density of the samples of lymph and blood serum, measured on the FÉK-256M photoelectric colorimeter (wavelength 656 nm). As a first step, to reduce the turbidity of the lymph, equal volumes of a 1% solution of sodium dodecylsulfate in distilled water were added to the samples. The optical density of the samples was measured in optical density units and expressed as percentages of the initial value.

The numerical results were subjected to statistical analysis by Student's two-way t test.

EXPERIMENTAL RESULTS

After injection of Dalargin into rats in doses of 30-1000 μg/kg the rate of elimination of Evans' blue from the pancreas was found to depend on dose (Table 1). When Dalargin was used in a dose of 1000 μg/kg the rate of disappearance of the dye from the depots was increased by 45.5%. Naloxone, a specific antagonist of opioid receptors, when given simultaneously with Dalargin, completely abolished its effect on the rate of elimination of the dye. Naloxone itself did not affect disappearance of Evans' blue from the depots.

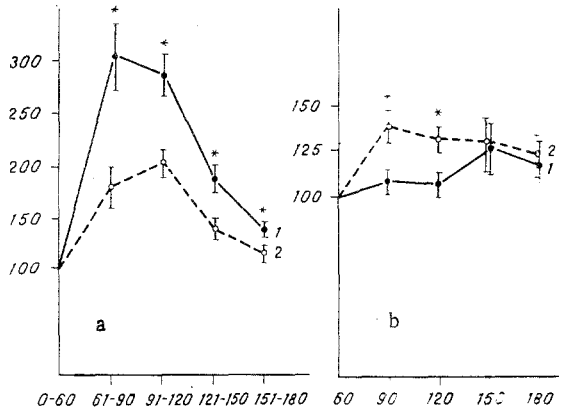

Fig. 1. Effect of Dalargin on concentration of Evans' blue, injected beneath the pancreatic capsule, in thoracic duct lymph (a) and blood plasma (b) of rats (based on changes in optical density). Continuous line — Dalargin; broken line — control. Abscissa, time of taking lymph (a) and blood (b), in min; ordinate, optical density (in %).

The action of Dalargin on pancreatic drainage was compared with the effect of agonists of various subpopulations of opioid receptors (Table 2). Dalargin, in a dose of 100 μg/kg, accelerated elimination of Evans' blue from beneath the pancreatic capsule by 52.6%. Injection of physiological saline alone accelerated elimination of the dye by 22.5%. Thus Dalargin itself accelerated elimination of the dye from the depots by 30.1%. This is about the same as the results of a previous experiment, in which dependence of the effect of Dalargin on dose were studied; it was shown that Dalargin, in a dose of 100 μg/kg, accelerated elimination of the dye by 34%. Meanwhile DAGO and SKF 10047, which are agonists of μ- and σ-opioid receptors, respectively, did not affect the rate of elimination of Evans' blue by comparison with the group of rats receiving physiological saline only. DADLE, an agonist of δ-opioid receptors, on the contrary, slowed elimination of the dye a little.

To determine the precise mechanisms of the stimulating action of Dalargin on pancreatic drainage function, its effect on the passage of Evans' blue, injected beneath the capsule of the dog pancreas, into the lymph and blood was studied (Fig. 1).

It follows from Fig. 1a that injection of Dalargin in a dose of 60-80 μg/kg led to a rapid and significant increase in the concentration of Evans' blue in the thoracic duct lymph, and this increased concentration lasted 2 h. The volume of lymph flowing out through the thoracic duct after injection of Dalargin was unchanged compared with the control (35.0 ± 4.25 and 36.1 ± 2.95 ml/h, respectively).

Data on the effect of Dalargin on passage of Evans' blue into the systemic blood flow are given in Fig. 1b. Clearly Dalargin reduced the concentration of Evans' blue in the blood plasma, mainly during the first hour after injection. The ratio of the optical density of the lymph to the optical density of the blood plasma 30 min after injection of Dalargin was 2.5, compared with 1.0 in the control group.

Thus Dalargin increased elimination of the dye from the pancreatic tissue on account of stimulation of its lymphatic drainage accompanied by simultaneous inhibition of the passage of the dye directly into the blood stream. The action of Dalargin was blocked by naloxone, a specific opioid receptor antagonist. It can accordingly be concluded that the stimulating effect of Dalargin on lymphatic drainage of the pancreas is due to interaction with opioid receptors. Meanwhile specific agonists of μ- and σ-opioid receptors did not affect pancreatic drainage in rats, whereas the δ-opioid receptor agonist actually reduced it. These data suggest that Dalargin affects the pancreatic microcirculation through a special population of opioid receptors, with which their known selective ligands interact only weakly.

LITERATURE CITED

1. V. A. Vinogradov and V. M. Polonskii, Byull. Vses. Kardiol. Nauch. Tsentra, No. 2, 62 (1986).

2. A. K. Georgadze, N. K. Permyakov, V. A. Penin, et al., Farmakol. Toksikol., No. 6, 66 (1986).
3. Yu. N. Levin, Principles of Therapeutic Lymphology [in Russian], Moscow (1986).
4. R. T. Panchenkov, I. V. Yarema, and N. N. Sil'manovich, Lymph Stimulation [in Russian], Moscow (1986).
5. V. G. Smagin, V. A. Vinogradov, and S. A. Bulgakov, Ligands of Opiate Receptors [in Russian], Moscow (1983).
6. V. G. Smagin, V. A. Vinogradov, S. A. Bulgakov, et al., Ter. Arkh., No. 2, 44 (1987).
7. V. K. Khugaeva and V. V. Suchkov, Byull. Vses. Kardiol. Nauch. Tsentra, No. 2, 92 (1980).
8. F. Appia, J. Chariot, C. Roze, et al., Eur. J. Pharmacol., 103, 71 (1984).
9. C. Roze, M. Dubrasquet, J. Chariot, and C. Vaille, Gastroenterology, 79, 659 (1980).