


REVIEW ARTICLE

Mitochondrial ion channels in pancreatic β -cells: Novel pharmacological targets for the treatment of Type 2 diabetes

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Pancreatic beta-cells are central regulators of glucose homeostasis. By tightly coupling nutrient sensing and granule exocytosis, beta-cells adjust the secretion of insulin to the circulating blood glucose levels. Failure of beta-cells to augment insulin secretion in insulin-resistant individuals leads progressively to impaired glucose tolerance, Type 2 diabetes, and diabetes-related diseases. Mitochondria play a crucial role in β -cells during nutrient stimulation, linking the metabolism of glucose and other secretagogues to the generation of signals that promote insulin secretion. Mitochondria are double-membrane organelles containing numerous channels allowing the transport of ions across both membranes. These channels regulate mitochondrial energy production, signalling, and cell death. The mitochondria of β -cells express ion channels whose physio/pathological role is underappreciated. Here, we describe the mitochondrial ion channels identified in pancreatic β -cells, we further discuss the possibility of targeting specific β -cell mitochondrial channels for the treatment of Type 2 diabetes, and we finally highlight the evidence from clinical studies.

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1 | INTRODUCTION

Type 2 diabetes is a chronic and progressive disease diagnosed by elevated levels of blood glucose. The condition develops as beta-cells of the endocrine pancreas becomes unable to compensate for insulin resistance in the target tissues. According to the last World Health

Organization report (2016) on diabetes mellitus, more than 400 million (8.5% of the adult population) of humans worldwide suffer from this pathology, and it is one of four non-communicable diseases with an urgent need to find novel approaches for the prevention and treatment.

Insulin is the only blood glucose lowering hormone and is produced in β -cells of the endocrine pancreas. Pancreatic β -cells together with other endocrine cells (α , δ , ϵ , and PP cells) are physically organized in the islets of Langerhans, micro-organs embedded in the exocrine pancreas (Figure 1a). These islets constitute only 1–2% of the total pancreas mass but are essential to maintain glucose homeostasis under fasting and fed states. Dysfunction or reduced

Abbreviations: CLICs, intracellular chloride channels; EMRE, essential MCU regulatory element; FADH₂, flavin adenine dinucleotide; Grp75, glucose-regulated protein 75; IMM, inner mitochondrial membrane; IMS, inter membrane space; K_{ATP} channel, ATP-sensitive potassium channel; MCU, mitochondrial calcium uniporter; OMM, outer mitochondrial membrane; PTP, permeability transition pore; VDAC, voltage-dependent anion channel.

Umberto De Marchi and Silvia Fernandez-Martinez contributed equally to the work.

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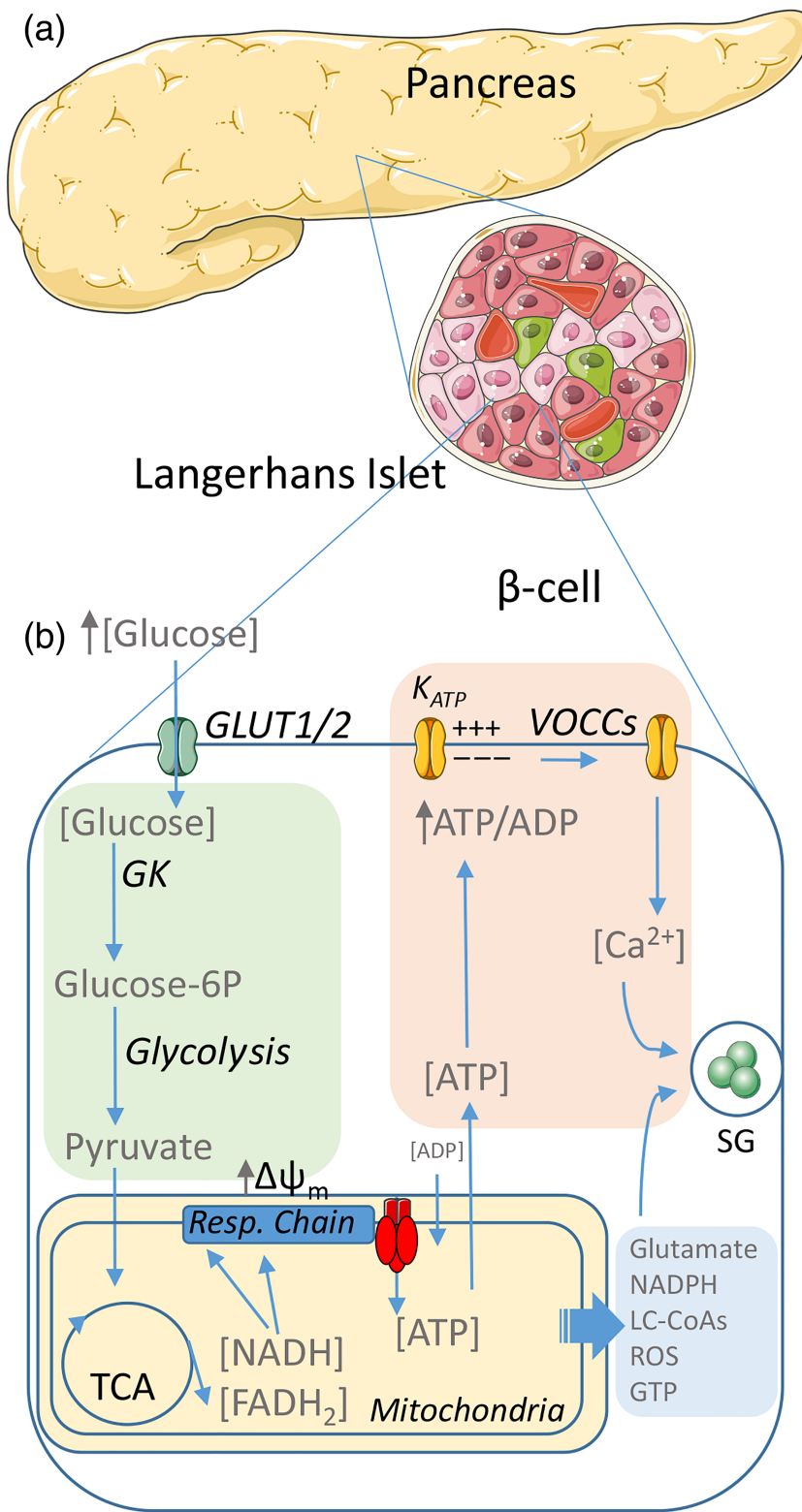


FIGURE 1 Pancreatic β -cells and metabolism-secretion coupling. (a) Pancreatic β -cells localize on the islets of Langerhans of the endocrine pancreas. (b) Essential role of β -cell mitochondria on metabolic-secretion coupling (described in the introduction). Glucose transporter (GLUT1/2); Glucokinase (GK); Tricarboxylic acid cycle (TCA); Long Chain Acyl-CoAs (LC-CoAs); Voltage Operated Calcium Channels (VOCCs)

mass of β -cells leads to insufficient insulin secretion, Type 2 diabetes, and a number of linked complications (Weir & Bonner-Weir, 2004). Therefore, approaches to maintain or expand the β -cell mass as well as to preserve or boost the capacity of β -cells to secrete insulin are valuable strategies to slow or prevent diabetes progression.

The mechanisms linking glucose metabolism to insulin exocytosis in β -cells have been termed “stimulus-secretion coupling” (Figure 1b).

During the postprandial phase, glucose rises in the blood and enters the β -cell mediated by **GLUT transporters** constitutively expressed at the plasma membrane. In contrast to muscle and fat, glucose uptake is not insulin dependent. Once in the cytosol, **glucokinase** rapidly phosphorylates glucose on the carbon -6 position and accelerates glycolysis to generate pyruvate. The transport of pyruvate into the mitochondria activates the tricarboxylic acid cycle promoting the

formation of **NADH** and **FADH₂**. These reducing equivalents are substrates for the mitochondrial respiratory chain complexes and activate ATP synthase-dependent respiration. Mitochondrial activation is thus closely linked to nutrient stimulation and respiratory activity essential for stimulus–secretion coupling. Elevated **ATP** synthesis and mitochondrial ATP export rapidly raise the cytosolic **ATP/ADP** ratio, triggering the closure of the **plasma membrane K_{ATP} channel**. The resulting plasma membrane depolarization increases the open probability of voltage-operated **Ca²⁺** channels, triggering a cytosolic **Ca²⁺** rise that promotes exocytosis of insulin containing secretory granules. In addition to the ATP produced by oxidative phosphorylation, a number of mitochondrial-derived metabolites generated during the metabolic activation of the β -cell have been proposed to enhance insulin secretion independent of the regulation of the **K_{ATP}** channel (Henquin, 2009). These metabolites, together with ATP, are known as metabolic coupling factors. Although much progress has been made regarding the mechanisms linking metabolism to insulin granule exocytosis, this remains a field of intensive research. In summary, glucose metabolism in β -cells is strictly coupled to the bioenergetic status of the mitochondria that in turn generates cytosolic signals that triggers insulin secretion. Mitochondria thus play a central role in the mechanisms leading to nutrient-induced insulin release.

Recent advances based on proteomics, molecular biology, and electrophysiology techniques are shedding light on the molecular nature of mitochondrial ion channels and their involvement in cell physiology and pathology (Szabo & Zoratti, 2014). β -cell mitochondria express many ion channels, but their roles in β -cell function are only beginning to be understood. In this review, we highlight the existence of pancreatic β -cell mitochondrial ion channels; we point to their role in mitochondrial bioenergetics, mainly in the context of nutrient-induced β -cell activation and cell death, and finally, we review clinical strategies targeting these channels for the treatment of Type 2 diabetes.

1.1 | Mitochondrial ion homeostasis, ion channels, and cellular roles

Mitochondria are separated from the cytosol by two lipid bilayers: the outer mitochondrial membrane (OMM) and the inner mitochondrial membrane (IMM), separated by the inter membrane space (IMS). The OMM is a semipermeable membrane to ions and low MW metabolites. Porins (voltage-dependent anion channel [VDAC]) constitute the most abundant proteins of the OMM, and their function is to facilitate the transport of ions and small solutes. The passive transport of ions across biological membranes is determined by the electrochemical gradients. Considering its high ion permeability, it is believed that ions rapidly equilibrate across the OMM following down the sum of their chemical and electrical gradients during physiological fluctuations of ions. The existence of a very small electrical potential across this membrane provided by immobile charges remains under debate. In contrast, the IMM is highly impermeable to ions, and the passive transport of ions through the IMM is mainly driven by the electric

component of the ion electrochemical gradient. The IMM of energized mitochondria holds $\Delta\psi_m \sim 160\text{--}200$ mV (negative inside) and $\Delta\text{pH} \sim 0.8$, maintained by the **H⁺** ejection activity of the respiratory chain complex as electrons flow from reduced substrates in the matrix to molecular oxygen in a low **H⁺** permeability membrane. Both ($\Delta\psi_m$ and ΔpH) drive the ATP synthase (Mitchell, 1961). In addition to ATP synthesis, both components of the proton motive force ($\Delta\psi_m$ and ΔpH) promote the transport respiratory substrates and mediate the exchange of ions and metabolites between the matrix and the IMS. The existence of **H⁺/cations antiporters** that in energized mitochondria promote the extrusion of the accumulated cations contributes to maintain the cation gradients across the IMM far from the Nernstian equilibrium. That is, $[\text{Na}^+]_{\text{mit}}$ is estimated in 5 mM, considering $\Delta\psi_m = -180$ mV and $[\text{Na}^+]_{\text{cyt}} = 10$ mM, the equilibrium would be reached at $[\text{Na}^+]_{\text{mit}} = 5$ M; $[\text{Ca}^{2+}]_{\text{mit}}$ is estimated in 100 nM. Considering $[\text{Ca}^{2+}]_{\text{cyt}} = 100$ nM, $[\text{Ca}^{2+}]_{\text{mit}}$ would have to reach ~ 100 mM to achieve equilibrium (Bernardi, 1999).

Both mitochondrial membranes contain channels mediating the passive transport of ions in and out of mitochondria (Szabo & Zoratti, 2014). Figure 2 gives an overview of the mitochondrial ion channels described up to date. Changes in ion fluxes across both membranes influence overall mitochondrial function in different but highly inter-related ways that we conceptually divided into three groups: bioenergetics, signalling, and cell death.

1.1.1 | Bioenergetics

As mentioned above, under resting conditions, the IMM potential (negative inside) generated by the **H⁺** pumping of the respiratory chain constitutes the main driving force for cation transport into the matrix. Consequently, the opening of cation channels causes immediate transport of the corresponding ions to the mitochondrial matrix. The net movement of positive charges to the matrix decreases the $\Delta\psi_m$. According to the chemiosmotic theory (Mitchell, 1961), this is balanced by an increase in NADH oxidation, electron flux, and **H⁺** export. Thus, the activity of the respiratory chain couples through the $\Delta\psi_m$ to the activity of IMM cation channels. In this manner, modification of IMM ion fluxes influence ATP synthesis and the energy status of the cell.

1.1.2 | Signalling

Mitochondrial ion channels are regulators of mitochondrial and cellular signalling, including **Ca²⁺** and ROS signalling events. Mitochondrial-produced ROS are well-recognized signalling molecules mediating the activation of specific transcriptional programmes or regulating translation among other cellular roles (Kobayashi et al., 2006; Topf et al., 2018). The mitochondrion is an important ROS signalling node due in part to the ability of the respiratory chain to generate superoxide, a precursor of most reactive oxidative species. Superoxide is constantly produced through the transfer of an electron from the

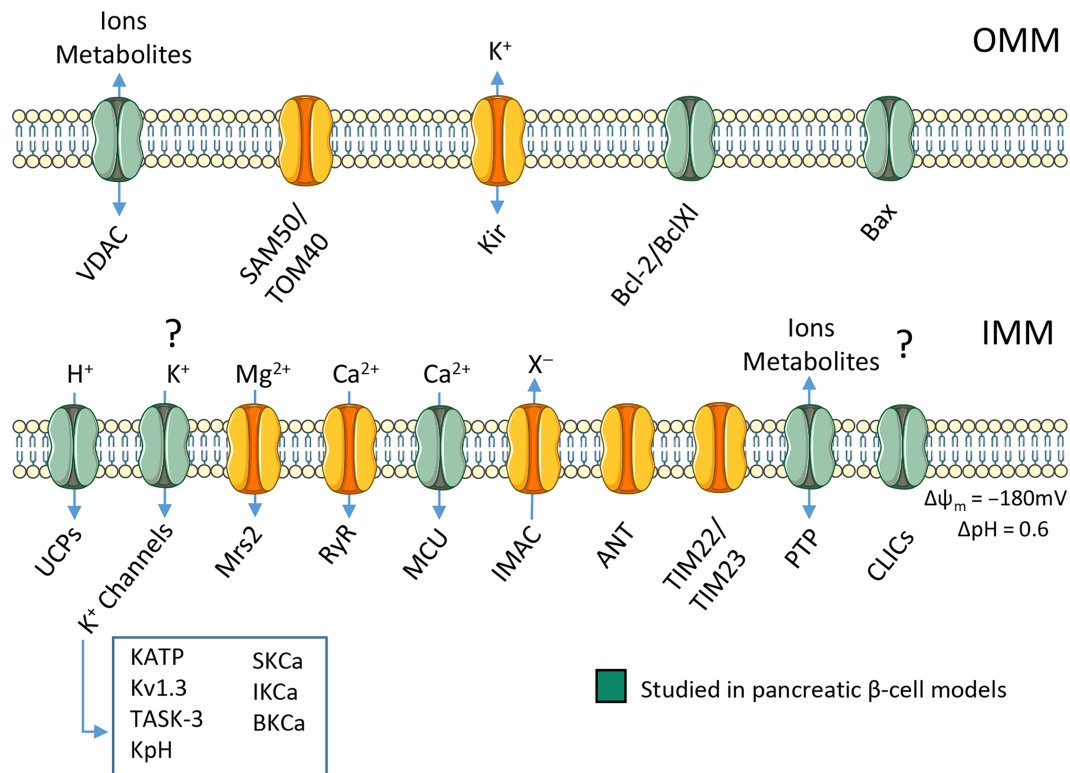


FIGURE 2 Mitochondrial ion channels studied in pancreatic β -cells. Inner (IMM) and outer (OMM) mitochondrial membrane channels. In the OMM: VDAC, voltage-dependent anion channel; SAM50 and TOM40 are outer mitochondrial membrane protein import systems; Kir, inward rectifying potassium channel; Bcl2 family proteins (Bcl2/BclXI and Bax). In the IMM: UCps, uncoupling proteins; potassium channels (including K_{ATP} , $K_v1.3$, SK_{Ca} , IK_{Ca} , BK_{Ca} , TASK-3, and KpH); Mrs2, magnesium permeable channel; RyR, ryanodine receptor; MCU, mitochondrial calcium uniporter; IMAC, inner membrane ion channel; ANT, adenine nucleotide exchanger; TIM22/TIM23, inner mitochondrial membrane protein import systems; PTP, permeability transition pore; CLICs, intracellular chloride channels. The electrophysiological characterization of these mitochondrial channels in pancreatic β -cells is not available. The scientific evidence supporting the existence and properties of β -cell mitochondrial channels is mainly pharmacological, biochemical, and from molecular biology. A query(?) marks those channels for which there is, at present, no biochemical or pharmacological definition

electron transport chain to molecular oxygen during oxidative phosphorylation, a phenomenon that is extremely sensitive to the proton motive force (Miwa & Brand, 2003). Thus, $\Delta\psi_m$ variations mediated by ion fluxes at the IMM control the rate of superoxide formation in the respiratory chain (Brand & Esteves, 2005). The ability of mitochondria to import Ca^{2+} from the cytosol also has important functional consequences. Mitochondrial Ca^{2+} uptake (mediated by the IMM channel mitochondrial Ca^{2+} uniporter [MCU]) shapes cytosolic Ca^{2+} signals and thus regulates the activity of the cytosolic Ca^{2+} effectors, that is, regulating the magnitude of the sub-plasma membrane high Ca^{2+} microdomains that control exocytosis in neuro endocrine cells (Montero et al., 2000). Additionally, mitochondrial Ca^{2+} uptake also links cytosolic signalling to the mitochondrial energy status through allosteric regulation of tricarboxylic acid cycle dehydrogenases and ATP synthase (Rutter et al., 1993).

1.1.3 | Cell death

Mitochondrial ion channels play an essential role regulating the release of pro-apoptotic factors to the cytosol under a number of

pathological conditions. Key mediators of this process are the non-selective mitochondrial permeability transition pore (PTP) together with other pore-forming outer mitochondrial proteins (Bcl2/Bax; Forte & Bernardi, 2006). The pore-forming activity of the PTP is itself strongly dependent on the bioenergetic status of the mitochondria. ROS, $[Ca^{2+}]_{mit}$, pH_{mit} levels, and $\Delta\psi_m$ are key regulators of the PTP activity, which are in turn dependent on the activity of other IMM channels (Bernardi et al., 2006). In addition, mitochondrial K^+ -channel modulation has been involved in cell-death regulation, and a direct pharmacological targeting of these channels has been recently suggested to selectively kill tumour cells in vivo (Leanza et al., 2017). Thus, the activity of the mitochondrial ion channels contributes to tightly tune cell survival and death process.

2 | CHANNELS OF THE IMM

2.1 | Uncoupling proteins

The chemiosmotic hypothesis establishes that mitochondrial ATP synthesis requires the formation of a proton (H^+) electrochemical gradient

($\Delta\mu_{\text{H}^+}^-$) across the IMM as substrates are oxidized. Both gradients ($\Delta\psi_{\text{m}}$ and ΔpH) are equally driven forces for the synthesis of ATP as H^+ flow back to the matrix through the ATP synthase. The concept of uncoupled mitochondria refers to the disconnection of substrate oxidation from the production of ATP by dissipating the proton motive force ($\Delta\mu_{\text{H}^+}^-$; Bouillaud, Alves-Guerra, & Ricquier, 2016). The channels responsible for this proton entry bypassing the ATP synthase are the uncoupling proteins (UCPs). The first UCP (**UCP1**) was described in brown adipose tissue, where the uncoupled mitochondria dissipate the proton gradient as heat whenever substrate and oxygen are available (Ricquier, 2017). Five UCPs have been reported in mammals (UCP1-5); among them, **UCP2** is the only member of the UCPs expressed in β -cells, although compared to other tissues, UCP2 expression in β -cells seems low (Azzu, Affouret, Breen, Parker, & Brand, 2008). UCP2 catalyse proton conductance when reconstituted in liposomes and causes uncoupling in isolated mitochondria (Jaburek & Garlid, 2003; Parker, Affouret, Vidal-Puig, & Brand, 2008). Nevertheless, other groups have not been able to validate the uncoupling activity of UCP2 (Couplan et al., 2002), even in pancreatic β -cells (Li, Karaca, & Maechler, 2017; Robson-Doucette et al., 2011). To test the idea that UCP2 causes mild uncoupling in pancreatic β -cells, a UCP2 KO mouse model was created. This model was generated in a mixed 129/SVJ x C57BL/6 background. Islets from these animals displayed increased insulin secretion (C. Y. Zhang et al., 2001). These findings are consistent with improved mitochondrial coupling between nutrient oxidation and ATP synthesis as a mechanistic explanation for improved insulin secretion. However, when the strain was backcrossed into another background, the effect disappeared, pointing to the fact that other mechanisms besides the lack of UCP2 underlie the generation of this phenotype (Pi et al., 2009). The contradictory results can be attributed in part to the UCP2 ablation in other cell types such as macrophages and pro-opiomelanocortin (POMC) neurons, also involved in response to glucose). Studies in cultured insulinoma cells (INS-1E) showed that upon UCP2 removal, insulin secretion increased significantly at high glucose concentrations (Affouret & Brand, 2008). In the INS-1E, the coupling efficiency is only 30% under resting glucose concentrations. The remaining 70% of the basal respiration drives proton leak instead of ATP production. This level of uncoupling is extraordinarily high compared with other mammalian cells (Affouret & Brand, 2009). If the proton leak was driven by UCP2, coupling efficiency should be improved in the KO model. The data were conclusive, the coupling efficiency in the UCP2 KO INS-1E cells reached 85–90%, supporting the hypothesis that UCP2 reduces the proton motive force, and thus insulin secretion (Affouret & Brand, 2009).

In mouse models of obesity-induced Type 2 diabetes, UCP2 expression is increased, providing a potential pathological mechanism to explain impaired β -cell function. Moreover, whole-body UCP2 ablation in the obese mouse model normalizes basal levels of insulin secretion and restores normal glycaemia (C. Y. Zhang et al., 2001). In contrast, other groups have reported that specific UCP2 ablation in β -cells triggers glucose intolerance, even if the ability of the β -cell to secrete insulin was significantly increased. The phenomenon was

attributed to increased β -cell ROS levels that (a) amplified the metabolic coupling in β -cells and (b) exacerbated glucagon production and secretion of α -cells (Robson-Doucette et al., 2011). To further complicate the picture, it has been recently demonstrated that β -cell UCP2 levels undergo a daily cycle that inversely controls the ability to secrete insulin during the course of the day–night cycle. *in vivo* experiments with the specific β -cell, UCP2 KO mouse revealed that UCP2 ablation impairs glucose tolerance only in the light phase of the daily cycle (Seshadri et al., 2017). Interestingly, polymorphisms in UCP2 have been directly linked with Type 2 diabetes in the Korean population. However, these variations seem not to affect Type 2 diabetes development in Europeans (Vimaleswaran et al., 2011). It can therefore be concluded that although some authors initially proposed that down-regulation of UCP2 expression might constitute a realistic strategy to maintain β -cell function in patients with Type 2 diabetes, this is currently under debate and much more experimental work is necessary to clarify the role of UCP2 in β -cell physiology and pathology.

2.2 | Mitochondrial Ca^{2+} uniporter

As glucose levels equilibrate across the plasma membrane of β -cells, the metabolic fluxes and ATP/ADP ratio increases, the plasma membrane depolarizes, and opening of **voltage-operated calcium channels** initiates cytosolic Ca^{2+} transients. Ca^{2+} ions cross both OMM and IMM to activate matrix dehydrogenases (Denton & McCormack, 1980) and ATP synthase (De Marchi, Thevenet, Hermant, Dioum, & Wiederkehr, 2014), and thereby accelerating oxidative phosphorylation. Therefore, mitochondrial Ca^{2+} uptake promotes stimulus–secretion coupling in pancreatic β -cell, further activating mitochondrial energy metabolism required for sustained insulin secretion (Wiederkehr et al., 2011).

In the OMM, VDAC facilitates the diffusion of Ca^{2+} , ions, and small solutes (Shoshan-Barmatz, Israelson, Brdiczka, & Sheu, 2006). However, mitochondrial Ca^{2+} influx is mainly regulated at the level of the IMM, where the transport is mediated by a protein complex named mitochondrial Ca^{2+} uniporter (MCU; De Stefani, Patron, & Rizzuto, 2015). From a functional point of view, MCU opening promotes the electrophoretic movement of Ca^{2+} ions into the mitochondrial matrix driven by the large gradient of membrane potential (-180 mV, negative inside). The MCU is activated by IMS Ca^{2+} , and kinetic studies of mitochondrial Ca^{2+} uptake as a function of the extra mitochondrial $[\text{Ca}^{2+}]$ display a sigmoid relationship, suggesting that mitochondrial Ca^{2+} uptake turns into a particularly efficient mechanism in the micromolar range (Csordas et al., 2013; Quesada et al., 2008). Electrophysiological recordings in whole-mitoplast configuration identified a highly selective Ca^{2+} current that is characterized by a I-V inwardly rectifying curve (Kirichok, Krapivinsky, & Clapham, 2004). Although this current was lately attributed to the protein MCU1 (Chaudhuri, Sancak, Mootha, & Clapham, 2013), alternative mitochondrial Ca^{2+} currents have been identified in heart cells (Michels et al., 2009).

Biochemical, genetic, functional, and structural studies provide evidences that the MCU complex is the most likely candidate to mediate mitochondrial Ca^{2+} uptake in various cell types (Sancak et al., 2013). The complex comprises a pore-forming unit and three accessory proteins or regulatory binding partners: (a) MCU1 forms the pore-forming subunits of the channel. The protein contains two transmembrane domains (TM1 and TM2) linked by a loop facing the IMS and flanked by coil-coil domains (Baughman et al., 2011; De Stefani, Raffaello, Teardo, Szabo, & Rizzuto, 2011). In 2018, three different studies including cryo-EM and X-ray crystallography approaches of fungi MCU and Zebra fish concluded MCU1 assembles into tetramers. Both transmembrane domains contribute to pore formation through the IMM, with TM1 located on the outside and TM2 facing the ion-conducting pore. The selectivity filter is located at the beginning of the second transmembrane domain (TM2). There, the aspartate and glutamate of the highly conserved DIME motif separated by one helical turn and oriented towards the central axis of the pore form two consecutive rings of acidic residues (Fan et al., 2018), a configuration that resembles the ion conduction model of the voltage-gated Ca^{2+} channels (Hess & Tsien, 1984). (b) MCU-b is a dominant-negative version of MCU1 and interferes with the ability of the channel to permeate Ca^{2+} (Raffaello et al., 2013). Thus, it has been hypothesized that different stoichiometry of MCU1/MCU-b would generate channels with different ability to transport Ca^{2+} in a cell type and tissue-specific manner. (c) MICU1 (Perocchi et al., 2010) and (d) MICU2 (Plovanich et al., 2013) constitute the Ca^{2+} -sensitive regulatory binding partners of the channel. MICU1 contributes to define the $[\text{Ca}^{2+}]$ threshold activation as well as Ca^{2+} cooperativity, which ensures respectively the proper closure of the channel at resting $[\text{Ca}^{2+}]_{\text{cyt}}$ and full activation of the channel at high $[\text{Ca}^{2+}]_{\text{cyt}}$ concentrations (Csordas et al., 2013; Patron et al., 2013). MICU2 also contributes to fix the Ca^{2+} activation threshold but does not stimulate uptake at higher $[\text{Ca}^{2+}]_{\text{cyt}}$. (e) Finally, quantitative mass spectroscopy in highly purified MCU samples identified EMRE, a resident protein of the complex essential for the activity of the channel (Sancak et al., 2013). In summary, although the MCU was initially described as a low affinity and high capacity mechanism of Ca^{2+} uptake, recent results have drawn a more complex picture from a functional point of view. The relative abundance of different MCU components in a particular cell type or physiopathological situation might define the specific functional characteristics of the channel: Ca^{2+} permeation across the pore, threshold activation, and Ca^{2+} cooperativity. Consistent with this accumulating evidence, it has been observed that both the relative abundance of MCU components (Markus et al., 2016) and MCU activity vary largely between cell types (Fieni, Lee, Jan, & Kirichok, 2012).

β -cell mitochondria import Ca^{2+} in response to the increase of cytosolic Ca^{2+} , such as observed after nutrient stimulation (Rutter et al., 1993; Wiederkehr et al., 2011; Kennedy et al., 1996). What are the characteristics of the β -cell mitochondrial Ca^{2+} uptake system? As in many other cell types β -cell $[\text{Ca}^{2+}]_{\text{mit}}$ levels in resting conditions are very close to the cytosolic Ca^{2+} levels, pointing a very tight regulation of the channel (Wiederkehr & Wollheim, 2008). However, permeabilized INS-1 cells display significant mitochondrial Ca^{2+} uptake when

perfused with buffers containing $[\text{Ca}^{2+}] \sim 150$ nM (Pitter, Maechler, Wollheim, & Spat, 2002), which is below the general threshold activation of the uniporter (Csordas et al., 2013) and indicates a slightly higher affinity configuration of the β -cell MCU. In addition, glucose stimulation generates cytosolic Ca^{2+} transients that rarely exceed 500 nM in terms of the bulk cytosolic Ca^{2+} (Rutter et al., 1993; Wiederkehr et al., 2011; Quesada et al., 2008). These data do not rule out the possibility of critically located mitochondria importing $[\text{Ca}^{2+}]_{\text{cyt}}$ from high Ca^{2+} microdomains generated close to the plasma membrane (Kennedy et al., 1996), whose presence has been measured (Hoppa et al., 2009; Quesada, Martin, & Soria, 2000). In fact, experiments in permeabilized β -cells expressing mitochondrial aequorin and perfused with a range of different Ca^{2+} buffers have shown that $[\text{Ca}^{2+}]_{\text{mit}}$ uptake is more efficient above 2 μM (Quesada et al., 2008). We conclude that although some authors certainly admit that β -cell mitochondria import significant amounts of Ca^{2+} in the nanomolar range, the mitochondrial uniporter of β -cells behaves as a low affinity and high capacity Ca^{2+} transport system, with properties similar to those reported in other tissues. High Ca^{2+} microdomains may not be fully required for $[\text{Ca}^{2+}]_{\text{mit}}$ uptake in pancreatic β -cells, but sub-plasma membrane is probably the main mitochondrial Ca^{2+} uptake sites during nutrient stimulation.

Interestingly, $[\text{Ca}^{2+}]_{\text{mit}}$ only reaches 600–800 nM (Wiederkehr & Wollheim, 2008; Kennedy et al., 1996; Wiederkehr et al., 2011) in glucose stimulated β -cells, mirroring the $[\text{Ca}^{2+}]_{\text{cyt}}$ events. Purinergic activation and potassium-induced depolarization generate cytosolic Ca^{2+} transients around 1 μM , with corresponding mitochondrial $[\text{Ca}^{2+}]_{\text{mit}}$ increases reaching close to 5 μM $[\text{Ca}^{2+}]_{\text{mit}}$ (Rutter et al., 1993). These results indicate that β -cell mitochondria, in average, hardly reach micromolar $[\text{Ca}^{2+}]_{\text{mit}}$ during physiological activation. This is in line with the fact that β -cell mitochondria marginally contribute to dissipate the increases in $[\text{Ca}^{2+}]_{\text{cyt}}$, a task that is mainly carried out by the sarcoendoplasmic and plasma membrane Ca^{2+} pumps (SERCA and PMCA; Chen, Koh, & Hille, 2003). This situation contrasts with the mitochondrial Ca^{2+} uptake characteristics of another type of neuroendocrine cell model, the chromaffin cells of the adrenal medulla. Chromaffin cell mitochondria take up significant amounts of $[\text{Ca}^{2+}]_{\text{cyt}}$ during cell depolarization, reaching up to 500 μM $[\text{Ca}^{2+}]_{\text{mit}}$ (Montero et al., 2000). During cell activation, mitochondria become the main cytosolic Ca^{2+} clearance system with significant functional consequences for the exocytosis of secretory granules (Garcia, Garcia-De-Diego, Gandia, Borges, & Garcia-Sancho, 2006).

The $[\text{Ca}^{2+}]_{\text{mit}}$ reached during glucose-induced cell activation is sufficient to stimulate matrix dehydrogenases. The $K_{0.5}$ of pyruvate dehydrogenase for Ca^{2+} activation is around 1 μM , and the $K_{0.5}$ of oxoglutarate dehydrogenase ranges from 0.2 to 2 μM depending on the ATP/ADP levels (Denton, 2009). The observed Ca^{2+} dependency provides evidence that mitochondrial Ca^{2+} levels just below the micromolar range are sufficient to accelerate mitochondrial metabolism during nutrient stimulation. These results outline a situation where mitochondrial Ca^{2+} uptake machinery in β -cell is optimized for nutrient-dependent mitochondrial metabolic activation with only a minor contribution to the shaping of cytosolic Ca^{2+} signals. Although,

as mentioned above, the stoichiometry of the different MCU subunits and their relative abundance is largely unknown in different tissues, it is nevertheless tempting to speculate that β -cell mitochondria are endowed with a particular configuration of the MCU that reduces the Ca^{2+} activation threshold.

The first genetic evidence for the physiological role of the MCU complex in the context of the pancreatic β -cell were recently obtained. In mouse pancreatic β -cells, MCU1 knockdown displayed strong reduction of the ability of mitochondria to import Ca^{2+} in response to depolarization-induced cytosolic Ca^{2+} transients. Consistent with this finding, with a stimulatory role of $[\text{Ca}^{2+}]_{\text{mit}}$ on mitochondrial metabolism, MCU1 knockdown cells were unable to increase the ATP levels to the same extent as wild-type cells (Tarasov et al., 2012). A similar set of experiments in INS-1 832/13 cells showed that either MCU1 or MICU1 knockdown diminished mitochondrial Ca^{2+} transients in response to cholinergic activation, glucose, or high potassium-induced depolarization. Invalidation of both genes reduced glucose-stimulated ATP levels and insulin secretion (Alam et al., 2012). Recently, the effects of MCU genetic down-regulation on stimulus–secretion coupling were confirmed in INS-1E cells. In this study, MCU1-deficient cells displayed diminished respiratory rates and lower levels of respiratory chain complexes. This link between the MCU and the expression of respiratory chain complexes has not been observed in other cell types and may be cell-specific. Interestingly, these cells failed to increase the mitochondrial pH gradient upon nutrient stimulation (Quan et al., 2015), a phenomenon that has been shown to be important for metabolic coupling (Wiederkehr et al., 2009). In summary, these studies confirm an important role for mitochondrial Ca^{2+} uptake to sustain stimulus–secretion coupling during nutrient activation of β -cells. Pharmacological activation of MCU is therefore a possible approach to improve β -cell function, thereby lowering glycaemia in Type 2 diabetic subjects. Surprisingly, despite the pleiotropic effects of MCU, the only MCU1 knockout mouse available does not show evidence of a metabolic disease phenotype (Pan et al., 2013).

Pharmacological inhibition of mitochondrial Ca^{2+} extrusion mechanisms might constitute another interesting alternative to enhance β -cell stimulus–secretion coupling. Inhibition of the **mitochondrial Na^{+} – Ca^{2+} exchanger** with CGP37157 increases mitochondrial metabolism and potentiates glucose-stimulated insulin secretion in INS-1E cells, isolated rat pancreatic islets, and in vivo (B. Lee et al., 2003).

2.3 | Permeability transition pore

The PTP is a large mitochondrial inner membrane channel, responsible for the so-called mitochondrial permeability transition (MPT). The MPT is a calcium-dependent, redox-dependent, and **cyclosporin A**-inhibited permeabilization of the IMM. Once open, the PTP will allow the exchange of solutes of molecular mass up to 1.5 kDa across the mitochondrial membranes (Zoratti & Szabo, 1995). The MPT plays an important role in the physiopathology of several tissues (Rasola & Bernardi, 2007), including pancreatic β -cells (Dufer, Krippeit-Drews,

Lembert, Idahl, & Drews, 2001; Fujimoto, Chen, Polonsky, & Dorn, 2010; Koshkin, Bikopoulos, Chan, & Wheeler, 2004; Lablanche et al., 2015). Although the molecular identity of the PTP is still uncertain (Baines & Gutierrez-Aguilar, 2018; Bernardi, 2018; Zoratti, Szabo, & De Marchi, 2005), the protein cyclophilin D has been demonstrated to be a regulator of the PTP (De Marchi, Basso, Szabo, & Zoratti, 2006). The generation of the cyclophilin D knockout mice contributed to elucidate its role and establish that cyclophilin D facilitates PTP opening and consequently MPT activation (De Marchi et al., 2006). The pharmacological PTP-inhibitor cyclosporin A prevented PTP activation in wild-type mice but not in cyclophilin D-knockout animals, demonstrating that cyclophilin D represents the target for PTP inhibition by cyclosporin A. Today, cyclophilin D knockout animals are likely to provide the best genetic model to study the MPT.

Although the effect of cyclosporin A on insulin secretion has been a matter of debate (Bugliani et al., 2009; Ebihara, Fukunaga, Matsumoto, Shichiri, & Miyamoto, 1996), several studies in pancreatic β -cells indicated the existence of MPT in this cell type (Barbu, Welsh, & Saldeen, 2002; Koshkin et al., 2004). They pointed its importance for β -cell secretory function (Dufer et al., 2001; Koshkin et al., 2004) and as a common effector of both apoptosis and necrosis (Barbu et al., 2002; Contreras et al., 2002). The characterization of MPT in semi-permeabilized cells from clonal pancreatic β -cell lines (MIN6 and INS-1) supported the existence of both, a calcium/phosphate-induced- and a thiol cross-linking-dependent and mitochondrial calcium-independent MPT (Koshkin et al., 2004). In that study (Koshkin et al., 2004) and consistently with previous observations (Dufer et al., 2001), inhibition of MPT opening with cyclosporin A was found to suppress glucose-induced insulin secretion, demonstrating a contribution of the MPT for physiological secretory function (Dufer et al., 2001; Koshkin et al., 2004). On the other hand, by preventing PTP opening, cyclosporin A decreased cell death caused by Pdx1 deficiency in mouse insulinoma MIN6 cells (Fujimoto et al., 2010). These results were confirmed in a genetic mouse model, after ablation of Ppif, the gene encoding cyclophilin D. Disruption of Ppif restored β -cell mass and decreased β -cell death. Moreover, genetic ablation of the Ppif gene normalized fasting glucose and glucose and insulin responses to an acute glucose challenge in adult mice previously maintained on a high-fat diet (Fujimoto et al., 2010). This study demonstrated that PTP is a critical regulator of pancreatic β -cell death. Lablanche and collaborators examined the involvement of PTP opening in INS-1 cell death induced by high levels of glucose or fructose. Cyclosporin A prevented PTP opening and protected from both glucose- and fructose-induced cell death (Lablanche et al., 2011). They proposed to target PTP, as a novel approach to preserve β -cell mass. Additional therapeutic opportunities for PTP inhibition have been highlighted in the context of islet transplantation (Lablanche et al., 2015). Lack of oxygen and metabolic substrates during islet isolation from donors and delayed vascularization are major challenges for the successful transplantation of islets and their ability to restore function. In vitro, energy substrate deprivation for 1 hr (but not the deprivation of oxygen for the same period of time) in INS-1 cells

caused oxidative stress, followed by PTP opening and β -cell death (Lablanche et al., 2015). Given that the opening of the PTP was inhibited by cyclosporin A, the authors suggested that pharmacological inhibition of the PTP during islet transplantation could improve islet cell survival and graft success.

In summary, PTP activation and inhibition appear to play dual roles in physiology and pathology of the pancreatic β -cell. While activation of PTP is required to promote insulin secretion during glucose stimulation (Dufer et al., 2001; Koshkin et al., 2004), PTP inhibition protects against glucotoxicity as well as hypoxia and lack of substrates during islet transplantation (Lablanche et al., 2011). Given the important role of the PTP in the pathophysiology of β -cells, the discovery of novel modulators of its function is a promising strategy for the protection of β -cells and the treatment of diabetes. Unfortunately, the PTP inhibitor cyclosporin A is not an ideal option because it is also a strong immunosuppressant. Several new PTP inhibitors that, unlike cyclosporin A, do not inhibit calcineurin are currently being developed (Leanza et al., 2019). The smart design of structure-based specific direct channel modulators is complicated by the fact that the molecular identity remains unknown. On the other hand, the electrophysiological identity of the PTP has been revealed and extensively characterized. These findings will hopefully lead to the molecular identification of the PTP and activators as well as inhibitors of the channel. Activators of β -cell PTP are expected to promote insulin secretion and, conversely, PTP inhibitors are promising agents for the protection of β -cells during islet transplantation or nutrient stress *in vivo*.

2.4 | Mitochondrial K^+ channels?

Mitochondrial K^+ channels are present on the IMM of different mammalian tissues (Szabo & Zoratti, 2014). In general, the electrophysiological characterization of those channels demonstrated that they are the mitochondrial counterpart of channels also present in other cellular membranes, especially the plasma membrane (Zoratti, De Marchi, Gulbins, & Szabo, 2009). Very few investigations have focused on the occurrence and properties of mitochondrial K^+ channels in β -cells. A K_{ATP} channel has been proposed to be present in various intracellular sites of β -cells, including mitochondria (Quesada & Soria, 2004), based on indirect evidence. The two subunits of the K_{ATP} channel, **K_{ir}6.2** (Suzuki, Fujikura, Inagaki, Seino, & Takata, 1997) and **SUR1** (Suzuki et al., 1999), have been shown to be endogenously expressed in most pancreatic islet cell types in mouse. In addition, the pharmacological effect of K_{ATP} channel modulators on mitochondrial function has been suggested to be related to mitochondrial K_{ATP} channel modulation (Smith, Proks, & Moorhouse, 1999), but functional activity was not demonstrated. Unfortunately, the limitation of this pharmacological approach is due to the lack of specificity, given that K_{ATP} channel modulators **tolbutamide** and **diazoxide** have been shown to be also efficient inhibitors of mitochondrial dehydrogenases in pancreatic β -cells (Lenzen & Panten, 1983; MacDonald, 1981). In addition, Varadi and collaborators failed to detect any immunoreactivity against either of the two classical β -cell K_{ATP} channel subunits (SUR1 and Kir6.2) on

mitochondrial membranes from mouse islets and MIN6 cells or any pharmacological evidence for a role of K_{ATP} channel on β -cell mitochondrial function (Varadi et al., 2006).

Even less is known about other mitochondrial K^+ channels in β -cells. Henquin and collaborators recently demonstrated that insulin secretion was increased in human islets by inhibiting K^+ channels other than the K_{ATP} channel (Henquin, Dufrane, Gmyr, Kerr-Conte, & Nenquin, 2017). Whether the target of **tetraethylammonium** (inhibitor of Kv or BK channels), ML-365 (inhibitor of **TASK-1** channels) and **TRAM-34** (inhibitor of the intermediate conductance Ca^{2+} -activated K^+ channel) was the plasma membrane or mitochondrial K^+ channel (or both) is not known.

In summary, the role of the several putative mitochondrial K^+ channels in pancreatic β -cell signal transduction remains to be studied. Very recently, the molecular identity of the mitochondrial K_{ATP} channel has been revealed (Paggio et al., 2019), opening new perspectives for pancreatic β -cell investigation and molecular intervention. For the other mitochondrial K^+ channels, in the absence of their molecular identity, a real possible approach to establish their existence and properties could be mitochondrial electrophysiology, which has already clarified the roles of other mitochondrial channels and could offer a solution also in the case of pancreatic β -cells.

3 | CHANNELS OF THE OMM

3.1 | VDAC

The VDAC is the most abundant protein present in the OMM (Kroemer & Reed, 2000). Three isoforms of this protein have been reported (VDAC1, VDAC2, and VDAC3). Pancreatic β -cells essentially express VDAC1 and VDAC2 (Ahmed, Muhammed, Kessler, & Salehi, 2010; E. Zhang et al., 2019). VDAC isoforms contains 19 β strands organized in a β -barrel structure with an N-terminal α -helix facing the interior of the pore. The presence of the VDAC in the OMM is responsible for the permeability of this membrane to solutes up to ~5,000 Da of molecular mass (Colombini, 1980; Zalman, Nikaido, & Kagawa, 1980). VDAC thus allows the transport of metabolites, including respiratory substrates, and inorganic ions between the IMS and the cytosol (Szabo & Zoratti, 2014). VDAC may, therefore, be a regulator of metabolic flux. In support of this hypothesis, permeabilized muscle fibres from VDAC1 and VDAC3 double KO mice exhibit reduced ADP-driven respiration, pointing to its importance for ADP transport (Anflous, Armstrong, & Craigen, 2001). Given that VDAC activity is modulated by cytosolic factors in multiple ways, it is tempting to speculate that VDAC regulation may also be relevant for β -cell metabolism-secretion coupling (Lemasters & Holmuhamedov, 2006). This possibility has not been tested experimentally to date.

In a pathological context, VDAC has been proposed to be part of the molecular machinery participating in mitochondrial-mediated apoptosis. VDAC certainly plays a role in the PTP opening, but the mechanisms involved are still quite controversial. VDAC1 and VDAC3 seem to have a less important role. When compared to control cells, the

single or double KO's do not display any difference in cytochrome-c release elicited by Bax overexpression, staurosporin, or TNF α stimulation (Baines, Kaiser, Sheiko, Craigen, & Molkenin, 2007). On the contrary, genetic and biochemical evidence support a role for VDAC2 in apoptosis. It has been demonstrated that VDAC2 exists in large complexes with Bak within the OMM and inhibits Bak activation and apoptosis (Lazarou et al., 2010). The functional interaction between the two proteins during apoptosis was elegantly demonstrated in lymphocytes. Deletion of VDAC2 in lymphocytes promotes cell death that can be rescued by concomitant genetic invalidation of Bak (Ren et al., 2009). A detailed description of these mechanisms is beyond the objective of this review. Several studies have explored the role of VDAC in β -cell apoptosis. By immunoprecipitation, it was shown that VDAC interacts with glucokinase in MIN6 cells and isolated islets. Exposure to high glucose under chronic conditions reduces the glucokinase–VDAC interaction and promotes Bax oligomerization, which promotes cytochrome c release and apoptosis (J. W. Lee et al., 2009).

The role of VDAC on β -cell dysfunction associated with Type 2 diabetes is just beginning to be elucidated. A proteomic study in INS-1E cells cultured under glucotoxic conditions (20-mM glucose culture) observed significant overexpression of VDAC1 and a reduction of VDAC2 (Ahmed et al., 2010), among other marked changes in the levels of several mitochondrial proteins. Recently, these findings were reproduced in islets from Type 2 diabetic patients (E. Zhang et al., 2019). According to their findings, VDAC1 is up-regulated under a glucose-toxicity-induced transcriptional programme that operates for years in pre-diabetes patients with suboptimal glycaemic indexes. The epigenetic modifier Ep300 in part mediates this programme. Overexpression of VDAC1 also results in the partial mis-targeting of VDAC1 to the plasma membrane. The mitochondrial pool of VDAC has been reported to be reduced in β -cells from Type 2 diabetic subjects (Thivolet, Vial, Cassel, Rieusset, & Madec, 2017). Plasma membrane-targeted VDAC mediates the export of cytosolic ATP, resulting in cellular ATP depletion. This loss of ATP has negative effects on stimulus–secretion coupling. Inhibition of VDAC targeted to the plasma membrane should therefore be able to rescue β -cell function. Indeed, treatment of db/db mice with the VDAC1 inhibitor VBIT-4 improved glucose tolerance and glucose stimulated insulin secretion in vivo. This evidence demonstrates that pharmacological inhibition of VDAC1 mis-targeted to the plasma membrane of β -cells may be a strategy to prevent Type 2 diabetes.

Beyond its function as an ion and metabolite channel, VDAC also contribute to physically tether ER and mitochondria at the ER–mitochondria contact sites. A number of different functions have been attributed to the ER–mitochondria contact sites, including lipid biosynthesis, ER to mitochondria Ca²⁺ transport, and response to cellular stress (Rieusset, 2017). Biochemical data suggest that at these sites, the molecular chaperone glucose-regulated protein 75 (Grp75) simultaneously interacts with VDAC and the inositol 3-phosphate receptor (IP₃R) on the ER membrane to form a protein complex that brings both organelles into close proximity (Szabadkai et al., 2006). Interestingly, liver ER–mitochondria contact sites are dynamically regulated

by nutrients and the cellular metabolic status (Sood et al., 2014). A recent study quantified the formation of VDAC–IP₃R complexes in human pancreatic islet from Type 2 diabetes and control donors as a readout of ER–mitochondria contact site formation. The study concluded the number VDAC–IP₃R complexes was significantly reduced in Type 2 diabetic donors compared to controls and hypothesizes that disruption of these complexes might contribute to the physiopathology of Type 2 diabetes in pancreatic β -cells (Thivolet et al., 2017).

4 | OTHER PUTATIVE MITOCHONDRIAL CHANNELS

4.1 | Bcl2 family proteins

There are two types of Bcl-2 family proteins with opposing effects on apoptosis progression: the anti-apoptotic (including Bcl-2 and Bcl-xL, Bcl-W, Mcl-1, Bfl1, and Bcl-B) and the pro-apoptotic (including Bax, Bak, Bok, and Bid; Hardwick & Soane, 2013). Some of these proteins have been reported to be able to form channels when they oligomerize. Electrophysiological recordings have demonstrated the formation of large pores in the OMM during apoptosis. These channels have specific electrophysiological properties and are known as Mitochondrial Apoptotic Channels. From a molecular point of view, these channels are formed by an oligomerization of Bax (Dejean et al., 2005). However, channel formation of Bcl-2 family proteins, including Bcl-xL, Bcl-2, Bax, tBid, and Bad (Bad, i.e., Schendel et al., 1997), has been reported mainly in artificial systems, especially in lipid bilayers or in isolated mitoplast and not under physiological conditions. Therefore, it is not clear if the function of these proteins is mediated by channel formation, and alternative hypotheses have been formulated. Bcl-2 family proteins are regulators of apoptosis in β -cells in the context of Type 2 diabetes (Tomita, 2016). Several different strategies targeting this family of proteins have been proposed to protect against β -cell apoptosis in diabetic patients in order to preserve β -cell mass (Ljubicic et al., 2015).

Bcl2 family proteins may have additional functions not related to the regulation of apoptosis in healthy β -cells. Evidence obtained in the Bad knockout mice indicates that Bad is important for the metabolic activation of the β -cell by glucose (Danial et al., 2008). Conversely and using a similar type of approach, Luciani and collaborators demonstrated that chemical and genetic loss of function of the anti-apoptotic Bcl-2 and Bcl-x(L) significantly promotes glucose-dependent metabolic activation and Ca²⁺ signalling in primary pancreatic β -cells (Luciani et al., 2013). Whether these functions are related or not with in vivo channel activity of these proteins was not addressed.

4.2 | Chloride channels (CLICs)

The intracellular chloride channel family (CLICs) comprises seven members that localize to different organelle membranes. These

chloride channels are essentially implicated in ion homeostasis. Interestingly CLIC4 (Fernandez-Salas, Sagar, Cheng, Yuspa, & Weinberg, 1999) and recently CLIC5 (Ponnalagu et al., 2016) have been demonstrated to localize partially to mitochondria. Mitochondrial-targeted CLIC4 may play a role in the regulation of the mitochondrial membrane potential. Overexpression of mtCLIC by transient transfection reduces the mitochondrial membrane potential but also releases cytochrome c into the cytoplasm. The latter process activates caspases and induces apoptosis (Fernandez-Salas et al., 2002). It has been reported that a variety of stressors induces translocation of the cytoplasmic pool of CLIC4 to the nucleus. Interestingly, only the overexpression of an engineered nuclear-targeted CLIC4 variant induced apoptosis in Apaf deficient mouse fibroblasts or in Bcl-2-overexpressing keratinocytes (Suh et al., 2004). These results suggest the role of CLIC4 in apoptosis is linked to the nuclear rather than the mitochondrial pool of the protein. In contrast with these pro-apoptotic functions, three independent studies found that suppression or inactivation of CLIC4 enhanced cell death induced by a variety of stressors in transformed cells (Xue et al., 2016).

The only CLIC member studied in pancreatic β -cells to date is CLIC4 (Patel, Ythier, Brozzi, Eizirik, & Thorens, 2015). CLIC4 seems to sensitize pancreatic β -cells to apoptotic stimuli. Pancreatic β -cells and mouse islets incubated with pro-inflammatory cytokines increase the expression of CLIC4. Silencing or inactivation of CLIC4 in both models partially prevented cytokine-induced apoptosis. This phenomenon was associated with an increase in the expression of the pro-survival proteins Bcl2 and p-BAD, which may provide a mechanistic explanation (Patel et al., 2015). It is worth mentioning that among interaction partners of CLIC4 identified by MS, none was mitochondrial. It is therefore uncertain whether CLIC4 localizes to mitochondria in pancreatic β -cells (Patel et al., 2015).

5 | PHARMACOLOGICAL TARGETING OF MITOCHONDRIAL ION CHANNELS IN TYPE 2 DIABETES: EVIDENCE FROM CLINICAL STUDIES

Although the evidence described above indicates that certain β -cell mitochondrial ion channels are interesting targets to prevent the onset and progression of Type 2 diabetes (MCU or VDAC), currently, there are no marketed drugs for the treatment of Type 2 diabetes, targeting these particular biological entities. In fact, the mitochondrial ion channels are largely under-represented in the pharmacopeia, a situation that contrasts with the plasma membrane ion channels, that constitute the most prominent group of molecular entities targeted by marketed drugs (Santos et al., 2017). This is partially explained by the fact that the molecular and functional characterization of mitochondrial ion channels is still in the initial stages compared with their plasma membrane counterparts, the multiple subcellular distribution of some of these channels, and the difficulty of establishing large targeted screening campaigns. However, the last 30 years have produced abundant information on the

pharmacological regulation of some of these channels (Leanza et al., 2019). Here, we explore whether some of these molecules have been clinically tested. In this section, we review clinical studies exploring the potential use of known mitochondrial ion channels modulators in the context of Type 2 diabetes (summarized in Table 1). Whether the metabolic effects of these molecules on Type 2 diabetic subjects is mediated by mitochondrial ion channels in β -cell is not demonstrated. Nevertheless, these studies provide evidence for the use of mitochondrial ion channel modulators in the management of Type 2 diabetes.

5.1 | VDAC modulators: Cannabidiol, catechin, and fluoxetine

5.1.1 | Cannabidiol

Cannabidiol is a VDAC inhibitor influencing lipid and glucose metabolism in preclinical models (Rimmerman et al., 2013). Unlike its synthetic analogues, **Abn-cannabidiol** and **O-1602**, which are potent inducers of insulin secretion both in vivo and in vitro (McKillop, Moran, Abdel-Wahab, & Flatt, 2013), the protective effects of cannabidiol seem to engage alternative mechanisms. For instance, cannabidiol treatment of Type 1 diabetic mice reduced the inflammatory changes associated with the condition and prevented the onset of the disease (Weiss et al., 2008). In a mouse model of Type 2 diabetes, cannabidiol treatment protected islet integrity, lowered blood glucose levels, and increased circulating insulin concentration (Ehud et al., 2012). The potential therapeutic effect of cannabidiol for the treatment of Type 2 diabetes in humans was investigated by Jadoon and collaborators (NCT01217112; Jadoon et al., 2016). Cannabidiol, at the dose evaluated in this pilot study, did not modify any glycaemic or lipid parameters in patients with Type 2 diabetes, although it seemed to decrease the concentration of resistin and increase the levels of the incretin hormone **GIP** compared to baseline concentrations. According to the authors, the lack of therapeutic effect showed by cannabidiol could be due to the dose evaluated in this study ($200 \text{ mg}\cdot\text{day}^{-1}$), which is relatively low compared to the doses tested for other human pathologies.

5.1.2 | Catechin

Catechin was recently shown to bind VDAC1 through Thr207 at the N-terminal region of the protein (Li et al., 2017). However, the direct effect of catechin on channel conductance was not assessed. Nagao and collaborators evaluated the effects of a 12-week continuous intake of a catechin-rich beverage (green tea containing 582.8 mg of catechins) on body fat and glucose metabolism in non-insulin-treated Type 2 diabetic patients (Nagao et al., 2009). All participants in the study were Japanese and close to 60 years of age. At Week 12, they observed a significant decrease in waist circumference in the catechin group compared to the control group. In addition, **adiponectin** was

TABLE 1 Summary of clinical studies exploring the potential benefits of well-known mitochondrial ion channels targeted drugs on glucose haemostasis of Type 2 diabetes patients

Compound	Published articles	NCT number	Outcome
VDAC modulators			
Cannabidiol	Jadoon et al., 2016	NCT01217112	Absence of effect
Catechin	Curtis et al., 2012, 2013	NCT00677599	Improvement in markers of insulin sensitivity
	Mellor 2013 (PhD thesis)	NCT01617603	Absence of effect
	Nagao et al., 2009	NA	Increased insulin and reduced HbA1c levels
	Hsu et al., 2011	NA	Within-group comparison show reduced HbA1c
Fluoxetine	Breum et al., 1995	NA	Decreased fasting glucose and C-peptide. Trend to reduce HbA1c
	Connolly et al., 1995	NA	Reduced HbA1c
	Daubresse et al., 1996	NA	Decreased fasting blood glucose and HbA1c levels
	Maheux et al., 1997	NA	Improved insulin sensitivity
PTP modulators			
Berberine	Yin et al., 2008	NCT00425009	Reduced HbA1c, fasting and postprandial blood glucose
	Zhang et al., 2008	NCT00462046	Reduced HbA1c, fasting and postprandial blood glucose
Cyclosporin A	Lorho et al., 2011	NCT00171717	Diabetogenic but prevent new onset of diabetes compared to tacrolimus-treated transplanted patients
	Rathi et al., 2015	NCT00171743	Diabetogenic but prevent new onset of diabetes compared to tacrolimus-treated transplanted patients
Ebselen	Beckman et al., 2016	NCT00762671	Absence of effect
Both VDAC and PTP modulators			
Curcumin	Chuengsamarn et al., 2012	NCT01052025	Reduced HbA1c and fasting blood glucose levels. Improved oral glucose tolerance test and insulin sensitivity
	Chuengsamarn et al., 2014	NCT01052597	–
	Wickenberg et al., 2010	NCT01029327	Increase in postprandial blood insulin levels without affecting glucose
	Na et al., 2013	NA	Reduced HbA1c and fasting blood glucose levels and improved insulin sensitivity

Note. Data obtained from Clinicaltrials.gov and PubMed. Only trials considered relevant and containing published data were included. NA applies for clinical studies not registered in clinicaltrials.gov.

significantly increased while free fatty acids, total ketone bodies, and total cholesterol levels decreased in the catechin group, indicating all together a reduced risk for the development of metabolic syndrome and diabetes. Similarly, the insulin levels at Week 12 were significantly higher in the catechin group compared to the control group, although there were not apparent changes in glucose and HbA1c. Interestingly, the difference in insulin levels between both groups at Week 12 was more evident in patients treated with insulinotropic agents, who in addition showed a significant decrease in HbA1c levels. These results suggest a potential therapeutic use of a catechin-rich beverage for obesity prevention, recovery of insulin-secretory ability and, in a way, to maintain low HbA1c levels in patients with Type 2 diabetes who do not yet require insulin therapy.

Supplementation of obese Type 2 diabetic patients with decaffeinated green tea extract corresponding to a daily dose of

856 mg of **epigallocatechin gallate** during 16 weeks did not lead to any statistically significant differences in the metabolic parameters measured (including fasting glucose, HbA1c, and fasting insulin) when compared to placebo (Hsu et al., 2011). Interestingly, a within-group comparison revealed a significant reduction in HbA1c, waist circumference, HOMA-IR (homeostasis model assessment for insulin resistance) index, and insulin after 16 weeks of treatment with the green tea extract compared to baseline. Again, within-group comparison also revealed significant increases in **ghrelin** levels in both groups, treatment and placebo. The patients in this study were exclusively Chinese, 20–65 years old.

Another clinical study (NCT00677599) assessed the effects of 1-year regular ingestion of flavonoid-enriched chocolate containing epicatechin and isoflavones on cardiovascular disease risk (Curtis et al., 2012) and cardiovascular function (Curtis et al., 2013) in

postmenopausal women with Type 2 diabetes on established statin and hypoglycaemic therapy. The daily dose of flavonoid-enriched chocolate contained 90 mg of epicatechin and 100 mg of isoflavones (Curtis et al., 2012; Curtis et al., 2013). The continuous intake of a combination of epicatechin and isoflavones for 1 year translated into a significant improvement in markers of insulin sensitivity. The dietary intervention also improved the lipoprotein status and decreased the estimated coronary heart disease 10-year risk in the flavonoid group compared to the placebo group (Curtis et al., 2012). However, this intervention did not generally improve the cardiovascular disease of postmenopausal Type 2 diabetic women versus placebo, although clinically relevant improvements in arterial stiffness were observed (Curtis et al., 2013). Overall, these findings indicate that flavonoids may contribute to the existing therapies for the management of the CVD risk in patients with Type 2 diabetes, although other studies are needed to evaluate the effects of both types of flavonoids independently and in other population of patients with Type 2 diabetes than postmenopausal women (Curtis et al., 2012; Curtis et al., 2013).

Cocoa and chocolate are among the most concentrated sources of the two flavonoids, catechin, and epicatechin (Gottumukkala, Nadimpalli, Sukala, & Subbaraju, 2014). A clinical trial sponsored by the food industry analysed the effects of polyphenol-enriched milk chocolate in patients with Type 2 diabetes, compared with dark chocolate rich in polyphenols and low polyphenol chocolate (NCT01617603). Participants ingested 20 mg of chocolate per day for 12 weeks, receiving thus 19.2 mg of epicatechin in the polyphenol-enriched milk chocolate, 19 mg in the dark chocolate (active comparator), and 2.7 mg in the low polyphenol chocolate (placebo) per day. At the doses used, only non-significant differences were observed between treatment groups in terms of insulin resistance, insulin, glucose, HbA1c, cholesterol, and triglycerides levels. The dose of epicatechin evaluated was lower than in previous studies, which may explain the lack of clear effect of flavonol-rich chocolate (both milk and dark formulations). However, the results showed a trend towards a benefit of milk chocolate enriched in flavonols for Type 2 diabetic patients without causing any harm.

5.1.3 | Fluoxetine

Fluoxetine is the active substance of Prozac, which is widely used to treat depression. In addition to inhibiting 5-HT reuptake in the synaptic cleft, it also inhibits VDAC (Nahon, Israelson, Abu-Hamad, & Varda, 2005). Although *in vitro* evidence indicates that fluoxetine inhibits glucose-stimulated insulin secretion by impairing mitochondrial energy metabolism (Elmorsy, Al-Ghafari, Almutairi, Aggour, & Carter, 2017), this molecule has also been explored as an agent able to control glucose levels in Type 2 diabetes patients. Daubresse and collaborators analysed the effect of 8-week treatment with fluoxetine (60 mg·day⁻¹) in obese Type 2 diabetic subjects (Daubresse et al., 1996). In this short-term study, patients treated with fluoxetine showed a significant weight reduction, better glycaemic control

(significant decrease in fasting blood glucose and a decrease in HbA1c levels), and lower serum triglyceride levels than those receiving placebo. These differences were noted already after 3 weeks of treatment. In a similar clinical study also in obese Type 2 diabetic patients, administration of fluoxetine (60 mg·day⁻¹) for 4 weeks did not reduce weight. However, compared to the placebo group, the fluoxetine-treated patients displayed improved insulin sensitivity, which was independent of weight loss (Maheux, Ducros, Bourque, Garon, & Chiasson, 1997). Fluoxetine also seemed to improve insulin sensitivity in obese individuals with Type 2 diabetes who were treated for a longer period of time (12 months; Breum, Bjerre, Bak, Jacobsen, & Astrup, 1995). In this study, patients under fluoxetine therapy showed an improvement of glycaemic control, with a significant decrease in fasting glucose and C-peptide compared to those in the placebo group. Moreover, a trend towards a larger reduction in insulin and HbA1c levels and a better glucose tolerance was also observed in the fluoxetine group (Breum et al., 1995). Finally, obese elderly patients receiving fluoxetine 60 mg·day⁻¹ for 6 months showed a significant weight loss together with better glycaemic control, which was measured as a decrease in HbA1c but without changes in fasting blood glucose (Connolly, Gallagher, & Kesson, 1995).

5.2 | PTP modulators: Berberine, cyclosporin A, and ebselen

5.2.1 | Berberine

Berberine has been shown to be an interesting chemotherapeutic agent inducing cyclophilin D-dependent PTP opening in two prostate cancer cell lines (L. Y. Zhang, Wu, Gao, & Guo, 2014). Interestingly, the therapeutic effects of berberine on diabetic patients have been investigated in two pilot studies of short duration (3 months) and including only Chinese subjects. Yin and collaborators (NCT00425009) studied the efficacy of berberine, alone or in combination with other hypoglycaemic agents, for the treatment of Type 2 diabetes in newly diagnosed and poorly controlled patients (Yin, Xing, & Ye, 2008). Zhang and collaborators (NCT00462046) tested berberine as a potential treatment for patients with newly diagnosed Type 2 diabetes and dyslipidemia (Y. Zhang et al., 2008). In both studies, newly diagnosed Type 2 diabetic patients treated with berberine showed significant decreases in HbA1c, fasting blood glucose, and postprandial blood glucose levels. In combination with other hypoglycaemic agents, berberine seemed to improve the glycaemic and lipidic parameters and insulin sensitivity (Yin et al., 2008). Zhang and collaborators also observed a significant decrease in BMI. In both studies, berberine produced non-serious gastrointestinal adverse events, and some patients required a dose reduction. Overall, the results from these studies seem to indicate that berberine has the potential to be an effective and safe treatment for Type 2 diabetes. Larger and longer studies, also in other ethnic groups, are needed to confirm these findings.

5.2.2 | Cyclosporin A

Cyclosporin A and **tacrolimus** are inhibitors of the PTP. Both agents also inhibit the Ca^{2+} -dependent phosphatase calcineurin and have diabetogenic activity. The risk of developing a new onset of diabetes after a (liver) transplant is (1.9-fold) higher with tacrolimus than with cyclosporin A (Lorho et al., 2011). Thus, most of the clinical studies conducted involving cyclosporin A and diabetes have focused on the regression of new onset of Type 1 diabetes in transplant patients after switching from tacrolimus to cyclosporin A. In two pilot studies conducted in liver transplant patients (NCT00171717; Lorho et al., 2011) and renal transplant patients (NCT00171743; Rathi et al., 2015) who developed a new onset of Type 1 diabetes after the transplant, conversion from tacrolimus to cyclosporin A led to a significant improvement of glucose control. In addition, even if the differences were not significant, in both studies, the cyclosporin A group showed a higher number of patients with resolution of the new onset of diabetes.

5.2.3 | Ebselen

The effect of ebselen in patients with Type 1 or Type 2 diabetes was investigated by Beckman and collaborators (NCT00762671). Their results revealed that treatment with ebselen did not improve the oxidative stress profile or the vascular function in diabetic patients (Beckman, Goldfine, Leopold, & Creager, 2016).

5.2.4 | Both VDAC and PTP modulators: Curcumin

Curcumin has been shown both, inhibit VDAC (Tewari et al., 2015) and induce PTP (Morin, Barthelemy, Zini, Labidalle, & Tillement, 2001). This molecule is the active component of the Asian plant *Curcuma longa* (turmeric) and constitutes 2% to 8% of the spice. In healthy subjects, treatment with 6 g of *C. longa* preparation produced an increase in postprandial serum insulin concentration without affecting plasma glucose, indicating that *C. longa* may have an effect on insulin secretion. Thus, the increased insulin response observed after ingestion of *C. longa* was probably due to the stimulation of β -cell function by curcumin (Wickenberg, Ingemansson, & Hlebowicz, 2010; NCT01029327). Curcumin also contributed to prevention of Type 2 diabetes in a study conducted in pre-diabetic individuals (Chuengsamarn, Rattanamongkolgul, Luechapudiporn, Phisalaphong, & Jirawatnotai, 2012; NCT01052025). After 9 months of treatment, the patients on curcumin therapy showed a significant reduction in HbA1c, fasting plasma glucose, and improved oral glucose tolerance test compared to placebo. Related to β -cell functions, they also showed a significant increase of HOMA- β , significantly lower blood levels of C-peptide and, although not significant, proinsulin/insulin ratio tended to be reduced in the curcumin-treated group. These results suggest an overall improvement of β -cell performance. Interestingly, also insulin sensitivity significantly

improved in the curcumin group at the end of the treatment. Moreover, 16.4% of the patients in the placebo group developed Type 2 diabetes after 9 months of treatment, whereas none of the patients in the curcumin-treated group developed the disease.

In another study performed by Chuengsamarn and collaborators (Chuengsamarn, Rattanamongkolgul, Phonrat, Tungtrongchitr, & Jirawatnotai, 2014; NCT01052597), a 6-month treatment with curcumin in a Type 2 diabetic cohort helped to improve relevant metabolic profiles in the curcumin-treated group and lower the atherogenic risk. In a similar way, 3-month supplementation with curcuminoids produced a significant decrease in HbA1c, fasting blood glucose, and insulin resistance in overweight/obese Type 2 diabetic patients (Na et al., 2013). Thus, again, curcuminoids seem to have a glucose-lowering effect in Type 2 diabetes patients potentially mediated by an improvement of β -cell performance.

6 | CONCLUSION

The OMM and IMM of β -cell mitochondria harbour a variety of ion channels with important roles for stimulus–secretion coupling and cell viability. The roles of MCU, PTP, and VDAC have been partly elucidated, including their effects on the physio-pathology of pancreatic β -cells. In contrast, the role of mitochondrial potassium channels, chloride channels (CLICs) and UCPs still remain to be clarified. Mitochondrial ion channels in pancreatic β -cells are thus interesting targets for the treatment of Type 2 diabetes. Some marketed drugs and natural bioactive compounds able to modulate the activity of mitochondrial ion channels, are already used in clinical trials aiming to prevent the onset of the disease or progression. However, the observed effects may be due to effects on both β -cells and insulin target tissues. The PTP and VDAC are the first targetable mitochondrial channels in β -cells, but other ion transporters are expected to influence β -cell health and may thereby improve glucose homeostasis. Stimulation of the MCU for example may improve β -cell energy metabolism during nutrient stimulation. Such a mechanism may explain how MCU activators could have a beneficial effect on β -cell stimulus–secretion coupling leading to improved glucose homeostasis. Whether other mitochondrial ion channels play a role on insulin secretion and glucose homeostasis is still an attractive possibility and should be further investigated.

6.1 | Nomenclature of target and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander, Fabbro et al., 2019; Alexander, Kelly, et al., 2019a, 2019b; Alexander, Mathie, et al., 2019).

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CONFLICT OF INTEREST

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