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Imaging exocytosis of single glucagon-like peptide-1 containing granules in a murine enteroendocrine cell line with total internal reflection fluorescent microscopy

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ABSTRACT

To analyze the exocytosis of glucagon-like peptide-1 (GLP-1) granules, we imaged the motion of GLP-1 granules labeled with enhanced yellow fluorescent protein (Venus) fused to human growth hormone (hGH–Venus) in an enteroendocrine cell line, STC-1 cells, by total internal reflection fluorescent (TIRF) microscopy. We found glucose stimulation caused biphasic GLP-1 granule exocytosis: during the first phase, fusion events occurred from two types of granules (previously docked granules and newcomers), and thereafter continuous fusion was observed mostly from newcomers during the second phase. Closely similar to the insulin granule fusion from pancreatic β cells, the regulated biphasic exocytosis from two types of granules may be a common mechanism in glucose-evoked hormone release from endocrine cells.

Introduction

Glucagon-like peptide-1 (GLP-1) is stored in secretory granules in intestinal L cells, and is secreted in response to dietary nutrients [1]. Oral administration of glucose and fat results in biphasic GLP-1 secretion [2–4]. GLP-1 is a potent incretin hormone that enhances the glucose-dependent insulin release from pancreatic β cells through receptor binding [1,5]. Using GLP-1-secreting cell lines such as GLUTag and STC-1, the mechanisms underlying GLP-1 release have been identified at the single cell level. A number of stimuli, including glucose and certain amino acids, result in membrane depolarization and Ca^{2+} entry through voltage-dependent Ca^{2+} channels [6]. Glucose triggers membrane depolarization both by closing ATP-sensitive potassium channels and through the uptake of glucose by Na^{+}-coupled glucose transporters [6]. Very recently, Reimann et al. clearly demonstrated using primary intestinal L cells from transgenic mice with L cell-specific expression of a fluorescent protein that glucose can directly stimulate L cells by triggering membrane depolarization, action potential firing, and voltage-dependent Ca^{2+} entry [7]. However, it is still unclear how GLP-1 containing granules exocytose and whether high glucose directly evokes biphasic GLP-1 release from the L cells.

Imaging techniques are powerful tools for detecting granule exocytosis in live cells. The use of total internal reflection fluorescent (TIRF) microscopy, which allows fluorescence excitation within a closely restricted domain close to the plasma membrane (within 100 nm) [8], has enabled us to observe single granules undergoing exocytosis. Indeed, we have previously reported the docking and fusion of a single insulin granule in primary cultured β cells using TIRF microscopy [9].

To image the GLP-1 granule exocytosis, the observation of granules labeled with fluorescent protein-tagged GLP-1 in primary cultured L cells may be the best method; however, it is technically difficult to prepare large numbers of pure intestinal L cells and to directly label GLP-1 which is liberated from the processing of proglucagon with other proglucagon-derived peptides [1,10]. To overcome this problem, we used an expression vector encoding enhanced yellow fluorescent protein (Venus) fused to human growth hormone (hGH–Venus) to label the GLP-containing granules in STC-1 cells. Transiently expressed hGH in various secretory cells including STC-1 cells [11] is well known to be localized predominantly in secretory granules in the regulated secretory pathway [12].

In the present study, we first confirmed that hGH–Venus is efficiently packaged in GLP-1 containing granules and co-secreted

Abbreviations: DMEM, Dulbecco's modified Eagle's medium; GFP, green fluorescent protein; GLP, glucagon-like peptide; hGH, human growth hormone; KRB, Krebs Ringer Buffer; TIRF, total internal reflection fluorescence; Venus, enhanced yellow fluorescent protein.

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with GLP-1. Next, we analyzed the docking and fusion of a single hGH–Venus tagged GLP-containing granules using TIRF microscopy. We found that high glucose stimulation caused biphasic GLP-1 granule exocytosis both from previously docked granules and newcomer granules. By analogy with glucose-induced insulin granule exocytosis from pancreatic β cells, regulated biphasic exocytosis from two types of granules may be a common mechanism in glucose-evoked hormone release from endocrine cells.

Materials and methods

Cells. Murine enteroendocrine cell line STC-1 cells [13] were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM) (Sigma) containing 25 mM glucose, 10% heat-inactivated fetal bovine serum (FBS) (GIBCO), 1% penicillin, and 1% streptomycin on polyethyleneimmine (Sigma)-coated high refractive index glass (Olympus) for TIRF imaging, at 37 °C in an atmosphere of 5% CO_2. Cells were transfected with the expression vector of hGH–Venus (a generous gift from Dr. M. Takahashi, Kitasato Univ., Japan) using Lipofectamine 2000 (Invitrogen). TIRF experiments were performed 2 days after the transfection. On the day of the experiments, the transfected cells were incubated with low glucose DMEM containing 5.5 mM glucose, 10% FBS for 3 h. The medium was replaced and preincubated with Krebs Ringer Buffer (KRB) containing 110 mM NaCl, 4.4 mM KCl, 1.45 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.3 mM calcium gluconate, 4.8 mM $NaHCO_3$, 2.2 mM glucose, 10 mM HEPES (pH 7.4), 5 μM forskolin, 5 μM isobutylmethylxanthine, and 0.1% bovine serum albumin for 30 min. Then the cells were transferred to the thermostat-controlled stage (37 °C) of a TIRF microscopy.

TIRF microscopy. The Olympus total internal reflection fluorescence system was used with a high-aperture objective lens (Apo 100× OHR; NA 1.65, Olympus) [14]. To observe the fluorescence of Venus, we used a 488-nm laser line for excitation and a 515-nm long-pass filter for the barrier. Images were then projected side by side onto a CCD camera (DV887DCSBV, ANDOR) at 300-ms intervals using Metamorph version 7.1 (Universal Imaging). Stimulation with glucose was achieved by the addition of 52 mM glucose-KRB into the chamber for a final concentration of 22 mM glucose. Stimulation with KCl was achieved by the addition of 100 mM KCl–KRB (NaCl was reduced to maintain the isotonicity of the solution) into the chamber (final = 50 mM KCl).

Diiodomethane sulfur immersion oil (n = 1.81, Cargille Laboratories) was used to make contact between the objective lens and the high refractive index cover glass. Light propagates through the cover glass at an angle of 65° and undergoes total internal reflection at the glass-cell interface. The refractive indices for the glass (n = 1.8 at 488 nm) and cells (n = 1.37) predict an evanescent field declining e-fold within 44 nm from the interface, and to ~10% within 100 nm. A granule 100 nm from the interface would be illuminated too dimly to be visible under these conditions. Thus, barely 100 nm into the cell can be seen, a distance comparable to the thickness of ultrathin sections cut for electron microscopy [15]. Most analyses, including tracking (single projection of different images) and area calculations were performed using Metamorph software. Fusion events were manually selected, and the average fluorescence intensity of individual granules in a 1 × 1 μm square placed over the granule center was calculated. The number of fusion events was manually counted while looping about 2000–4000 frame time-lapses. Quantified data are presented as means ± SE.

GLP-1 and hGH release batch-assay. The transfected cells in 35 mm-dishes were incubated with low glucose DMEM for 3 h. The medium was replaced and preincubated with KRB for 30 min and then challenged for 30 min with 2.2 mM glucose (base), 22 mM glucose, or 50 mM KCl. At the end of the stimulation period, the media were collected and the cells were disrupted by sonication. The aliquots of media and cell extracts were analyzed by GLP-1 enzyme-linked immunosorbent assay (ELISA) kit (Shibayagi) and hGH ELISA Kit (Roche), respectively. The released GLP-1 and hGH are expressed as percentages of the total cellular content.

Measurement of Fura-2 fluorescence. Cells in glass-bottom culture dishes were incubated with low glucose DMEM for 3 h. The cells were loaded with 10 μM Fura-2 acetoxymethyl ester (Fura-2 AM: Molecular Probes) for 30 min at 37 °C in KRB (2.2 mM glucose), then washed and incubated for an additional 15 min with KRB. The dishes were mounted on an ARGUS/HiSCA system (Hamamatsu Photonics). Fura-2 fluorescence was detected by a cooled CCD camera after excitation at 340 nm (F340) and 380 nm (F380), and the ratio image (F340/F380) was calculated with the ARGUS/HiSCA system.

Immunostaining. Transfected cells were fixed, made permeable with 2% paraformaldehyde/0.1% Triton X-100 and processed for immunocytochemistry as described previously [14]. The intrinsic fluorescence of Venus was maintained in this condition. GLP-1 was probed with anti-GLP-1 antiserum (1:100) (Yanaihara Institute) and revealed with Alexa Fluor-546-conjugated anti-rabbit IgG (1:200) (Molecular Probes). Immunofluorescence was detected by TIRF microscopy.

Immuno-electronmicroscopy. Immuno-electron microscopy was carried out as described previously [16]. STC-1 cells were fixed in 4% paraformaldehyde–PBS for 1 h at 4 °C. They were embedded in LR White (London Resin) and sectioned. Double-immunogold

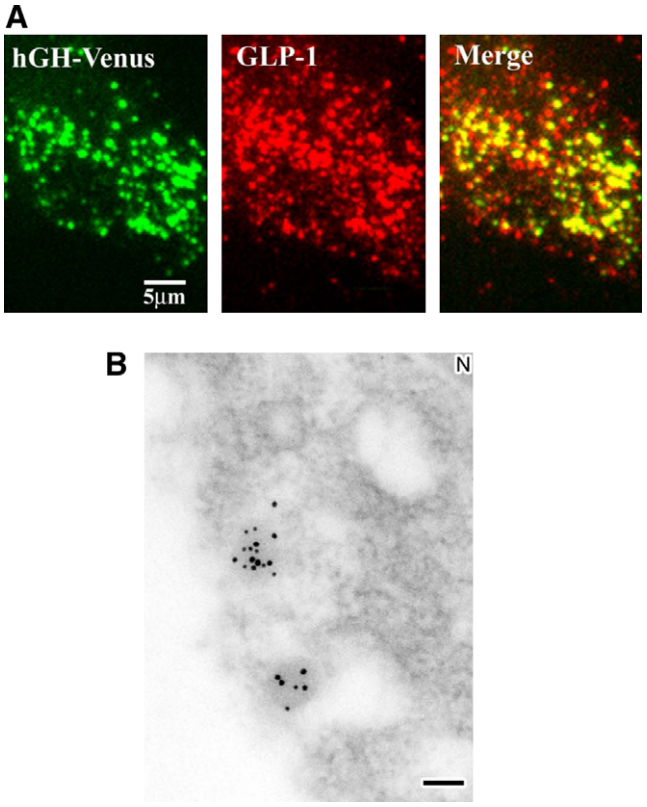

Fig. 1. Colocalization of hGH–Venus and GLP-1 in STC-1 cells. (A) After STC-1 cells were transfected with the expression vector encoding hGH–Venus, they were fixed with paraformaldehyde and immunostained with anti-GLP-1 antibody as described in Materials and methods. There was significant overlap (yellow) of the hGH–Venus (green) and GLP-1 (red) fluorescence intensity. Scale bar: 5 μm. (B) Double-labeling immuno-electronmicroscopy indicated that hGH immunoreactivity (large gold particles) is associated with GLP-1-containing (small gold particles) granules. N, nucleus; Bar = 100 nm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

labeling was performed. One grid face was incubated with rabbit anti-GLP-1 antiserum (1:100) (Yanaihara Institute) and then labeled with 12 nm colloidal gold-conjugated donkey anti-rabbit antibody (1:50) (Jackson Immunoresearch). After drying, the grid was turned over and the other grid face was incubated with rabbit anti-growth hormone antiserum (AFP5672099) (1:100) (National Hormone and Pituitary Program) and then labeled with 18 nm colloidal gold-conjugated donkey anti-rabbit antibody (Jackson Immunoresearch). Sections were stained with uranyl acetate and lead citrate and examined with JEM-1010C (JEOL).

Results and discussion

Colocalization of GLP-1 and hGH–Venus in STC-1 cells

First, to examine whether hGH–Venus transiently expressed in STC-1 cells is localized to GLP-1 containing granules, the localization of the fluorescence of hGH–Venus was compared with that of GLP-1 in fixed STC-1 cells. The intense punctuate fluorescence of Venus was localized to 92.1 ± 2.6% of GLP-1 containing granules stained with anti-GLP-1 antibodies as detected by immunohistochemistry (Fig. 1A). Furthermore, double-labeling immuno-electronmicroscopy also revealed that hGH was associated with GLP-1 containing granules (Fig. 1B). These results indicate that most of the hGH–Venus expressed in STC-1 cells was localized to GLP-1 containing granules.

Fusion events induced by KCl stimulation originate mostly from previously docked granules in STC-1 cells

Stimulation with high KCl (50 mM) has been shown to induce GLP-1 release from STC-1 cells by Ca^{2+} influx through L-type voltage-dependent Ca^{2+} channels [17]. In fact, 50 mM KCl stimulation induced a rapid and marked increase, and a quick decrease in the cytosolic Ca^{2+} concentration was observed in almost 90% of the cells (Fig. 2A). Fig. 2B shows the net endogenous GLP-1 and hGH release from hGH–Venus expressing STC-1 cells exposed to 50 mM KCl solution measured by release batch-assay and ELISA. The net hGH release was significantly observed in a similar proportion to endogenous net GLP-1 release upon KCl stimulation suggesting that hGH in GLP-1 containing granules is in the regulated secretory pathway.

We then used TIRF microscopy to monitor the real-time docking and fusion process of single GLP-1 containing granules labeled with hGH–Venus near the plasma membrane. Fusion events evoked by KCl stimulation (50 mM) were almost always seen during the first 60 s (Fig. 2C). It is noted that most fusion occurred from morphologically previously docked granules that were visible before stimulation (red column in Fig. 2C). As shown in the sequential images of single granules, the fluorescent spot remained nearly constant, as if it were docked at the plasma membrane, then suddenly brightened and vanished (red boxes in Supplementary Fig. S1). Some of the fusion occurred from newcomer granules

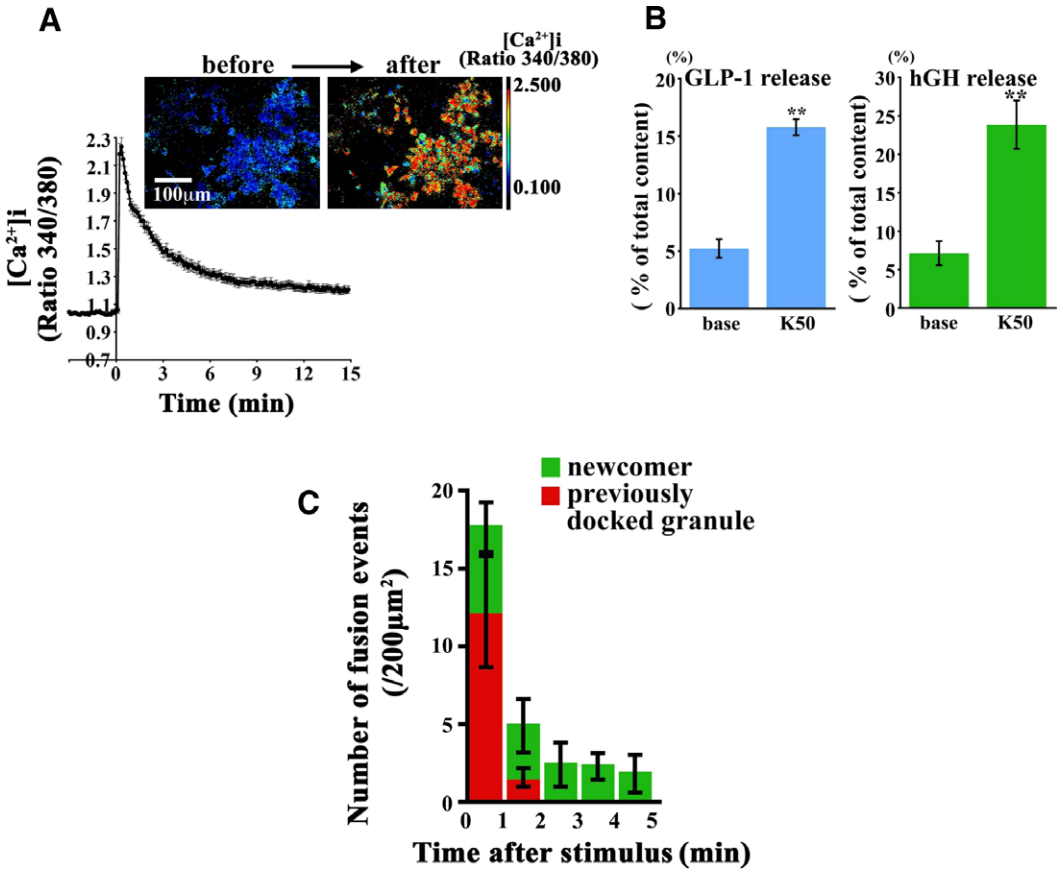

Fig. 2. TIRF images and analysis of single GLP-1 containing granules labeled with hGH–Venus in STC-1 cells during high KCl (50 mM) stimulation. (A) 50 mM KCl-induced changes in $[Ca^{2+}]_i$ in STC-1 cells. Changes in $[Ca^{2+}]_i$ were measured by 10 μM Fura-2 AM. Time 0 indicates when high KCl was added. The typical pseudocolor $[Ca^{2+}]_i$ images before and after (0.35 min after) KCl stimulation were superimposed. Note that KCl-responsive cells were 89.2 ± 2.2 % of total STC-1 cells (n = 8 microscope field). Data are the means ± SE (n = 8 cells). (B) The net high KCl-evoked secretion of endogenous GLP-1 and hGH from STC-1 cells measured by release batch-assay and ELISA. Data are the means ± S.E. (n = 5 each). **, $P < 0.001$ versus base. (C) Histogram showing the number of fusion events in granules labeled with hGH–Venus (per 200 μm^2) at 1-min intervals after high KCl stimulation measured by TIRF microscopy. Data are the means ± SE (n = 4 cells). Time 0 indicates the addition of 50 mM KCl. The red column shows fusion events from previously docked granules, and the green column shows those from newcomers. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

which approached from the inside (being absent before stimulation), reached the plasmalemma and then were quickly fused (green column and boxes in Fig. 2C and Supplementary Fig. S1). We previously reported insulin granule exocytosis in pancreatic β cells by TIRF imaging of GFP-tagged insulin granules docking and fusion [9]. Interestingly, the insulin granule fusion evoked by high KCl with rapid and marked Ca^{2+} influx also occurred mainly from previously docked granules, with only some of the fusion occurring in newcomer granules in pancreatic β cells [18]. Thus, the KCl-induced exocytosis of GLP-containing granules seems to be regulated in the same manner as insulin granule exocytosis in pancreatic β cells.

Two types of granules are associated with glucose-evoked biphasic release from STC-1 cells

In STC-1 cells, it has been reported that glucose, an agent that inhibits K_{ATP} channels by increasing the cytosolic ATP levels, produces both plasma membrane depolarization and a significant increase in $[Ca^{2+}]_i$ through voltage-dependent Ca^{2+} channels [19]. we examined the docking and fusion process of GLP-1-containing granules during physiological secretagogue, glucose stimulation. As shown in Fig. 3A, a 22 mM glucose-evoked rise in $[Ca^{2+}]_i$ was seen in about 27 % of the STC-1 cells in our experiment, but the reason for the cell heterogeneity in the glucose-induced Ca^{2+} response is unknown at present. in the glucose-responsive cells, $[Ca^{2+}]_i$ showed a biphasic pattern: a marked rise and slow decrease, then a sustained concentration. Fig. 3B shows the net endogenous GLP-1 and hGH release from hGH–Venus expressing STC-1 cells exposed to 22 mM glucose measured by release batch-assay and ELISA. Reflecting the glucose responsiveness to $[Ca^{2+}]_i$ rise, the net hGH was slightly but significantly released in a similar proportion to endogenous net GLP-1 upon glucose stimulation.

We then examined the docking and fusion of GLP-1-containing granules labeled with hGH–Venus during high glucose stimulation in the glucose-responsive cells. As shown in Fig. 3C, 22 mM glucose stimulation evoked biphasic granule exocytosis with fusion from two types of granules (previously docked granules and newcomers). During the first phase (within 3 min) that was immediately seen after glucose stimulation, fusion events occurred from both previously docked granules and newcomers, and thereafter a continuous fusion was observed mostly from newcomers during the second phase (over 15 min) (Supplementary Fig. S2).

Thus, we found that high glucose directly evoked biphasic GLP-1 granule exocytosis from single GLP-1 secreting cells *in vitro*. In insulin exocytosis from pancreatic β cells, such fusion from two types of granules was also observed during biphasic glucose-evoked release. during the first phase, fusion originated from previously docked granules with some of the fusion from newcomers, whereas granule fusion during the second phase arose mostly from newcomers [9]. Analogously with the glucose-induced insulin granule exocytosis, regulated biphasic exocytosis from two types of granules may be a common mechanism in glucose-evoked hormone release from endocrine cells.

The mechanisms underlying the biphasic granule exocytosis are still not fully understood, but in β cells we and others recently suggested that the phasic Ca^{2+} concentration may contribute to the biphasic insulin granule exocytosis with two types of granules [18,20]. Pedersen and Sherman, using a kinetic model of insulin granule exocytosis, reported that newcomer granules are from a

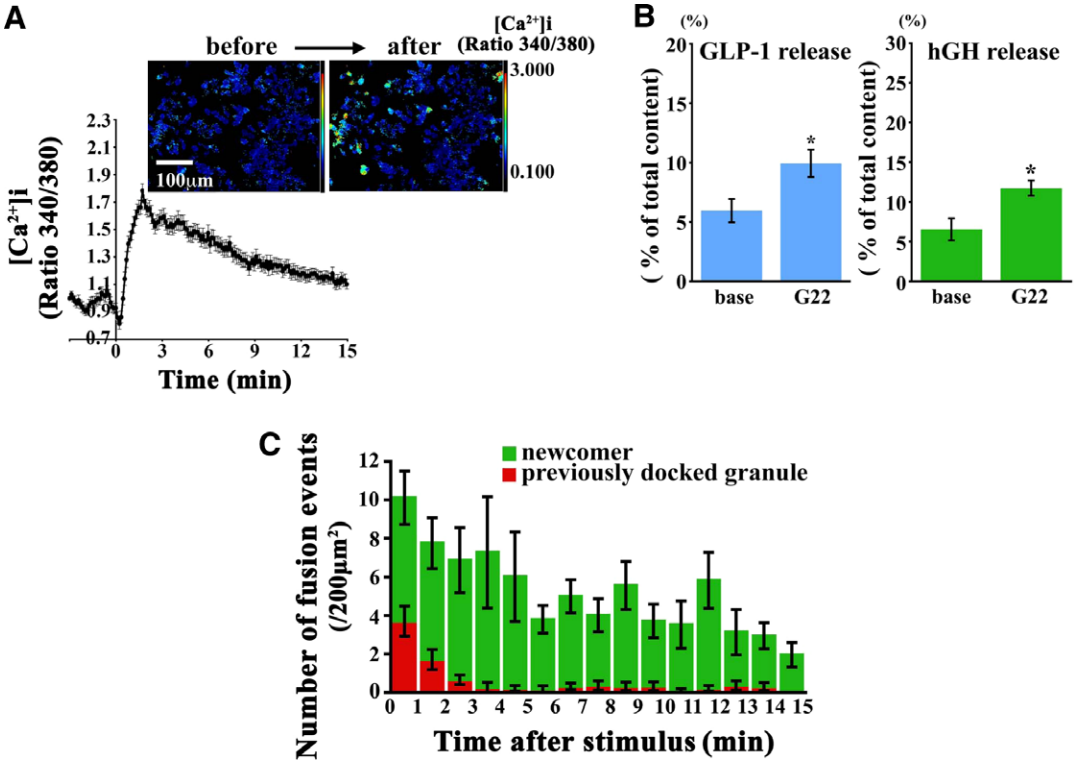

Fig. 3. TIRF images and analysis of single GLP-1 containing granules labeled with hGH–Venus in STC-1 cells during high glucose (22 mM) stimulation. (A) 22 mM glucose-induced changes in $[Ca^{2+}]_i$ in STC-1 cells. Changes in $[Ca^{2+}]_i$ were measured by 10 μM Fura-2 AM. Time 0 indicates when high glucose was added. The typical pseudocolor $[Ca^{2+}]_i$ images before and after (1.72 min after) glucose stimulation were superimposed. Note that glucose-responsive cells were 27.0 ± 1.9 % of total STC-1 cells (n = 13 microscope field). Data are the means ± SE (n = 8 cells). (B) The net high glucose-evoked secretion of endogenous GLP-1 and hGH from STC-1 cells measured by release batch-assay and ELISA. Data are the means ± SE (n = 5 each). *, $P < 0.05$ versus base. (C) Histogram showing the number of fusion events in granules labeled with hGH–Venus (per 200 μm^2) at 1-min intervals after high glucose stimulation measured by TIRF microscopy. Data are the means ± SE (n = 12 cells). Time 0 indicates the addition of 22 mM glucose. The red column shows fusion events from previously docked granules, and the green column shows those from newcomers. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

highly Ca^{2+} sensitive pool, whereas docked granules are from a lower Ca^{2+} sensitive immediately releasable pool, suggesting that the Ca^{2+} concentration determines whether the fusion events are from previously docked granules or newcomers [20]. We experimentally showed that the rise in the Ca^{2+} concentration directly determined the type of fusing insulin granules [18]. Indeed, the glucose-induced subplasma membrane Ca^{2+} concentration ($[Ca^{2+}]_{PM}$) rise displayed a biphasic pattern: during the 1st phase, the rapid and marked $[Ca^{2+}]_{PM}$ rise caused fusion events from previously docked granules with some from newcomers; during the 2nd phase, the slow and sustained $[Ca^{2+}]_{PM}$ rise caused fusion events mostly from newcomers. Thus, the glucose-evoked biphasic pattern of the Ca^{2+} concentration may be important in forming the biphasic insulin release via direct triggering of each type of granule fusion. Interestingly, the glucose-induced $[Ca^{2+}]_i$ rise also showed a biphasic pattern in STC-1 cells (Fig. 3A). Thus, the common biphasic Ca^{2+} concentration by a glucose sensing mechanism involving ATP-dependent K_{ATP} channels, membrane depolarization and Ca^{2+} influx through voltage-dependent Ca^{2+} channels may contribute to the biphasic exocytosis from two types of granules in both STC-1 cells and β cells. Of course, our data do not exclude the possibility of effects of other signals, e.g., cAMP on biphasic granule exocytosis in both cells, thus, more experiments will be required to confirm our hypothesis.

In conclusion, this is the first report to image the motion of GLP-1 granule. In future, TIRF analysis using hGH–Venus tagged granule system may help to reveal the molecular mechanism of GLP-1 granule exocytosis in L cells evoked not only by glucose, but also by other secretagogues.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.09.043.

References

[1] J.J. Holst, The physiology of glucagon-like peptide 1, Physiol. Rev. 87 (2007) 1409–1439.
[2] R.M. Elliott, L.M. Morgan, J.A. Tredger, S. Deacon, J. Wright, V. Marks, Glucagon-like peptide-1 (7–36) amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns, J. Endocrinol. 138 (1993) 159–166.
[3] M.A. Nauck, M.M. Heimesaat, C. Orskov, J.J. Holst, R. Ebert, W. Creutzfeldt, Preserved incretin activity of glucagon-like peptide 1 [7–36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus, J. Clin. Invest. 91 (1993) 301–307.
[4] C. Herrmann, R. Goke, G. Richter, H.C. Fehmann, R. Arnold, B. Goke, Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients, Digestion 56 (1995) 117–126.
[5] G. Tolhurst, F. Reimann, F.M. Gribble, Nutritional regulation of glucagon-like peptide-1 secretion, J. Physiol. 587 (2009) 27–32.
[6] F. Reimann, P.S. Ward, F.M. Gribble, Signaling mechanisms underlying the release of glucagon-like peptide 1, Diabetes 55 (Suppl. 2) (2006) S78–S85.
[7] F. Reimann, A.M. Habib, G. Tolhurst, H.E. Parker, G.J. Rogers, F.M. Gribble, Glucose sensing in L cells: a primary cell study, Cell Metab. 8 (2008) 532–539.
[8] D. Axelrod, Total internal reflection fluorescence microscopy, Methods Cell Biol. 30 (1989) 245–270.
[9] M. Ohara-Imaizumi, C. Nishiwaki, T. Kikuta, S. Nagai, Y. Nakamichi, S. Nagamatsu, TIRF imaging of docking and fusion of single insulin granule motion in primary rat pancreatic beta-cells: different behaviour of granule motion between normal and Goto-Kakizaki diabetic rat beta-cells, Biochem. J. 381 (2004) 13–18.
[10] G.E. Lim, P.L. Brubaker, Glucagon-like peptide 1 secretion by the L-cell, Diabetes 55 (Suppl. 2) (2006) S70–S77.
[11] K. Matsumura, T. Miki, T. Jhomori, T. Gonoi, S. Seino, Possible role of PEPT1 in gastrointestinal hormone secretion, Biochem. Biophys. Res. Commun. 336 (2005) 1028–1032.
[12] P.F. Wick, R.A. Senter, L.A. Parsels, M.D. Uhler, R.W. Holz, Transient transfection studies of secretion in bovine chromaffin cells and PC12 cells. Generation of kainate-sensitive chromaffin cells, J. Biol. Chem. 268 (1993) 10983–10989.
[13] G. Rindi, S.G. Grant, Y. Yiangou, M.A. Ghatei, S.R. Bloom, V.L. Bautch, E. Solcia, J.M. Polak, Development of neuroendocrine tumors in the gastrointestinal tract of transgenic mice. Heterogeneity of hormone expression, Am. J. Pathol. 136 (1990) 1349–1363.
[14] M. Ohara-Imaizumi, T. Fujiwara, Y. Nakamichi, T. Okamura, Y. Akimoto, J. Kawai, S. Matsushima, H. Kawakami, T. Watanabe, K. Akagawa, S. Nagamatsu, Imaging analysis reveals mechanistic differences between first- and second-phase insulin exocytosis, J. Cell Biol. 177 (2007) 695–705.
[15] D. Zenisek, J.A. Steyer, W. Almers, Transport, capture and exocytosis of single synaptic vesicles at active zones, Nature 406 (2000) 849–854.
[16] Y. Akimoto, G.W. Hart, L. Wells, K. Vosseller, K. Yamamoto, E. Munetomo, M. Ohara-Imaizumi, C. Nishiwaki, S. Nagamatsu, H. Hirano, H. Kawakami, Elevation of the post-translational modification of proteins by O-linked *N*-acetylglucosamine leads to deterioration of the glucose-stimulated insulin secretion in the pancreas of diabetic Goto-Kakizaki rats, Glycobiology 17 (2007) 127–140.
[17] J. Eiki, K. Saeki, N. Nagano, T. Iino, M. Yonemoto, Y. Takayenoki-Iino, S. Ito, T. Nishimura, Y. Sato, M. Bamba, H. Watanabe, K. Sasaki, S. Ohyama, A. Kanatani, T. Nagase, T. Yada, A selective small molecule glucagon-like peptide-1 secretagogue acting via depolarization-coupled Ca(2+) influx, J. Endocrinol. 201 (2009) 361–367.
[18] M. Ohara-Imaizumi, K. Aoyagi, Y. Nakamichi, C. Nishiwaki, T. Sakurai, S. Nagamatsu, Pattern of rise in subplasma membrane Ca^{2+} concentration determines type of fusing insulin granules in pancreatic beta cells, Biochem. Biophys. Res. Commun. 385 (2009) 291–295.
[19] A.W. Mangel, V. Prpic, N.D. Snow, S. Basavappa, L.J. Hurst, A.I. Sharara, R.A. Liddle, Regulation of cholecystokinin secretion by ATP-sensitive potassium channels, Am. J. Physiol. 267 (1994) G595–G600.
[20] M.G. Pedersen, A. Sherman, Newcomer insulin secretory granules as a highly calcium-sensitive pool, Proc. Natl. Acad. Sci. USA 106 (2009) 7432–7436.