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EFFECT OF THE HEXAPEPTIDE DALARGIN ON ORNITHINE DECARBOXYLASE ACTIVITY IN THE DUODENAL MUCOSA OF RATS WITH EXPERIMENTAL DUODENAL ULCER

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Previously the authors showed that several endogenous opioid peptides and their synthetic analogs have marked antiulcerative activity in rats with an experimental model of cysteamine-induced duodenal ulcer [1, 2].

The substance with the strongest antiulcerative potential was found to be a hexapeptide with the structure Tyr-D-Ala-Gly-Phe-Leu-Arg, which was synthesized in the Laboratory of Peptide Synthesis, Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR (Director M. I. Titov), and which was called dalargin. It differs from the N-terminal fragment of dinorphine in replacement of Gly by D-Ala in position 2. Dalargin has now been successfully used for the treatment of duodenal ulcer in man [3], but the precise mechanisms of its antiulcerative action have not been established.

The aim of this investigation was to study the effect of dalargin on ornithine decarboxylase (ODC; ED 4.1.1.17) in homogenates of the duodenal ulcer from rats with experimental duodenal ulcer induced by cysteamine.

EXPERIMENTAL METHOD

Experiments were carried out on 150 male Wistar rats weighing 200-250 g. The animals were given a single subcutaneous injection of cysteamine hydrochloride (Fluka, Switzerland) in a dose of 350 mg/kg. Immediately thereafter and 12 h later the rats received an injection of dalargin in doses of 12.5 (group 1) and 5000 μg/kg (group 2) or of physiological saline (group 3), also subcutaneously, 12 h later. Group 4 consisted of animals which received a subcutaneous injection of naloxone in a dose of 1 mg/kg simultaneously with dalargin in a dose of 12.5 μg/kg. Rats of group 5 received an injection of physiological saline only. Some rats were decapitated 24 h after the beginning of the experiment, the duodenum was removed, and by means of a binocular loupe, the state of the mucosa was assessed. The parameters of ulcer formation were the ulcer index (UI), the frequency of involvement (FI), and the severity of the lesion (SL), which were determined by the method described previously [2]. Activity of ODC was then determined in homogenates prepared from scrapings of the mucosa [11]. Some animals from each group were decapitated 6 h after the beginning of the experiment and their duodenum was taken for determination of ODC activity in the mucosa before the development of ulcers. ODC activity was determined by the method described pre-

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TABLE 1. Effect of Dalargin on Incidence of Duodenal Ulcers in Rats

Group of animals	No. of animals	Parameter of ulcer formation		
		SL (points)	FI	UI
1) Control (cysteamine)	16	1,87±0,22	0,78	3,43
2) Dalargin, 12.5 μg/kg	16	0,32±0,19*	0,10*	0,52
3) Dalargin, 5000 μg/kg	16	1,73±0,21	0,67	3,07
4) Dalargin, 12.5 μg/kg, naloxone, 1 mg/kg	16	2,14±0,50	0,86	3,86

Legend. Here and in Table 2: *P < 0.05: difference compared with control group significant at the 95% level.

TABLE 2. Effect of Dalargin on ODC Activity in Experimental Ulcer Formation

Group of animals	No. of animals	ODC activity (pmoles $^{14}CO_2$/mg protein/h)
1) Control (cysteamine)	6	8,55±0,64
2) Dalargin, 12.5 μg/kg	6	23,06±4,39*
3) Dalargin, 5000 μg/kg	6	7,45±1,22
4) Dalargin, 12.5 μg/kg, naloxone, 1 mg/kg	6	5,74±0,67*
5) Animal house control	6	7,23±0,77

previously [12]. Activity of the enzyme was expressed in pmoles $^{14}CO_2$/mg protein/h. Protein was determined by Lowry's method. The results were subjected to statistical analysis by Student's t test for ODC activity and SL and also by Pearson's Chi-square test for FI.

EXPERIMENTAL RESULTS

It follows from Table 1 that dalargin in a dose of 12.5 μg/kg sharply reduced duodenal ulcer formation, so that UI fell by 85%. In a dose of 5000 μg/kg dalargin had no effect on ulcer formation. The antiulcerative action was completely blocked by naloxone.

No significant changes in ODC activity compared with intact animals were observed 6 h after administration of cysteamine in any of the groups (results not given). Dalargin in a dose of 12.5 μg/kg, preceded by cysteamine, increased ODC activity 24 h after the beginning of the experiment by 3.2 and 2.7 times, respectively, compared with the control level and the level in rats receiving cysteamine alone, respectively. Naloxone completely blocked this

effect of dalargin; ODC activity was depressed even below the control level (Table 2). Dalargin in a dose of 5000 μg/kg, given after cysteamine, had no effect on ODC activity (Table 2).

ODC is an enzyme which controls the rate of synthesis of the natural polyamines spermidine and spermine, which participate in the regulation of many intracellular processes [15]. We know that the concentration of polyamines and ODC activity in the tissues are increased during growth and regeneration and in other situations when protein biosynthesis is intensified. The present results suggest that the antiulcerative effect of dalargin may be linked with stimulation of protein synthesis in the duodenal mucosa, i.e., ultimately with stimulation of its regenerative potential.

Movements, secretion, and other functions of the gastrointestinal tract are known to be controlled by central and peripheral opioidergic mechanisms [13]. Since dalargin, in doses inhibiting ulcer formation in rats does not pass through the blood-brain barrier [5], it can be tentatively suggested that stimulation of ODC in the duodenal mucosa is effected through peripheral mechanisms. Enkephalins have been found in the small intestine by immunohistological methods in the bodies of nerve cells of the myenteric plexus and in nerve fibers running in the composition of the myenteric, deep muscular, and submucous nerve plexuses and penetrating the circular muscles [7, 9, 14]. In the large intestine and stomach the distribution of enkephalins is roughly the same, with certain quantitative differences [9, 14]. Endocrine cells containing enkephalins have been found in the stomach [8]. Receptors for ^{3}H-D-Ala^{2}, D-Leu^{5}-enkephalin (which interacts mainly with opioid receptors of the "delta" subtype and rather less well with receptors of the "mu" subtype) have been found autoradiographically in the mucosa, nerve plexuses in the submucosa, and in the deep intermuscular plexuses [13]. Enterocytes have been shown to be able to bind specifically with enkephalins [10] and naloxone [6]. Incubation of enterocytes in the presence of Leu^{5}-enkephalin [10] or dalargin [4] leads to activation of adenylate cyclase; in the case of dalargin, the effect is reduced in high concentrations of the peptide and in the presence of naloxone. All these findings indicate that stimulation of ODC and the antiulcerative effect of dalargin may be due to direct interaction of the peptide with cells of the intestinal mucosa and, in particular, with enterocytes.

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