

CHINESE MEDICAL SCIENCES JOURNAL

SHORT COMMUNICATION

Observation of Insulin Exocytosis by a Pancreatic β Cell Line with Total Internal Reflection Fluorescence Microscopy

Zhao-ying Fu^{1*}, Ya-ping Wang¹, and Yu Chen²

¹College of Medicine, Yanan University, Yanan 716000, China

²Institute of Biophysics, Chinese Academy of Science, Beijing 100101, China

Key words: total internal reflection fluorescence microscopy; exocytosis; insulin; kiss-and-run

INSULIN secretion was traditionally measured with biochemical and immunological methods such as enzyme linked immunosorbant assay and radio-immunoassay. However, these methods can only tell the amount of insulin secreted; they give no information about the secretion process or mechanism of exocytosis. In recent years, an imaging technique known as total internal reflection fluorescence (TIRF) microscopy has been employed to study insulin secretion.¹⁻⁴ This imaging technique can explore events taking place near or on live cell membrane, such as secretory granule movement, exocytosis, vesicle content release, and membrane fusion.⁵⁻¹⁰ In the present paper, we applied TIRF microscopy to the observation of insulin exocytosis by the pancreatic β cell line Ins-1.

MATERIALS AND METHODS

Materials

RPMI 1640 medium, fetal calf serum, 2-mercaptoethanol, trypsin, and EDTA were GIBCO products (Invitrogen Corporation, Carlsbad, CA, USA). Sodium pyruvate, poly-L-lysine, NaCl, KCl, Na₂HPO₄, KH₂PO₄, CaCl₂·2H₂O, MgSO₄·7H₂O, HEPES, MgCl₂·6H₂O, CaCl₂·2H₂O, glucose, and bovine sera

albumin were Sigma products (St. Louis, MO, USA). Ins-1 cell line (pancreatic β cell-derived or insulinoma-originated) and expression plasmid containing cDNA for fused vesicle associated membrane protein 2 and enhanced green fluorescent protein (VAMP2-eGFP) were from Institute of Biophysics, Chinese Academy of Science. Round glass coverslips (n=1.518, d=25 mm) were from Menzel-glaser (Braunschweig, Germany). The 25 cm culture flasks and 12 well culture plates were BD Falcon products (Sparks, USA). Lipofectamione 2000 was from Invitrogen Corporation.

Cell culture

Ins-1 cells were cultured with 5 mL of culture medium (90 mL RPMI 1640 medium, 10 mL fetal calf serum, 11 mmol/L glucose, 1 mL sodium pyruvate, and 2-mercaptoethanol) in a 25 cm culture flask at 37°C. The culture medium was changed every 2 days. The cell culture was passed to a new passage when the cells came into contact with each other or grew to a single layer, which usually took 4-5 days.

Transfection

Ins-1 cells were transfected with VAMP2-eGFP cDNA expression-plasmids by using Lipofectamione 2000. The day before transfection, Ins-1 cells were transferred from a 25 cm flask to a 12 well culture plate. Cells were allowed to grow to a density of 80%-90% before transfection was carried out. Transfection was performed in accordance with manufacture's instructions. Six hours after transfection,

Received for publication November 23, 2010.

*Corresponding author Tel: 86-13259326181, E-mail: zhaoyingfu@tom.com

culture medium was changed with fresh medium as mentioned earlier. Twelve hours after transfection, the cells were examined under an inverted fluorescent microscope to see if VAMP2-eGFP proteins had been expressed.

Transfer of transfected cells to glass coverslip

Twenty-four hours after transfection, the successfully transfected Ins-1 cells were transferred to a poly-L-lysine coated glass coverslip that was then placed in a 35 mm culture dish. Coverslips were treated beforehand with chromic acid for over 6 hours, cleaned with deionized water, and soaked in absolute ethyl alcohol for use. After 20 minutes at 37°C, when the cells were attached to the coverslip, fresh culture medium was added to the culture dish and the cells were incubated at 37°C. After 24 hours of incubation, the cells were ready for insulin secretion induction and TIRF microscopic examination.

Induction of insulin secretion

The coverslip mentioned above was mounted to a special TIRF microscopic chamber and the chamber was filled with Krebs-Ringer bicarbonate buffer (3 mmol/L glucose, 136 mmol/L NaCl, 4.70 mmol/L KCl, 5.00 mmol/L Na_2HPO_4 , 1.20 mmol/L KH_2PO_4 , 2.50 mmol/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.20 mmol/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 10.0 mmol/L HEPES, 1 g/L bovine sera albumin; pH 7.2, osmosis 290-310 mmol/kg). Insulin secretion was induced by replacing Krebs-Ringer bicarbonate buffer in the chamber with a solution containing high concentration of glucose and potassium chloride (15.0 mmol/L glucose, 70.0 mmol/L KCl, 57.8 mmol/L NaCl, 2.60 mmol/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.20 mmol/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 10.0 mmol/L HEPES; pH 7.2, osmosis 295-312 mmol/kg). The experiments were performed at room temperature.

TIRF microscopic examination of insulin exocytosis

TIRF microscope setup was constructed using an Olympus IX71 inverted microscope (Olympus Co., Tokyo, Japan) based on the prism-less and through-the-lens configuration. TIRF microscopic examination of insulin-containing vesicle exocytosis was performed by using incidence laser beams of 488 nm wavelength (argon ion laser). Successive images were collected every 0.3 second before stimulation (100 images) and after stimulation with the solution containing high concentration of glucose and potassium (500 images).

RESULTS

TIRF microscopic imaging before glucose-potassium stimulation

Before the Ins-1 cells were induced to secrete insulin with the solution containing high concentration of glucose (15.0 mmol/L) and potassium chloride (70.0 mmol/L), Brownian-like motion of fluorophores was viewed within the Ins-1 cells; no obvious exocytosis (the appearance of bright spots on the cell membrane) could be seen during a certain period of time (Fig. 1).

TIRF microscopic imaging of glucose-potassium stimulated insulin exocytosis

After stimulating Ins-1 cells with high concentration of glucose and potassium, bright spots could be observed in numerous cells moving to or approaching the cell membrane and then fading away, resembling the kiss-and-run model of insulin exocytosis (Fig. 2). Some bright spots emerged and then stayed on the cell membrane continuously; these should be the results of the full membrane fusion exocytosis, the established way of insulin secretion (Fig. 2).

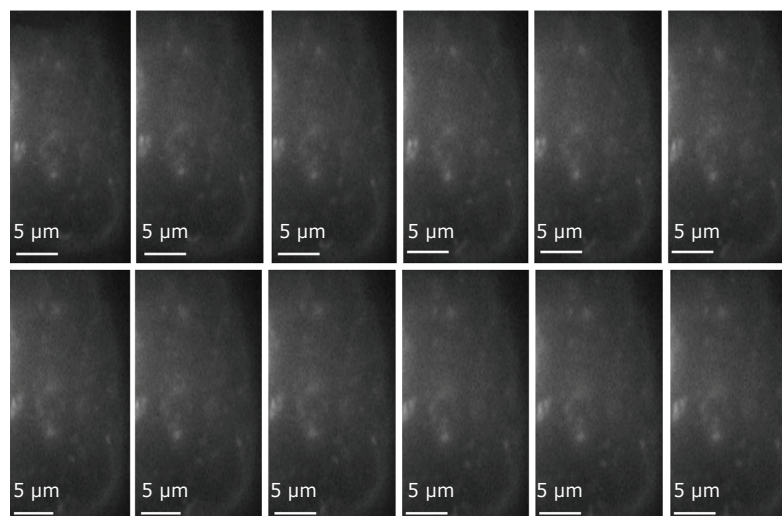


Figure 1. Total internal reflection fluorescence (TIRF) microscopic images of an Ins-1 cell before glucose-potassium stimulation.

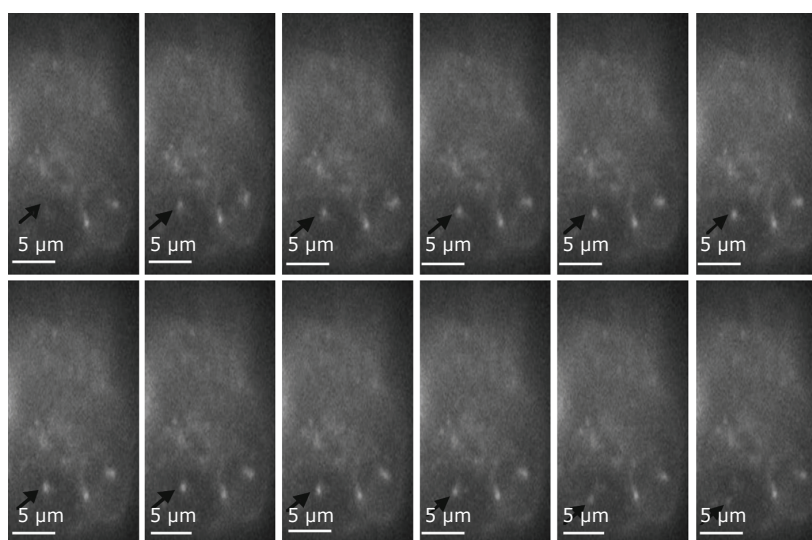


Figure 2. TIRF microscopic images of an Ins-1 cell after glucose-potassium stimulation.

The arrow in the first image shows the place where a fluorophore (insulin-containing vesicle) will appear, those in the 2nd to the 11th images indicate the insulin-containing vesicle has been near or on the cell membrane; and that in the last image shows the vesicle has just faded away from the membrane. These together suggest the kiss-and-run mode of exocytosis. The two bright spots in the lower right of the cell should be the sites where two insulin-containing vesicles have just fused with the cell membrane (full fusion exocytosis, the classical way of insulin secretion).

DISCUSSION

When light passes through a higher refractive index medium (e.g. glass) into a lower refractive index medium (e.g. tissue), and when the incident angle is greater than a critical value, the light is totally reflected into the first medium. The reflected light generates an evanescent field or waves that penetrate into the second medium. The intensity of the evanescent field decreases exponentially with distance. Usually, the thickness of effective penetration is less than 100 nm. TIRF microscopy, also known as evanescent field (or wave) microscopy, utilizes the evanescent field illumination to detect fluorophores that are very close to the specimen-glass interface. The most significant advantages of this technique are: (1) its greatly increased signal to noise ratio (S/N), which makes this technique remarkably superior to other similar techniques such as wild field microscopy or confocal laser scanning microscopy, (2) its usefulness in examination of living specimens, and (3) the real time observation of life processes.⁷⁻¹⁰ One of the most frequent applications of TIRF microscopy is the observation of the events taking place in the vicinity of cell membrane such as exocytosis and endocytosis.⁵⁻⁹ In the present study, we applied TIRF microscopy to the examination of the mechanism of insulin exocytosis/secretion.

The classical model of insulin secretion involves the full

merge or fusion of the secretory vesicle membrane with the plasma membrane (as well as the complete release of the vesicle contents) and is known as full fusion or total fusion exocytosis. In recent years, an alternative mechanism has been proposed about insulin secretion; it is called kiss-and-run or transient fusion exocytosis.¹¹⁻¹⁴ According to this new model, the secretory vesicle makes contact with the plasma membrane only transiently, forming a fusion hole, releasing its contents partially, detaching from the cell membrane and retracting into the cytoplasm with its membrane intact. The physiological significance of the kiss-and-run mode of exocytosis may lie in facilitating hormone refilling and speeding up successive or continued secretion. In our experiment, we examined the process of insulin secretion by a pancreatic β cell-derived cell line with TIRF microscopy; our results supported the kiss-and-run model of insulin exocytosis. To further investigate the kiss-and-run mechanism of insulin secretion, we may quantitatively measure and compare the two modes of insulin exocytosis with TIRF microscopy. We may also use siRNA technique to knock out some of the proteins or overexpress some proteins implicated in cellular exocytosis to identify the important elements involved in the kiss-and-run exocytosis.

REFERENCES

1. Fish KN. Total internal reflection fluorescence (TIRF) mi-

- croscopy. In: Robinson JP, editors. Current protocols in cytometry. New York: John Wiley & Sons Inc.; 2009 .p. 1801-13.
2. Nagamatsu S. TIRF microscopy analysis of the mechanism of insulin exocytosis. *Endocr J* 2006; 53:433-40.
 3. Nagamatsu S, Ohara-Imaizumi M. Imaging exocytosis of single insulin secretory granules with TIRF microscopy. *Methods Mol Biol* 2008; 440:259-68.
 4. Ohara-Imaizumi M, Nagamatsu S. Insulin exocytotic mechanism by imaging technique. *J Biochem (Tokyo)* 2006; 140:1-5.
 5. Bowser DN, Khakh BS. Two forms of single-vesicle astrocyte exocytosis imaged with total internal reflection fluorescence microscopy. *Proc Natl Acad Sci USA* 2007; 104:4212-7.
 6. Holz RW. Analysis of the late steps of exocytosis: biochemical and total internal reflection fluorescence microscopy (TIRFM) studies. *Cell Mol Neurobiol* 2006; 26: 439-47.
 7. Toomre D, Axelrod D. Total internal reflection fluorescence microscopy. In: Celis JE, editors. *Cell biology, a laboratory handbook*. 3rd ed. Amsterdam: Elsevier Inc., 2006 .p.19-28.
 8. Trache A, Meininger GA. Total internal reflection fluorescence (TIRF) microscopy. In: Coico R, Kowalik T, Quarles JM, editors. *Current protocols in microbiology*. New York: John Wiley & Sons Inc.; 2008 .p.201-22.
 9. Axelrod D. Total internal reflection fluorescence microscopy. In: Allen T, editors. *Methods in cell biology*. Amsterdam: Elsevier Inc., 2008. p.169-221.
 10. Letinic K, Sebastian R, Barthel A. Deciphering sub-cellular processes in live imaging data sets *via* dynamic probabilistic networks. *Bioinformatics* 2010; 26:2029-36.
 11. Tsuboi T, McMahon HT, Rutter GA. Mechanisms of dense core vesicle recapture following "kiss and run" ("cavicapture") exocytosis in insulin-secreting cells. *J Biol Chem* 2004; 279:47115-24.
 12. MacDonald PE, Braun M, Galvanovskis J, et al. Release of small transmitters through kiss-and-run fusion pores in rat pancreatic β cells. *Cell Metab* 2006; 4:283-90.
 13. Rutter GA, Hill EV. Insulin vesicle release: walk, kiss, pause...then run. *Physiology (Bethesda)* 2006; 21:189-96.
 14. Hanna ST, Pigeau GM, Galvanovskis J, et al. Kiss-and-run exocytosis and fusion pores of secretory vesicles in human β -cells. *Pflugers Arch* 2009; 457:1343-50.