Nanoscale Dynamics of Growth Hormone Secretion in Pigs

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Summary and Implications

The anterior pituitary gland contains cells that produce and secrete growth hormone (GH) into the circulating blood that causes muscle accretion, lipolysis, and lean growth in the pig. GH-secretagogues, such as L-692,585, stimulate GH release by action at the pituitary in a dose dependent manner in vivo, and by isolated porcine GH cells in culture. We used atomic force microscopy (AFM) to identify cytoplasmic structures at the plasma membrane of GH-secreting cells of the pituitary and implicate their involvement in hormone release. New cellular structures at the plasma membrane called "pits" and "depressions" were identified where membrane-bound secretory vesicles dock and fuse to release vesicular contents. Pits containing 100- to 200- nanometer in diameter depressions or "fusion pores" were identified in unstimulated (resting) GH-secreting cells. After stimulation of secretion with L-692,585, the size of depressions enlarged and gold-tagged GH antibody were found to bind to the pit structures in the stimulated GH cells. This study documents, for the first time, the presence of these structures and their involvement in hormone secretion in a neuroendocrine cell.

Introduction

Growth hormone (GH) produced by specialized cells of the anterior pituitary gland is stored in secretory vesicles within the cytoplasm of the cell. On stimulation of the GH cell with a GH-secretagogue (i.e., L-692,585), the secretory vesicles containing the hormone dock and transiently fuse with the plasma membrane to release the GH through the bilipid membrane to the exterior of the cell. In the living animal, the GH would be immediately collected in nearby blood sinusoids for distribution in the circulating blood to cause muscle accretion and lean growth. The focus of the present investigation was to determine the dynamics of GH-containing vesicles in their release of GH through the plasma membrane of the pituitary cell using AFM. Results from this study demonstrate the presence of pits and depressions in GH-secreting cells of the porcine pituitary and their involvement in hormone release (Cho et al., 2002).

Materials and Methods

Experimental animals

Yorkshire pigs, raised at the Iowa State University Animal Nutrition Farm, were used for these experiments. Newborn pigs, 1 to 8 days of age, were killed with electricity and decapitated. Pituitary glands were immediately removed and collected in cold sterile Earle's balanced salt solution (EBSS) solution (4°C). Anterior lobes of the glands were transferred to a sterile cold (4°C) minimal essential medium (MEM) (0.1% bovine serum albumin (BSA) medium).

Isolation and stimulation of GH-secreting cells

Primary pituitary cell cultures from pig anterior pituitary were established by modification of a procedure for neuronal cultures. Tissue was incubated with 2.5% papain solution for 40 minutes at 37°C. After incubation, tissue was mechanically dispersed in 1 ml of culture medium by triturating through a 1-ml fire-polished glass pipette 8 to 10 times and plated on poly-L-lysine (1 mg/ml) coated dishes. Cells were incubated overnight at 37°C in a humidified 5% CO_2/95% air atmosphere to allow them to adhere. Culture medium consisted of Eagle's MEM supplemented with 10% fetal blood serum (FBS) and 40 mM glucose, 2-mM L-glutamine, 1 mM pyruvate, 14 mM sodium bicarbonate, and penicillin/streptomycin. Cells in phosphate-buffered saline (PBS) were stimulated with 20 μM of the GH-secretagogue L-692,585.

Atomic Force Microscopy (AFM)

Pits and depressions at the plasma membrane in live and fixed GH secreting cells (n=24) in PBS, pH 7.5, were imaged by the AFM (Bioscope III; Digital Instruments) by using both contact and tapping mode. All images were obtained in the "tapping" mode in fluid, using silicon nitride tops with a spring constant of 0.06 Nm1/n, and an imaging force of <400 nN. Images were obtained at line frequencies of 1 Hz, with 512 lines per image, and constant image gains. Topographical dimensions of pits and depressions at the cell plasma membrane were analyzed using software supplied by Digital Instruments.

Immunogold AFM

After stimulation of secretion using 20 μM L-692,285, live GH-secreting cells of the pituitary were exposed to 1:200 dilution of GH-specific antibody, and washed in PBS, before AFM imaging in PBS at room temperature.

Cell fixation and immunogold localization

After stimulation of secretion using 20 μM L-692,585, live GH-secreting cells were fixed for 30 min by using ice-cold 2.5% paraformaldehyde in PBS. Cells were then washed in PBS, followed by labeling with 1:200 dilution of GH-specific antibody, and 30-nm gold conjugated secondary antibody, washed in PBS, before AFM imaging in PBS at room temperature.

Results and Discussion

Examination of unstimulated (resting) and stimulated GH cells demonstrated no detectable changes after fixation. AFM of resting GH cells revealed the presence of pits and depression at the plasma membrane (Figure 1). Depressions in resting cells measure 154 ± 4.5 nm (mean ± standard error). However, after exposure of GH cells to the secretagogue, L-692,585, a 40% increase in the size (215 ± 4.6 nm; $P<0.01$) of depressions is demonstrated. When stimulated live cells were exposed to 30-nm gold-tagged GH-antibody, gold particles were found to decorate pit and depression structures. From our studies using pancreatic acinar cells, it was determined 1-h treatment of ice-cold 2.5% paraformaldehyde in PBS, pH 7.5, was ideal in retaining structural integrity of pits and depressions. No detectable changes were identified in live GH cells after fixation. In conformation with our observation in live GH cells, AFM images of the immunolabeled fixed cells, demonstrate specific localization of immunogold at depressions, implicating them to be secretory sites at the cell plasma membrane. In agreement with earlier studies in pancreatic acinar cells, the GH-secreting neuroendocrine cells of the pituitary demonstrate the presence of pits and depressions at the plasma membrane, where secretory vesicles dock and fuse to release vesicular contents.

Implications

Growth hormone (GH) is one of the most important hormones affecting growth of pigs. GH improves performance with greater feed efficiency and carcasses with reduced fat. AFM has revealed for the first time the presence of pits and depressions in both exocrine and neuroendocrine cells suggests that these structures may be universal to secretory cells, where exocytosis occurs.

Reference

Cho, Sang-Joon, Ksenija Jeftinija, Aleksandra Glavaski, Srdija Jeftinija, Bhanu P. Jena, and Lloyd L. Anderson. 2002. Structure and dynamics of the fusion pores in live GH-secreting cells revealed using atomic force microscopy. *Endocrinology* **143**:1144-1148.

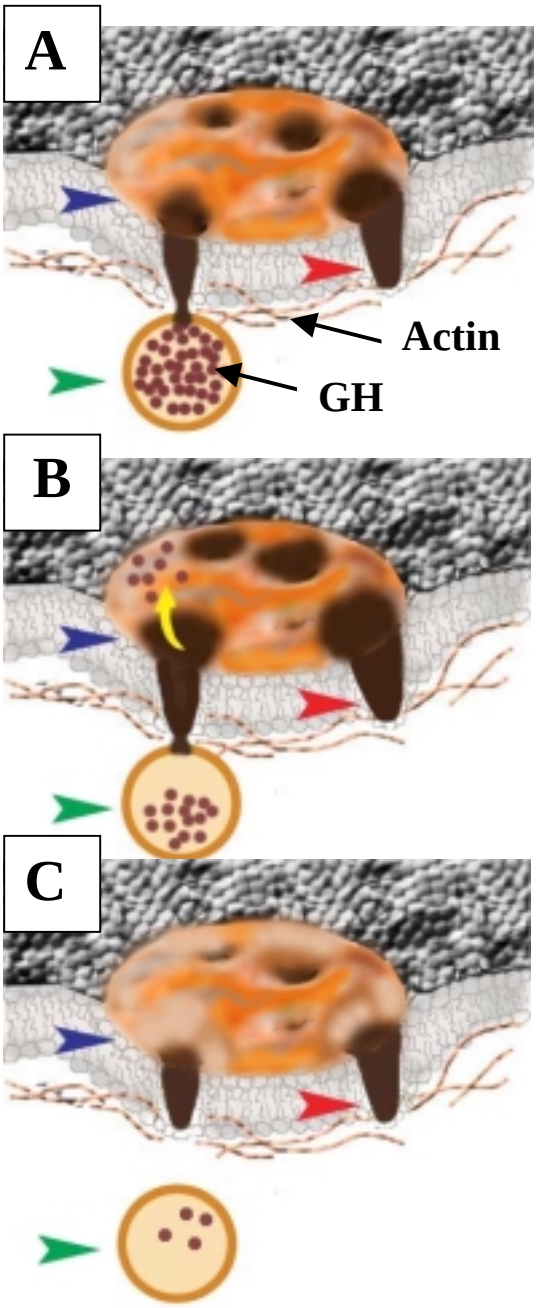

Figure 1. Schematic diagrams depict cross sectional views at cell plasma lipid membrane of a pit (blue arrowhead) and depression (red arrowhead) as well as a vesicle (green arrowhead) that contains hormone (GH), within the cell cytoplasm. Immediately after GH-secretagogue stimulation, the secretory vesicle-containing hormone docks and fuses with the depression (A) and then releases hormone (B) through the fusion pores of the depression (yellow arrow). After hormone release from the vesicle (C), the depression becomes smaller and the vesicle returns to the cellular cytoplasm.