Review Article

Chloride transporters and channels in β-cell physiology: revisiting a 40-year-old model

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It is accepted that insulin-secreting β-cells release insulin in response to glucose even in the absence of functional ATP-sensitive K+ (KATP)-channels, which play a central role in a 'consensus model' of secretion broadly accepted and widely reproduced in textbooks. A major shortcoming of this consensus model is that it ignores any and all anionic mechanisms, known for more than 40 years, to modulate β-cell electrical activity and therefore insulin secretion. It is now clear that, in addition to metabolically regulated KATP-channels, β-cells are equipped with volume-regulated anion (Cl–) channels (VRAC) responsive to glucose concentrations in the range known to promote electrical activity and insulin secretion. In this context, the electrogenic efflux of Cl– through VRAC and other Cl– channels known to be expressed in β-cells results in depolarization because of an outwardly directed Cl– gradient established, maintained and regulated by the balance between Cl– transporters and channels. This review will provide a succinct historical perspective on the development of a complex hypothesis: Cl– transporters and channels modulate insulin secretion in response to nutrients.

Introduction

Stimulus-secretion coupling in β-cells is a complex process with multiple facets that cannot be simply incorporated in any single comprehensible model. [1]

In a widely accepted consensus mechanism for insulin secretion, ATP-sensitive K+ (KATP)-channels play a key role [1]. In this model, when the circulating glucose is low, β-cell plasma membrane (PM) KATP-channels remain open allowing a high K+ conductance and maintaining the PM potential (Em) hyperpolarized (approximately −70 mV) [2]. An increase in blood glucose concentration triggers signaling paths summarized as follows: glucose enters β-cells via facilitative transporters, e.g. Slc4a2 (Glut2) and is immediately phosphorylated by glucokinase [3]. Subsequently, glucose is metabolized generating ATP and decreasing ADP through the Krebs’ cycle. This change in the ATP/ADP ratio inactivates/closes KATP-channels, resulting in a gradual β-cell PM depolarization to a threshold where voltage-gated Ca2+ channels open and action potentials are triggered. The influx of Ca2+, necessary for β-cell exocytosis, causes the release of granules containing insulin [4]. This model is strengthened by the fact that inactivating mutations affecting either of the two subunits of the human KATP-channel, i.e. ABCC8/Sur1 or KCNJ11/Kir6.2, genes [5,6] result in insufficient insulin secretion leading to neonatal diabetes [7]. Conversely, activating mutations in Sur1/Kir6.2 result in unregulated insulin responses independent of the level of glucose present, leading to hyperinsulinaemic hypoglycaemia [8].

While the simplicity of this model is attractive and presents the essentials of the triggering pathway, it is restricted by failing to include anionic (Cl–) mechanisms known, for more than 40 years, to modulate β-cell electrical activity and insulin secretion [9–11]. Clearly, unless an inward background current exists to drive Em away from the K+ equilibrium potential [12], the simple closure of KATP-channels is not enough to increase PM potential (depolarize) to the activating threshold for voltage-gated Ca2+ channels to open. As originally discussed by Henquin et al. [1] and Best et al. [10].
accumulating experimental evidence suggests that glucose triggers insulin secretion by modulating a background inward Cl$^-$ current necessary to cause β-cell depolarization when K$_{ATP}$-channels are fully closed. The slow systematic dissection of the complex anionic mechanism(s), modulating β-cell electrical activity, has provided evidence for the regulation of intracellular Cl$^-$ concentration ([Cl$^-$]) and Cl$^-$ fluxes by glucose metabolism consistent with a more inclusive hypothesis: Cl$^-$ transporters and channels can modulate insulin secretion in response to nutrients.

**Overview of β-cell Cl$^-$ physiology**

Regulation of intracellular Cl$^-$ concentration

Some cells actively extrude Cl$^-$, others actively accumulate it, but few cells ignore it. [13]

Insulin-secreting β-cells, like immature neurons, maintain their [Cl$^-$], higher than expected for passive distribution across the PM [9,14,15] due to the predominance of Cl$^-$ loaders relative to extruders. This outwardly directed Cl$^-$ gradient in β-cells is likely to be set, maintained and regulated by multiple Cl$^-$ transporters and channels [16]. The active movement of Cl$^-$ ions, in and out of cells, is carried out by members of the Cl$^-$ transporter families, Slc12a, Slc4a, Slc26a, that can maintain [Cl$^-$], above thermodynamic equilibrium, whereas Cl$^-$ channels allow constitutive and regulated fluxes that dissipate the gradient. Theoretically, when a cell without Cl$^-$ transporters and an $E_m = −70$ mV is incubated in media with physiological [Cl$^-$]$_o$, i.e. ∼120 mM, Cl$^-$ will passively distribute across the PM to reach thermodynamic equilibrium where the net movement of Cl$^-$ ions equals zero. Under these conditions, [Cl$^-$], will settle at ∼10 mM, the concentration predicted by the Nernst equation. In β-cells, however, [Cl$^-$], is kept above that Nernstian value by the net action of Cl$^-$ loaders. Therefore, the opening of any Cl$^-$ channel will allow for efflux, rather than influx, of Cl$^-$, as shown in Figure 1. This naturally electrogenic and depolarizing efflux of Cl$^-$ is expected to contribute to insulin secretion, even in the absence of functional K$_{ATP}$-channels [17,18].

**Evolution of the anionic mechanism of insulin secretion**

Cl$^-$ fluxes and insulin secretion

Electrophysiological experiments, performed in the 1970s using mouse β-cells, demonstrated that a reduction in [Cl$^-$]$_i$, had a very small effect on their resting $E_m$ and insulin secretion in response to glucose [2]. These experiments, and others performed a decade or so later, erroneously assumed that Cl$^-$ fluxes in β-cells follow $E_m$, concluding that Cl$^-$ passively distributes across the PM [19], while disregarding previous evidence suggesting otherwise. Indeed, Sehlin [9] showed for the first time that Cl$^-$ distributes in a non-passive way across mouse β-cells' PM and suggested that Cl$^-$ could be transported into the β-cell against its electrochemical gradient by mechanisms requiring cellular metabolism.

In analogy to GABAergic action on immature neurons, the opening of Cl$^-$ channels in β-cells is depolarizing, an early hypothesis supported by data from db/db diabetic β-cells that exhibit an altered regulation of Cl$^-$ permeability, $E_m$ and insulin secretion [20,21]. Additional support for that hypothesis is provided by experiments using rat islets incubated in very low Cl$^-$ concentrations (to reduce [Cl$^-$], in islet cells) which established a decrease in both secretory phases [22]. As an additional line of support, at least partially, Tamagawa et al. demonstrated an ∼66% reduction in the second phase of glucose-stimulated insulin secretion (GSIS) in rat islets incubated in low Cl$^-$ [23]. As it became apparent that [Cl$^-$], and Cl$^-$ fluxes had a role in insulin secretion, it was difficult to reconcile, in a single picture, the complexity of the secretory response using data involving anions, cations and secretagogues [23]. Therefore, a clearer model of insulin secretion mainly focused on K$^+$ channels and few other players rapidly progressed [24–27], while Cl$^-$ channels and transporters were altogether disregarded [28].

The role of [Cl$^-$], in β-cell physiology regained momentum a decade or so after the introduction of the consensus model when Kinard and Satin [29] and Best et al. [30] in 1995 demonstrated that β-cells are equipped with a volume-sensitive anionic (Cl$^-$) conductance, thus providing, for the first time, a functional link between hypotonic β-cell swelling [31,32], transient insulin secretion [31] and β-cell electrical activity [29,30,33,34]. Furthermore, the demonstration of a close relationship between β-cell electrical activity, glucose and [Cl$^-$], by Best [18] allowed him and collaborators to put forward the ‘VRAC hypothesis’ that a volume-regulated anion channel contributes to PM depolarization, electrical activity and insulin secretion [10]. This now 10-year-old...
hypothesis has been experimentally tested and elegantly demonstrated at the molecular, functional and in vivo levels very recently [11,35].

**Cl\(^–\) transporters and insulin secretion**

**Slc12a family of Cl\(^–\) loaders and extruders**

The Slc12a family of genes encode at least seven secondary active cation-Cl\(^–\) cotransporters [36], all extensively characterized at the molecular, pharmacological and functional levels and considered to be key regulators of cellular volume and [Cl\(^–\)]\(_i\) [37].

The presence, in β-cells, of a depolarizing Cl\(^–\) conductance requires that [Cl\(^–\)]\(_i\) be maintained above thermodynamic equilibrium by Cl\(^–\) transport mechanisms operating in a net uptake mode. In the early 1980s, such Cl\(^–\) transport mechanisms, sensitive to diuretics such as bumetanide and furosemide, were identified in β-cells [38–45]. These diuretics are extensively used in the clinic and were long suspected to interfere with glucose homeostasis in humans, as summarized by Giugliano et al. [46]. Low concentrations of these diuretics inhibit insulin secretion, Ca\(^{2+}\) and Cl\(^–\) uptake from β-cells [39,40,43] and impair glucose tolerance in mice [41,42,47]. This early pharmacological evidence supported the existence of Cl\(^–\) loaders in β-cells. The subsequent demonstration of diuretic-sensitive K\(^+\)-Cl\(^–\) extruder mechanisms involved in osmotic volume regulation [48,49] and the

Figure 1. [Cl\(^–\)]\(_i\) β-cell regulation.

β-cells exhibit an [Cl\(^–\)]\(_i\) ≈ 34 mM, i.e. ∼3.4-times above the predicted thermodynamic equilibrium. Therefore, the functional prevalence of Cl\(^–\) loaders over Cl\(^–\) extruders makes possible the efflux of the anion upon Cl\(^–\) channel opening. The expression pattern of some of the Cl\(^–\) transporters and channels already identified and others in β-cells are currently being mapped. Shown are those partially/fully supported by experimental evidence (e.g. Nkccs, Kccs, Ano1/2, Cfr, GABA\(_A\), GlyR and VRAC).
fact that osmotic β-cell swelling promoted insulin secretion [31] further highlighted the importance of Cl⁻ cotransport systems in mouse β-cells [45]. More recent molecular studies [50–53] have confirmed that β-cells express several splice variants of the prototypical Cl⁻ transporters of the Slc12a family, i.e. loaders Slc12a2 (Nkcc1a-b), Slc12a1 (Nkcc2a) [52], and extruders Slc12a4 (Kcc1), Slc12a5 (Kcc2a-b), Slc12a6 (Kcc3a-d), Slc12a7 (Kcc4) and a Kcc2a variant, Kcc2a-S25, lacking exon 25 [53]. Our recent work has shown that pharmacological inhibition of Kcc2 influences the efficacy of GSIS in vitro [53].

**Slc4a and Slc26a families of anion exchangers**

β-cell transcriptome profiling and quantitative proteomic analysis identified an assorted repertoire of Cl⁻ transporters [54–56] including members of the Slc4a and Slc26a families. Based on their recognized function in several tissues and cells, some of them can be considered as electroneutral Cl⁻ loaders. Indeed, Slc4a1, Slc4a2, Slc4a3, Slc26a3, Slc26a4, Slc26a6, Slc26a7 or Slc26a9 can function as Cl⁻/HCO₃⁻ exchangers [57,58]. These transporters are functionally sensitive to changes in intracellular pH ([pH]ᵢ), thus contributing to its regulation by extruding intracellular bicarbonate in exchange for extracellular Cl⁻. They also contribute to cell volume homeostasis and $E_{\text{m}}$ through modulation of the PM Cl⁻ gradient in many cell types studied [59]. In β-cells, however, virtually nothing is known about the potential physiological roles of any of these electroneutral Cl⁻/HCO₃⁻ exchangers. However, their presence in β-cells might be indirectly inferred by the fact that (i) pHᵢ modulates β-cell electrical activity [60], (ii) glucose increases β-cell pHᵢ [61] and (iii) β-cells do regulate its pHᵢ [61], at least via Slc4a4, which encodes electrogenic Na⁺/HCO₃⁻ cotransporters [62] and through Slc4a8, a Na⁺-dependent Cl⁻/HCO₃⁻ exchanger [63].

**Cl⁻/H⁺ exchanger Clc3**

The electrogenic 2Cl⁻/H⁺ exchanger Clc3 was the first and last of a large family of anion transporters and channels [64] to be associated with insulin secretion [65,66]. Clc3 was considered to be expressed in mouse and human β-cells localized to large insulin-containing dense-core vesicles, where it was proposed to play a physiological role in the maturation and acidification of these vesicles [65–67]. However, the use of knockout-validated Clc3 antibodies previously demonstrated a different Clc3 localization; β-cell synaptic-like macrovesicles [68], thus generating an important controversy [69] that will come to fruition with new experiments. Furthermore, it remains unknown whether β-cells express other members of the Clc family of Cl⁻ channels and exchangers.

**Cl⁻ channels and insulin secretion**

**Volume-regulated anion channel, VRAC**

Although inferred from previous results [9], the first description of Cl⁻ channels in β-cells was made by Kinard and Satin [29] and by Best and collaborators [30,33]. Using the whole-cell patch clamp technique, these authors demonstrated swelling (volume)-regulated, ATP-dependent and outwardly rectifying Cl⁻ currents in rodent β-cells. This Cl⁻ current was sensitive to classic anion channel blockers, like 4,4′-di-isothiocyanato-2,2′-stilbenedisulfonic acid. Further biophysical characterization demonstrated an ionic selectivity sequence very close to that known for the Cl⁻ channel cystic fibrosis transmembrane conductance regulator (Cftr), but suggested that the volume-activated Cl⁻ current recorded in β-cells was mediated by a different channel or group of channels, all permeable to several anions [34]. Activation of this current in intact β-cells was linked to the generation of a depolarizing Cl⁻-selective efflux which promoted electrical activity [33,70] and stimulated insulin secretion [33], providing a functional explanation for the early observation that hyperosmolarity increases insulin secretion [31]. However, a question remained: Does glucose activate a depolarizing Cl⁻ current? This has been partially answered: glucose and hypotonicity induced cell swelling promote a similar pattern of transiently depolarizing Cl⁻ currents [71] and β-cells swell in response to increased glucose concentrations within the physiological range [32,72,73]. Reiterating that $K_{\text{ATP}}$-channel closure is not enough to fully depolarize β-cells, unless an inward current existed [12], a new model for insulin secretion was conceived, whereby glucose decreases the magnitude of the outward K⁺ current due to the inhibition of $K_{\text{ATP}}$-channels while increasing the inwardly directed Cl⁻ current, promoting β-cell PM depolarization, electrical activity and secretion [34]. VRAC was the only fairly studied Cl⁻ channel at the end of the 1990s and was the one included in the model. This ‘VRAC’ model was experimentally tested and strengthened after the confirmation that (i) VRAC activity does not depend on that of $K_{\text{ATP}}$-channels [74,75], (ii) VRAC carries a depolarizing Cl⁻ current (efflux) which promotes insulin secretion in response to osmotic swelling [17,32,72,76–78], (iii) β-cell electrical activity in response to glucose is reliant on [Cl⁻], [18] and (iv)
K$_{ATP}$-channel-independent modalities of β-cell electrical activity and secretion are clearly present in β-cells lacking K$_{ATP}$-channels [79]. Indeed, β-cells from knockout mice lacking either subunit of the K$_{ATP}$-channel (Sur1$^{KO}$ or Kir6.2$^{KO}$) are depolarized and exhibit increased [Ca$^{2+}$]$_{o}$ as predicted by the consensus model of insulin secretion. However, these β-cells had well-defined Ca$^{2+}$ oscillations and modulated [Ca$^{2+}$]$_{i}$ in response to glucose [79–81], an observation not anticipated by the consensus model providing additional evidence for some of the shortcomings in the model [1]. The new hypothesis, introduced by Best et al., in 2010, thus proposed that glucose metabolism activates VRAC, generating a depolarizing Cl$^{-}$ efflux, which requires [Cl$^{-}$], kept above its electrochemical equilibrium [10].

The discovery of the molecular identity of VRAC, i.e. leucine-rich repeat-containing family 8 (Lrrc8a-c) proteins [82,83] paved the road for detailed biophysical and structural characterization of this channel [84–89]. Conditional animal models lacking the essential VRAC subunit Lrrc8a only in β-cells provided a direct test of the VRAC hypothesis [10]. Kang et al. [35] confirmed the original observations by Best et al. [30,33] using shRNA-mediated silencing, CRISPR/Cas9 technology or Cre/Lox-mediated elimination of Lrrc8a in MIN6 or primary β-cells. Loss of Lrrc8a reduced Cl$^{-}$ currents in response to cell swelling and blunted insulin secretion in response to glucose [35]. In more controlled experiments Stuhlmann et al. [11] confirmed that glucose swells β-cells triggering VRAC currents that rely on Lrrc8a to provoke electrical excitation. However, these responses were delayed and insulin secretion was decreased [11], but not abolished, as suggested by the results of Kang et al. [35]. Therefore, VRAC may not be the only Cl$^{-}$ channel β-cells require to reach a PM depolarization threshold for voltage-gated Ca$^{2+}$ channel-dependent Ca$^{2+}$ entry and insulin secretion, a concept entirely in line with the notion that VRAC may form part of a ‘Cl’ machinery composed of many Cl$^{-}$ channels. Once more, Stuhlmann et al. [11] demonstrated heterogeneous expression of the five VRAC subunits in mouse islets with Lrrc8a, Lrrc8c and Lrrc8d relatively abundant, less Lrrc8b and minute amounts of Lrrc8e. These findings may have further physiological relevance; Lrrc8d confers VRAC with permeability to the osmotically active metabolite taurine [90], which may be involved in insulin secretion [91] as well as that of the osmolyte myo-inositol, GABA or lysine [92] which have been also implicated in the secretory response [15,93–95].

Obviously, the physiological role of VRAC in β-cells has added new tools to study islet biology including the proposed paracrine/autocrine effects [11] of glucose metabolites or neurotransmitters, such as GABA. Finally, the differential stoichiometry of VRAC subunits within the different islet cells may modulate autocrine/paracrine islet responses and the ability of VRAC to act as a Cl$^{-}$ channel.

**Cystic fibrosis conductance regulator, CFTR**

In addition to Cl$^{-}$ transporters, β-cells also express an impressive array of Cl$^{-}$ channels [54–56] whose characterization has advanced slowly amidst controversy due, in part, to their low expression and perceived functional relevance in the islet. The presence of Cfr in β-cells, as in many other non-epithelial cells, is low and expression is heterogeneous making its study challenging [96,97]. In fact, Cfr is considered not expressed at all in mouse β-cells based on RNAseq analysis [98] or at physiologically irrelevant levels [99–101]. However, it is known that ‘omics’ approaches exclude low expressed genes, particularly in heterogeneous populations of cells [102] like islet β-cells [103,104] or α- and β-cell lines [105]. Although some investigators implicated Cfr in insulin and glucagon secretion [106–108], others did not [99,101]. Similarly, few studies performed in neurons, where Cfr is expressed at low levels, have suggested a role for Cfr in the regulation of [Cl$^{-}$], [109,110], including glucose sensing by hypothalamic neurons [111,112]. Importantly and contrary to general awareness, Cfr does not need to be an abundant PM-confined Cl$^{-}$ channel to have a function; Cfr is active in the trans-Golgi network [113], endosomes and lysosomes [114] and in acidic organelles/granules [115]. Moreover, it has been suggested that Cfr regulates exocytosis in β-cells [107], a hypothesis recently tested and extended to a potential role of Cfr in insulin processing [116], a phenomenon of the acidified insulin granule [117]. Indeed, exocytosis events and the number of insulin-containing granules docked at the PM were reduced in β-cells lacking functional Cfr, whereas stimulation of Cfr increased granular acidification [116]. Furthermore, and to keep in mind, Cfr, as many other proteins, may have channel-independent roles, which await discovery. At any rate, the critical analysis of available experimental evidence suggests that Cfr may have a role in the secretory response under certain conditions. It also remains possible that Cfr, as many other islet proteins, may be expressed in a subpopulation of β-cells. At any rate, its ‘absence of evidence is not evidence of absence’ additional experiments are needed to dissect the potential physiological relevance of Cfr in the islet.
Ca\(^{2+}\)-activated Cl\(^-\) channels, Ano1/2

The same issues are applicable to other Cl\(^-\) channels expressed at low levels in the islet, Ano1/2 are examples. Kozak and Logothetis [118] first described the biophysical properties of a Ca\(^{2+}\)-dependent Cl\(^-\) current in guinea pig β-cells. A current that reversed at ~22 mV giving an estimated [Cl\(^-\)] of ~65 mM in these cells, well above that predicted by thermodynamic equilibrium. These biophysical properties are comparable to those of the recently characterized Ca\(^{2+}\)-dependent Cl\(^-\) channels Ano1 and/or Ano2 [119] and now implicated in the secretory response [107,120]. Crutzen et al. established that (i) Ca\(^{2+}\)-activated Cl\(^-\) channels are required to sustain glucose-stimulated membrane potential oscillations and insulin secretion in dispersed β-cells, (ii) Ano1 is expressed at the mRNA and protein levels and (iii) reducing the Cl\(^-\) driving force by decreasing [Cl\(^-\)], through inhibition of Cl\(^-\) uptake mechanisms impairs Ano1-dependent depolarizing currents [120]. Additionally, Ano1 gene expression is responsive to glucose, whereas siRNA-mediated silencing of Ano1 inhibits insulin secretion in human islets [121,122]. The physiological role(s) and expression of Ano1/Ano2 in the islet have been questioned and used to argue against the specificity TAO1, an inhibitor of Ano1, used to study the involvement of Ca\(^{2+}\)-sensitive Cl\(^-\) channels in β-cells [98]. However, TAO1 [107], like siRNA-mediated Ano1 silencing [121] does inhibit insulin secretion from human islets [107], supporting the hypothesis that Ca\(^{2+}\)-activated Cl\(^-\) channels participate in β-cell secretory responses.

Gamma-aminobutyric acid receptor-A, GABA\(_A\)

The observation that GABA, which binds and activates pentameric Cl\(^-\) channels, i.e. the ionotropic GABA receptor-A (GABA\(_A\)) in other cells, has the potential to modulate the β-cell secretory response is also controversial [123].

We have known, since the early 1970s, that rodent β-cells [124] contain high levels of the neurotransmitter GABA [125–127] distributed in the cytoplasm, and potentially in insulin-containing granules and other cell organelles [128,129]. These results suggested the possibility that β-cells released the neurotransmitter, a concept first demonstrated in rodent β-cells in 1995 [130] and extended to primary human β-cells a decade later [131]. Although it has been already shown that exogenous GABA inhibits first-phase insulin secretion in response to arginine [132] or glucose [133], attributing this effect to the activation of GABA\(_A\) receptors, which are Cl\(^-\) channels, would have been complex to interpret based on the hypothesis that opening Cl\(^-\) channels depolarize/stimulate β-cells [9]. Therefore, if GABA\(_A\) receptors are not involved in GABA actions in rodent β-cells, then the G-coupled GABA receptor-B (GABA\(_B\)) might. In support, the GABA\(_B\) receptor agonist baclofen inhibited islet insulin release [132] but not that of islets lacking GABA\(_B\) [134]. Furthermore, the GABA\(_A\) agonist muscimol did not affect insulin release from the isolated and perfused rat pancreas [135] or rodent islets [136], consistent with the proposal that functional GABA\(_A\) Cl\(^-\) channels are undetectable in rodent β-cells [98]. However, clonal mouse and rat β-cells do express different subunits of GABA\(_A\) receptor [137,138]. In fact, GABA\(_A\) receptor activation in rat INS1 β-cells stimulates or inhibits insulin secretion following changes in glucose levels [138], whereas glucose lowers GABA content in mouse [139] and human islets [140]. Therefore, the role of GABA in rodent islet function remains disputed.

In human β-cells, muscimol activation of GABA\(_A\) receptors increased insulin secretion in human insulinoma cells [141], whereas the application of GABA to human β-cells resulted in PM depolarization, increased action potential firings and insulin secretion by mechanisms involving GABA\(_A\) [15]. Taneera et al. [142] subsequently confirmed an active role for GABA in human islets and provided a detailed expression analysis of GABA\(_A\) subunits. Therefore, it seems that human islets are endowed with a GABA system which can be considered an integral part of the secretory machinery.

Other Cl\(^-\) channels expressed in the islet

Glycine receptors (GlyRs) are strychnine-sensitive Cl\(^-\)-selective channels gated by the amino acid and neurotransmitter glycine [143]. GlyRs were originally inferred to be present in pancreatic β-cells by Weaver et al. [144], but their molecular/functional confirmation required two decades [140,145]. Human β-cells do conduct depolarizing Cl\(^-\) currents and promote action potentials in a GlyR-dependent manner [145]. In addition, human β-cells actively uptake glycine and release this neurotransmitter, whereas insulin appears to enhance GlyR-mediated Cl\(^-\) currents. Therefore, Yan-Do et al. [145] proposed that β-cells are endowed with an autocrine positive feedback loop that enhances insulin secretion. Although further experiments are needed to corroborate that proposal, it is worth mentioning that activation of GlyR did not promote Ca\(^{2+}\) uptake in a small
proportion of human β-cells [145]. These results suggest that a sub-set of these cells may exhibit [Cl\textsuperscript{−}], below equilibrium. Should that be the case, the opening of a Cl\textsuperscript{−} channel would result in β-cell PM hyperpolarization, decreased Ca\textsuperscript{2+} uptake and inhibition of insulin secretion in response to glucose. This hypothesis, if tested and demonstrated, would add an extra layer of complexity to the picture of insulin secretion, but would reaffirm the concept that [Cl\textsuperscript{−}], regulates the secretory response.

**Figure 2. Hypothesis model: Cl\textsuperscript{−} transporters and channels regulate insulin secretion.** Represented is a β-cell expressing glucose transporter (Glut), K\textsubscript{ATP}-channels, voltage-gated Ca\textsuperscript{2+} channels (VGCC), Cl\textsuperscript{−} loaders, extruders and channels, including VRAC. Upon facilitative transport of glucose into the β-cell, the sugar is metabolized producing ATP and osmotically active metabolites, including lactate [32], hypothesized to promote the uptake of water to increase cell volume. The former closes K\textsubscript{ATP}-channels, leading to reduced K\textsuperscript{+} permeability and PM depolarization, and the latter activates a VRAC-mediated inward Cl\textsuperscript{−} current that depolarizes the PM as well. Additional Cl\textsuperscript{−} channels are expected to contribute to Cl\textsuperscript{−} currents, which altogether are sufficient to activate VGCC provoking Ca\textsuperscript{2+} entry, action potentials, electrical activity and insulin secretion.

**Perspectives**

- The importance of the field: ‘Models are useless if they are not regularly challenged and counterproductive when they become dogmatic’ [1]. The current consensus model of insulin secretion is important, but incomplete and must be challenged as new information arises. In Figure 2, we revise the current model to include a version of a 40-year-old hypothesis [9].

- Summary of the current thinking: Cl\textsuperscript{−} channels and transporters do contribute to the secretory response complementing K\textsubscript{ATP}-channel function. However, the apparent functional redundancy and cooperativity of the Cl\textsuperscript{−} machinery on the regulation of insulin release, remains far from being understood.
Abbreviations
GlyRs, glycine receptors; GSIS, glucose-stimulated insulin secretion; $K_{ATP}$, ATP-sensitive K$^+$; PM, plasma membrane; VRAC, volume-regulated anion channel.

Author Contribution
L.A.-B. and M.D.F. drafted, critically read and edited the article. Both authors approved the final version.

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Competing Interests
The Authors declare that there are no competing interests associated with the manuscript.

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Future directions: Since global knockout mice did not offer a clear vision on the role that a single Cl$^-$ transporter or channel may play in islet biology [146–148], it is now critical to generate conditional mice models harboring $\beta$-cell-specific deletion of one, as previously done [11,35], or two, or more components of the machinery involved in the regulation of [Cl$^-$], and carefully track their phenotypic characterization.
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