EFFECT OF THE ANTIULCERATIVE ACTION (CENTRAL OR PERIPHERAL) OF THE SYNTHETIC ENKEPHALIN ANALOG DALARGIN IN EXPERIMENTAL CYSTEAMINE-INDUCED DUODENAL ULCER IN RATS

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In previous investigations the writers showed that many endogenous opioid peptides and their synthetic analogs possess high antiulcerative activity in rats with experimental cysteamine-induced duodenal ulcers [1, 2], a condition which closely resembles duodenal ulcer in man. An encephalin analog, Dalargin, which has the structure Tyr-D-Ala-Gly-Phe-Leu-Arg and possesses the strongest antiulcerative activity under experimental conditions, has now been successfully used for the treatment of duodenal ulcer in man [4], although the precise mechanisms of its protective action on the duodenal mucosa have not been finally settled. The aim of this investigation was to establish the role of central and peripheral opiate receptors in the antiulcerative action of Dalargin.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 160-200 g. Duodenal ulcers were produced with the aid of cysteamine hydrochloride (from "Fluka," Switzerland), which was injected subcutaneously in a single dose of 350 mg/kg. Dalargin also was injected in a single dose, either of 10 μg/kg subcutaneously or of between 0.02 and 2 μg into the lateral cerebral ventricle simultaneously with cysteamine. The rats were decapitated 24 h later, the stomach and duodenum removed, and the severity of the lesion (SL) of the mucosa was estimated visually, with the aid of a binocular loupe, using a point system suggested by the writers previously [1]; the frequency of appearance of ulcers (FU) also was determined as the ratio of the number of animals with ulcers to the total number of animals in the group. For an integral evaluation of ulcer formation in each group the ulcer index (UI) was used, and was calculated by the equation:

$$UI = SL + 2FU.$$

A separate experiment was undertaken to study the passage of Dalargin through the blood-brain barrier. For this purpose rats were anesthetized with pentobarbital (25 min) and 5 μ Ci of ^{3}H-D-Ala^{2},D-Leu^{5}-enkephalin (^{3}H-DADL) in 20 μl of physiological saline was injected into the cisterna magna. The rats were decapitated 10 min after the end of injection of the preparation, the medulla was quickly removed and homogenized in 5 ml of physiological saline in a "Polytron" homogenizer, aliquots of 0.5 ml of the homogenate were filtered through GF/C filters (Whatman), the filters were washed with cold physiological saline, and the radioactivity retained by the filters was measured. Dalargin (in doses of 0.1 μg/kg to 10 mg/kg) was injected intraperitoneally 5 min before the beginning of injection of the label.

The results were subjected to statistical analysis, for SL by Student's t test, and for FU by Pearson's chi-square test. The level of significance of differences was 95%.

EXPERIMENTAL RESULTS

The dose of Dalargin (10 μg/kg) for peripheral administration was chosen as optimal on the basis of the results of a previous investigation [4], which showed that the antiulcerative action of Dalargin in low doses is dose-dependent in character, but with an increase in

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TABLE 1. Effect of Peripheral Administration of Dalargin on Ulcer Formation in Rat Duodenum

Parameter of ulcer formation	Control	Dalargin
SL, points	3.00±0.37	0.5±0.33*
FU	1.0	0.25*
UI	5.0	1.00

Legend. Asterisk indicates that difference from control is significant at the 95% level ($p < 0.05$).

TABLE 2. Effect of Central Administration of Dalargin on Ulcer Formation in Rat Duodenum

Parameter of ulcer formation	Control	Dalargin, μg		
		0,02	0,2	2
SL, points	2,33±0,37	2,29±0,18	2,25±0,16	0,89±0,35*
FU	0,89	1,0	1,0	0,44*
UI	4,11	4,29	4,25	1,77

Legend. Asterisk indicates that difference from control is significant at the 95% level ($p < 0.05$).

TABLE 3. Suppression of ^{3}H-DADL Binding in Medulla by Peripherally Injected Dalargin

Dose of Dalargin, μg/kg	^{3}H-DADL binding, % of control
0	100
0.1	96
1	106
10	97
100	90
500	65
1 000	31
5 000	18
10 000	15

dose above 10 μg/kg the antiulcerative action was weakened and then disappeared completely. A dose-effect curve of similar character was observed by other workers when studying activity of opioids in other experimental models [5]. The causes of this "sliding" of the effect of the opioids with an increase in dose has not been satisfactorily explained but has been interpreted as the result of complex interaction between opioids and various populations of opiate receptors [7].

Peripheral administration of Dalargin caused a sharp reduction in the severity of ulcer formation in the rat duodenum (Table 1). Under these circumstances SL was reduced by six times, FU by four times, and UI by five times. After central (intraventricular) administration, the antiulcerative activity of Dalargin fell (Table 2). For instance, in doses of 0.02 and 0.2 μg Dalargin had no effect on ulcer formation. In a dose of 2 μg Dalargin lowered the index of ulcer formation by 2-2.5 times compared with the control group. It can thus be postulated that the antiulcerative action of Dalargin is mediated primarily by peripheral opiate mechanisms, and the effect of the maximal dose of Dalargin when injected centrally can be explained by its passage into the systemic circulation [6].

The ability of Dalargin, injected peripherally, to penetrate into the brain through the blood-brain barrier was investigated by studying its ability to inhibit binding of centrally administered ^{3}H-DADL in the medulla. A preliminary experiment showed that peripherally injected naloxone (0.5 and 1 mg/kg) reduced binding of ^{3}H-DADL by 75-80%. Since 65-70% of the radioactivity from the homogenates of the medulla retained by the filters was in the form of ^{3}H-DADL and its N-terminal tetrapeptide, it can be tentatively suggested that binding of ^{3}H-DADL in the medulla is due to its interaction with opiate receptors. It will be clear from Table 3 that in a dose of 0.1-100 μg/kg Dalargin had virtually no effect, but in a dose of 500 μg/kg to 10 mg/kg it dose-dependently blocked binding of ^{3}H-DADL, i.e., starting from a dose 50 times greater than optimal.

When these results are assessed, it can be postulated that the antiulcerative action of Dalargin, when injected peripherally in small doses, is due to interaction with peripheral opiate receptors. With a marked increase in the dose of Dalargin, it is able to pass through the blood-brain barrier and to interact with central opioid mechanisms. The absence of antiulcerative activity under these circumstances may be linked with activation of central opioid

mechanisms, leading to inhibition of the peripheral protective effects of Dalargin. This hypothesis is indirectly confirmed by the fact that rats with depressed sensitivity to pain, due most probably to increased activity of the central opioidergic system, are predisposed to the development of cysteamine-induced duodenal ulcers [3]. It can be postulated on the basis of these results that the disturbed relations between peripheral and central opioid activity may play an essential role in the pathogenesis of peptic ulcers. In that case Dalargin, as an opioid peptide with peripheral action, can be regarded as a remedy with a true pathogenetic action on the course of peptic ulcer.

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