

## Review Article

# Chloride transporters and channels in $\beta$ -cell physiology: revisiting a 40-year-old model

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It is accepted that insulin-secreting  $\beta$ -cells release insulin in response to glucose even in the absence of functional ATP-sensitive  $K^+$  ( $K_{ATP}$ )-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  $\beta$ -cell electrical activity and therefore insulin secretion. It is now clear that, in addition to metabolically regulated  $K_{ATP}$ -channels,  $\beta$ -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  $\beta$ -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  $\beta$ -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  $K^+$  ( $K_{ATP}$ )-channels play a key role [1]. In this model, when the circulating glucose is low,  $\beta$ -cell plasma membrane (PM)  $K_{ATP}$ -channels remain open allowing a high  $K^+$  conductance and maintaining the PM potential ( $E_m$ ) hyperpolarized (approximately  $-70$  mV) [2]. An increase in blood glucose concentration triggers signaling paths summarized as follows: glucose enters  $\beta$ -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  $K_{ATP}$ -channels, resulting in a gradual  $\beta$ -cell PM depolarization to a threshold where voltage-gated  $Ca^{2+}$  channels’ open and action potentials are triggered. The influx of  $Ca^{2+}$ , necessary for  $\beta$ -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  $K_{ATP}$ -channel, i.e. *ABCC8/Sur1* or *KCJN11/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 hyperinsulinemic hypoglycemia [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  $\beta$ -cell electrical activity and insulin secretion [9–11]. Clearly, unless an inward background current exists to drive  $E_m$  away from the  $K^+$  equilibrium potential [12], the simple closure of  $K_{ATP}$ -channels is not enough to increase PM potential (depolarize) to the activating threshold for voltage-gated  $Ca^{2+}$  channels to open. As originally discussed by Henquin et al. [1] and Best et al. [10],

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accumulating experimental evidence suggests that glucose triggers insulin secretion by modulating a background inward  $\text{Cl}^-$  current necessary to cause  $\beta$ -cell depolarization when  $\text{K}_{\text{ATP}}$ -channels are fully closed. The slow systematic dissection of the complex anionic mechanism(s), modulating  $\beta$ -cell electrical activity, has provided evidence for the regulation of intracellular  $\text{Cl}^-$  concentration ( $[\text{Cl}^-]_i$ ) and  $\text{Cl}^-$  fluxes by glucose metabolism consistent with a more inclusive hypothesis:  $\text{Cl}^-$  transporters and channels can modulate insulin secretion in response to nutrients.

## Overview of $\beta$ -cell $\text{Cl}^-$ physiology

### Regulation of intracellular $\text{Cl}^-$ concentration

*Some cells actively extrude  $\text{Cl}^-$ , others actively accumulate it, but few cells ignore it. [13]*

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

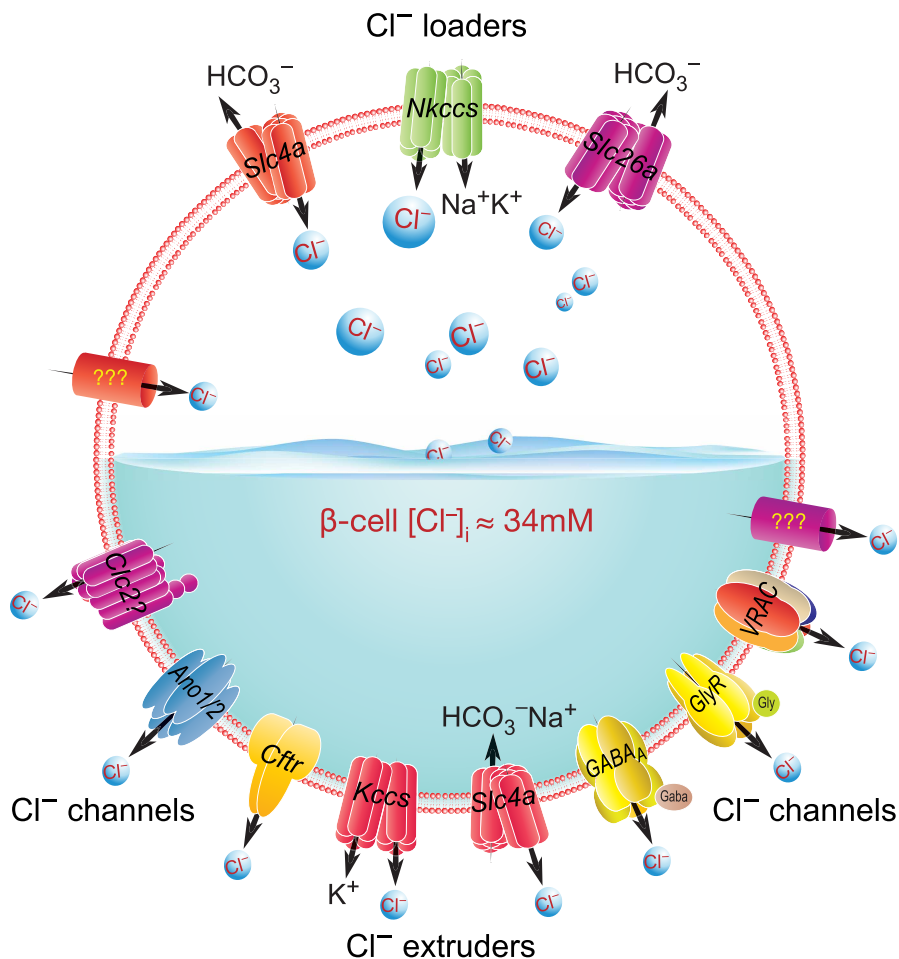
## Evolution of the anionic mechanism of insulin secretion

### $\text{Cl}^-$ fluxes and insulin secretion

Electrophysiological experiments, performed in the 1970s using mouse  $\beta$ -cells, demonstrated that a reduction in  $[\text{Cl}^-]_i$  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  $\text{Cl}^-$  fluxes in  $\beta$ -cells follow  $E_m$ , concluding that  $\text{Cl}^-$  passively distributes across the PM [19], while disregarding previous evidence suggesting otherwise. Indeed, Sehlin [9] showed for the first time that  $\text{Cl}^-$  distributes in a non-passive way across mouse  $\beta$ -cells' PM and suggested that  $\text{Cl}^-$  could be transported into the  $\beta$ -cell against its electrochemical gradient by mechanisms requiring cellular metabolism.

In analogy to GABAergic action on immature neurons, the opening of  $\text{Cl}^-$  channels in  $\beta$ -cells is depolarizing, an early hypothesis supported by data from *db/db* diabetic  $\beta$ -cells that exhibit an altered regulation of  $\text{Cl}^-$  permeability,  $E_m$  and insulin secretion [20,21]. Additional support for that hypothesis is provided by experiments using rat islets incubated in very low  $\text{Cl}^-$  concentrations (to reduce  $[\text{Cl}^-]_i$  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  $\sim 66\%$  reduction in the second phase of glucose-stimulated insulin secretion (GSIS) in rat islets incubated in low  $\text{Cl}^-$  [23]. As it became apparent that  $[\text{Cl}^-]_i$  and  $\text{Cl}^-$  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  $\text{K}^+$  channels and few other players rapidly progressed [24–27], while  $\text{Cl}^-$  channels and transporters were altogether disregarded [28].

The role of  $[\text{Cl}^-]_i$  in  $\beta$ -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  $\beta$ -cells are equipped with a volume-sensitive anionic ( $\text{Cl}^-$ ) conductance, thus providing, for the first time, a functional link between hypotonic  $\beta$ -cell swelling [31,32], transient insulin secretion [31] and  $\beta$ -cell electrical activity [29,30,33,34]. Furthermore, the demonstration of a close relationship between  $\beta$ -cell electrical activity, glucose and  $[\text{Cl}^-]_i$  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



**Figure 1.  $[Cl^-]_i$   $\beta$ -cell regulation.**

$\beta$ -cells exhibit an  $[Cl^-]_i \approx 34$  mM, i.e.  $\sim 3.4$ -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  $\beta$ -cells are currently being mapped. Shown are those partially/fully supported by experimental evidence (e.g. *Nkccs*, *Kccs*, *Ano1/2*, *Cftr*, *GABA<sub>A</sub>*, *GlyR* and *VRAC*).

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  $\beta$ -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  $\beta$ -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  $\beta$ -cells [39,40,43] and impair glucose tolerance in mice [41,42,47]. This early pharmacological evidence supported the existence of  $Cl^-$  loaders in  $\beta$ -cells. The subsequent demonstration of diuretic-sensitive  $K^+Cl^-$  extruder mechanisms involved in osmotic volume regulation [48,49] and the

fact that osmotic  $\beta$ -cell swelling promoted insulin secretion [31] further highlighted the importance of  $\text{Cl}^-$  cotransport systems in mouse  $\beta$ -cells [45]. More recent molecular studies [50–53] have confirmed that  $\beta$ -cells express several splice variants of the prototypical  $\text{Cl}^-$  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**

$\beta$ -cell transcriptome profiling and quantitative proteomic analysis identified an assorted repertoire of  $\text{Cl}^-$  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  $\text{Cl}^-$  loaders. Indeed, *Slc4a1*, *Slc4a2*, *Slc4a3*, *Slc26a3*, *Slc26a4*, *Slc26a6*, *Slc26a7* or *Slc26a9* can function as  $\text{Cl}^-/\text{HCO}_3^-$  exchangers [57,58]. These transporters are functionally sensitive to changes in intracellular pH ( $[\text{pH}]_i$ ), thus contributing to its regulation by extruding intracellular bicarbonate in exchange for extracellular  $\text{Cl}^-$ . They also contribute to cell volume homeostasis and  $E_m$  through modulation of the PM  $\text{Cl}^-$  gradient in many cell types studied [59]. In  $\beta$ -cells, however, virtually nothing is known about the potential physiological roles of any of these electroneutral  $\text{Cl}^-/\text{HCO}_3^-$  exchangers. However, their presence in  $\beta$ -cells might be indirectly inferred by the fact that (i)  $\text{pH}_i$  modulates  $\beta$ -cell electrical activity [60], (ii) glucose increases  $\beta$ -cell  $\text{pH}_i$  [61] and (iii)  $\beta$ -cells do regulate its  $\text{pH}_i$  [61], at least via *Slc4a4*, which encodes electrogenic  $\text{Na}^+/\text{HCO}_3^-$  cotransporters [62] and through *Slc4a8*, a  $\text{Na}^+$ -dependent  $\text{Cl}^-/\text{HCO}_3^-$  exchanger [63].

### **$\text{Cl}^-/\text{H}^+$ exchanger *Clc3***

The electrogenic  $2\text{Cl}^-/\text{H}^+$  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  $\beta$ -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;  $\beta$ -cell synaptic-like macrovesicles [68], thus generating an important controversy [69] that will come to fruition with new experiments. Furthermore, it remains unknown whether  $\beta$ -cells express other members of the *Clc* family of  $\text{Cl}^-$  channels and exchangers.

## **$\text{Cl}^-$ channels and insulin secretion**

### **Volume-regulated anion channel, VRAC**

Although inferred from previous results [9], the first description of  $\text{Cl}^-$  channels in  $\beta$ -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  $\text{Cl}^-$  currents in rodent  $\beta$ -cells. This  $\text{Cl}^-$  current was sensitive to classic anion channel blockers, like 4,4'-di-isothiocyano-2,2'-stilbenedisulfonic acid. Further biophysical characterization demonstrated an ionic selectivity sequence very close to that known for the  $\text{Cl}^-$  channel cystic fibrosis transmembrane conductance regulator (*Cftr*), but suggested that the volume-activated  $\text{Cl}^-$  current recorded in  $\beta$ -cells was mediated by a different channel or group of channels, all permeable to several anions [34]. Activation of this current in intact  $\beta$ -cells was linked to the generation of a depolarizing  $\text{Cl}^-$ -selective efflux which promoted electrical activity [33,70] and stimulated insulin secretion [33], providing a functional explanation for the early observation that hyposmolarity increases insulin secretion [31]. However, a question remained: Does glucose activate a depolarizing  $\text{Cl}^-$  current? This has been partially answered: glucose and hypotonically induced cell swelling promote a similar pattern of transiently depolarizing  $\text{Cl}^-$  currents [71] and  $\beta$ -cells swell in response to increased glucose concentrations within the physiological range [32,72,73]. Reiterating that  $\text{K}_{\text{ATP}}$ -channel closure is not enough to fully depolarize  $\beta$ -cells, unless an inward current existed [12], a new model for insulin secretion was conceived, whereby glucose decreases the magnitude of the outward  $\text{K}^+$  current due to the inhibition of  $\text{K}_{\text{ATP}}$ -channels while increasing the inwardly directed  $\text{Cl}^-$  current, promoting  $\beta$ -cell PM depolarization, electrical activity and secretion [34]. VRAC was the only fairly studied  $\text{Cl}^-$  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  $\text{K}_{\text{ATP}}$ -channels [74,75], (ii) VRAC carries a depolarizing  $\text{Cl}^-$  current (efflux) which promotes insulin secretion in response to osmotic swelling [17,32,72,76–78], (iii)  $\beta$ -cell electrical activity in response to glucose is reliant on  $[\text{Cl}^-]_i$  [18] and (iv)

$K_{ATP}$ -channel-independent modalities of  $\beta$ -cell electrical activity and secretion are clearly present in  $\beta$ -cells lacking  $K_{ATP}$ -channels [79]. Indeed,  $\beta$ -cells from knockout mice lacking either subunit of the  $K_{ATP}$ -channel (*Sur1*<sup>KO</sup> or *Kir6.2*<sup>KO</sup>) are depolarized and exhibit increased  $[Ca^{2+}]_i$ , as predicted by the consensus model of insulin secretion. However, these  $\beta$ -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  $[Cl^-]_i$  kept above its electrochemical equilibrium [10].

The discovery of the molecular identity of VRAC, i.e. leucine-rich repeat-containing family 8 (*Lrrc8a-e*) 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  $\beta$ -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  $\beta$ -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  $\beta$ -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  $\beta$ -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 importance of VRAC in  $\beta$ -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,  $\beta$ -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 *Cftr* in  $\beta$ -cells, as in many other non-epithelial cells, is low and expression is heterogeneous making its study challenging [96,97]. In fact, *Cftr* is considered not expressed at all in mouse  $\beta$ -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  $\beta$ -cells [103,104] or  $\alpha$ - and  $\beta$ -cell lines [105]. Although some investigators implicated *Cftr* in insulin and glucagon secretion [106–108], others did not [99,101]. Similarly, few studies performed in neurons, where *Cftr* is expressed at low levels, have suggested a role for *Cftr* in the regulation of  $[Cl^-]_i$  [109,110], including glucose sensing by hypothalamic neurons [111,112]. Importantly and contrary to general awareness, *Cftr* does not need to be an abundant PM-confined  $Cl^-$  channel to have a function; *Cftr* is active in the trans-Golgi network [113], endosomes and lysosomes [114] and in acidic organelles/granules [115]. Moreover, it has been suggested that *Cftr* regulates exocytosis in  $\beta$ -cells [107], a hypothesis recently tested and extended to a potential role of *Cftr* 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  $\beta$ -cells lacking functional *Cftr*, whereas stimulation of *Cftr* increased granular acidification [116]. Furthermore, and to keep in mind, *Cftr*, as many other proteins, may have channel-independent roles, which await discovery. At any rate, the critical analysis of available experimental evidence suggests that *Cftr* may have a role in the secretory response under certain conditions. It also remains possible that *Cftr*, as many other islet proteins, may be expressed in a subpopulation of  $\beta$ -cells. At any rate, as 'absence of evidence is not evidence of absence' additional experiments are needed to dissect the potential physiological relevance of *Cftr* in the islet.



## Ca<sup>2+</sup>-activated Cl<sup>-</sup> channels, *Ano1/2*

The same issues are applicable to other Cl<sup>-</sup> channels expressed at low levels in the islet, *Ano1/2* are examples. Kozak and Logothetis [118] first described the biophysical properties of a Ca<sup>2+</sup>-dependent Cl<sup>-</sup> current in guinea pig β-cells. A current that reversed at -22 mV giving an estimated [Cl<sup>-</sup>]<sub>i</sub> 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<sup>2+</sup>-dependent Cl<sup>-</sup> channels *Ano1* and/or *Ano2* [119] and now implicated in the secretory response [107,120]. Crutzen et al. established that (i) Ca<sup>2+</sup>-activated Cl<sup>-</sup> 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<sup>-</sup> driving force by decreasing [Cl<sup>-</sup>]<sub>i</sub> through inhibition of Cl<sup>-</sup> 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<sup>2+</sup>-sensitive Cl<sup>-</sup> 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<sup>2+</sup>-activated Cl<sup>-</sup> channels participate in β-cell secretory responses.

## Gamma-aminobutyric acid receptor-A, GABA<sub>A</sub>

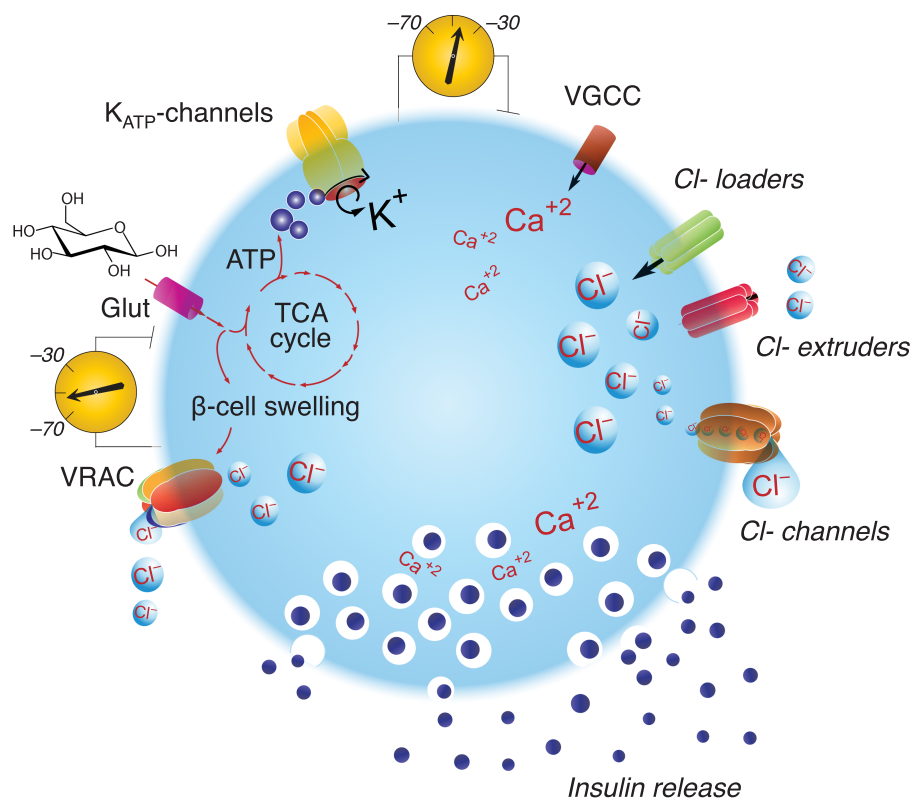
The observation that GABA, which binds and activates pentameric Cl<sup>-</sup> channels, i.e. the ionotropic GABA receptor-A (GABA<sub>A</sub>) in other cells, has the potential to modulate the β-cell secretory response is also controversial [123].

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<sub>A</sub> receptors, which are Cl<sup>-</sup> channels, would have been complex to interpret based on the hypothesis that opening Cl<sup>-</sup> channels depolarize/stimulate β-cells [9]. Therefore, if GABA<sub>A</sub> receptors are not involved in GABA actions in rodent β-cells, then the G-coupled GABA receptor-B (GABA<sub>B</sub>) might. In support, the GABA<sub>B</sub> receptor agonist baclofen inhibited islet insulin release [132] but not that of islets lacking GABA<sub>B</sub> [134]. Furthermore, the GABA<sub>A</sub> 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<sub>A</sub> Cl<sup>-</sup> channels are undetectable in rodent β-cells [98]. However, clonal mouse and rat β-cells do express different subunits of GABA<sub>A</sub> receptor [137,138]. In fact, GABA<sub>A</sub> 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<sub>A</sub> 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<sub>A</sub> [15]. Taneera et al. [142] subsequently confirmed an active role for GABA in human islets and provided a detailed expression analysis of GABA<sub>A</sub> 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<sup>-</sup> channels expressed in the islet

Glycine receptors (GlyRs) are strychnine-sensitive Cl<sup>-</sup>-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<sup>-</sup> 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<sup>-</sup> currents. Therefore, Yan-Do et al. [145] proposed that β-cells are endowed with an auto-crine 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<sup>2+</sup> uptake in a small



**Figure 2. Hypothesis model: Cl<sup>-</sup> transporters and channels regulate insulin secretion.**

Represented is a  $\beta$ -cell expressing glucose transporter (Glut),  $K_{ATP}$ -channels, voltage-gated  $Ca^{2+}$  channels (VGCC),  $Cl^-$  loaders, extruders and channels, including VRAC. Upon facilitative transport of glucose into the  $\beta$ -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  $K_{ATP}$ -channels, leading to reduced  $K^+$  permeability and PM depolarization, and the latter activates a VRAC-mediated inward  $Cl^-$  current that depolarizes the PM as well. Additional  $Cl^-$  channels are expected to contribute to  $Cl^-$  currents, which altogether are sufficient to activate VGCC provoking  $Ca^{2+}$  entry, action potentials, electrical activity and insulin secretion.

proportion of human  $\beta$ -cells [145]. These results suggest that a sub-set of these cells may exhibit  $[Cl^-]_i$  below equilibrium. Should that be the case, the opening of a  $Cl^-$  channel would result in  $\beta$ -cell PM hyperpolarization, decreased  $Ca^{2+}$  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  $[Cl^-]_i$  regulates the secretory response.

## 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:  $Cl^-$  channels and transporters do contribute to the secretory response complementing  $K_{ATP}$ -channel function. However, the apparent functional redundancy and cooperativity of the  $Cl^-$  machinery on the regulation of insulin release, remains far from being understood.

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

## Abbreviations

GlyRs, glycine receptors; GSIS, glucose-stimulated insulin secretion; K<sub>ATP</sub>, ATP-sensitive K<sup>+</sup>; 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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