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Peptide-Containing Nerve in the Rat Stomach: An Immunohistochemical Study

Hisao ITO, Yoshiro KATO, Hiroshi YOKOZAKI and Eiichi TAHARA

The First Department of Pathology, Hiroshima University School of Medicine, 1-2-3, Kasumi, Minami-ku, Hiroshima 734 Japan

ABSTRACT

The distribution of peptide-containing nervous systems in the rat glandular stomach was studied immunohistochemically by use of the cryostat sections and whole mount preparations. A variety of peptide-containing nerve fibers made a dense network in the mucosa, submucosa, smooth muscle layers and intramural ganglia. Distribution density was the highest for nerve fibers containing vasoactive intestinal peptide (VIP) followed by calcitonin gene-related peptide, bombesin, and substance P. Peptide histidine isoleucine was detected in a few VIP-containing nerve fibers. Galanin, beta-endorphin and neuropeptide Y were also found in a variable density, mainly in the muscle layers. Somatostatin and gastrin were localized in the gut endocrine cells in contrast to their absence in the nervous systems. Truncal vagotomy did not affect the density nor distribution of various peptide-containing nerve fibers. In view of their distribution, the possible physiological roles of these peptides in the nervous systems on the regulation of the rat stomach were discussed.

Key words: *Neuropeptide, Rat stomach, Nervous systems, Immunohistochemistry*

The gastrointestinal tract is rich in neuropeptides which may play an important role on the regulation of gastrointestinal functions. These neuropeptides are present in both the gut endocrine cells and nervous systems throughout the gastrointestinal tract. The distribution of peptide-containing neuronal elements has been studied immunohistochemically mainly in the intestine[3,5-8], but only a few studies have been made on the stomach[9,10,14]. Conventional neurotransmitters, such as acethylcholine and norepinephrine are well known as the neurotransmitters of the digestive tract. On the other hand, the physiological significance of peptide-containing nerves is not yet well elucidated, although pharamacologic examinations have been made on individual peptides[1,12,18].

The purpose of the present study was to ascertain the distribution of various peptide-containing neuronal elements in the rat stomach using cryostat sections and whole mount preparations. The possible role and physiological significance of these neuronal elements on the regulation of the rat stomach are also discussed.

MATERIALS AND METHODS

A total of 15 adult Wistar rats of both sexes weighing 180—260g were used in this study. Three rats were subjected to subdiaphragmatic truncal vagotomy and were killed 7 days later.

Preparation of Tissue:

Animals were anesthetized with diethyl ether and perfused via the heart with ice-cooled saline. The stomach was removed, opened along the greater curvature and then immersed in Zamboni's fixative for 24 hrs. After thorough rinsing by 0.1M phosphate buffer with 10% sucrose for at least 24 hrs, the specimens were frozen and sectioned in a cryostat at 12—16 μm in thickness. After allowed to dry in air at room temperature for at least 2 hrs the sections were immunostained by indirect method after Nakane[16].

Five rats were examined for the distribution of the nerve fibers containing calcitonin gene-related peptide (CGRP) and vasoactive intestinal peptide (VIP) by whole mount preparations as reported by Costa et al[4]. Briefly, the stomachs were stretched in a 1 × 1 cm segment and pinned flat on a piece of balsa wood with the mucosal side facing the wood. The specimens were fixed with Zamboni's solution for 24 hrs and then washed in 80% ethanol for 24—48 hrs until cleared of picric acid. They were cleared in 100% xylol for 30 min after dehydration, and then were rehydrated through 100%, 80%, 50% ethanol to PBS. The outer (longitudinal) and inner (circular) muscle layers and mucosa-submucosa were separated from each other with a pair of forceps under a stereoscopic microscope.

Immunohistochemistry:

The cryostat sections and whole mount preparations were incubated with primary antibodies at room temperature for 2 and 24 hrs, respectively.

After washing in PBS four times each for 20 min, the sections were incubated with peroxidase labeled secondary antibody for 1 hr.

Antibodies:

The following antibodies were used as primary antibodies in this study. Rabbit antibodies to vasoactive intestinal peptide (VIP; working dilution 1:15000), peptide histidine isoleucine (PHI; 1:1500), neuropeptide Y (NPY: 1:1200), peptide YY (PYY; 1:6000), galanin (1:1800), substance P (SP; 1:700), bombesin (Bom; 1:7500) and beta-endorphin (1:1000) were purchased from MILAB (Sweden). Anti-somatostatin antibody was obtained from DAKO Immunoglobulins (Copenhagen, Denmark) and employed at a 1:2000 dilution. Antibody to calcitonin gene-related peptide (CGRP) was obtained from Amersham (Buckinghamshire, England) and employed at a 1:2000 dilution. Peroxidase labeled anti-rabbit IgG (γ chain L-chain specific)/goat Fab was prepared by MBL Company (Nagoya, Japan).

For negative controls, nonimmune rabbit IgG at a 1:100 dilution was utilized in place of primary antibodies. The control slides were invariably negative for immunostaining. Cross-reaction with other peptides or proteins containing amino acid sequences recognized by the different antibodies can not be excluded. It is appropriate, therefore, to express the immunohistochemical finding as nerve fibers containing VIP-like, bombesin-like immunoreactivity, and so on. For simplicity, however, the shorter terms are used henceforth.

RESULTS

All the antibodies used except for somatostatin,

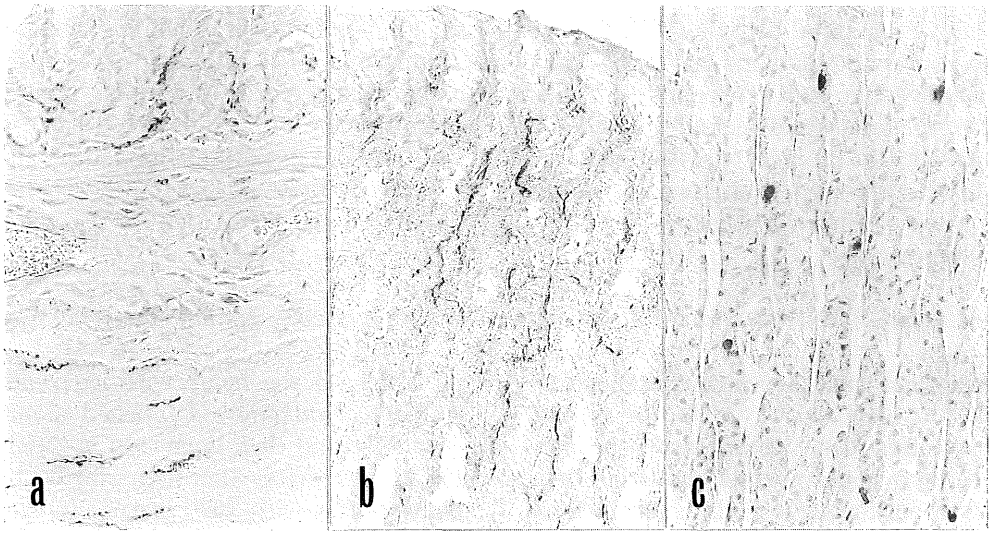

Fig. 1. a) VIP-containing nerve fibers around pyloric glands and in muscle layer. × 220 b) Bombesin-containing nerve fibers in the oxyntic mucosa. × 220 c) Somatostatin-containing (D) cells in the oxyntic mucosa. × 220

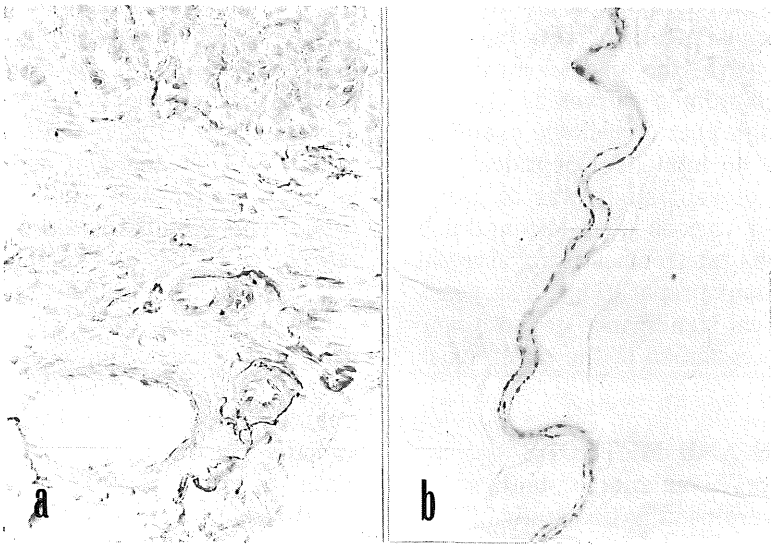

Fig. 2. Calcitonin gene-related peptide-containing nerve fibers in the submucosa of the rat stomach. They are mainly present around the small arteries (a) and along the capillary wall (b). a) × 220 b) Whole mount preparation, × 500

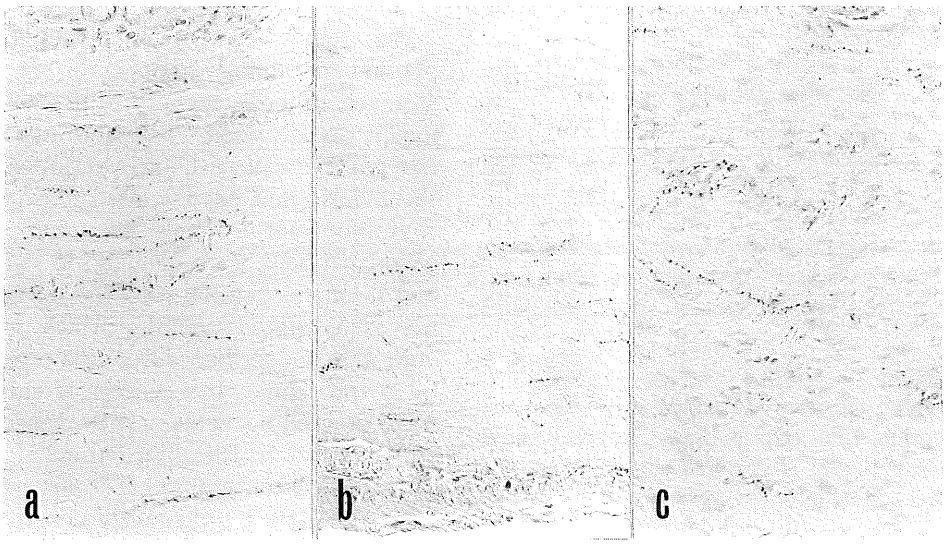

Fig. 3. Nerve fibers containing bombesin (a), PHI (b) and substance P (c) in the muscle layer. a) b) and c) × 220

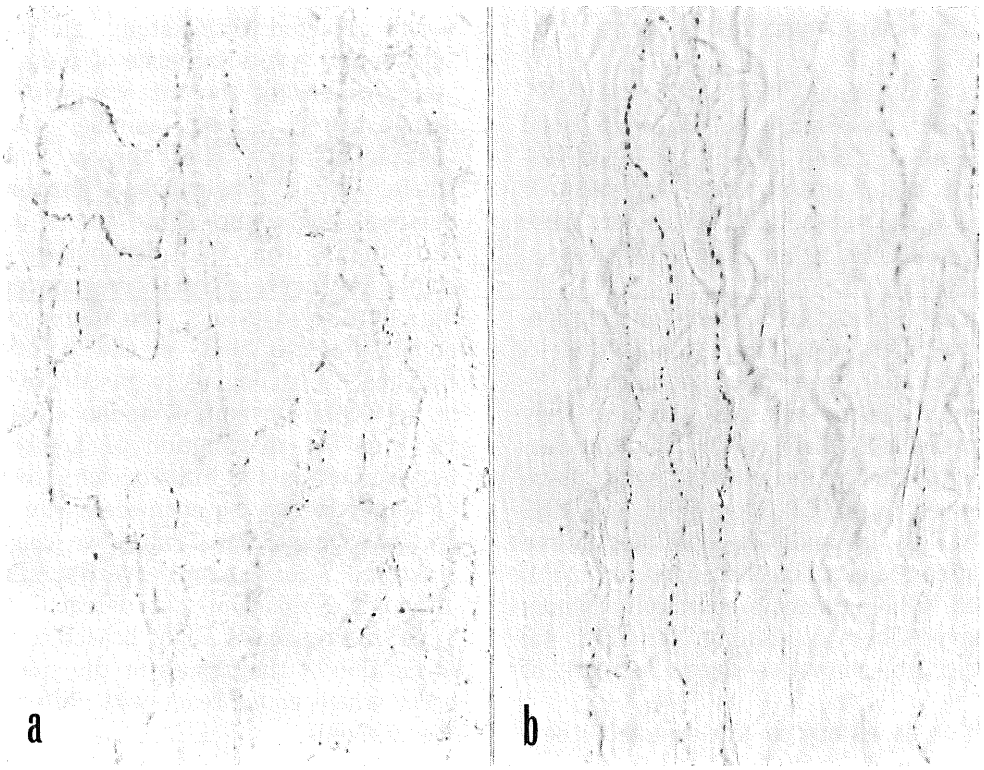

Fig. 4. Whole mount preparation with the nerve fibers containing VIP (a) and CGRP (b) in the muscle layer. a) and b) × 340

gastrin and peptide YY reacted with neuronal elements in the gastric wall, although the localization and distribution density were different in each peptide-containing neuronal element. Nerve fibers were demonstrated as a fine granular string with a delicate bead-like configuration.

1. Distribution of peptide-containing nerve fibers

1) Mucosa

In the lamina propria, a variety of peptide-containing nerve fibers were present along the gastric glands and pits. Of these, the distribution density was highest for nerve fibers containing vasoactive intestinal peptide (VIP), bombesin (Bom) and calcitonin gene-related peptide (CGRP) (Figs. 1a and b), followed by nerve fibers containing peptide histidine isoleucine (PHI), substance P (SP) and galanin (Gal), in the order given. Beta-endorphin (End) and neuropeptide Y (NPY)-containing nerve fibers were not found. PHI and VIP immunoreactivities were identified in the same fibers by mirror section immunohistochemistry, although the number was apparently smaller in PHI-fibers than

Table 1. Distribution of the Peptide-Containing Nerve Fibers of the Rat Stomach

Peptides	Mucosa	Submucosa	Muscle Layers
CGRP*	+++**	+++	+++
VIP	+++	++	+++
PHI	+	+	++
Bombesin	+++	+	++
Galanin	+	+	++
Substance P	+	+	++
Beta-endorphin	−	+	+
Neuropeptide Y	−	+	+

*: CGRP; calcitonin gene-related peptide, VIP; vasoactive intestinal peptide, PHI; peptide histidine isoleucine
**: +; a few nerve fibers, ++; moderate number of nerve fibers, +++; abundant nerve fibers

VIP-fibers. They were present evenly in the pyloric and oxyntic mucosa except for Bom-containing nerve fibers, which were obviously greater in number in the oxyntic than in pyloric mucosa. A small number of CGRP-containing endocrine cells were found in pyloric mucosa (data not shown).

2) Submucosa

Nerve fibers containing CGRP (Fig. 2a), VIP, PHI, Bom, Gal, SP, beta-End, and NPY were found in a variable density, mainly around the blood vessels including arteries, veins and capillary walls. Of these, VIP and CGRP-containing nerve fibers were most frequently detected around the blood vessels.

3) Muscle layers

In the muscle layers, a variety of peptide-containing nerve fibers were found in both the circular and longitudinal layers and the former appeared to contain a larger number of fibers. They ran parallel to the muscle fibers. Distribution density was the highest for VIP-containing nerve fibers (Fig. 1a), followed by CGRP, Bom (Fig. 3a), PHI (Fig. 3b), SP (Fig. 3c) and Gal-containing nerve fibers. A few fibers containing beta-End and NPY were also noted. Whole mount preparations demonstrated the nerve fibers containing VIP (Fig. 4a) and CGRP (Fig. 4b)' showed a dense network of nerve fibers.

The distribution of various peptide-containing neuronal systems in the rat stomach is summarized in Table 1.

2. Effect of the truncal vagotomy

Truncal vagotomy did not show any obvious changes on the density and distribution of various peptide-containing nerve fibers in the entire rat gastric walls.

3. Distribution of other peptides

Gastrin and somatostatin (Fig. 1c) were found in a good number of endocrine cells distributed in the antral and/or fundic mucosa, but they were not detected in the nervous systems in this study. Peptide YY were demonstrated in neither the endocrine cells of the gastric mucosa nor in nervous elements.

DISCUSSION

We demonstrated the distribution of various peptide-containing nerves in the rat glandular stomach of both the cryostat sections and whole mount preparations. They were numerous in the entire gastric wall without overt differences between the pyloric and oxyntic area except for bombesin-containing nerve fibers. As a rule, different types of gut peptides were detected in gut endocrine cells and nervous systems with the exception of calcitonin gene-related peptide, which were present in both the endocrine cells and nervous systems.

On the whole, the results of the present study were in good agreement with previous descriptions with respect to the distribution of nerve fibers in rat stomach containing calcitonin gene-related peptide (CGRP)[15], vasoactive intestinal peptide (VIP)[9,10,19], bombesin or gastrin-releasing peptide (GRP)[2,9,10], neuropeptide Y (NPY)[9,11], galanin[8,9,13] and substance P (SP)[10,14,19]. However there were minor differences in the distribution of peptides. First, somatostatin-containing nerve fibers could not be detected in this study. This does not necessarily imply a total absence of somatostatin in nervous systems of the rat stomach. Ekblad et al[9] demonstrated a small number of somatostatin-containing nerve fibers in the muscle. Second, Buffa et al[2] observed a larger number of bombesin-containing nerve fibers in the antrum. Our finding on the distribution of bombesin-containing nerve fibers corresponded to the findings of Ekblad et al[9], who found more numerous bombesin-nerve fibers in the oxyntic gland area. Bombesin is analogous to gastrin releasing peptide in its COOH-terminal 7 amino acid sequence[2]. In view of the distribution of bombesin-containing nerve fibers in the rat stomach, however, the role of bombesin may be not necessarily limited to gastrin releasing effect. Third, we could not demonstrate NPY-containing nerve fibers in the mucosa, although Ekblad et al[9] detected a few fibers. These discrepancies might be due to the differences in fixation of the tissues or the specific antibodies used, which could react with different epitope of the antigens.

Pharmacologic effects of the peptides on the gut nervous systems have been well examined. For instance, SP and bombesin cause gastric contraction, and VIP relaxes the smooth muscle[2,3,5,18]. These effects seem to be compatible with the immunohistochemical findings in the present study, which demonstrated abundant nerve fibers containing SP and VIP in muscle layer. Blood vessels in the submucosa received a rich supply of CGRP and VIP-containing nerve fibers, suggesting neural control of blood flow. VIP and CGRP are known to be a potent vasodilator[1,18], whereas bombesin contracts the blood vessels. Thus, the smooth muscle and blood vessels of the rat stomach are regulated by a wide variety of peptides, which exhibit different

or contrary effects. On the other hand, the physiologic effects of these peptide on the epithelial cells of the gastric mucosa and the interaction with the gastric endocrine cells are not fully understood. Recently, Lenz et al[12] reported that the pentagastrin stimulated gastric acid secretion in conscious dog decreased by intracerebroventricular administration of several kinds of peptides including CGRP, calcitonin, neurotensin, endorphin corticotropin-releasing factor and bombesin. Bombesin is well known to have powerful effects on mammalian gastric acid secretion. This discrepancy might be due to the difference of the administration site of bombesin.

A vast majority of the peptide-containing nerve fibers within the rat gastric wall have been demonstrated to be of intrinsic origin with a few exceptions. Minagawa et al[14] demonstrated reduction of SP-fibers in the myenteric plexus by bilateral dorsal spinal gangliotomy from Th5 to L2, indicating the intrinsic and extrinsic origin of SP-containing never fibers of the rat stomach. Ekblad et al[9] reported disappearance of the mucosal and perivascular NPY-containing fibers by upper abdominal sympathectomy. Lee et al[11] also demonstrated that the majority of NPY-fibers in the myenteric plexus and along the blood vessels in muscle layers originated from NPY cell bodies located in the celiac ganglion. On the other hand, truncal vagotomy in the present study did not remarkably affect the density and distribution of various peptide-containing nerve fibers. This finding had been previously demonstrated by Ekbald et al[9]. They indicated in the recent study[8] that the nerve fibers in the mucosa originate from the submucosal ganglia and nerve fibers from muscle layers of myenteric ganglia. Most of the peptide-containing nerve fibers in the rat stomach may have a closed circuit, independent of the central nervous system.

The significance of various peptide-containing nerve fibers in the development of chronic gastritis, intestinal metaplasia, peptic ulcer and tumor remains to be elucidated in the future. Analysis of the interaction between peptide-containing nerve fibers and endocrine cells will contribute much to the general knowledge of the hormonal and neural regulations of the gastrointestinal tract.

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