JOURNAL CLUB

TSPAN-7 as a key regulator of glucose-stimulated Ca2+ influx and insulin secretion

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The human body is a complex machinery that requires tight control of blood glucose levels for normal function. The pancreas plays a major role in glucose homeostasis as it is responsible for the secretion of the blood sugar-lowering hormone insulin. In pancreatic β-cells, an increase in intracellular Ca^{2+} concentration contributes to glucose-stimulated insulin secretion (GSIS) (Roder *et al.*, 2016). GSIS is a finely tuned process and dysfunctions can lead to glucose-associated conditions such as hyperglycaemia. Although the molecular steps that couple the increment in extracellular glucose to insulin release is now well established, the regulation of GSIS remains unclear.

The consensus model for GSIS proposes that glucose uptake into pancreatic β-cells through glucose transporters (GLUT) is metabolised into the mitochondria by glycolysis and oxidative phosphorylation to produce ATP. Increase in ATP:ADP ratio promotes closing of ATP-sensitive K^+ (K_{ATP}) channels and subsequently causes membrane depolarisation. The change in membrane potential elicits Ca^{2+} influx through the activation of L-type voltage-dependent Ca^{2+} channels ($Ca_v1.2$ and $Ca_v1.3$) and the rise in intracellular Ca^{2+} triggers secretion of the insulin-containing secretory granules to control blood glucose levels. Ca_v channels are key players in tuning insulin secretion and their activity can be modulated by auxiliary proteins (e.g. $\alpha_2\delta$-, β- and γ-subunits). These subunits can influence channel trafficking, localisation, gating and activation/inactivation kinetics. Tetraspanins (TSPANs) are transmembrane proteins that are structurally similar to the γ-subunit of Ca_v channels and TSPANs can regulate many biological processes, including intracellular Ca^{2+} handling (Termini & Gillette, 2017). They are composed of four transmembrane domains and two extracellular loops, involved in protein-protein interactions. As an example, TSPAN-13 is an important regulatory protein for the N-type $Ca_v2.2$ channel and it can modulate neuronal function such as neurotransmitter release. By interacting with the CaV voltage-sensing domain (VSD), TSPAN-13 accelerates activation and activation kinetics of $Ca_v2.2$, therefore decreasing the voltage sensitivity and channel opening probability (Mallmann *et al.*, 2013). Another example is TSPAN-7, a highly expressed TSPAN in mouse and human pancreatic islets, which can regulate the surface expression of AMPAR in neurons and modulate F-actin dynamics. However, the role of TSPANs in controlling islet Ca^{2+} handling and insulin secretion remains unexplored.

In their paper entitled "Tetraspanin-7 regulation of L-type voltage-dependent Ca^{2+} channels controls pancreatic β-cell insulin secretion" (Dickerson *et al.*, 2020), the authors used a well-designed and well-conducted multidisciplinary approach combining genetics, protein

biochemistry, Ca^{2+} imaging and electrophysiology to demonstrate that TSPAN-7 is a novel, key regulator of L-type Ca_v channels and modulates GSIS in pancreatic cells.

Using immunofluorescence staining, Dickerson *et al.* showed that TSPAN-7 is highly expressed is mouse and human pancreatic α-/β-cells, whereas it is not present in surrounding acinar tissue. Using Ca^{2+} imaging (fluorescence dyes and genetically encoded Ca^{2+} sensors), they investigated the effect of TSPAN-7 knock-down (KD) on glucose- and depolarisation-stimulated Ca^{2+} influx in pancreatic islets. The authors developed and validated a lentiviral-based shRNA delivery system to knock-down TSPAN-7 expression in dispersed mouse/human islet β-cells with an efficiency of over 60%. For their imaging experiments, Dickerson *et al.* used a combination of intensometric and ratiometric Ca^{2+} indicators (Cal- dyes, Fura-2, and GCaMP). Ratiometric indicators have the advantage of being independent of dye loading, expression levels or focus drift. The authors observed similar changes in calcium responses mediated by TSPAN-7 when using single-wavelength or ratiometric calcium indicators. Their results strongly suggest that TSPAN-7 KD enhanced glucose-stimulated Ca^{2+} influx in both mouse and human β-cells, and increased depolarisation-stimulated Ca^{2+} influx in human β-cells. In addition, they demonstrated that TSPAN-7 KD accelerated Ca^{2+} oscillation frequency in mouse β-cells when stimulated with high level of glucose. Interestingly, in human β-cells TSPAN-7 KD had a larger impact on glucose-stimulated Ca^{2+} influx than on depolarisation-stimulated Ca^{2+} entry. It could be explained by TSPAN-7 being involved in glucose transport which is a hypothesis that the authors are keen to investigate.

To determine whether the increase of Ca^{2+} influx was controlled by Ca_v channels and more specifically L-type Ca^{2+} channels, the authors combined patch clamp electrophysiology with genetics and pharmacological agents. Dickerson *et al.* showed that TSPAN-7 KD in mouse and human β-cells significantly increased depolarisation-induced L-type Ca^{2+} currents whereas non-L-type currents remained unaffected. Using a heterologous overexpression system in HEK cells, the authors demonstrated that TSPAN-7 significantly decreased depolarisation-stimulated Ca^{2+} influx for $Ca_v1.2$ and $Ca_v1.3$. In addition, they showed that Ca^{2+} conductance was reduced for $Ca_v1.2$ (~30%) and $Ca_v1.3$ (~65%), whereas K_{ATP} current densities remained unchanged. Interestingly, TSPAN7 was differentially affecting the kinetics of the Ca_v channels with decreasing activation kinetics for $Ca_v1.2$ by ~50%, whereas it decreased inactivation kinetics for $Ca_v1.3$ by ~30%. Recovery from voltage-dependent inactivation (VDI) was accelerated only for $Ca_v1.2$. Ca^{2+}-dependent inactivation (CDI) was not investigated. Altogether, this data demonstrates that TSPAN-7 plays an important role in intracellular Ca^{2+} handling through the modulation of L-type Ca_v channels activity.

To determine whether TSPAN-7 was directly interacting with the Ca_v channels, Dickerson *et al.* conducted pull-down experiments from human islet cell lysates and HEK heterologous overexpressing systems. They confirmed that TSPAN-7 interacts with both $Ca_v1.2$ and $Ca_v1.3$ without affecting Ca_v channels trafficking and localisation. However, it is not clear whether this is a direct interaction or if accessory proteins are involved. To address this question, the authors are considering using mass spectrometry to determine TSPAN-7 interactome as well as surface plasmon resonance (SPR) using synthetic TSPAN-7 and Ca_v peptides to better characterise the interaction.

Using insulin secretion assays, the authors showed that TSPAN-7 KD significantly increased insulin secretion in human β-cells under both basal (5.6 mM) and high (14 mM) glucose conditions. The activation of $Ca_v1.2$ and insulin secretion at low glucose concentrations suggest a left-shift in glucose concentration required for Ca_v channel activation.

In summary, the author presented compelling evidence that TSPAN-7 is a new regulatory subunit of Ca_v channels, with an important role in Ca^{2+} handling and GSIS in pancreatic β-cells. Their approach is innovative and the methodology strong. They showed that TSPAN-7 limits glucose-stimulated Ca^{2+} influx, decreases Ca^{2+} oscillation frequency, which subsequently reduces insulin secretion. They demonstrated that TSPAN-7 interacts with $Ca_v1.2$ and $Ca_v1.3$ complexes to decrease channel conductance and differentially affect activation/inactivation kinetics as well as recovery from VDI. TSPAN-7 regulates insulin secretion levels and may play an important role is defining the set-point at which GSIS occurs. A follow-up study investigating the role of TSPAN-7 in human β-cells as a function of gender, body mass index (BMI), age, exercise activity, diabetes would be very interesting. A better understanding of the physiological role of TSPAN-7 could have implications in developing new therapeutic strategies for the treatment of impaired glucose-stimulated insulin secretion.

Competing interests
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Cav1.2, GSIS, insulin secretion, pancreatic beta-cell, tetraspanin-7

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