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Previews

Insulin Release: Shedding Light on a Complex Matter

Michele Solimena[1,3,*] and Stephan Speier[1,2,*]
[1]Paul Langerhans Institute Dresden
[2]Center for Regenerative Therapies Dresden
School of Medicine, Dresden University of Technology, 01307 Dresden, Germany
[3]Max Planck Institute of Molecular Cell Biology and Genetics, 01307 Dresden, Germany
*Correspondence: michele.solimena@tu-dresden.de (M.S.), stephan.speier@crt-dresden.de (S.S.)
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SNAP25 and syntaxin-1 are core proteins of the exocytosis machinery for insulin granules. Takahashi et al. (2010) show that the distribution and kinetics of the SNAP25/syntaxin-1 complex assembly play a key role in regulating the first and second phase of insulin release.

Pancreatic β cells produce and release insulin, the key hormone for lowering blood glucose levels. β cells store insulin within granules, from which the hormone is secreted extracellularly when glycemia is >5.5 mM. Insulin release to a stepwise glucose challenge occurs normally in two phases: the first phase has a rapid onset and is brief (2–5 min), while the second lasts until fasting glycemia is restored (usually <2 hr). Accordingly, inadequate insulin supply relative to metabolic needs causes hyperglycemia and diabetes mellitus. In type 2 diabetes, the first phase of insulin release is reduced, and islets from type 2 diabetic patients and the Goto-Kakizaki rat, a model of type 2 diabetes, express lower levels of SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins (Nagamatsu et al., 1999; Ostenson et al., 2006). In general, SNARE proteins are either vesicle associated (v-SNAREs; such as VAMP2/synaptobrevin-2) or target membrane bound (t-SNAREs; such as syntaxin-1 and SNAP25). Formation of a ternary complex between the cytosolic tails of v- and t-SNAREs drives the juxtaposition and fusion of opposing membranes—an essential process in all eukaryotic cells that is often triggered by an increase in free Ca^{2+} (Südhof and Rothman, 2009), as the fusion of insulin granules with the plasma membrane.

In this issue of *Cell Metabolism*, Takahashi et al. (2010) present data regarding the kinetics of binary complex formation between SNAP25 and syntaxin-1 (t-SNARE complex) in relationship to granule exocytosis. They show that membrane regions rich in preassembled t-SNARE complexes are the preferred sites for granule exocytosis immediately after glucose stimulation. This remarkable achievement was possible by measuring fluorescence resonance energy transfer (FRET) in mouse islets imaged with two-photon laser scanning microscopy. A FRET signal is generated when two fluorescent dyes with overlapping spectra are less than 10 nm apart. Takahashi et al. developed a new FRET probe, termed SLIM (SNAP25 reporter linker mutant), in which the conformational change induced by the binding of SNAP25 to syntaxin-1 reduces the distance between two fluorochromes inserted within SNAP25, thereby enhancing FRET. Notably, the SLIM probe, unlike a similar construct described previously (An and Almers, 2004), is insensitive to the interaction of t-SNAREs to the v-SNARE VAMP2 on the granules. This characteristic enabled the authors to measure the kinetics and distribution of t-SNARE assembly before and after glucose stimulation by FRET, while simultaneously monitoring granule exocytosis by imaging the uptake of an extracellular polar (membrane-impermeable) fluorescent tracer. Before stimulation, the areas with low FRET signals, interpreted as areas of unassembled t-SNAREs, outnumbered those with stable high FRET signals, i.e., assembled t-SNAREs, 2:1, indicating that syntaxin-1/SNAP25 complexes are unevenly distributed throughout the plasma membrane. Exocytosis occurred preferentially in high-FRET areas during the first phase of glucose stimulation, whereas during the second phase, it was equally frequent in high- and low-FRET areas. Notably, FRET values did not change during exocytosis in high-FRET regions, but increased ~3 s before detection of exocytosis in low-FRET regions, suggesting that t-SNARE assembly is enhanced by glucose stimulation and precedes exocytosis. Overall, granule fusion was three times more frequent in high-FRET areas compared to low-FRET areas. Persistence of maximal FRET values at exocytotic sites further suggests that SNAREs neither diffuse nor immediately disassemble after granule fusion.

To gain insight into the kinetics of t-SNARE complex assembly in relationship to exocytosis, intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) were rapidly increased by uncaging a caged-Ca^{2+} compound. With this increase in $[Ca^{2+}]_i$, the time constant for induction of exocytosis was ~1 s in high-FRET areas, whereas in low-FRET areas, the increases of FRET values had a latency of ~10 s and preceded exocytosis by ~3 s. These data strengthen the conclusion that t-SNARE assembly is a prerequisite for exocytosis, its preassembly prior to stimulation being key for the first phase of insulin secretion and its assembly after stimulation being the rate-limiting step for the second phase.

Previous studies showed clustering of SNARE proteins in discrete membrane areas in neuroendocrine cells (Lang et al., 2001), including β cells, where they are the preferred sites of granule exocytosis (Ohara-Imaizumi et al., 2007). Strikingly, the latter study found that neither syntaxin-1A nor other syntaxins were involved in the second phase of insulin secretion. Hence, a lower expression of syntaxin-1 in type 2 diabetic islets could account for their impaired first phase of insulin release. This scenario is at odds with the new data by the Kasai group, unless it is assumed that SNAP25 forms fusion complexes with proteins other than syntaxins and VAMP2—a proposition

that seems unlikely. Could it then be that the critical factor leading to the selective deficit of insulin secretion in type 2 diabetes is a decrease in the number of *preassembled* t-SNARE complexes (Figure 1)? This hypothesis could be readily tested by employing SLIM to investigate t-SNARE assembly in β cells of type 2 diabetic patients and the Goto-Kakizaki rat. Preassembled t-SNARE complexes could be reduced in diabetic β cells due to various factors, including the lower expression of SNARE proteins, but also alterations of membrane lipid microdomains. t-SNARE proteins and interacting ion channels such as $Ca_V1.2$ and $K_V2.1$ have been shown to preferentially partition in cholesterol-rich membrane areas (Xia et al., 2004). The high levels of circulating free fatty acid commonly found in diabetic patients could perturb this protein-lipid order.

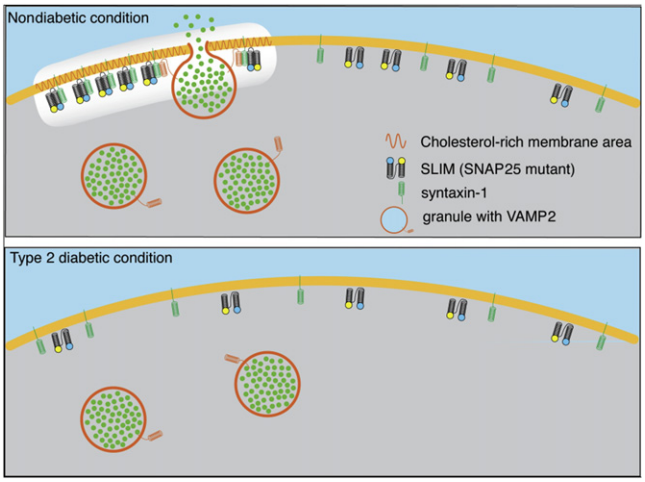

Figure 1. Reduced t-SNARE Complex Preassembly as a Possible Mechanism for Secretion Deficit in Diabetic β Cells

The graphic illustrates the distribution of unassembled t-SNARE proteins SNAP25 and syntaxin-1 and preassembled SNAP25/syntaxin-1 complexes (white shaded area) in the plasma membrane of β cells in physiological conditions (top panel) and in the context of type 2 diabetes (lower panel). Lower expression of t-SNAREs and alterations of the membrane lipid composition occurring in type 2 diabetes could lead to fewer preassembled t-SNARE complexes and thereby to the loss of the first phase of insulin release—a characteristic phenotypic alteration of the disease.

The new data by Takahashi et al. provide valuable insight into parts of the kinetics regulating exocytosis, but leave open numerous questions. According to the prevailing model, the first phase of insulin release is mainly due to the exocytosis of granules that, prior to glucose stimulation, reside in direct contact with the cell membrane ("docked granules") and are competent for fusion ("primed granules"). The second phase, instead, reflects the exocytosis of granules that are recruited from intracellular stores to the plasma membrane at a later time (Rorsman and Renström, 2003). Evidence also suggests, however, that granules newly approaching the plasma membrane also contribute to the first phase (Shibasaki et al., 2007). Therefore, it will be important to clarify the spatial and temporal relationship of these various granule pools with the assembly states of t-SNAREs and SNAREs in general. To this aim, additional FRET probes for the v-SNARE VAMP2 could be used. Cargo peptides of insulin granules can also be fluorescently labeled. This approach, in particular, can ensure the exclusive imaging of insulin granule trafficking and exocytosis in β cells. It might also allow for elucidating whether the observed different kinetics for exocytosis between high-FRET (~1 s) and low-FRET (~3 s) areas after stimulation and complex assembly reflects the time necessary for granules to dock and/or be primed for fusion. Finally, the use of fluorescence lifetime imaging microscopy (FLIM), a FRET technique that is unaffected by probe concentrations, tissue light scattering, or the emission light path, and the application of super-resolution microscopy could further improve the analysis of t-SNARE complex assembly (Rickman et al., 2010). As elegantly shown here by Takahashi and coworkers, new bright ideas and tools are bringing us ever closer to untangling complex matters such as insulin secretion and, hopefully, the reason for its deficit in type 2 diabetes.

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