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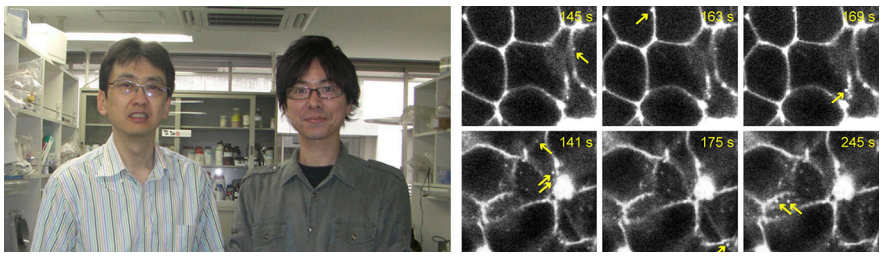
A reason to avoid SNAP judgments

Study reveals that the SNARE protein SNAP23 has opposing functions in exocrine and endocrine pancreatic cells.

FOCAL POINT



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Akihiro Harada (left), Masataka Kunii (right), and colleagues investigate the function of SNAP23 and find that the SNARE protein has opposing roles in the exocrine and endocrine cells of the pancreas. Whereas SNAP23 promotes the exocytosis of secretory granules in exocrine acinar cells, it reduces the secretion of insulin-containing granules from endocrine β cells by competitively inhibiting the assembly of its more-efficient homologue, SNAP25, into fusion-competent SNARE complexes. Time-lapse imaging of pancreatic islets labeled with a fluorescent, fluid-phase marker show that, compared with control cells (top), insulin granule exocytosis (arrows) is increased in β cells lacking SNAP23 (bottom). The researchers also identify a small molecule that enhances insulin secretion by inhibiting SNAP23, offering a potential new approach to treating diabetes.

Secretory vesicles release their contents from the cell by fusing with the plasma membrane, a process catalyzed by SNARE proteins in the vesicle and plasma membranes. Kunii et al. now report that, although the SNARE protein SNAP23 promotes the release of secretory granules from pancreatic acinar cells, it inhibits the secretion of insulin-containing granules from β cells. Moreover, the researchers identify a compound that binds to SNAP23 and enhances insulin release in mice, raising the possibility of a new approach to treating diabetes (1).

The SNARE protein SNAP25 promotes insulin secretion from β cells, an endocrine cell type in the pancreas, by forming a complex with the SNAREs VAMP2 and syntaxin1A. SNAP23 is a ubiquitously expressed homologue of SNAP25 that can mediate insulin secretion, albeit less efficiently, when SNAP25 is inhibited (2). SNAP23 has also been shown to mediate secretory granule exocytosis in a variety of cell types that don't express SNAP25. It is involved, for example, in the secretion of digestive enzyme-containing zymogen granules from acinar cells, the exocrine cells of the pancreas (3). Relatively little is known about SNAP23's function in vivo, however; SNAP23-deficient mice die early in embryogenesis. Akihiro Harada and colleagues at Osaka University in Japan therefore generated a conditional knockout mouse strain that would allow them to specifically remove SNAP23 from individual tissues.

Led by Assistant Professor Masataka Kunii, the researchers decided to delete SNAP23 from the different cell types of the pancreas (1). As expected, mice lacking SNAP23 in their acinar cells showed impaired secretion of digestive enzymes such as amylase, although, as previously seen in mice lacking another SNARE involved in zymogen granule fusion (3), the animals' overall health was largely unaffected.

Next, the researchers deleted SNAP23 from pancreatic β cells, expecting that insulin secretion would be impaired, leading to reduced glucose tolerance. "So we were surprised when we saw lower blood glucose levels in the knockout mice after glucose stimulation," Harada says. Furthermore, serum insulin levels were increased in these animals, suggesting that insulin secretion was increased in the absence of SNAP23. "In collaboration with Dr. Haruo Kasai's group at the University of Tokyo, we used two photon microscopy to observe insulin secretion from isolated SNAP23-deficient islets," Harada explains. "We were thrilled to see that insulin secretion was enhanced."

> **"[The SNAP23 inhibitor] might be useful in the treatment of diabetes."**

Because SNAP23–VAMP2–syntaxin1A complexes are less efficient at mediating membrane fusion than SNAP25–VAMP2–syntaxin1A complexes (4, 5), this surprising finding could be explained if SNAP23 competes with SNAP25 for the other two SNAREs. In the absence of SNAP23, the number of SNAP25-containing complexes would be increased, enhancing the efficiency of insulin granule exocytosis. In support of this idea, Kunii et al. found that, in vitro, recombinant SNAP23 inhibited SNAP25–VAMP2–syntaxin1A complex assembly in a dose-dependent manner, and that the amount of SNAP25 bound to VAMP2 was increased in SNAP23-deficient β cells.

The researchers then screened a RIKEN chemical compound library for small molecules that could specifically bind to SNAP23, and identified one, MF286, that specifically inhibited the SNARE protein's assembly with VAMP2 and syntaxin1A. "MF286 enhanced insulin secretion from a β cell–derived cell line and isolated islets," says Harada. It also increased insulin levels in mice, who showed improved glucose tolerance without displaying any adverse side effects.

"So MF286 might be useful in the treatment of diabetes," says Harada, adding that the drug has two significant advantages. "It could be used in conjunction with other drugs because its point of action—SNAP23—is different. And, because SNAP23 β cell–specific knockout mice have normal blood glucose levels in the absence of food intake or glucose stimulation, MF286 is less likely to cause hypoglycemia."

In keeping with SNAP23's opposing function in exocrine acinar cells, MF286 inhibited amylase secretion, suggesting that the drug might also be used to treat pancreatitis. Kunii et al. now want to use their conditional knockout mice to investigate SNAP23's functions in other tissues of the body.

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3. Wang, C.C., et al. 2004. *Dev. Cell*. 7:359–371.
4. Sørensen, J.B., et al. 2003. *Cell*. 114:75–86.
5. Montana, V., et al. 2009. *J. Physiol.* 587:1943–1960.