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$[Na^+]_i$, $[K^+]_i$ and $[Cl^-]_i$ Regulation of Exocytosis in Guinea-Pig Antral Mucous Cells

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Effects of intracellular Na^+, K^+ and Cl^- on Ca^{2+}-regulated exocytosis activated by 10 μM acetylcholine (ACh) were studied in guinea-pig antral mucous cells which are permeabilized by nystatin treatment. Ca^{2+}-regulated exocytotic events were modulated by $[Na^+]_i$, $[K^+]_i$ and $[Cl^-]_i$ via mediation of PTX-sensitive G proteins. Increases in $[Na^+]_i$ and PTX inhibit G protein (G^{Na}), which suppressed the exocytosis. Increases in $[K^+]_i$ caused the exchange of G proteins (from G^{Na} to G^K) to increase, and GK evoked activation of the exocytosis and was inhibited by PTX. Increases in $[Cl^-]_i$ and PTX inhibit G protein (G^{Cl}), which stimulates exocytotic events. Based on these observations, the exocytosis in antral mucous cells were modulated by intracellular ions, concentration of which were increased or decreased by cell volume changes caused by Ach.

Introduction

Effects of intracellular Na^+, K^+ and Cl^- on Ca^{2+}-regulated exocytosis activated by 10 μM acetylcholine (ACh) were studied in guinea-pig antral mucous cells which are permeabilized by nystatin treatment.

Methods

The control solution contained (in mM): NaCl 146, KCl 4.5, $MgCl_2$ 1, $CaCl_2$ 1.5, HHEPES 10 and glucose 5, and the KCl solution contained (in mM): KCl 150.5, $MgCl_2$ 1, $CaCl_2$ 1.5, HHEPES 10 and glucose 5. Solutions were gassed with 100% O_2 at 37℃. Guinea-pigs were anesthetized by inhalation of ether and sacrificed by cervical dislocation. The experiments were approved by the Animal Research Committee of Osaka Medical College, and animals were cared according to the guidelines of this committee. The procedures for cell preparation have already been described in detail previously (1). The stripped antral mucosa was minced and incubated with 0.1% collagenase at 37℃. The digested mucosa was filtered through a nylon mesh (150 μM^2) and the cells were suspended in control solution (4℃) containing 2% BSA and used for experiments within 3 hr. The isolated cells were incubated in control solution containing PTX (200 ng/mL) for 2 hr to inhibit G proteins, and then used for the experiments. Cells were mounted on a coverslip precoated with neutralized Cell-Tak and the coverslip with cells was set on the stage of a microscope. The frequency of exocytotic events were measured by a video-microscopy (ARGUS-10, Hamamatsu Photonics) (1). Isolated cells were loaded with SBFI-AM or SPQ for 25 min at room temperature (22-24℃) and $[Na^+]_i$ or $[Cl^-]_i$ was measured by an image analysis system.

Key Words: *Gastric Mucin; Acetylcholine; Nystatin; Pertussis Toxin; G Protein*

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Results and Discussion

The frequencies of ACh-evoked exocytotic events were increased by addition of 50 mM NaCl (hyperosmotic stress), but not by addition of 100 mM sucrose. Intracellular Na^+ concentrations, $[Na^+]_i$, were significantly increased by addition of 50 mM NaCl but not by addition of 100 mM sucrose. Cell shrinkage caused by Cl^-- free solution or addition of bumetanide potentiated the frequency of ACh-evoked exocytotic events. Cell shrinkage significantly decreased $[Cl^-]_i$. Thus, increases in $[Na^+]_i$ and decreases in $[Cl^-]_i$ are likely to potentiate ACh-evoked exocytotic events. Treatment of nystatin (50 μM) allowed us to control $[Na^+]_i$ or $[Cl^-]_i$ by changing extracellular ion compositions in ACh-stimulated antral mucous cells.

Na^+ concentration of perfusion solution was altered by replacing Na^+ to Cs^+. Frequencies of ACh-evoked exocytotic events were decreased as $[Na^+]_o$ decreased from 146 mM to 5 mM in nystatin treated cells. Thus, frequencies of ACh-evoked exocytotic events were dependent on $[Na^+]_i$, however, the frequencies of ACh-evoked exocytotic events remained to be constant in cells treated with PTX, when $[Na^+]_o$ decreased from 146 mM to 5 mM in nystatin treated cells.

K^+ concentration of perfusion solution was increased by replacing Na^+ to K^+ in PTX-treated cells. Frequencies of ACh-evoked exocytotic events in PTX-treated cells

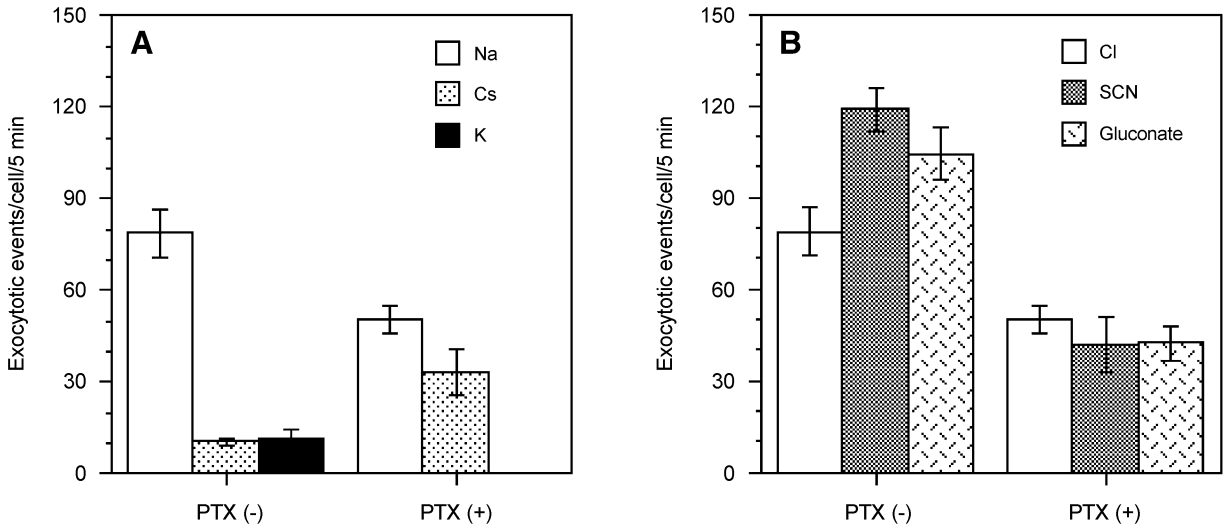

Fig. 1. Exocytotic events evoked by 10 μM ACh. Cells were perfused with test solutions containing 50 μM nystatin, and then stimulated with 10 μM ACh for 5 min. ACh stimulation evoked a transient increase in the frequency of exocytotic events. Experiments were performed in cells with and without PTX treatment. **A:** Na of the control solution was replaced by Cs or K. **B:** Cl of the control solution was replaced by SCN or gluconate.

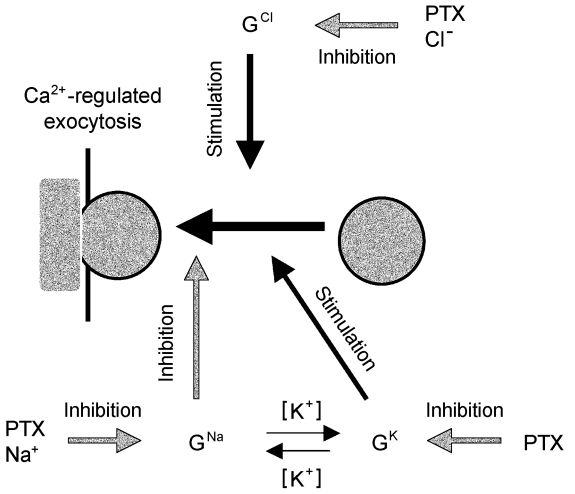

Fig. 2. $[Na^+]_i$, $[K^+]_i$ and $[Cl^-]_i$ regulation of exocytosis in antral mucous cells.

decreased as $[K^+]_o$ increased from 4.5 mM to 152 mM. Thus, frequencies of ACh-evoked exocytotic events, which are PTX-sensitive, are dependent on $[K^+]_i$. $[K^+]_i$ is suggested to alter number of PTX-sensitive G protein.

Cl^- concentration of perfusion solution was altered by replacing Cl^- to SCN^-. Frequencies of ACh-evoked exocytotic events were increased as $[Cl^-]_o$ decreased from 156 mM to 5 mM in nystatin treated cells. Thus, frequencies of ACh-evoked exocytotic events were dependent on $[Cl^-]_i$. However, these increases in the frequency of exocytotic events were no longer noted in cells treated with PTX, when $[Cl^-]_o$ decreased from 156 mM to 5 mM in nystatin treated cells.

These results were summarized in Fig. 1. $[Na^+]_i$, $[K^+]_i$ and $[Cl^-]_i$ modulated Ca^{2+}-regulated exocytotic events mediated via G proteins. Increases in $[Na^+]_i$ and PTX inhibit G protein (G^{Na}), which suppressed the exocytosis. Increases in $[K^+]_i$ caused G protein (G^K) to increase, which activates exocytosis and inhibited by PTX. Increases in $[Cl^-]_i$ and PTX inhibit G protein (G^{Cl}), which stimulates exocytotic events (Fig. 2). Based on these observations, the exocytosis in antral mucous cells were modulated by intracellular ions, concentrations of which were increased or decreased by cell volume changes caused by ACh.

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