Physiology and Pharmacology of the Gut Nutrient Perception

Immunohistochemical and Morphologic Basis for Glutamate Signaling in the Rat Stomach

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Physiologic studies conducted in rats have demonstrated that afferent fibers of the gastric branch of the vagus nerve increase their firing rate with the intragastric administration of the amino acid glutamate, and the increased firing rate is blocked by the depletion of serotonin (5-HT), administration of the blocker for the serotonin type-3 receptor (SR3), or nitric oxide synthase (NOS). To understand glutamate signaling in the gastric mucosa at the cellular level, we have been studying rats as an animal model using anatomic and immunohistochemical procedures. Our results have indicated that 5-HT-immunoreactive (ir) cells are present in the superficial part of the gastric mucosal epithelium and in the base of the fundic glands, whereas immunoreactivity for SR3 is localized in the neck and its vicinity of the fundic glands. Further, NOS1/neuronal NOS-ir cells with a bipolar shape are located in the lamina propria where a dense network of neuronal cells is present. These results suggest that complex cellular events take place during intragastric glutamate signaling.

Key words serotonin receptor type 3; nitric oxide synthase; fundic gland; rat

1. INTRODUCTION

The stomach is a sac-like organ between the esophagus and duodenum. It is generally considered to be a site where food is stored and partially digested.1,2 Recently, Uneyama et al.2 have demonstrated that the administration of the amino acid glutamate into the rat stomach increased the firing rate of the gastric branch of vagal afferent nerve fibers, suggesting the presence of glutamate signaling within the stomach. The activation of the vagal nerve fibers as inhibited by the depletion of serotonin (5-HT), administration of the blocker for the serotonin type-3 receptor (SR3), or nitric oxide synthase (NOS). To understand glutamate signaling in the gastric mucosa at the cellular level, we have been studying rats as an animal model using anatomic and immunohistochemical techniques. We present here a summary of our recent immunohistochemical data and propose a working hypothesis to investigate further the mechanisms of gastric glutamate signaling.

2. GENERAL HISTOLOGY OF THE RAT STOMACH

To facilitate understanding of our findings, the histologic organization of the corpus of stomach is shown in Fig. 1. Surface epithelial linings that contain solely mucus-producing cells invaginate to form gastric pits, each with a few fundic glands. A region containing dividing and undifferentiated cells, called the “isthmus,” leads to the neck region of fundic glands. Mucous neck cells are localized in the neck region of fundic glands. Parietal cells that secrete HCl are distributed throughout the glands, whereas chief cells that secrete pepsinogen are mainly located in their base.

3. SR3

Using a polyclonal antibody to N-terminal residues of SR3, we demonstrated that SR3-immunoreactive (ir) cells are localized in the neck region and vicinity of fundic glands. They are relatively small, angular in shape, and clearly distinguishable from parietal cells that are much larger and polyhedral in shape (Fig. 2). Interestingly, the same antibody to SR3 does not immunostain cells in the duodenal mucosa, suggesting that SR3 cells in the stomach play a specific role in stomach-specific functions. However, using an antibody to C-terminal residues of SR3, Glatzle et al.3 reported SR3 immunoreactivity in different cell types from those found by us, i.e., the neuronal population of the rat gastric corpus. We need to resolve this discrepancy by using different antibodies and gene histologic methods.

4. 5-HT

A typical gastric mucosal section demonstrating immunofluorescence for serotonin (5-HT) is shown in Fig. 3. 5-HT-ir cells are present both in the surface epithelium and in the base of fundic glands. Their density is greater in the former. 5-HT-ir cells in the stomach are pleomorphic; some are flask-shaped, ovoid, fungoid, or spindle shaped. The distribution pattern of 5-HT-ir cells demonstrated by us is similar to that by Yu et al.2

6. NOS1

Neuronal NOS or NOS1-ir cells are present in the lamina propria; spindle-shaped somata with long processes are seen (Fig. 4A). The outer muscle coat contains NOS1-ir cells as...
well. NO produced and released by these cells plays a critical role in glutamate signaling in the stomach, as demonstrated by Uneyama et al. 2) Baccari et al. 6) reported that in the mouse gastric fundus NOS1 immunoreactivity is almost exclusively detected in structures featuring neurons and nerve fibers of the intramural neural plexi. Thus the NOS1-immunoreactive cells found in the rat gastric corpus appear to be neurons, although double-labeling immunohistochemical experiments using antibodies to NOS1 and neuronal markers should be carried out.

Fig. 1. General Structure of the Corpus of Rat Stomach
Histologic section stained with hematoxylin and eosin (left) and schematic diagram (right) in which histologic and cellular components are indicated. The nomenclatures are based on a textbook of histology. 1)

Fig. 2. SR3 Immunofluorescence in the Rat Stomach
SR3-immunoreactive (ir) cells are localized in the neck region and its vicinity of fundic glands (white arrows). Inset: differential interference contrast image showing SR3-ir cell (yellow arrow) between parietal cells (arrowheads).

Fig. 3. 5-HT Immunofluorescence in the Rat Stomach
5-HT-ir cells are localized within the surface epithelium and fundic glands. The enlargement of the boxed area is shown in the inset. Inset: 5-HT-ir cells (arrowheads) exhibit varied morphology.

Fig. 4. Confocal Images of Immunofluorescence for NOS1 (A), PGP 9.5 (B), MAP1b (C), and MAP2 (D) in the Lamia Propria of the Rat Stomach
Ir-somata (arrows) and processes (arrowheads) are indicated in A—D. Cell nuclei exhibit blue fluorescence. * Soma of enteroendocrine cell.

7. PRESENCE OF NERVE PLEXUS IN THE LAMINA PROPIRA

Protein gene product (PGP) 9.5, which is a cytosolic marker of neurons and neuroendocrine cells, 7) has been utilized as a marker for fine nerve fibers in the skin 8) and nasal 9) and lingual mucosa. 10) Using an antibody to PGP 9.5, dense
nerve plexi are visualized in the lamina propria of the gastric mucosa (Fig. 4B). Microtubule-associated proteins 1b (MAP1b) and 2 (MAP2) are major proteins in axons and dendrites, respectively.[11] Using these antibodies, we demonstrated that a complex nerve plexus is present within the lamina propria of the gastric mucosa (Figs. 4C, D).

8. CONCLUSION AND FUTURE PERSPECTIVES

Cell types and their locations described in this review are illustrated in Fig. 5. Our preliminary observations suggest that all PGP 9.5-ir cells in the lamina propria of the gastric mucosa exhibit immunoreactivity to MAP1b. It is necessary to clarify whether NOS1 is colocalized with PGP 9.5, MAP1b, and MAP2.

Possible cellular events necessary for gastric glutamate signaling are shown in Fig. 6. First, glutamate binds to a receptor; one of the candidates is metabotropic glutamate receptor 1 (mGlur1).[12] It is not known which types of signals are utilized between the glutamate receptor-expressing and NOS1-expressing cells (step 1 in Fig. 6). Second, NO produced by NOS1 cells stimulates 5-HT cells (step 2). Third, 5-HT cells release 5-HT, which binds to SR3 cells (step 3). Then, it is necessary to identify the signal cascade between SR3 cells and the neuronal cells present in the lamina propria of the gastric mucosa (step 4). Finally, signals transmitted from those neuronal cells to other neuronal cells present in the muscularis mucosae (step 5) and in the outer muscle coat are necessary to increase the firing rate of the gastric branch of the vagus nerve. The nature of these signals needs to be determined as well.

So far, we have only obtained fragmented knowledge on cell candidates involved in glutamate signaling in the stomach. Further analyses using various techniques, such as immunoelectron microscopic, biochemical, gene histochemical, and molecular biological procedures are necessary to understand the glutamate signaling mechanism in the mammalian stomach.

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REFERENCES