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Supplementary information

Mechanisms controlling pancreatic islet cell function in insulin secretion

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Supplementary Box 1. β-cell secretory granule trafficking

Insulin secretory granules, like synaptic vesicles, dock to the plasma membrane and then undergo priming to become readily-releasable granules. Loading of granules at the plasma membrane (PM) is accomplished by a set of four proteins, three soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins and sec1/Munc18, which assemble into a quaternary complex to place the granule at the PM[1]. The three SNARE proteins involved in the quaternary complex are syntaxin 1A, synaptosome-associated protein of 25 kDa (SNAP25), and vesicle associated protein (VAMP)-2. Phosphorylation of Munc18 by PKC causes activation of syntaxin 1A, facilitating its binding to SNAP25 on the PM and VAMP2 on the secretory granule. The priming function of MUNC18 is assisted by the DAG- and MAG-binding protein Munc13-1. Synaptotagmin VII clamps the quaternary complex at low Ca^{2+} and induces a conformational change in the complex in response to increases in intracellular Ca^{2+}. These changes culminate in the formation of a fusion pore through which insulin stored in secretory granules is released to the cell exterior[1]. These events are further regulated by other ancillary proteins, including complexin-2, which binds to VAMP2 to suppress synaptotagmin-induced granule fusion with the PM, and tomosyn which interacts with SNAP25 and syntaxin1A. At basal glucose levels, access of an intracellular pool of insulin containing secretory granules to the PM SNARE proteins is blocked by the cortical actin cytoskeleton. Stimulation with glucose induces remodelling of the actin cytoskeleton and subcortical actin network, allowing access of the intracellular pool of granules to the PM SNARE proteins, a process that is potentiated by fatty acids[1,2].

Supplementary Box 2. Mechanisms of cAMP signalling in β-cells

cAMP serves as both a priming factor required for normal glucose- and nutrient-stimulated insulin secretion, and as a potentiating agent that enhances insulin secretion at stimulatory glucose levels. Eliminating hormone-stimulated cAMP generation in β-cells by deletion of Gαs produces insulin deficiency and hyperglycemia in mice[3,4]. In the absence of proglucagon-derived peptides, cAMP is increased in β-cells by two mechanisms, one dependent on Ca^{2+} and the other tied to changes in glucose metabolic rate. The operation of the Ca^{2+} mechanism was revealed in islets exposed to low, non-stimulatory glucose concentrations in the presence of the K_{ATP} channel opener diazoxide and a depolarizing concentration of K^+, which activates Ca^{2+} influx and increases cAMP levels. Addition of stimulatory glucose increases cAMP levels above those achieved with K^+ and diazoxide treatment alone. This additional pathway may be tied to glucose metabolism-driven increases in ATP, the substrate of the cAMP generating adenylate cyclase enzymes, or possibly to a lowering of AMP, a cyclase inhibitor[5,6].

Changes in β-cell cAMP translate to increases in insulin secretion by two main signalling nodes--activation of protein kinase A (PKA) and binding to Exchange Protein Activated by cAMP 2A (EPAC2A), a guanine nucleotide exchange factor[5,6]. Separate inhibition of these two nodes during dynamic insulin secretion in mouse islets revealed that the EPAC pathway potentiates first phase insulin secretion, while both PKA and EPAC contribute to second phase insulin secretion[7]. Activation of PKA results in phosphorylation of the SUR1 subunit of the K_{ATP} channel, as well as the Cav1/2 voltage-gated Ca^{2+} channel, contributing to inactivation of the former, and activation of the latter, thus enhancing Ca^{2+} influx and insulin secretion[8]. PKA subunits also bind to insulin secretory granules via association with A-kinase anchoring proteins, thereby becoming physically juxtaposed with multiple granule trafficking proteins[9]. Finally, glutamate uptake into secretory vesicles via the VGLUT1 transporter requires cAMP and activation of PKA, although the specific PKA substrates that mediate this effect have not been reported.

Signalling of cAMP through EPAC2A involves a separate set of events, initiated by cAMP binding to EPAC2A to cause a conformational change that facilitates interaction with Rap1, a small GTPase[5]. The Epac2A/Rap1 complex enhances recruitment of secretory granules to the readily releasable pool. Epac2A also interacts with Rim2α, a Rab3-interacting protein that functions as a scaffold for proteins that mediate granule docking and fusion to the plasma membrane for exocytosis, and with Piccolo, which stabilizes the Epac2A/Rim2α complex. All of these proteins (Epac2, Rim2a, Piccolo and Rab3) are required for cAMP-regulated granule exocytosis[5]. Epac2 also interacts with the K_{ATP} channel to alter its sensitivity to the inhibitory effects of ATP[10].

Treatment of islets with proglucagon-derived peptides, or with the related gastrointestinal hormone GIP, leads to an increase in cAMP levels above those measured in the presence of stimulatory glucose alone. Cultured islets from proglucagon knockout mice have dramatic decreases in cAMP levels, accompanied by reduced phosphorylation of PKA substrates and impaired insulin secretion responses to all depolarizing stimuli, highlighting a critical role of proglucagon-derived peptides in driving β-cell cAMP signalling[11]. The extent to which the PKA-mediated versus the Epac2-mediated signalling pathways are engaged during stimulation with glucose alone versus with the combination of stimulatory glucose + hormones is not precisely defined and remains as an area for further investigation.

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