Commentary

Insulin Secretion: A High-affinity Ca²⁺ Sensor After All?

Sebastian Barg[1] and Patrik Rorsman[2]

[1]The Vollum Institute, Oregon Health and Sciences University, Portland, OR 97239
[2]Oxford Centre for Diabetes, Endocrinology, and Metabolism, University of Oxford, Oxford OX3 7LJ, UK

Insulin is secreted from the β-cells of the pancreatic islets by Ca^{2+}-dependent exocytosis of large dense core vesicles (LDCVs) (Ämmälä et al., 1993), a process that is triggered by glucose-induced electrical activity (Henquin and Meissner, 1984). In response to a step elevation of glucose, insulin secretion follows a characteristic biphasic time course (Curry et al., 1968): an initial transient first phase of secretion, which is completed within 10–15 min, is followed by a slowly developing and sustained second phase. It has been estimated that the first phase of secretion is due to the rapid release of a total of 40–80 LDCVs per β-cell, after which secretion proceeds at a rate of five vesicles per β-cell per minute (Rorsman and Renström, 2003).

The Ca^{2+} channel density in β-cell plasma membranes is very low, only about one-twentieth of that in chromaffin cells (Barg et al., 2001). Yet, the β-cell is capable of remarkably high rates of exocytosis. Capacitance measurements have suggested that secretion transiently may proceed at rates as high as 500 LDCV per second (Barg et al., 2001). It therefore has been proposed that the β-cell exocytosis is efficiently coupled to Ca^{2+} entry via the assembly of a functional complex consisting of Ca^{2+} channels and exocytotic proteins (Wiser et al., 1999) so that exocytosis is triggered by the large increases in the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) occurring at the inner mouth of the Ca^{2+} channels (Fig. 1 A). In accordance with such a scenario, the rates of exocytosis that can be elicited by voltage-clamp depolarizations require elevation of $[Ca^{2+}]_i$ by several tens of micromolar, as estimated from experiments using photolytic release of caged Ca^{2+} (Takahashi et al., 1997; Barg et al., 2001). The latter experiments suggested that exocytosis is sigmoidally related to $[Ca^{2+}]_i$ with a K_d of ~20 μM and a Hill coefficient (n) as high as 5. By contrast, measurements of insulin secretion from permeabilized cells have indicated that insulin secretion is activated already at submicromolar $[Ca^{2+}]_i$ (Yaseen et al., 1982; Wollheim et al., 1987; Okazaki et al., 1994) and capacitance increases have also been observed at such low Ca^{2+}

concentrations (Proks et al., 1996). Indeed, the latter type of measurements indicate that exocytosis at Ca^{2+} concentrations as low as a few hundred nanomolar proceeds at rates 10,000-fold higher than that expected from the Ca^{2+} dependence quoted above. To date, there has been no explanation of the widely different Ca^{2+} dependencies of exocytosis indicated by these two experimental paradigms. Two complementary studies appearing in this issue (Yang and Gillis, 2004; Wan et al., 2004) may explain this conundrum.

Recently, a small pool of LDCVs, capable of release in response to moderate (low micromolar) elevations of $[Ca^{2+}]_i$ was documented in two types of endocrine cells: adrenal chromaffin cells (Yang et al., 2002) and pituitary gonadotropes (Zhu et al., 2002). Exocytosis of these vesicles (termed the highly calcium-sensitive pool [HCSP]) proceeds in parallel with the previously described low-affinity Ca^{2+}-dependent exocytosis. Release of HCSP is rapid (rate constant: $10–100\ s^{-1}$) and at $[Ca^{2+}]_i < 10$ μM, exocytosis of HCSP is actually faster than the rapid initial components previously described. Whereas this could be indicative of separate pathways, both forms of exocytosis involve SNARE proteins. Importantly, the existence of HCSP is not confined to endocrine cells and it has also been found in the rod photoreceptor synapse, where it was postulated to contribute to the linearity of the responses (Thoreson et al., 2004).

Using capacitance measurements, Yang and Gillis (2004) and Wan et al. (2004) now report the presence of HCSP also in rat insulin-secreting insulinoma and primary β-cells. They demonstrate that HCSP granules are released by global (i.e., throughout the cytosol) rather than localized increases in $[Ca^{2+}]_i$. This suggests that the vesicles in the HCSP are not located in the immediate vicinity of the Ca^{2+} channels (Fig. 1 A). One explanation for this phenomenon could be the existence of two types of vesicles in the β-cell: synaptic-like microvesicles (SLMVs) in addition to the LDCVs (compare Kasai, 1999; Braun et al., 2004). However, Yang and Gillis rule out this possibility by using car-

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Abbreviations used in this paper: HCSP, highly calcium-sensitive pool; LDCV, large dense core vesicles; RRP, readily releasable pool.
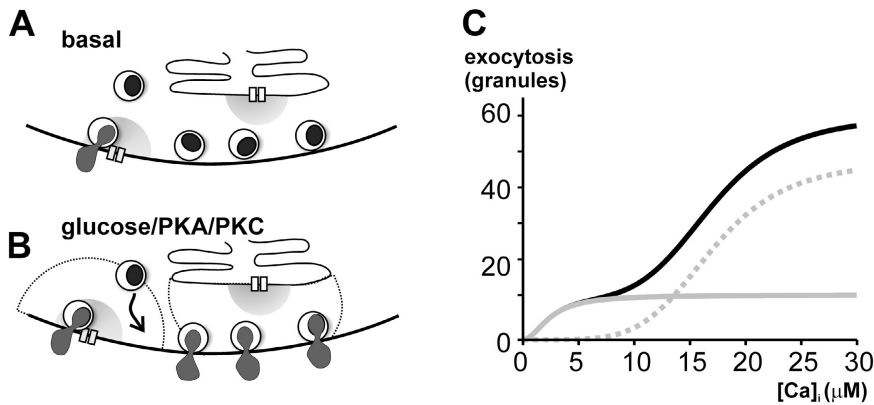
FIGURE 1. (A and B) Schematic illustrating the presence of two populations of granules in β-cells with distinct Ca²⁺ sensitivities. Granules with a low-affinity Ca²⁺ sensor need to be associated with the voltage-gated Ca²⁺-channels, whereas those equipped with a high-affinity Ca²⁺ sensor (HCSP) undergo exocytosis in response to a global elevation of cytoplasmic Ca²⁺. A and B show the situation under basal conditions and following activation of PKA or PKC, respectively. In A, only granules close to the Ca²⁺ channels are exposed to sufficiently high Ca²⁺ concentrations to undergo exocytosis (gray areas). In B, moderate but global increases in cytoplasmic Ca²⁺ (dashed lines; both by Ca²⁺ influx through the plasma membrane Ca²⁺ channels and release from intracellular Ca²⁺ stores) suffice to trigger exocytosis of HCSP granules, which need not be associated with the Ca²⁺ channels. In addition to the Ca²⁺-dependent processes discussed here that are involved in the triggering of exocytosis, Ca²⁺-dependent steps have also been described for more upstream events such as priming/mobilization (indicated by arrow in B). (C) Exocytosis as a function of cytoplasmic Ca²⁺ concentration for the low-affinity (dashed line) and high-affinity (HCSP; gray line) processes and the sum of the two (i.e., HCSP plus RRP; black line). Note that HCSP attains it maximum already at Ca²⁺ concentrations <10 μM. Data are derived from Barg et al. (2001) assuming a depolarization lasting 50 ms (corresponding to the β-cell action potential) and Wan et al. (2004). The sum of the two predicts a gradual stimulation of exocytosis over a wide range of calcium concentrations (0.5–30 μM).

bon fiber amperometry to measure exocytosis. They found an excellent correlation between the HCSP capacitance increases and release of the amine. Because serotonin selectively accumulates in LDCVs (Aspinwall et al., 1999), this provides strong evidence that the early component of capacitance increase does in fact reflect exocytosis of LDCVs.

The presence of HCSP in β-cells explains how insulin secretion can proceed at low Ca²⁺ concentrations. Why then does secretion in the β-cell (or indeed any of the cells in which HCSP has been described) require two mechanisms of Ca²⁺ sensing? It is attractive to propose that this feature provides the β-cell with a means to linearize its secretory response to the stimulus, in a way reminiscent of the situation in the rod photoreceptor (Thoreson et al., 2004). As illustrated schematically in Fig. 1 C, the combination of a size-limited high-affinity (HCSP) and a large low-affinity, readily releasable pool (RRP) component allows the β-cell to regulate its secretory response over a wide range of Ca²⁺ concentrations, which in turn is proportional to the stimulus intensity.

Under basal conditions, the HCSP is limited to as few as 10 granules, or about one-tenth of the RRP of granules. It is essential that the HCSP is of limited size to prevent too much insulin from being released in response to low and moderate stimulus intensities. Insulin secretion is well known to be regulated by reversible protein phosphorylation/dephosphorylation (Ämmälä et al., 1994). For example, agents that promote protein phosphorylation by activation of protein kinases A and C are strong stimuli of insulin secretion. Examples of physiological regulators that act via these protein kinases include the islet hormone glucagon

(Renström et al., 1997), the incretin hormone GLP-1 (Lester et al., 1997), and acetylcholine (PKC) (Gao et al., 1994). Exactly how activation of PKA or PKC promotes secretion in the β-cells remains unclear but their effects involve an increase in RRP. The results of Yang and Gillis (2004) and Wan et al. (2004) are significant also in this context, as they demonstrate that the size of HCSP increases several-fold (up to fourfold) in the presence of PKA/PKC activators as well as glucose, the major physiological stimulus of insulin secretion. Because of this effect, these compounds will appear to sensitize the secretory machinery to Ca²⁺, as first proposed 20 yr ago (Rorsman and Abrahamsson, 1985; Jones et al., 1986). The present studies make it likely that this effect results from an increase in HCSP size and not in the Ca²⁺ sensitivity of the release machinery itself. This represents a very significant advance of our knowledge about the control of insulin secretion. The data also explain the ability of the PKC activator PMA to stimulate insulin secretion even at subthreshold glucose concentrations when there is no electrical activity and [Ca²⁺]ᵢ is low (Bozem et al., 1987). The present data suggest this is because HCSP is released at a low rate already at resting [Ca²⁺]ᵢ in the presence of the PKC activator.

What is the way forward? It is becoming increasingly evident that high-affinity Ca²⁺-dependent exocytosis is a ubiquitous feature encountered in numerous (if not all) cells capable of regulated Ca²⁺-dependent exocytosis. Although it will certainly remain worthwhile to expand the catalog of cells in which HCSP contributes to the plasticity of secretion, it now becomes important to focus on the molecular mechanisms. One essential

question that needs to be resolved is the differences in the Ca^{2+}-sensing mechanisms involved in exocytosis of RRP (with a K_d of ~ 20 μM) and HCSP ($K_d \sim 1$ μM). As already noted above, both HCSP and RRP exocytosis involves SNARE proteins; but the widely different Ca^{2+} sensitivities of the two pathways suggest that they may not involve the same isoform of the calcium sensor synaptotagmin (Syt). It is in this context pertinent that rat insulin-secreting Ins1- and β-cells contain the isoforms Syt III (Mizuta et al., 1994), V, and Syt IX (Iezzi et al., 2004). Future work should address the roles of these synaptotagmins in the two forms of Ca^{2+}-induced exocytosis. One intriguing possibility is that phosphorylation of SNARE proteins somehow produces a switch from a low- to a high-affinity synaptotagmin isoform.

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