ENDORPHIN-CONTAINING CELLS IN THE ANTRAL MUCOSA OF THE STOMACH IN DUODENAL ULCER

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Endorphins belong to the class of endogenous opioid peptides and, depending on the size of their molecule, they are subdivided into α-, β-, and γ-endorphins. Endorphins are known to be contained in the pituitary gland and in the central and peripheral nervous system, where they are formed from their biosynthetic precursor pro-opiomelanocortin [8]. Other peptide hormones also are formed from pro-opiomelanocortin: ACTH and α-, β-, and γ-melanocyte-stimulating hormones [1]. All these degradation products of pro-opiomelanocortin are found in the gastrointestinal tract; immunoreactive ACTH is found, moreover, in the G-cells of the antral portion of the stomach [6], along with immunoreactive methionine-enkephalin [13]. Endogenous opioids have a protective action on the gastric and duodenal mucosa in which pathological changes have been induced experimentally [2, 4].

The object of this investigation was an immunohistochemical study of endorphin-producing cells in the antral mucosa of the stomach in patients with duodenal ulcer.

EXPERIMENTAL METHOD

The investigation was conducted on 22 men aged from 22 to 47 years, 12 of whom had duodenal ulcer in a stage of exacerbation, whereas the other 10, forming the control group, had superficial gastritis. The duration of the duodenal ulcer varied from a few months to 17 years.

During endoscopy two biopsy specimens were taken from the antral portion of the stomach. The specimens were fixed in a mixture of a saturated solution of picric acid and neutral formalin (3:1). Serial sections 4 μ thick were stained with hematoxylin and eosin and also immunohistochemically by means of a peroxidase—antiperoxidase complex (PAP, from Miles Laboratories, England), by Sternberger's method [11]. Antisera against α- and γ-endorphins were used as "first" antibodies. These antisera were obtained by immunizing rabbits with synthetic endorphins (synthesized in the Laboratory of Peptide Synthesis — Head M. I. Titov — All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR) after conjugation with bovine serum albumin by the aid of bis-diazotized benzidine [1]. The antisera possessed high specificity: antibodies against γ-endorphin did not interact with other endorphins, with enkephalins, or with LH releasing hormone, gastrin, neurotensin, or cholecystokinin. Antibodies against α-endorphin likewise did not interact with the above peptides, but they had cross-reactivity with γ-endorphin (about 10%, as shown by radioimmunochemical testing).

The stained cells were counted by means of a special grid in 7-10 fields of vision in each preparation and their number was expressed per square millimeter. Statistical analysis was carried out by Students' t test at a 95% level of significance ($P < 0.05$).

EXPERIMENTAL RESULTS

The mucosa of the subjects of the control group was normal in structure. The lamina propria was infiltrated by lymphocytes and plasma cells. Normally cells producing γ-endorphin were oval or pyramidal in shape (Fig. 1) and were distributed in the epithelium of the neck portion and upper third of the pyloric glands. Their number varied from 84 to 181/mm^2 (mean

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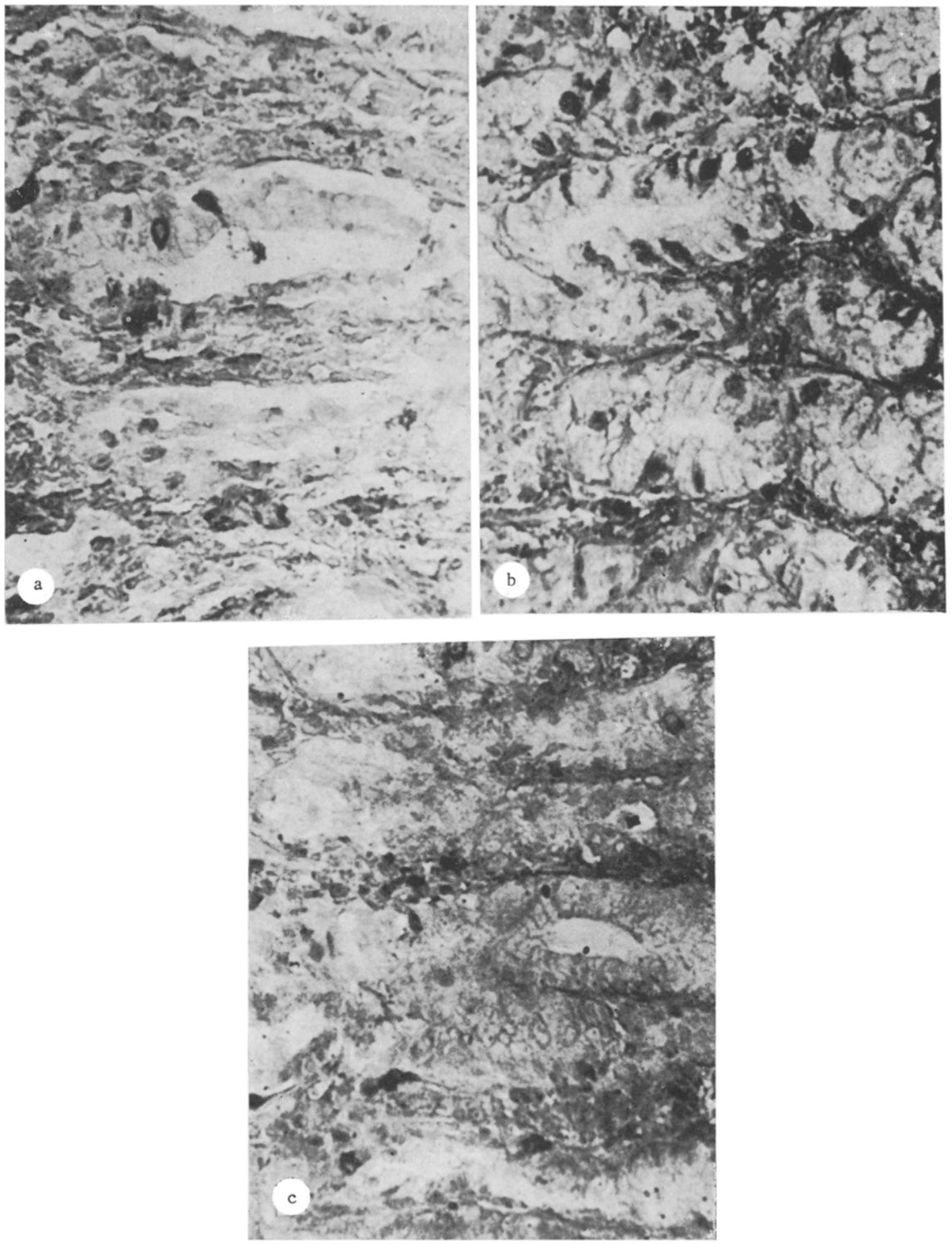

Fig. 1. Endorphin-producing cells in mucosa of antral portion of stomach. a) γ-Endorphin-producing cells of control group, b) γ-endorphin-producing cells in duodenal ulcer, c) α-endorphin-producing cells in stroma and epithelium. Immunohistochemical method of staining with PAP complex. 375×.

118.0 ± 10.0/mm^2). These cells were similar both in shape and in localization with gastrin-producing G cells. Enkephalins [13] and ACTH [6] are known to be found in G cells. The discovery of γ-endorphin by means of our antiserum in these (or similar) cells may be evidence that the cells of the antral portion of the stomach contain pro-opiomelanocortin [9, 12] and that it can be hydrolyzed to γ-endorphin.

Cells producing α-endorphin were found both in the epithelium of the upper third of the pyloric glands and in the lamina propria of the mucous membrane. Their average number was 11.0 ± 2.0 cells/mm^2. These cells were mainly oval in shape (Fig. 1). Cells in the stroma were mainly large in size, oval in shape, with a central nucleus (Fig. 1c), and less frequently they were smaller, but with the nucleus similarly positioned. They were found chiefly in the zone from the middle third of the glands to the muscular lamina propria of the mucous membrane. The number of cells varied from 4 to 34, on average 14.5/mm^2. It is not yet possible to characterize cells of this type exactly. They are considered to be plasma cells [5]. The presence of immunoreactivity of α-endorphin in the pyloric glands can be explained by cross interaction between antibodies and other breakdown products of pro-opiomelanocortin, but the possibility cannot be ruled out that, besides enkephalins and γ-endorphin, α-endorphin may also be formed in several such cells.

In the patients with duodenal ulcer an almost twofold increase in the number of cells producing γ-endorphin was found (Fig. 1c). The average number of these cells was 216.0 ± 28.0/mm^2 (from 81 to 362/mm^2), significantly more than in the control ($P < 0.05$). The increase in the number of γ-endorphin-producing cells in duodenal ulcer may play a compensatory role, for opioid peptides and, in particular, γ-endorphin has been shown to have a protective action on the mucous membrane of the stomach and intestine under conditions of experimental ulcer formation [3]. Opioids also have an acid-inhibiting effect [4], which may also be compensatory in its importance. Very probably endorphins are formed in the epithelium of the glands in the same cells as gastrin [7, 13] and they may limit its liberation in a similar manner to the mechanism of catecholamine secretion in the adrenal medulla under the influence of intracellular opioids [10]. In that case the increase in the number of endorphin-producing cells in peptic ulcer may have a protective role.

The number of cells stained with antiserum against α-endorphin in the epithelium of the gastric mucosa was considerably reduced in peptic ulcer to 4.5 ± 1.6/mm^2. This fact is evidence in support of the presence of α-endorphin in cells of the gastric epithelium, for in the case of cross reactions of antiserum with γ-endorphin the number of these cells must be increased parallel with the increase in the number of γ-endorphin-containing cells. The physiological significance of this change is not yet clear.

The number of cells containing α-endorphin in the stroma of the mucous membrane was the same as in the control (13.8 ± 2.3/mm^2).

The use of an immunohistochemical method thus revealed the presence of endocrine cells containing α- and γ-endorphins in the antral mucosa of the stomach. Some cells of the stroma also contain α-endorphin. The number of γ-endorphin-containing cells was increased in duodenal ulcer, and this may be of compensatory importance, bearing in mind the antisecretory and protective effect of endorphins on the stomach and duodenum [3, 4].

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EFFECT OF NEONATAL SYMPATHECTOMY ON SYSTEMIC HEMODYNAMICS AND MYOCARDIAL CONTRACTILITY IN SPONTANEOUSLY HYPERTENSIVE RATS

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A study of the role of the sympathetic nervous system in the genesis and development of spontaneous (hereditarily determined) hypertension in rats [13, 14] showed that at least one of the genetic defects responsible for the development of spontaneous hypertension consists of increased activity of the peripheral part of the sympathetic nervous system and a decrease in the inhibitory noradrenergic influences of the brain [14]. To study this problem more recently, besides determining catecholamines (CA), various methods of total sympathectomy also have been used, including immunologic and chemical methods [4, 6, 7, 9], with the aid of guanethidine and 6-hydroxydopamine (6-OH-DA) [5]. However, the results have been very contradictory. According to some workers, chemical or immunologic sympathectomy alone is insufficient to destroy sympathetic neurons and nervous pathways, and the two methods have to be combined for this to be done [7]. Morever, in most investigations the effect of sympathectomy was judged purely by changes in arterial pressure (BP) and heart rate (HR) [9, 11]. Changes in the systemic hemodynamics in spontaneously hypertensive rats (SHR) subjected to immunologic sympathectomy have been described in only a few publications [6]. The present writers showed previously that the early hypertensive stage in SHR is characterized by a hyperkinetic type of circulation. Activation of adrenergic influences on the heart plays an important role under these circumstances [2, 3].

In the investigation described below the effect of neonatal chemical sympathectomy by 6-OH-DA on the systemic hemodynamics and myocardial contractility in SHR was studied. Two problems were to be solved: 1) Is an intact sympathetic nervous system essential for the development of hypertension in SHR and also to maintain the normal BP level in normotensive animals? 2) What are the hemodynamic effects of sympathectomy due to 6-OH-DA in the rats of the above-mentioned two groups?

EXPERIMENTAL METHOD

Neonatal sympathectomy was induced in normotensive rats (NR) and SHR by means of 6-OH-DA-HCl. On the 1st and 2nd days after birth of the animals the compound was injected subcutaneously in a dose of 100 μg/g body weight per animal, and on the 8th and 15th days the injection of the compound was repeated, but this time in a dose of 250 μg/g. The rats were used in the experiments at the age of 10-12 weeks. NR and SHR with an intact sympathetic nervous system served as the control. Under pentobarbital anesthesia (50-60 mg/kg) and with artificial respiration by means of a "Harvard" respirator (model 860, USA), thoracotomy was performed and the transducer of an RT-400 electromagnetic flow meter (Narco Biosystems, USA) was applied to the ascending aorta to record the cardiac output (after deduction of the coronary blood flow). BP was measured through a catheter introduced into the common carotid artery by means of a Statham P23-1D electromagnetic transducer. Myocardial contractility was judged by analysis of the systolic ejection curve, using a special ED600G differentiator (Ni-

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