

Visualization and quantification of the exocytosis of gastric paracrine cells

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Objective: The aim of the study is to visualize the exocytosis of paracrine cells in fundic mucosa and to quantify the rapid frequency change of exocytosis.

Methods: Specimens of fundic mucosa of adult mice stomachs from which the muscle layer had been stripped off, were observed from the serosal side using video-enhanced microscopy. Video images were digitized and subtracted between adjacent images in order to emphasize exocytosis of paracrine cells.

Results: The paracrine cells revealed spontaneous exocytosis of vesicles attached the cell membrane. The frequency of exocytosis was enhanced by gastrin and acetylcholine in 78% and 25%, respectively, of cells examined. Temporal analysis revealed the long-lasting effect of gastrin and short effect of acetylcholine. Occasionally when the exocytosis was bursting, intact vesicles were released from the cell surface and fell into extracellular space without rupturing.

Conclusions: Using video-enhanced microscopy and the digital subtraction technique of the video images, the rapid frequency change of exocytosis in the fundic paracrine cells were detected.

Key words: exocytosis, paracrine cells, video-enhanced microscopy, Scion Image

Introduction

Histamine and somatostatin are released from paracrine cells located in the serosal fundic region of the stomach.^{1,2} These messenger molecules are known to regulate gastric acid secretion from parietal cells that are located deep in the fundic mucosa. In particular, histamine, which has been identified to be mainly secreted from enterochromaffin-like (ECL) cells definitely located in the fundic region, is a direct stimulator of acid secretion via H₂ receptors.¹ During the last several decades, the H₂ receptors have been highlighted as a target of a cure of gastric and duodenal ulcers.^{3,4} As generally recognized in the majority of secreting manners of chemical messengers, these molecules packed to secretory vesicles are released outside the cell, by the rupture of the vesicle membrane, so-called exocytosis. In this study, I have performed the realtime observation of the exocytosis of the paracrine cells located at the fundus of the gastric gland from the serosal side using video-enhanced microscopy.⁵ Sequential images, each of which were enhanced by a video camera and analyzer, were digitized to analyze the temporal and spatial dynamics of

exocytosis. The regulation of the exocytosis in the fundic paracrine cells by the hormone- and neuron-derived secretagogues for gastric acid, gastrin and acetylcholine, were also examined.

Materials and Methods

From 6- to 8-week-old male ddY mice were decapitated, and the fundic region of their stomachs was removed. After peeling off the smooth muscle layer, the submucosal surface was exposed. These tissues were kept in O₂-saturated Tyrode solution (pH 7.4) composed of 140 mM Na⁺, 3 mM K⁺, 1.2 mM Ca²⁺, 1.2 mM Mg²⁺, 119 mM Cl⁻, 21 mM HCO₃⁻, 0.6 mM H₂PO₄⁻, 2.4 mM HPO₄²⁻, 10 mM glucose at 4 °C. The dissected mucosa was held between two coverslips, the edges of which were sealed with waterproof tape. The gap between the two coverslips was filled with GIBCO™, HEPES-buffered solution (Invitrogen Corp., Carlsbad, CA, USA) (pH 7.4, 37 °C) comprising 135 mM Na⁺, 5 mM K⁺, 1 mM Ca²⁺, 1 mM Mg²⁺, 144 mM Cl⁻, 10 mM HEPES and 5.5 mM D-glucose.

The preparation was mounted onto the stage of an

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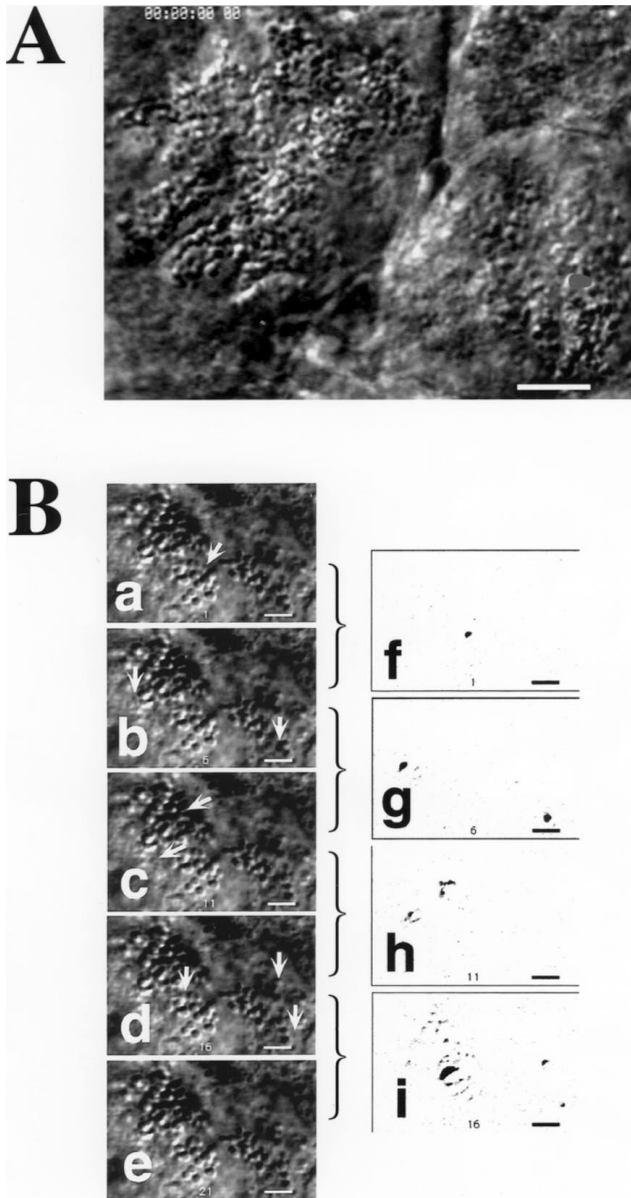


Figure 1. (A) An enhanced video image of mouse stomach fundic mucosa. Several paracrine cells were clustered on the fundus of the gastric gland. The exocytosis of paracrine cells could only be observed from the serosal side of a specimen from which the muscle layer had been stripped off. Scale bar, 10 μm . (B) Time-lapse sequence of enhanced video images. Spontaneous exocytosis in control condition, bathed in physiological saline solution, in successive 5-second intervals. (a-e) The arrowhead points out an intracellular vesicle that will rupture within the next second. (f-i) Four successive video images, digitally subtracted (b) from (a), (c) from (b), (d) from (c), and (e) from (d), show clearly ruptured vesicles per second. Scale bar, 2 μm .

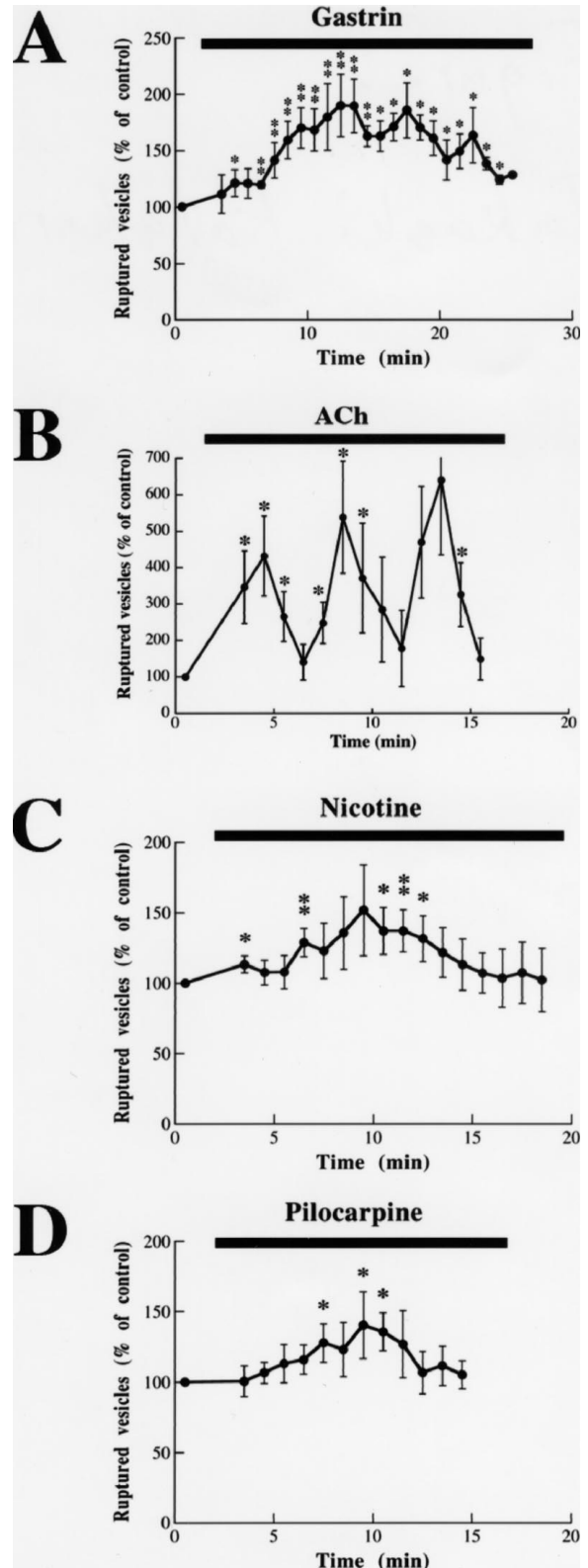


Figure 2. The effects of (A) pentagastrin (10^{-6} M, $n = 5$), (B) acetylcholine (10^{-6} M, $n = 5$), (C) nicotinic agonist, nicotine (10^{-5} M, $n = 4$) and (D) muscarinic agonist, pilocarpine (10^{-6} M, $n = 3$) on exocytosis frequency of paracrine cells in fundic mucosa. Each point with bar represent the mean and SD of the values obtained from 3 to 5 experiments. Statistically significant difference from the control by Mann-Whitney's U test is expressed as * $P < 0.05$ and ** $P < 0.005$. Ordinate: Percentile change in exocytosis frequency of the control. Abscissa: Time in minutes.

inverted Zeiss Axiomat microscope (Carl Zeiss, Oberkochen, Germany), with $\times 20$ and oil-immersed plan-apochromat $\times 100$ 1.30 objective (Carl Zeiss). When the test solution was applied after the control period, the solution (0.5 ml) was injected into one opened edge between coverslips using a Pasteur pipette, and the perfused solution was removed from another opening using a filter paper. This procedure and small chamber volume (0.1 ml) enabled complete solution changes to occur within 10 seconds. The gastrin, acetylcholine, nicotine, and pilocarpin, each dissolved in the HEPES-buffered saline, was applied at the concentration indicated in the text and figure legends.

Images were obtained from the fundic region of the mouse stomachs using video-enhanced microscopy. They were displayed on a video monitor (C1846, Hamamatsu Photonics, Hamamatsu). Serial images were enhanced by a DVS20 (Hamamatsu Photonics) and were stored on a video recorder (PVW-2800, Sony, Tokyo). The dimension of video image by $20\times$ objective was $80\ \mu\text{m}$

wide and $60\ \mu\text{m}$ high.

Video signals were captured by video capture board (LG3, Scion Corp., Frederick, MD, USA) on a Macintosh computer (Mac OS 9.1). The digitized images were compiled into a tiff-formatted stack file using Scion Image 1.62, which is the non-commercial development of NIH Image by the Scion Corporation. I wrote a tiny stack macroprogram which makes a difference between two successive video slices in order to visualize ideally only the vesicles ruptured within a period of two slices.

Results

As shown in Figure 1A, there were clusters of vesicles on the serosal face of cells that comprise the gastric gland fundus in the dissected mouse. Cells revealed the spontaneous exocytosis of vesicles on their surface.¹² The spontaneous exocytosis was observed in about 30% of cells in this dissected preparation. The vesicles were rupturing at an average frequency of 0.5/sec/video screen

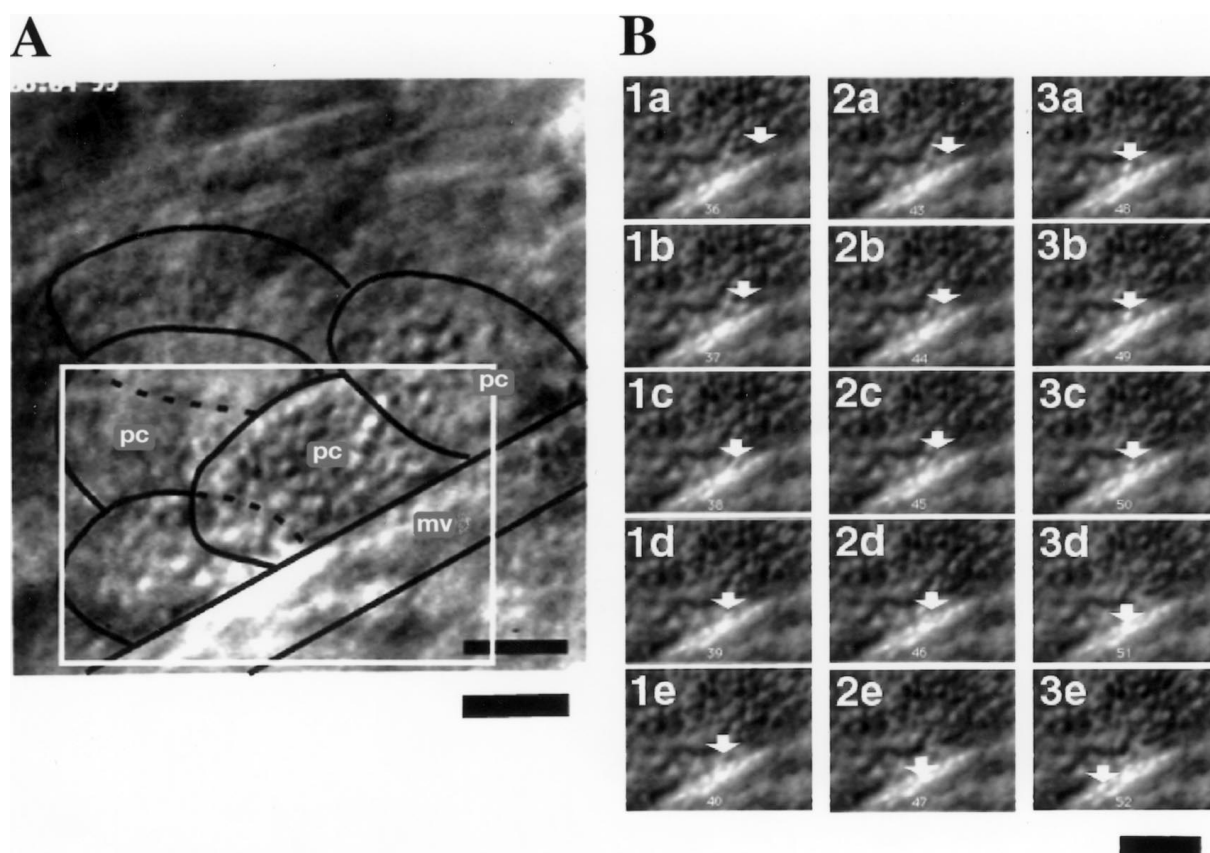


Figure 3. Time-lapse sequence (a-e) of enhanced video images of paracrine cells. (A) An video image of releasing intracellular vesicles into extracellular space. pc, paracrine cells; mv, microvessel. The white framed area is the same area shown in Figure 3B 1a-3e. Scale bar, $5\ \mu\text{m}$. (B) Acetylcholine ($10^{-6}\ \text{M}$) not only enhanced the exocytosis at the cell surface but also prompted the release of the vesicles from the cell surface to the extracellular space without rupturing. The photo shows 3 sets of successive video frames of 0.5-second intervals (1a-e, 2a-e, 3a-e). The arrowheads indicate such a vesicle movement. Scale bar, $3\ \mu\text{m}$.

(4,800 μm^2) under the control condition. We examined the dynamics of exocytosis and its regulatory mechanisms in cells showing spontaneous exocytosis. Application of pentagastrin induced an increase in the frequency of exocytosis in 78% (35/45) of cells tested. The remaining cells (22%, 10/45) were insensitive to gastrin. When acetylcholine was applied, the frequency was increased in 25% (32/129) of cells, and was decreased in <1% (1/129) of cells. The remaining 74% (96/129) of cells did not respond to acetylcholine.

We further analyzed the time course of the change in frequencies of the exocytosis induced by gastrin and acetylcholine. Application of pentagastrin (10^{-6} M) induced gradual increase in the frequency of exocytosis (Figure 2A). The effect was maximized 10-20 minutes after the application, and this enhancement was sustained for more than 30 minutes. The value of frequency subsequently gradually restored to the initial level. Acetylcholine (10^{-6} M), on the contrary, immediately increased the frequency of exocytosis, and the initial peak came at 4 minutes after the application (Figure 2B). Another two peaks were followed about 4-5 minutes interval and diminished within 20 minutes. The agonist of N-type of acetylcholine receptors nicotine (10^{-5} M) mimicked the facilitatory effect of acetylcholine (Figure 2C). The agonist of the acetylcholine M-type receptor pilocarpine (10^{-6} M) slightly increased the exocytosis (Figure 2D).

In some cases of acetylcholine administration, when the frequency of exocytosis abruptly increased, the vesicles were dropped off from the cell surface and fell into extracellular space without rupturing (Figure 3A, B).¹²

Discussion

In the present study, the dynamics of the vesicular release of mouse gastric paracrine cells located at the serosal side of the fundic region were visualized by video-enhanced microscopy.⁵ This technique can provide a display of realtime dynamics of vesicular release of cells and thereby its quick responses to external signals that are not detected by other experimental techniques. When the frequency of exocytosis was analyzed, two thirds of the paracrine cells were sensitive to gastrin, and one third of the cells were sensitive to acetylcholine. These results seem to be consistent with the immunocytochemical observation in the rodent fundic region.⁶⁻⁸ Those studies revealed that 60% of paracrine cells located in the fundic region are histamine releasing ECL cells and a few percent of the fundic paracrine cells are somatostatin

releasing D cells (others are unknown) in mice⁸ as well as in rats.^{6,7} Moreover, studies on rat-cultured ECL or D cells have shown that histamine secretion is stimulated by gastrin and acetylcholine and that somatostatin secretion is stimulated by gastrin but inhibited by acetylcholine.¹ In my experiments, acetylcholine enhanced the exocytosis in fundic paracrine cells. Therefore, most of the fundic paracrine cells observed seem to be histamine-releasing ECL cells.

In the present study, temporal analysis revealed that the stimulatory effect of gastrin on the spontaneous exocytosis was sustained for more than 30 minutes while the exocytosis in the cell exposed to acetylcholine raised immediately and finished within 15 minutes. Such different temporal patterns of changes in the exocytosis frequency may contribute to the sophisticated regulation of acid secretion in response to hormonal and neuronal signals. My observation also indicated that the nicotinic receptor agonist mimicked the effect of acetylcholine, and the muscarinic agonist slightly increased the frequency of exocytosis. The muscarinic receptor-mediated regulation on gastric functions is well known. In contrast, the role of nicotinic receptors on these gastric functions has rarely been reported. However, some previous studies have revealed the importance of nicotinic receptor-mediated effect on the motility of stomach and gastric acid secretion.^{10,11} Here, my observation also provides evidence that nicotinic receptors are involved in the regulatory mechanisms of histamine release.

In conclusion, I found an unexpected event for vesicular release in the fundic paracrine cells.¹² When the exocytosis is bursting from the cells, the vesicles were directly released into extracellular space without rupturing. Although it has been believed that secretion of molecules is performed by endocytosis or exocytosis, the observations outlined in the present report represent another way of vesicular release. This newly observed releasing manner in fundic paracrine cells may contribute to the spatiotemporal regulation of gastric acid secretion.

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12. Digital QuickTime videos, were made from video records in the present study; copyright, Kitasato Medical Society. The videos will be provided on request from Kitasato Igakukai. 1-15-1, Kitasato, Sagamihara, Kanagawa 228-8555, Japan. Fax: +81-42-778-9176.