A new possible method to detect the rate of insulin release from mouse pancreatic islet cells using Zinquin, a specific fluorescent probe for zinc

Jumhee Kim

Laboratory of Physiology
Department of Biomedical Sciences
School of Veterinary Medicine
Hokkaido University, Sapporo 060-0818, Japan

In the present experiments, an attempt was made to detect the rate of insulin release from isolated islets using Zinquin, a specific probe for zinc, as zinc is co-stored and co-released with insulin by exocytosis. Changes in Zinquin fluorescence were examined with incubation solutions of isolated mouse islets with various stimuli for exocytosis. Various secretagogues which stimulate insulin release also increased Zinquin fluorescence, i. e. zinc concentration, indicating co-release of both substances.

A significant correlation was obtained between zinc measured with the Zinquin method and insulin concentration measured by RIA.

This report was the first demonstration that Zinquin fluorescence increased in association with insulin release with a significant correlation and may propose future possibility of utilization of this method for semi-quantitative detection of insulin release under experimental conditions.

Photodynamic modulation of exocytosis in rat peritoneal mast cells

Sayaka Hashikura

Laboratory of Physiology, Department of Biomedical Sciences,
School of Veterinary Medicine,
Hokkaido University, Sapporo 060-0818, Japan

Photodynamic action which produces singlet oxygen by transferring photon energy from a phtosensitizer to ground state molecular oxygen can modify various cellular responses. It has been reported that membrane -localized phtosensitizers such as PLMGdB (gadolinium porphyrin-like macrocycle B) and SALPC (sulfonated aluminum phthalocy- and cardiomyocytes, $[Ca^{2+}]_i$ is increased by photodynamic action. A photosensitizer could induce contraction of smooth muscle cells or elicite amylase secretion in isolated rat pancreatic acinar cells while it inhibited amylase release from AR 4-2 J cells. These effects are assumed to be caused by singlet oxygen -induced permanent conformational changes

cretory cells, the present experiments were conducted using rat peritoneal mast cells. Qualitative experiments were carried out to investigate photodynamic modification of exocytosis by directly observing exocytosis with conventional microscope and with sulforhodamine B. The latter is one of exocytotic probes which bind with intragranular matrix protein after exocytosis and produces fluorescence. In addition, $[Ca^{2+}]_i$ was also monitored with indo-1.

1. Photostimulation of SALPC (5 μM)-loaded mast cells (20,000 lux, 2 min) itself caused neither exocytosis nor $[Ca^{2+}]_i$ increase in mast cells. This result is incompatible with that reported in other cell types such as pancreatic acinar cells.

2. Stimulation with compound 48/80 (50 μg/ml), a direct G-protein activator, induced massive exocytosis which was easily detectable under conventional microscope. The fluorescent granules stained with sulforhodamine B were found to be numerous on the perimetry of or around mast cells, confirming occurrence of exocytosis. The stimulation also increased $[Ca^{2+}]_i$ and cell volume before exocytosis.

3. Pretreatment of the cells with photodynamic action with SALPC inhibited the compound 48/80-induced exocytosis but the $[Ca^{2+}]_i$ increase and increase of cell volume were unaffected.

4. NaN_3 at 0.5 mM could relieve the photodynamic action-induced inhibition of exocytosis.

These results indicate that, unlikely to other secretory or contractile cells, photodynamic action with SALPC does not directly affect exocytotic machinery but modulates some functional proteins involved in signal transduction process which may be posterior to G-protein activation in mast cell. A singlet oxygen may be involved in the photodynamic action-induced modulation. A possible target protein can be a protein in the cell membrane which binds with a protein of a granular membrane durng the course of exocytosis. A further experiments remain to be conducted to clarify the site of action in mast cell.