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Review

Fusion pore in exocytosis: More than an exit gate? A β-cell perspective

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ABSTRACT

Secretory vesicle exocytosis is a fundamental biological event and the process by which hormones (like insulin) are released into the blood. Considerable progress has been made in understanding this precisely orchestrated sequence of events from secretory vesicle docked at the cell membrane, hemifusion, to the opening of a membrane fusion pore. The exact biophysical and physiological regulation of these events implies a close interaction between membrane proteins and lipids in a confined space and constrained geometry to ensure appropriate delivery of cargo. We consider some of the still open questions such as the nature of the initiation of the fusion pore, the structure and the role of the Soluble N-ethylmaleimide-sensitive-factor Attachment protein REceptor (SNARE) transmembrane domains and their influence on the dynamics and regulation of exocytosis. We discuss how the membrane composition and protein-lipid interactions influence the likelihood of the nascent fusion pore forming. We relate these factors to the hypothesis that fusion pore expansion could be affected in type-2 diabetes via changes in disease-related gene transcription and alterations in the circulating lipid profile. Detailed characterisation of the dynamics of the fusion pore *in vitro* will contribute to understanding the larger issue of insulin secretory defects in diabetes.

1. Introduction

Pancreatic islet β-cells occupy a unique position in the physiological regulation of nutrient homeostasis [1]. Although several other organs (such as gut, brain, liver, adipocytes and muscle) are required for fine-tuning of metabolism and its adaptation to environmental cues [2–4], the β-cells of the pancreatic islets arguably represent the body's principal nutrient sensor. The β-cell exerts its function by secreting insulin, the only hormone of the body capable of lowering the plasma glucose levels. Thus, the β-cell plays both the part of the sensor and the actuator. The functional significance of the β-cell and insulin is illustrated by the devastating metabolic consequences of type 1 diabetes (T1D), an autoimmune disease associated with the destruction of the insulin-producing β-cells. However, T1D accounts for only a small fraction of diabetes (10%) and most diabetic patients instead have type 2 diabetes (T2D).

In T2D, the β-cells remain viable but they fail to dispense insulin with the right kinetics and at the right level to maintain plasma glucose levels in the normal range. The underlying disturbance remains obscure but it is now clear that the insulin secretion defect develops gradually over many years [5,6] as a result of a conspiracy between the genetic predisposition and lifestyle factors. Because of its central role in fuel homeostasis and the evidence implicating defective insulin secretion as a cause of T2D, it is perhaps not surprising that the β-cells represent the target of many of the diabetes medicine currently in clinical use (such as sulfonylureas and GLP-1). Currently, more than 100 gene variants increasing T2D risk have been identified but – with a few exceptions – the mechanism(s) by which they act remain to be established. In some cases, the evidence points to 'unusual suspects' such as the very last steps of the insulin release process.

2. β-cell physiology and insulin secretion via exocytosis

The concept 'exocytosis' denotes the fusion of intracellular vesicles with the plasma membrane to release either soluble cargo or to insert new molecules (such as lipids or proteins) into the target membrane (Fig. 1). Physiologically, exocytosis is of very broad significance, spanning from renewal of the plasma membrane and its components to cell migration and cell division, maintenance of the membrane integrity and intercellular communication [7–9]. The term "exocytosis", was first coined in the 1960s [10] in chromaffin cells, observed at the ultrastructural level in the 1970s there [11] as well as at synapses [12] and described molecularly (at a relatively detailed level) in the 1990s (and onwards) [13–15].

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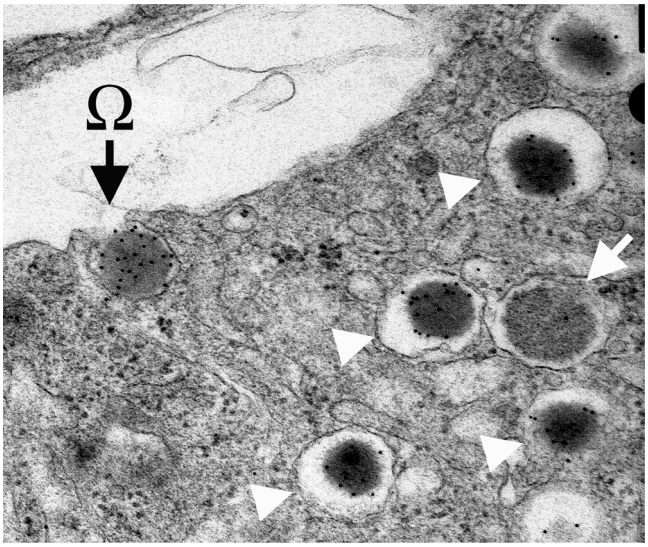

Fig. 1. Insulin exocytosis in rodent pancreatic β-cells.
Note the presence of immature, mainly pro-insulin containing secretory granules (white arrows) and of mature secretory granules (arrowheads) characterized by a denser core due to proinsulin conversion to insulin and subsequent crystallisation. A vesicle fused with the plasma membrane is visible on the upper left with the typical omega configuration (Ω) of the fusion pore, already considerably enlarged here. Note that the core is less dense than in non-fused mature granules indicating partial solvation of the insulin hexamers

Insulin secretion involves exocytosis of large dense core vesicles (LDCVs) and show great similarity to the release of catecholamines from adrenal chromaffin cells (the archetypal neuroendocrine cells). Indeed, β-cells and insulin secretion now represent a widely used model system to study LDCV exocytosis [16]. Studies of insulin exocytosis and its regulation are of particular interest given the link to T2D and its clinical implications [1].

The process of nutrient-induced insulin secretion is well understood and will not be described in great detail here. Briefly, insulin secretion is elicited by nutrients such as glucose, lipids or certain amino acids [17]. Their metabolism, subsequent to transporter-mediated entry into the cytosol generates a number of coupling factors linking metabolism to hormone secretion. Whereas changes in the ATP/ADP ratio are the best characterized example, other are also important such as increases in cyclic AMP or specific lipids [18–21]. Changes in ATP/ADP ratio lead to closure of ATP-regulated K^+ (K_{ATP}) channels [22]. K_{ATP} channel activity maintains membrane potential at the resting potential (−70 mV) at low glucose concentrations which is not associated with stimulation of insulin secretion. Closure of these channels results in membrane depolarization and the initiation of action potential firing due to opening of voltage-dependent Ca^2 channels. The associated increase in cytoplasmic Ca^{2+} ($[Ca^{2+}]_i$) triggers insulin exocytosis [3] (Fig. 1).

Insulin secretion follows a characteristic biphasic time course and consists of an initial but transient 1st phase followed by a sustained/slowly developing 2nd phase. Although first described 50 years ago [23] and preserved in all species investigated (including man [24]), the cellular background of biphasic insulin secretion remains debated. It has been attributed to both sequential release of distinct pools of secretory granules and time-dependent changes in β-cell electrical activity [17,25–27].

It is interesting to correlate the kinetics of biphasic insulin secretion with the physiological actions of insulin. Whereas insulin action on the liver is maximal after 15 min [28], a considerable further delay in insulin action on other organs is caused by the time required for transepithelial delivery of the peptide hormone to attain its site of action [29]. These considerations suggest that the biphasic release subserves a physiological function.

Impaired nutrient homeostasis is a hallmark of T2D [30]. Insulin resistance in target tissues as well as β-cell dysfunction are recognized as the major pathogenic factors. However, the view that islet failure develops mainly secondary to hormone resistance is no longer tenable on the base of functional, epidemiological and genetic data [1,31–34].

Interestingly, T2D is associated with the loss of normal biphasic insulin secretion: 1st phase insulin secretion is nearly abolished and 2nd phase insulin secretion strongly reduced [35]. The underlying causes are not known but may be secondary to impaired metabolism [36], electrical activity or exocytosis [37–40]. T2D has also been shown to be accompanied by a loss in β-cells, especially in long-standing cases, but control and patient's post-mortem sample values largely overlap and differences are not pronounced enough to explain the progression to the disease [35,41–43]. Thus, it seems reasonable to conclude that impaired β-cell secretory function must be taken into account in the aetiology of T2D. Of course, the two concepts are not mutually exclusive and T2D may well involve both reduced β-cell function and mass.

3. Insulin secretion as paradigm for LDCV exocytosis

3.1. Vesicles and pools

Exocytosis encompasses the last steps in hormone secretion (Fig. 1). It can be divided into several distinct steps [44]. The movement to and tethering of the secretory granule to the plasma membrane is referred to as 'docking' [12,45–48]. Once the granules have docked, they undergo additional biochemical reactions to attain release competence in a process termed 'priming' [49,50]. To allow release of the cargo, the two membranes (the plasma membrane and the secretory granule) must overcome membrane repulsion and merge [51] (Fig. 2A). Insulin containing LDCVs are formed in the trans-Golgi Network and have to undergo several maturation steps [16,52,53]. In this process, pro-insulin is converted into insulin [54]. The diameter of mature granules has been a matter of debate. Chemical fixation yields a diameter of around 350 nm, however, this may well be an artefact and rapid freezing techniques concluded upon a diameter of some 250 nm [55–57]. Granule maturation also involves membrane remodelling and insertion of proteins involved in the exocytotic process including certain SNARE proteins (see below) such as syntaxin 6 or VAMP4 as well as calcium-sensing synaptotagmin 4 [58–61].

The β-cell contains some 5.000–10.000 secretory vesicles [55,62]. Of these, only 1% are immediately available for release ("readily releasable pool" or RRP) (see Fig. 1). The RRP granules have been proposed to underlie the first phase of biphasic insulin secretion. The remaining 99% of granules constitute a 'reserve pool' of granules [25,26]. Reserve granules must undergo a series of priming reactions and even physical translocation within the β-cell to attain release competence. It has been proposed that this gives rise to 2nd phase insulin secretion [63]. As in other cases of LDCV exocytosis, both the final fusion step and the transit between pools are Ca^{2+}-dependent processes, albeit with distinct affinities [64,65].

3.2. The β-cell SNARE machinery

Membrane fusion is driven by an evolutionary conserved set of proteins, the Soluble N-ethylmaleimide-sensitive-factor Attachment protein REceptor (SNARE) complex [13–15,66–68]. The core of this supramolecular complex encompasses two cytosolic proteins with transmembrane domains (see Fig. 2A): VAMP/synaptobrevin on the vesicles (v- or R-SNARE, according to the central presence of an Arg in its cytosolic helix [67,69]), syntaxin on target plasma membranes and lipid-anchored SNAP-25 (target t-SNAREs or Q-SNAREs, according to the central presence of an Gln in their cytosolic helices [67,69]). The interaction of the cytosolic domains of these three proteins gives rise to a coiled coil [69,70]. Zippering of the helix exerts a mechanical force