Ion Channels in Endocrine Pancreatic Cells and their Role in Diabetes

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Maintaining glucose homeostasis is an important factor in mammalian physiology, and is controlled to a large extent by the pancreas. Specialized beta cells residing in the Islets of Langerhans sense the blood glucose levels and secrete insulin, which signals these levels to the rest of the body. Ion channels play a major role in many aspects of this cellular process and serve as targets for pharmacological interventions that treat diseases in which glucose homeostasis is impaired. In type-2 diabetes, the tight link between glucose sensing and insulin secretion is impaired due to mutations (as well as other genetic and acquired changes) in a specific K+ channel and most medications for this condition target this channel. This short review describes the mechanisms underlying the physiology and pathophysiology of insulin secretion with emphasis on the role ion channels play in controlling and modulating it.

The Pancreas

The main function of the pancreas, a gland attached to the intestine, is to control blood glucose levels. However, most of the pancreas is comprised of exocrine tissue, which secretes enzymes into the intestine, while only about 1% of the tissue consists of endocrine cells, which are clustered in the Islets of Langerhans.

Most of the endocrine cells in the islet are called beta cells. These respond to an increase in the plasma glucose level by secreting insulin into the blood circulation, which in turn allows cells (mainly in muscle and liver) to absorb glucose. (For more information see the website mentioned in Ref. 1)

Other cell types in the pancreas further control the plasma glucose levels. Alpha cells secrete glucagon, which stimulates mitochondrial respiration in target cells, when the glucose level is low. Delta cells secrete somatostatin onto the islet, which inhibits both insulin and glucagon secretion. Lambda cells secrete GLP-1, which causes an increase in glucagon and insulin secretion. All these cell types contribute to synchronization in the “whole islet” response.

Cells in the Islet of Langerhans are Excitable

Like neurons, pancreatic endocrine cells respond to their relevant stimulation with plasma membrane depolarization (caused by changes in the activity of ion channels) and action potential firing, which leads to vesicle exocytosis (controlled by elevation of Ca2+ concentration, mediated by ion channels) and hormone secretion.

Thus, glucose stimulation leads to depolarization, which is often oscillatory and is accompanied by oscillations in Ca2+ and NADH concentrations and in mitochondrial membrane potential, often leading to oscillatory insulin secretion. Ion channels play a major role in a network of cellular and molecular feedback mechanisms that produce these dynamics.

Resting Potential and Initiation of Hormone Secretion

The mechanism that links glucose sensing to hormone secretion has been extensively studied...
in beta cells and involves the activity of several ion channels at key points in the cascade (for detailed reviews see Refs. 2, 8-9).

K+ channels that are sensitive to ATP are plasma membrane protein complexes composed of four Kir6.2 (KCNJ11) pore-forming subunits surrounded by four SUR1 (sulphanylurea receptor, of the ABC transporter protein family) auxiliary subunits. These protein complexes sense the amount of glucose entering a beta cell since the activity of KATP channels depends on the amount of ATP in the cytoplasm, which in turn depends on the amount of glucose absorbed by the beta cell. The activity of KATP channels is negatively correlated to the amount of ATP, i.e. increases in ATP levels tend to reduce the activity of the channel.3

KATP channels are the main channels that are open during resting conditions and therefore their activity sets the resting membrane potential. Closure of KATP channels by increased ATP concentration leads to membrane depolarization, which causes opening of voltage dependent Ca2+ (CaV) channels, leading to Ca2+ influx.4 The exact molecular make-up of CaV channels in beta cells probably differs between species and is still somewhat controversial.10 However, the main CaV channels that control insulin secretion are L-type channels of the CaV1 subfamily (CaV1.2 and/or CaV1.3).11-13 In addition, using a CaV1.3 knockout mouse it was demonstrated that this channel is essential for the normal development of the pancreas.14 Other CaV channels, members of the CaV2 (P/Q, N and R types) channel subfamily have been detected in these cells and in several cases their functional contribution was demonstrated.15-17 Members of the CaV3 (T-type) channels were also detected in some beta cell preparations.10

The mechanism of coupling between depolarization and secretion is very similar in beta cells and in neuronal synaptic transmission. Further emphasis on the major role played by CaV1.3 channels in insulin secretion is given by demonstrations of physical and functional interaction of CaV1 channels with synaptic proteins (such as syntaxins and SNAP-25) that control vesicle exocytosis.18-20

The above-mentioned channels and pathways comprise the principle route for transduction of the blood glucose level into blood insulin signal (Fig. 1). However, many more channels are involved in fine-tuning and regulation of this
signal transduction pathway and these are briefly described below.

Voltage dependent $K^+$ (Kv) channels also sense the depolarization initiated by KATP closure and their delayed activation leads to repolarization and termination of exocytosis. In beta cells, $K_{v2.1}$ channels were demonstrated to be the main Kv channel that mediates this termination,21-26 which in part is based on their interactions with synaptic proteins.27,28 Other $K_v$ channels (see below) are expressed in beta and other endocrine cells but their exact functional role is still not fully resolved.23,26

$Ca^{2+}$ activated $K^+$ channels open in response to intracellular $[Ca^{2+}]_i$ elevation and in turn cause repolarization of the membrane potential, often leading to attenuation of the $[Ca^{2+}]_i$ signal. Via such a feedback loop, $K_{Ca2}$ and/or $K_{Ca3}$ channels (of the small/intermediate conductance protein family) contribute to oscillations in membrane potential and $[Ca^{2+}]_i$.29,30 The presence of $K_{Ca1.1}$ (large conductance, BK, Slo) channels was confirmed but they did not contribute to glucose induced electrical activity.31

Several Kir3 (GIRK or G protein activated inward rectifier $K^+$ channels) which are structurally similar to the Kir6.2 subunit of KATP, were detected in islet cells. Kir3 (mainly Kir3.4 but also Kir3.2, GIRK4 and GIRK2) channels were found in alpha as well as beta cells. These G-protein activated $K^+$ channels contribute to hyper and/or repolarization of the membrane potential, once they are opened. Their exact role is not completely resolved, but they probably contribute to reduction in

**Fig. 2: Effects of Ca$_v$ Channel Modulators on Ca$_v$ Currents and [Ca$^{2+}$], in RIN Cells**

Left: Traces of the effects of (from left to right) 5 µM (±)-Bay K 8644 (#B-350), 2 µM TaiCatoxin (#T-800) and 1 µM ω-Conotoxin GVIA (#C-300) on whole cell Ca$_v$ currents. In all cases, the black trace represents control currents and the colored trace represents the Ca$_v$ current during perfusion of the indicated drug. In all experiments, membrane potential was held at -50 mV and a test pulse of 40 ms to 0 mV was delivered every 10 sec.

**Right: Percentage change in current amplitude caused by these three Ca$_v$ modulators (n=10, 3 and 2 for 5 µM (±)-Bay K 8644, 2 µM TaiCatoxin and 1 µM ω-Conotoxin GVIA, respectively).**

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**Effects of (±)-Bay K 8644, TaiCatoxin, ω-Conotoxin GVIA on Ca$_v$ Currents in RIN Cells**

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**FS-2 Inhibits High K$^+$ or Glucose Induced L-Type Ca$^{2+}$ Influx in RIN Beta Cells**

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**(-)-Bay K 8644 Promotes L-Type Ca$^{2+}$ Influx in RIN Beta Cells**

Ca$^{2+}$ traces from Fura-2 AM loaded RIN Beta cells; stimulated with 35 mM K$^+$ (left) or 20 mM glucose (right) in presence or absence of 1 or 2 µM FS-2 (#F-700). Time of stimulation represented by the arrow.

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**Calcicludine Inhibits Glucose Induced L-Type Ca$^{2+}$ Influx in RIN Beta Cells**

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**Immunohistochemical staining of Kir3.4 in rat pancreas was visualized using Anti-Kir3.4 antibody (#APC-027) (1:50).**

Paraffin embedded sections of rat pancreas showing both endocrine (Islets of Langerhans) and exocrine areas. Strong and highly specific staining is evident only in endocrine cells (arrow). Universal Immuno-alkaline-phosphatase Polymer followed by New Fuchsin Substrate (Histofine, Nichirei corp) was used for the color reaction. Counterstain is Hematoxilin.
insulin release, in response to hormones and transmitters such as Ach, epinephrine and somatostatin.13,35

In addition, a few $K_{v}$ channels have been detected in the whole pancreas at the RNA level (PCR). $K_{v}5.1$ (TASK-2) was found to be expressed in the islet as well as exocrine tissue, and $K_{v}10.1$ (TREK-2) is expressed in the insulinoma cell line MIN6.34

TRP channels are sensitive to various stimuli and their activation modulates both [Ca$^{2+}$] and membrane potential, therefore contributing to regulation of secretion. The TRPM5 channel, which is permeable to monovalent cations and gated by internal Ca$^{2+}$ is expressed in the pancreas, where it might contribute to membrane potential oscillations.37 Recently, the involvement of the Ca$^{2+}$ permeable TRPM2 in insulin secretion was reported. It involves ADP ribose activation of this channel as well as temperature dependence.38 The activation by ADP ribose might be related to oscillations in NADH concentration that have been reported to occur in beta cells in response to glucose activation. In addition, two splice variants of TRPC4 which were cloned from mouse insulinoma cell lines have been suggested to act as heteromeric (with other TRPC) Ca$^{2+}$ channels.39,40

Intracellular RyR and/or IP, R channels on the ER membrane contribute to shaping Ca$^{2+}$ signals and therefore, insulin secretion.41,42 Another proposed mechanism involves insulin secreted from beta cells, which reduces the alpha cell $K_{ATP}$ channels' sensitivity to ATP inhibition. This means that glucose in now less effective in inducing glucagon secretion from alpha cells.43

Some evidence suggests that islet cells may under certain conditions (low glucose for example) respond to ATP (which may have been co-released with insulin) via P2X channels.44 Such a mechanism would further increase secretion, as these ATP receptors respond to ATP binding by opening a Ca$^{2+}$ permeable pore. However, expression of P2 receptors in the pancreas is developmentally regulated, and for example, P2X7 (in alpha cells) is expressed only in adults and might be related to aging.19 This might be in correlation to the suggested role for this channel in supporting apoptosis.

**Repertoire of Ion Channels in Different Types of Endocrine Cells**

Alpha cells respond to glucose by decreasing glucagon secretion. This might be achieved by glucose acting on beta cells to release GABA, which in turn activates GABA(A)R on alpha cells and causes inhibition of glucagon secretion. However, once the glucose level is low, GABA is not released from beta cells and alpha cells go on secreting glucagon.44 Another proposed mechanism involves insulin secreted from beta cells, which reduces the alpha cell $K_{ATP}$ channels' sensitivity to ATP inhibition. This means that glucose in now less effective in inducing glucagon secretion from alpha cells.45

In comprehensive studies that have addressed this issue, alpha cells are reported to express the following channels: $K_{v}3.1$, the silent subunits $K_{v}6.1$ and possibly $K_{v}9.2$.46 At the level of a single cell RT-PCR and electrophysiology experiments have suggested the following Ca$^{2+}$ and Na$^{+}$ channels as mediators of voltage dependent inward currents in alpha cells: Ca$_{v}1.2$ and Ca$_{v}1.3$ (L-type), Ca$_{v}2.1$ (P/Q-type), Ca$_{v}2.3$ (R-type) and Na$_{v}1.7$ (TXT-sensitive).17

Beta cells respond to increase in glucose concentration by insulin secretion. Beta cells are reported to express the following $K_{v}$ channels: $K_{v}2.1$, K$_{v}3.2$ and the silent subunits $K_{v}6.2$ and $K_{v}9.3$.47 Reports that $K_{v}11.1$ (erg1) is also expressed and is involved in glucose induced insulin release are based on PCR and some pharmacology.48 Shaker type $K_{v}$ channels were found along with $K_{v}$2 channels, but these probably do not contribute to secretion.47 RT-PCR and electrophysiology experiments at the level of the single cell suggested that the following Ca$^{2+}$ and Na$^{+}$ channels mediate voltage dependent inward currents in beta cells: Ca$_{v}1.2$ (L-type), Ca$_{v}2.1$ (P/Q-type), Ca$_{v}2.2$ (N-type), Ca$_{v}2.3$ (R-type) and Na$_{v}1.7$ (TXT-sensitive).17

Delta cells secrete somatostatin, which inhibits both alpha and beta cell secretion. One possible mechanism for this inhibitory action is via inhibition of oxidative respiration in beta cells.19 Another proposed mechanism is the activation of $K_{v}3$ channels by somatostatin receptors on alpha and beta cells.50 Delta cells are reported to express $K_{v}2.2$ and possibly the silent $K_{v}9.2$.20 Another report implies that the $K_{v}3.4$ channel acts as the major delayed rectifier and shows electrical activity following glucose stimulation and Na$_{v}$

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**K$_{v}$2.1 Expression in Rat Pancreas**

![Immunofluorescent staining of K$_{v}$2.1 in rat pancreas was visualized using Anti-K$_{v}$2.1 antibody (#APC-012) (1:50). Paraffin embedded sections of rat pancreas showing both endocrine (islets of Langerhans) and exocrine areas. Strong and highly specific staining is evident only in endocrine cells (red). Secondary antibody is goat anti-rabbit Alexa 555 (1:400). Counterstain is Hoechst 33342 (blue).](image)

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**Fig. 3: Stromatoxin-1 Inhibits K$_{v}$ Currents in RIN Cells**

![Inhibition of K$_{v}$ currents in RIN Beta cells by rStromatoxin-1 (rScTx-1).](image)
Use of Specific Channel Modulators and Neurotoxins in the Study of Endocrine Physiology

K_\text{ATP} channel blockade leads to insulin secretion when openers of K_\text{ATP} block insulin secretion; both approaches are used clinically to tackle diabetes and hyperinsulinism, respectively. Extensive reviews cover this important topic and it will not be discussed here in detail.50,51

Pharmacological manipulation of Ca_\text{L} (L-type) channels modulate secretion. DHPs (Dihydropyridines) are widely used, relatively specific L-type modulators. (+)-Bay K 8644 is a potent enhancer of L-type current and is often used to induce [Ca\^{2+}]_\text{i} elevations and insulin secretion.52 Several snake venom toxins, TaT-Catoxin, Calcinudine, Calciscinete and FS-2 are specific and potent inhibitors of L-type channels, some of which have been shown to be effective in beta cells (Figure 2).53

As mentioned before, the main Ca_\text{L} channel involved in insulin secretion is one of the Ca_\text{L} family members. However, although other Ca_\text{L} channels have been detected in beta cells, their role is not fully resolved. The role played by these channels might be to regulate and/or fine tune the release process. A nice example is illustrated by an experiment in which SNX-482, a specific Ca_{2,3} channel blocker, was shown to inhibit only the second phase of insulin release.16

In accordance with its major role in depolarization and termination of exocytosis, K_{2,1} channel blockade increases secretion. This was demonstrated with a few spider toxins such as Hanatoxin and Guangxitoxin, which are specific blockers of this channel.21,22 We demonstrate that rStromatoxin-1 (rScTx-1) is a potent inhibitor of K_{2,1} currents recorded in RIN insulinoma cells (Fig. 3).

Synaptic neurotoxins have been used to affect secretion. α-Latrotoxin application induced both ion channel modulation (in a direction favoring increased exocytosis) as well as direct enhancement of insulin containing vesicle exocytosis.52,53 We demonstrate that α-Latrotoxin potently affects Ca_{2,1} dependent enzyme phosphorylation in RIN cells (Fig. 4).

Channelopathies Related to Diabetes

Mutations and polymorphism in K_{2,1} channel components cause a few congenital as well as acquired conditions (via increased susceptibility).54,55

Gain of function mutations usually make K_{2,1} less sensitive to ATP blockage, resulting in increased channel activity, decreased insulin release and diabetes.

Loss of function mutations either eliminate K_{2,1} channels from the plasma membrane or increase their sensitivity to ATP block. This causes decreased channel activity, leading to increased insulin secretion, hyperinsulinism and hypoglycemia. This depolarized beta cell state may eventually lead to apoptosis, beta cell death and diabetes.

Recently two gain of function mutations in Ca_{1,2} (Timothy syndrome)56 and in Na_{1,7} (familial erythermalgia)57 were described, as these two channels are expressed in islet cells, mutations in their coding might influence the normal functioning of the pancreas.

For humans, the consequences of glucose imbalance might be severe. Currently the principle way to tackle such conditions in the clinic is with pharmacophores that bind to K_{2,1} and shift their ATP sensitivity. However, many ion channels participate in the control of secretion in pancreatic endocrine cells and therefore, in the maintenance of glucose homeostasis. Therefore, the fact that the modulation of secretion can be fine tuned renders it amenable to many possible interventions.

References:

5. Gilin, P. et al. (1999), Biol. Chem. 279, 2097.
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