Review

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Role of TRPV channels in regulating various pancreatic β-cell functions: Lessons from *in vitro* studies

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Summary **Pancreatic β-cell functions are regulated by a variety of endogenous and exogenous factors. Calcium is one of the most potent triggers of β-cell growth, insulin production and exocytosis. Recently, others and we showed that TRPV channels are expressed in insulin producing cell lines and/or primary β-cells. These channels modulate calcium ions, insulin secretion and cell proliferation. Besides the classical roles of TRPV channels in the sensory system, there are also novel functions described in non-excitable cells such as in insulin-producing β-cells. This review summarises the current knowledge about the expression and the role of TRPV channels in controlling β-cell functions based upon studies performed in isolated primary β-cells as well as permanent β-cell models.**

Keywords: Apoptosis, beta cell, calcium, insulin, TRPV, proliferation

1. Introduction

Pancreatic islets are composed of several types of endocrine cells including α, β, δ, ε and PP cells, which produce glucagon, insulin, somatostatin, ghrelin and pancreatic polypeptide, respectively (*1*). Approximately 80% of endocrine cells composing pancreatic islet are represented by β-cells (*2*). Insulin is the major hormone responsible for maintenance of normoglycemia by promoting glucose transport to insulin-depended tissues, suppression of gluconeogenesis and enhancing glycogenesis (*3*). Furthermore, insulin has pleiotropic effects such as modulation of learning, memory and reproduction processes (*4*). The loss of pancreatic β-cells and impaired insulin production are hallmarks of type 1 and type 2 diabetes (*5*). Late complications of persistent chronic hyperglycemia and other metabolic derangements are nephropathy, neuropathy, retinopathy or other cardiovascular diseases (*6,7*). Notably, the prevalence of both types of diabetes is currently increasing (*8*). There is growing evidence that changes in intracellular calcium levels play a prominent physiological role in β-cells. Ca^{2+} ions modulate insulin secretion, expression and proliferation as well as β-cell growth and apoptosis (*9,10*). Calcium changes related to β-cell death or insulin exocytosis were reported in beta cells derived from diabetic subjects as well as in insulinomas (a neuroendocrine tumor derived from β-cells). It is well-known that in β-cells Ca^{2+} influx depends on the activity of a large number of ion channels. However, voltage-operated Ca^{2+} channels (VOCCs) play a predominant role (*11*). In addition, recent studies in pancreatic primary β-cell and insulin producing permanent β-cell lines indicate that Ca^{2+} ions entrance is regulated by transient receptor potential vanilloid (TRPV) channels. In the present work, we review the current knowledge about the role of these channels in controlling primary β-cell functions including calcium homeostasis, insulin secretion as well as cell growth and death.

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2. TRPV as subfamily of TRP channels

Transient potential receptor (TRP) channels are members of a superfamily of cation channels. They are subdivided into six groups with differences in their amino acid sequences, activation factors and modulatory mechanisms. TRP channels (TRPs) were firstly described in *Drosophila,* where *trp* gene mutation carried a characteristic defect in phototransduction (*12*). All TRPs consists of six putative transmembrane protein subunits with the pore loop between the fifth and sixth segment (S5-S6). They differ in cytoplasmic amino and carboxy termini (*13,14*). The 28 mammalian TRP channels are divided into six different subfamilies based on sequence homology: canonical (TRPC), melastatin (TRPM), vanilloid (TRPV), polycystin (TRPP), ankyrin (TRPA), and mucolipin (TRPML1-3) receptors. TRPs are activated by wide range of stimuli including mechanical and osmotic changes, temperature or intracellular ligands. The TRPV channel family consists of six members (TRPV1-6). All of them contain 3-6 intracellular N-terminal ankyrin repeats domain, common protein-protein interaction motif. They can form homomeric and/or heteromeric, tetrameric configurations. TRPVs can be subdivided into two groups: TRPV1-4 and TRPV5-6. TRPV1-4 are thermosensitive, non-selective calcium channels having permeability ratio P_{Ca}/P_{Na} between 5 and 10, whereas TRPV5-6 are highly Ca^{2+}-selective ion channels, with P_{Ca}/P_{Na} over 100 (*15-17*). TRPV1 was the first TRPV subtype to be identified and is therefore the most extensively characterized channel. TRPV1 is activated by vanilloid compounds such as capsaicin (an active ingredient in hot chili peppers), noxious heat (≥ 43°C) (*18*), low pH (≤ 5.9) or voltage (*19,20*). TRPV1 is not only involved in classical pain sensations *via* nociceptor activation (*21*), but also contribute to novel functions in non-neuronal or tumor cells (*22*). TRPV1 activity can be modulated by protein kinase A (*23,24*), protein kinase C (*25*) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) phosphorylation (*26*). Another regulatory molecule of TPRV1 is PIP2, which binds to the C- and N-terminal region of TRPV1 and thus regulates this channel activation (*27,28*). TRPV2 is approx. 50% identical to TRPV1, but is insensitive to capsaicin and low pH. Very recent results indicated a link between TRPV1 and VEGF in benign tumor cells (*29*). The high-heat sensor TRPV2 is activated by noxious heat with an activation threshold greater than 52°C (*30*). Moreover, TRPV2 is activated by physical stimuli including mechanical stretch and osmotic swelling (*31*). This channel is highly expressed in various tissue types such as nervous system, immune-related tissues and cells, vascular smooth muscle, and endothelial cells. TRPV3 is a moderate heat-activated TRP channel with 40% identity to TRPV1 (*32*). It's temperature activation threshold in the physiological temperatures is in the range of 32 to 39°C. TRPV3 can be also activated by camphor, thymol and 2- aminoethoxydiphenyl borate (2-APB). This channel plays a major role in regulating physiological skin homeostasis by regulating proliferation and apoptosis in epidermal keratinocytes (*33*). In addition, it has been reported that TRPV3 is involved in hairlessness combined with dermatitis (*34*). TRPV4 can be activated by physical stimuli such as cell swelling, moderate heat (> 24-27°C), shear stress and chemical ligands (*e.g*. phorbol esters, endocannabinoids, arachidonic acids or synthetic compound GSK1016790) (*35-37*). This channel is widely expressed in various tissues such as brain, vascular endothelium, bladder, kidney and multiple excitable, and non-excitable peripheral cell types including eye surface cells (*38*). TRPV5 and TRPV6 are the only calcium selective TRP channels. Although they share 74% amino acids identity, they are only 22-24% identical to other TRPVs. TRPV5 and TRPV6 maintain calcium homeostasis in epithelial tissues (*39*). Both channels can be activated by fluid shear force (*40*). TRPV5 is highly expressed in the kidney, where it regulates Ca^{2+} excretion into the urine (*41,42*). It is regulated by Klotho, a β-glucuronidase that hydrolyzes extracellular sugar residues on TRPV5 (*43*). TRPV6 is involved in the active Ca^{2+} absorption and is predominantly expressed in the small intestine (*44*). Increased expression of TRPV6 has been observed in various human tumors, including prostate cancer (*45*).

3. Expression of TRPVs in pancreatic β-cells and insulin producing cell lines and their role in controlling insulin secretion

3.1. *TRPV1*

Initially, TRPV1 mRNA expression was detected in rat INS-1 and RINm5F insulin producing (insulinoma) β-cell lines (*46*). Immunofluorescence staining revealed that TRPV1 is expressed in endocrine cells of rat islets. Furthermore, it was reported that TRPV1 activation by the agonist capsaicin stimulated insulin secretion from INS-1 cells (*46*). Capsaicin-induced insulin secretion was attenuated by EGTA or the TRPV1 blocker capsazepine. In the same study, insulinotropic action of capsaicin was confirmed in animal experiments. It was found that in rats s.c. injection of capsaicin resulted higher plasma insulin levels as compared with vehicle-treated animals. The presence of TRPV1 protein was also identified in rat insulinoma INS-1E cells (cell line derived from INS-1 cells) (*47,48*).

Consistent with previous data, it was reported that the TRPV1 agonists capsaicin and AM404 are able to rise intracellular calcium levels in INS-1E cells, which depends upon TRPV1 channel activation (*47*). However, the same research group failed to detect TRPV1 immunoreactivity in human primary β- as well as human insulinoma cells (*47*). In agreement with these

observations, capsaicin did not increase Ca^{2+} levels in human primary β-cells. Furthermore, capsaicin failed to affect calcium influx in rat primary β-cells (*47*). Consistent with the incapability of capsaicin to affect calcium levels in rat primary β-cells, others reported that rat pancreatic islets lack TRPV1 (*49*). Similar data were obtained by Diaz-Garcia *et al*. who studied whether TRPV1 is expressed in rat pancreatic β-cells (*50*). This study showed that primary β-cells isolated from adult rats do not express TRPV1 mRNA. To further identity factors that can induce TRPV1 expression in β-cells, the same group tested whether TRPV1 can be induced by the nerve growth factor (NGF). It was found that NGF stimulates TRPV1 mRNA expression. However, NGF failed to induce TRPV1 protein production. The lack of TRPV1 expression was also reported in β-cells isolated from neonate rats (*50*). Furthermore, this research laboratory was not able to detect TRPV1 protein production in RINm5F cells, which was inconsistent with previous work (*46*). Overall, these data indicate that TRPV1 protein and mRNA are present in rat insulinoma cells (INS-1 and INS-1E), whereas TRPV1 is absent in rat and human primary β-cells as well as human insulinoma. Moreover, it appears that TRPV1 directly stimulates insulin secretion from rat insulinoma cells but not from human or rodent primary β-cells or human insulinoma. However, contribution of TRPV1 in modulating insulin secretion *in vivo* cannot be completely excluded. For instance, animal studies indicated that pancreatic islets do not express TRPV1 but they are innervated by TRPV1-expressing sensory nerve fibers and systemic capsaicin treatment potentiated glucose induced insulin secretion (*49*). Thus, it is likely TRPV1 may modulate insulin levels despite its absence in β-cells.

3.2. *TRPV2*

Both TRPV2 mRNA and protein were detected in murine insulin producing min6 cell line as well as in primary β-cells (*51*). The vast part of data describing the role of TRPV2 in controlling intracellular calcium levels and insulin secretion was obtained using cells with TRPV2 downregulation. Hisanaga *et al*. found that insulin stimulates intracellular calcium levels in min6 cells, which is mediated through insulin-induced TRPV2 translocation to the plasma membrane (*51*). Suppression of TRPV2 protein production by shRNA or blockade by tranilast (an inhibitor of TRPV2) inhibited insulin-induced Ca^{2+} influx in min6 cells (*51*). Furthermore, this study reported that transilast supressed high glucose- and potassium-induced insulin secretion from min6 cells. Importantly, the same work found that insulin-induced TRPV2 translocation from cytoplasm to the plasma membrane accurse also in mouse primary β-cells. In addition, downregulation of TRPV2 protein production resulted in lower rate of glucose- and potassium-induced insulin secretion in mouse primary β-cells. The contribution of insulin to the translocation of TRPV2 to the plasma membrane was confirmed by another study, which showed that this action is mediated *via* PI3K. Furthermore, this study showed also that TRPV4 is involved in first but not second phase of insulin release (*52*). Another work investigated the role of Klotho gene in controlling insulin secretion from min6 cells. It was found that overexpression of Klotho leads to increased plasma membrane retention of TRPV2 in min6 cells (*53*). By contrast, Klotho knockout reduced plasma membrane retention of TRPV2. Furthermore, pharmacological inhibition of TRPV2 by tranilast supressed both, Klotho-induced calcium influx and glucose-induced insulin secretion in min6 cells. In summary, these data collectively showed that TRPV2 stimulates insulin secretion from min6 and mouse primary β-cells. Furthermore, there is convincing evidence that its translocation to the plasma membrane is controlled by insulin as well as Klotho-dependent manner.

3.3. *TRPV3*

According to our knowledge data indicating functional expression of TRPV3 in primary β-cells or insulin producing cells are not available.

3.4. *TRPV4*

Expression of TRPV4 mRNA was found in min6, INS-E cells and rat pancreatic islets (*54,55*). There are two studies addressing the question whether TRPV4 modulates intracellular calcium levels in insulin producing cells. Casas *et al*. using min6 cells reported that human islet amyloid polypeptide (hIAPP) increases that intracellular calcium level in min6 cells (*54*). This effects of hIAPP was supressed by addition of gadolinium a non-specific TRP channel inhibitor. Another experiments showed that the hIAPP-induced intracellular Ca^{2+} rise in min6 cells can be also suppressed by ruthenium red, which is a non-competitive pan inhibitor of TRPs, in particularly TRPV channels including TRPV4 (and also TRPV1) (*56,57*). To assess whether TRPV4 mediates hIAPP-induced intracellular Ca^{2+} rise the authors of this study utilised siRNA technique. These experiments demonstrated that elevation of Ca^{2+} by hIAPP was supressed in cells with TRPV4 protein downregulation. Overall, this study showed for the first time that TRPV4 activation leads to increase Ca^{2+} levels in min6 cells. Recently, we studied the effects of TRPV4 activation on intracellular Ca^{2+} levels in INS-1E cells. We found that TRPV4 activation by moderate heating, hypotonic challenge as well as its agonist 4αPDD increased Ca^{2+} levels (*55*). Stimulation of Ca^{2+} elevation was attenuated in INS-1E cells with TRPV4 protein downregulation suggesting these effects are mediated *via* TRPV4. Moreover, we found elevation

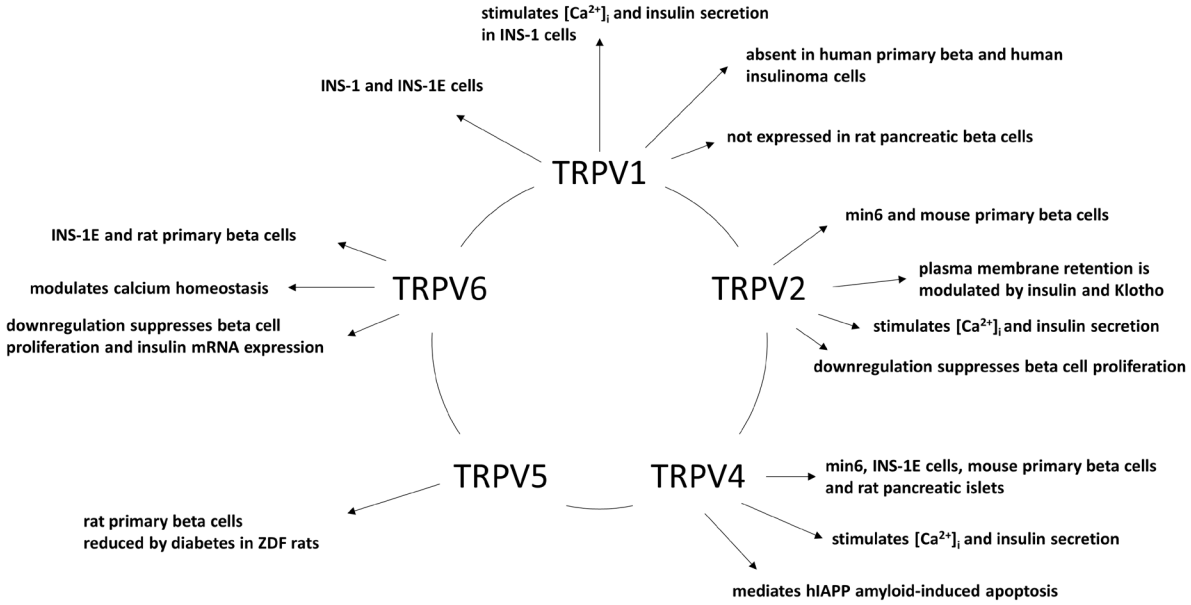

Figure 1. Expression and potential function of TRPV channels in the endocrine pancreas.

of Ca^{2+} influx induced by 4αPDD was accompanied by increased insulin secretion. Stimulation of insulin secretion by 4αPDD was additionally confirmed in isolated rat pancreatic islets. In summary, these data revealed that TRPV4 modulates calcium homeostasis and insulin secretion in insulin producing INS-1E cells, suggesting its influence on insulin secretion in primary β-cells. Nevertheless, whether TRPV4 contribute to insulin secretion *in vivo* remains an open question.

3.5. *TRPV5*

Janssen *et al*. reported that TRPV5 mRNA is present in RNA isolated from whole rat pancreas (*58*). The authors of this work also studied immunofluorescence localization of this channel in pancreatic islets. Double immunofluorescence studies revealed that TRPV5 colocalize with β- but not α-cells. It was also observed that TRPV5 expression decreases during the progression of diabetes in ZDF Rats (*58*). In contrast to rat islets, others reported no expression of TRPV5 in mouse islets (*59*). To our best knowledge, there are no data demonstrating contribution of TRPV5 to calcium homeostasis and insulin secretion in β-cells.

3.6. *TRPV6*

Human studies appeared that TRPV6 expression is restricted only to exocrine pancreas (*60*). Recently, however, we reported that TRPV6 mRNA is expressed in rat INS-1E cells and rat pancreatic islets (*61*). Using double immunofluorescence staining we detected that TRPV6 is expressed in rat β-cells. Furthermore, we found that TRPV6 downregulation is associated with impaired calcium influx in INS-1E cells. On the other hand, we did not find any difference in basal and glucose-induced insulin secretion between cells with normal and supressed TRPV6 protein production. However, we showed that suppression of TRPV6 expression leads to suppression of insulin mRNA expression in INS-1E cells. These data suggest that TRPV6 modulates insulin mRNA expression but not secretion. However, since these data were obtained using INS-E cells further studies are needed to elucidate the role of TRPV6 in primary β-cells.

4. TRPVs in controlling β-cell growth and death

Keeping mind the pivotal role of calcium ions in modulating cell proliferation and apoptosis, several studies investigated whether selected TRPVs are involved in pancreatic β-cell proliferation or death. Hisanaga *et al*. found that suppression of TRPV2 protein production in min6 is associated with impaired glucose- and serum-induced cell proliferation but not enhanced cell death (*51*). Their results showed that TRPV2 is required to maintain min6 cell proliferation. A promitogenic role of TRPVs in insulin producing cells is also supported by our recent data. We found that suppression of TRPV6 protein production by siRNA technique resulted in reduced cell proliferation and viability in INS-1E cells (*61*). Likewise previous studies, these effects were detected in the presence of glucose and serum. Studying potential mechanism by which TRPV6 may control cell growth, we identified that decreased cell proliferation in TRPV6 siRNA transfected cells was accompanied by reduced activity of calcineurin/NFAT

signalling and ERK1/2 phosphorylation. Since these pathways were involved in β-cell proliferation (*62,63*), these data strongly suggested that TRPV6 may trigger INS-E cell growth *via* calcineurin/NFAT- and ERK1/2-dependent mechanisms. Notably, similar data indicating the link between TRPV6/NFAT signalling and cell proliferation were described in human neuroendocrine pancreatic BON cells (*64*). By contrast, to promitogenic effects of TRPV2 and TRPV6 in insulin producing cells, it was demonstrated that TRPV4 activation by hIAPP stimulate β-cell death. Casas *et al*. found that activation of TRPV4 by hIAPP induces the endoplasmic reticulum (ER) stress response and apoptosis in min6 and murine primary β-cells (*54*). Since increased accumulation of hIAPP aggregates associates with β-cell death in type 2 diabetic patients (*65*), these re*sul*ts suggest that TRPV4 can contribute to β-cell loss in type 2 diabetes. The role of other TRPVs in controlling β-cell growth remains unknown.

The TRPV channel expression and function in the endocrine pancreas are shown in Figure 1.

5. Concluding remarks

In summary, there is evidence that TRPV1, TRPV2, TRPV4 and TRPV6 are expressed in rodent insulinoma cell lines which are involved in calcium regulation. TRPV1, TRPV2 and TRPV4 were implicated in insulin secretion in rodent insulin producing cells. Furthermore, there is evidence that TRPV2 controls Ca^{2+} influx and insulin secretion in mouse primary β-cells. By contrast, TRPV1 is rather not expressed in rat primary β-cells or human insulinoma. The loss of TRPV2 protein production in murine min6 cells and TRPV6 in rat INS-1E cells leads to supressed cell proliferation. On the other hand, TRPV4 activation by hIAPP results in min6 and mouse primary β-cell apoptosis.

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