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EFFECT OF β-ENDORPHIN ON G CELLS IN RATS WITH EXPERIMENTAL DUODENAL ULCER

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UDC 616.342-002.44-02:547.943:547.95/-092.9

KEY WORDS: β-endorphin; experimental duodenal ulcer; Grimelius-positive cells; G cells.

β-endorphin is an endogenous opioid substance that is distributed mainly in parts of the CNS responsible for pain sensation and emotions. Endorphins have recently been found in the gastrointestinal tract. We now know that the discovery of opioid peptides in the alimentary tract is not accidental, for they have a protective action on the gastric and duodenal mucosa in rats with cysteamine-induced duodenal ulcer [1] and they also inhibit gastric secretion [3] and affect the blood gastrin level [2].

The aim of the present investigation was accordingly to study the effect of β-endorphin on gastrin producing cells in the mucosa of the antral portion of the stomach in rats with experimental duodenal ulcer.

EXPERIMENTAL METHOD

Experiments were carried out on 40 male Wistar rats weighing 150-200 g. The rats were divided into four groups with 10 animals in each group. Rats of group 1 (control) received 0.2 ml of physiological saline, animals of group 2 received a single subcutaneous injection of 350 mg/kg of cysteamine hydrochloride, rats of group 3 received cysteamine and also β-endorphin in a dose of 300 nmoles/kg twice a day (4 times altogether), and animals of group

Fourth Main Board, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 1, pp. 28-31, January, 1984. Original article submitted March 25, 1983.

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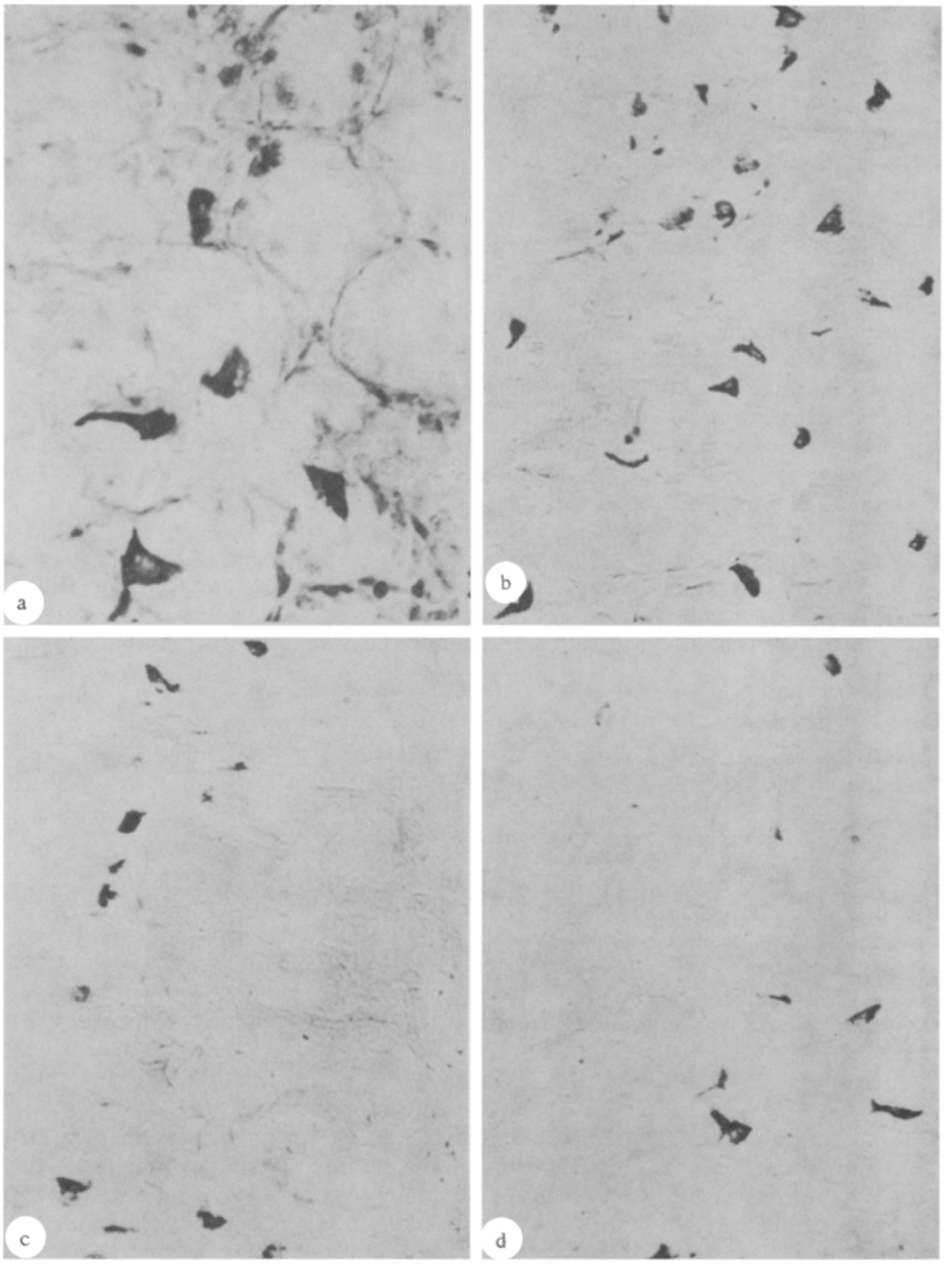

Fig. 1. Endocrine cells positively stained by Grimelius' in the antral portion of the rat stomach: a) control, b) 48 h after injection of cysteamine; c) 48 h after injection of cysteamine and β-endorphin; d) 48 h after injection of cysteamine, β-endorphin, and naloxone. Staining by Grimelius' method. Magnification: a) 600, b-d) 3.75.

4 received naloxone in a dose of 500 nmoles/kg simultaneously with β-endorphin. The animals were decapitated 48 h after receiving cysteamine, the antral portion of the stomach was removed and, after preliminary orientation, it was fixed in a mixture consisting of a saturated solution of picric acid and neutral formalin (3:1), and embedded in paraffin wax. Serial sections 4 μ thick were stained with hematoxylin and eosin, impregnated with silver by Grimelius' argyrophilic method, which stains nearly all types of endocrine cells [12], and stained immunohistochemically with peroxidase—antiperoxidase complex (PAP, from Miles, England), by Sternberger's method [11]. Cells producing gastrin-17 were demonstrated by Sternberger's method in the mucosa of the antral portion of the rats' stomach. Antiserum against gastrin was obtained by immunizing a rabbit with synthetic gastrin-17 after conjugation with bovine serum albumin with the aid of *bis*-diazotized benzidine [4]. The antiserum possessed

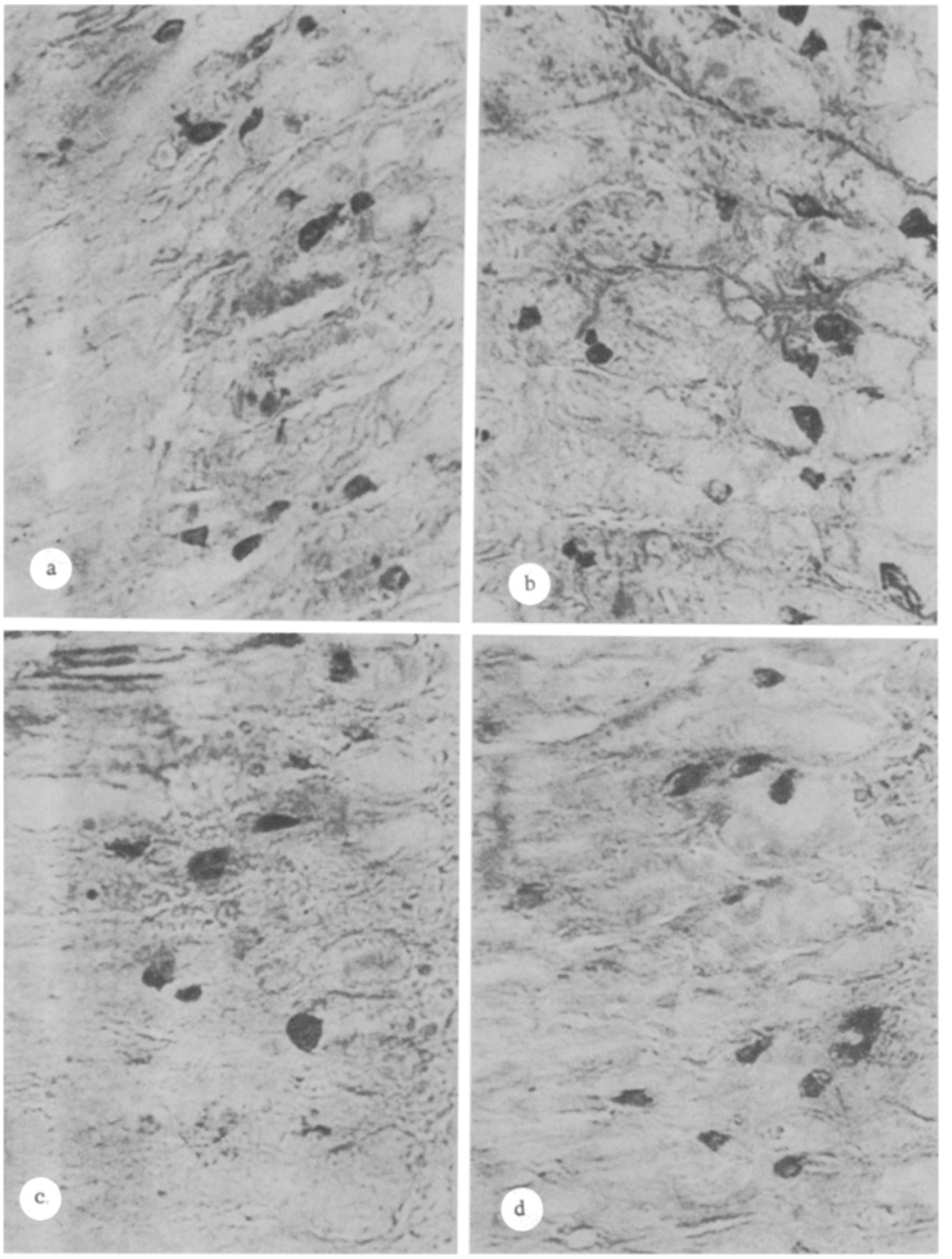

Fig. 2. G-cells in antral portion of rat stomach: a) control; b) 48 h after injection of cysteamine; c) 48 h after injection of cysteamine and β-endorphin; d) 48 h after injection of cysteamine, β-endorphin, and naloxone. Immunohistochemical staining with PAP complex. Magnification: 375.

high specificity: Antibodies against gastrin-17 did not interact with cholecystokinin or cerulein and cross-reacted with gastrin-34 to the extent of only 2% (radioimmunoassay data).

Endocrine cells were counted in an area of 1 mm^2 of gastric mucosa in each preparation in 7-10 fields of vision. The numerical results were subjected to statistical analysis by Student's t test; differences were taken to be significant at the 95% level ($P < 0.05$).

EXPERIMENTAL RESULTS

In the control group endocrine cells stained by Grimelius' method (Fig. 1a) were located in the cervical portion and upper half of the pyloric glands of the stomach. They were pyramidal, triangular, or oval in shape. Their mean number was 285.0 ± 10.0/mm^2. G cells in the antral portion were mainly oval or pyramidal in shape (Fig. 2a) and were located in the cervical portion and upper third of the glands. Their number varied from 165 to 297/mm^2 (mean 220.0 ± 20.0/mm^2).

During the development of duodenal ulcers after injection of cysteamine the number of endocrine cells (Fig. 1b) in the gastric mucosa increased to 342.0 ± 17.0/mm^2, significantly more than in the control. The focal arrangement of the endocrine cells was disturbed with a tendency toward diffuse distribution. The number of gastrin-producing cells also was increased on average 283.0 ± 13.0/mm^2 (Fig. 2b; $P < 0.05$).

The total number of endocrine cells in the group of animals receiving β-endorphin varied from 173.0 to 349.0/mm^2, on average 263.0 ± 21.0/mm^2 (Fig. 1c). The number of G cells was the same as in the control, namely 230.0/mm^2 (Fig. 2c). The opioid peptide thus blocked the increase in the number of endocrine cells in the gastric mucosa caused by cysteamine.

In rats receiving β-endorphin together with naloxone, the number of endocrine cells stained in the silvering reaction consisted on the average of 240.0 ± 12.0/mm^2 (Fig. 1d). The number of cells producing gastrin was found to be within normal limits (159.0 to 305.0/mm^2) and constituted on the average 236.0 ± 17.0/mm^2 (Fig. 2d).

The effect of β-endorphin on endocrine cells of the stomach in rats with experimental duodenal ulcer was thus discovered for the first time.

Staining by Grimelius' method is known to reveal mainly EC- and G-cells in the antral portion of the stomach; the antral G-cells, which produce somatostatin, are not revealed [12]. By a combination of this staining method with immunocytochemical assay of G-cells it is thus possible to detect changes both in EC-cells and in G-cells in cysteamine-induced ulcer.

Injection of cysteamine sharply reduces the somatostatin concentration in many tissues, including the stomach [10], and as a result, gastrin production may be increased. The increase in the number of G-cells found in this investigation is probably linked with the more intensive synthesis of gastrin and an increase in its content in the endocrine cells. There is a parallel rise in the blood gastrin concentration [1], accompanied by increased gastric secretion, and this is one factor in the pathogenesis of cysteamine ulcers [6]. The number of EC cells calculated by subtracting the number of gastrin-producing cells from the total number of Grimelius-positive cells was unchanged under these circumstances.

The increase in the number of G-cells was blocked by β-endorphin and this effect was not abolished by naloxone, an antagonist of opiate receptors, probably on account of a special population of receptors. Incidentally, naloxone-resistant effects of endorphins have been observed in various peripheral tissues and, in particular, in the gall bladder [5] and stomach [8]. A special population of ε-receptors, with which β-endorphin interacts specifically, has now been described [7].

The action of β-endorphin on endocrine cells may be due to several causes: increased somatostatin formation, switching of synthesis in G-cells to the formation of other peptides, such as ACTH and enkephalins [9]. However, the most likely cause is depression of gastrin synthesis, for we know that endogenous opioids block the rise in its blood level in cysteamine-induced ulcer [1], and lower the secretion of hydrochloric acid in the stomach [3]. The decrease observed in the number of gastrin-producing cells under the influence of endorphin is in agreement with data on its protective effect on the duodenal mucosa in rats with cysteamine-induced ulcer [2].

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