Effect of Vagotomy on Expression of Neuropeptides and Histamine in Rat Oxyntic Mucosa

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The effect of vagotomy and pyloroplasty on the density of nerve fibers containing bombesin/gastrin-releasing peptide (GRP), calcitonin gene-related peptide (CGRP), and galanin as well as histamine-, 5-hydroxytryptamine (5-HT)-, and somatostatin-containing cells in the oxyntic mucosa of the rat stomach was studied. Ten days after vagotomy and pyloroplasty the density of histamine-containing cells in the oxyntic mucosa was increased by 70% (P < 0.05), and these cells were larger and showed more extensive cell processes than in control animals. The density of 5-HT-immunoreactive (IR) cells and somatostatin-IR cells were not affected. A marked decrease in the density of CGRP-IR nerve fibers and a slighter decrease in the density of GRP-IR nerve fibers was observed in the mucosal layer, while only a minor reduction of CGRP-IR fibers, and no reduction of GRP-IR fibers was seen in the muscular layer. The density of galanin-IR nerve fibers was not affected. The height of the oxyntic mucosa was reduced by about 25% (P < 0.05). Thus, a striking effect on the histamine-IR cells was seen, supporting the view that these cells are regulated by the vagus nerve. The study also indicates that a major portion of the CGRP-IR nerve fibers, and part of the GRP-IR nerve fibers, in the mucosal layer of the fundic region are of vagal origin or regulated by normal vagus nerve activity.

KEY WORDS: gastric mucosa; vagotomy; bombesin/gastrin-releasing peptide; calcitonin gene-related peptide; galanin; histamine; 5-hydroxytryptamine; somatostatin.
function, and the evidence for direct and indirect influence of vagal nerve activity on this regulation, new interest is raised about the effect of vagotomy on the gastric mucosa. Therefore, in the present study the effects of vagotomy on mucosal structure and on the number of mucosal GRP-, CGRP-, and galanin-containing nerve fibers as well as 5-HT-, histamine-, and somatostatin-containing cells in the rat stomach were investigated.

MATERIALS AND METHODS

Subdiaphragmatic bilateral truncal vagotomy with pyloroplasty was performed in 25 Wistar rats (250-300 g) under ether anesthesia. Sixteen unoperated and 20 sham-operated rats served as controls. After a postoperative period of 10 days, the rats were processed for immunocytochemical demonstration of GRP, CGRP, GAL, somatostatin (SOM), 5-HT, and histamine. Before sacrifice the animals were fasted overnight (having free access to water).

Immunohistochemical Procedure. The operated and control rats were anesthetized with sodium pentobarbital (Mebunat, Orion), 40 mg/kg body weight intraperitoneally, and perfused through the left ventricle of the heart either with 4% paraformaldehyde, or, for the demonstration of histamine, with 4% carbodiimide in 0.1 M sodium phosphate buffer, pH 7.4. The stomach was dissected out and opened along the major curvature, the antral portion was removed under a dissection microscope, and the fundic portion flattened against a wax plate. Thereafter 1- to 2-mm-wide slices of fundic mucosa were cut in three systematic directions, the first direction being selected at random (17). The slices were divided in 5-mm-long segments, and from this set of segments every fourth was systematically sampled and immersed in the same fixative for 2 hr (paraformaldehyde) or overnight (carbodiimide) at 4°C. After fixation, the specimens were transferred into 0.1 M sodium phosphate buffer, pH 7.4, containing 20% sucrose, for at least 24 hr at 4°C. Tissue specimens were frozen with carbon dioxide ice, and 10-120 μm cryostat sections were obtained under a dissection microscope, and the sections were mounted on chrome-alum-gelatin-coated glass slides and processed for indirect immunofluorescence. The sections were then rinsed twice in PBS for 10 min and mounted in a mixture of glycerol and PBS (1:1). The specimens were examined with a Leitz Aristoplan fluorescence microscope equipped with epillumination and a specific filter block I 2 for FITC.

In control incubations the primary antiserum was omitted or replaced with normal rabbit serum. Preabsorption of the peptide antiserum with 1 μM of the corresponding peptide totally abolished immunostaining. Preabsorption of the CGRP, GRP, and galanin antisera with 1-10 μM substance P did not affect immunostaining.

Evaluation of nerve fiber density in the gastric wall specimens was performed visually by three independent observers. The number of immunoreactive fibers in the different sections were ranked from - to +, +++, +++++, or ++++++, and the codes of studied glasses were opened later. The photographs were taken on Kodak T-Max film using an automatic Vario-Orthomat microscope camera.

Morphometry. In order to measure the area of the oxyntic mucosa, the opened stomach, after removal of the antrum, was flattened against a wax plate, a transparent plastic sheet was overlaid, and the borders of the oxyntic mucosa were outlined. The area was determined from these tracings by planimetry.

For the determination of mucosal thickness, the sections were photographed with a phase-contrast microscope under low magnification, and the height of the mucosal layer measured from three systematically selected locations in each projected negative.

Cell density was estimated by counting the number of immunoreactive cells from the enlarged negatives of micrographs taken from four areas selected systematically from the set of sections (eyepiece ×5, objective ×25) (19). As the height of the mucosa was affected by vagotomy, a field (600 μm × 900 μm) was chosen that contained the whole mucosal layer in normal animals. The same field was used for all determinations, although the mucosa occupied a varying proportion of this field. This gives an estimate of cell density related to linear length of the basis of the mucosa. The value is determined by cell number, but also cell size and shape, as cellular enlargement tends to increase the number of cell transections in individual sections (20).

Statistical comparison between groups was made with Student's t test.

RESULTS

Control Animals

Unoperated and sham-operated rats were used as control animals. GRP-, CGRP-, and galanin-IR nerve fibers were detected in different layers of gastric wall in all control animals. No differences in immunohistochemical distribution of these neuropeptides were observed between these two groups.

GRP Immunoreactivity. GRP-IR nerve fibers were observed in the oxyntic mucosa, submucosa,