

Review

The Role of cAMP in Beta Cell Stimulus-Secretion and Intercellular Coupling

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Abstract:

Pancreatic beta cells secrete insulin in response to stimulation with glucose and other nutrients, and impaired insulin secretion plays a central role in development of diabetes mellitus. Pharmacological management of diabetes includes various antidiabetic drugs, including incretins. The incretin hormones, glucagon-like peptide-1 and gastric inhibitory polypeptide, potentiate glucose-stimulated insulin secretion by binding to G protein-coupled receptors, resulting in stimulation of adenylate cyclase and production of the secondary messenger cAMP, which exerts its intracellular effects through activation of protein kinase A or the guanine nucleotide exchange protein 2A. The molecular mechanisms behind these two downstream signaling arms are still not fully elucidated and involve many steps in the stimulus-secretion coupling cascade, ranging from the proximal regulation of ion channel activity, to the central Ca²⁺ signal, and the most distal exocytosis. In addition to modifying intracellular coupling, the effect of cAMP on insulin secretion could also be at least partly explained by the impact on intercellular coupling. In this review, we systematically describe the possible roles of cAMP at these intra- and inter-cellular signaling nodes, keeping in mind the relevance for the whole organism and translation to humans.

Keywords: cAMP; beta cells; stimulus-secretion coupling; intercellular coupling; PKA; Epac2A;

1. Introduction

Insulin secreted by pancreatic beta cells regulates storage and usage of nutrients, and its relative or absolute lack results in diabetes mellitus that affects more than 460 million people around the world, a number that is expected to increase to 700 million by 2045 [1]. If we consider also undiagnosed diabetes, the picture is even worse. The disease burden is immense and includes both public and personal costs [2], with total annual global health expenditures for diabetes estimated to more than 760 billion USD [1]. More than 95 % of all people with diabetes have type 2 diabetes mellitus (T2DM), which is characterized by obesity, insulin resistance, and insufficient insulin secretion [3]. Numerous antidiabetic drugs with different molecular mechanisms are available for T2DM treatment, among which sulfonylureas (SUs) are the most widely prescribed. They are very potent antidiabetic drugs, sufficient to activate the so-called triggering pathway, but bear a risk of

hypoglycemia and weight gain [4]. The triggering pathway of insulin secretion in beta cells is typically activated by glucose metabolism, closing of ATP-dependent K^+ (K_{ATP}) channels, membrane depolarization, opening of voltage-dependent Ca^{2+} (VDCC) channels, and Ca^{2+} influx which triggers insulin secretion. SUs stimulate insulin secretion by closing K_{ATP} channels by binding to their SU receptor SUR1 [5]. Besides the risk of hypoglycemia, chronic treatment with certain SUs could lead to the progressive failure of beta cells, since tolbutamide and glibenclamide were found to induce beta cell apoptosis in rat [6] and human islets [7].

Beside SUs, the incretin-related drugs are increasingly being used for stimulation of insulin secretion. The incretin hormones secreted from the gut augment insulin secretion observed after an oral glucose intake compared with that observed after identical elevation of plasma glucose with a controlled intravenous infusion of glucose [8,9]. Among incretins, the glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like polypeptide-1 (GLP-1) are the most investigated [10]. GIP and GLP-1 are released into the bloodstream from enteroendocrine K and L cells, respectively, following meal ingestion [11,12]. In beta cells, they act through the so-called neurohormonal amplifying pathway. Incretins have almost no effect on insulin secretion in low glucose [13,14], making them a much safer option in terms of risk of hypoglycemia compared with SUs. GIP and GLP-1 bind to the specific guanine nucleotide-binding protein-coupled receptors (GPCR) GIPR and GLP-1R, respectively [15-17], and this results in interaction with the Gas subunit, with subsequent activation of adenylyl cyclase (AC) and consecutive production of cAMP. In beta cells, cAMP acts on intracellular targets via protein kinase A (PKA) and the guanine nucleotide exchange factor Epac2A to amplify insulin secretion elicited by the triggering signal. It has also been shown recently that PKA-Ria could act independently of the cAMP signaling mechanism [18]. On the other hand, Epac2A is also directly activated by various SUs, except for gliclazide, and activation of Epac2A signaling actually seems to be required for SU-induced insulin secretion [19-21]. Furthermore, binding properties of various SUs to Epac2A have been quantified and it was found that cAMP and SUs cooperatively activate Epac2A [22,23]. This molecular mechanism provides a possible explanation for additive effects of combination therapies of incretin-related drugs and SUs, although many important questions still need to be answered in this regard.

While in healthy humans, oral administration of glucose triggers higher insulin secretion than a comparable glucose challenge intravenously due to the incretin effect, in T2DM, this phenomenon is partly lost, but the attenuated insulinotropic action is observed only for GIP. On the other hand, the actions of GLP-1 remain relatively preserved, although the levels of GLP-1 are significantly decreased [24-26]. Therefore, therapeutic approaches are mainly oriented at enhancing GLP-1 action through degradation-resistant GLP-1R agonists (incretin mimetics) or through inhibitors of the enzyme dipeptidyl-peptidase-4, which is responsible for a rapid degradation of incretin hormones. The effect of GLP-1 administration in humans was described over 20 years ago [27], leading to development of GLP-1 based therapies for T2DM [28], and improved drugs with GLP-1R agonist activity are currently being developed [29]. In mice, GLP-1 or GLP1-R agonists clearly improve glucose tolerance and increase GSIS, their effects ranging from normal mice [29] to the alloxan-induced diabetic model [21]. Accordingly, the oral glucose tolerance test (oGTT), known to elicit release of incretins, results in increased blood glucose levels in GLP1-R KO mice, corroborating the importance of GLP1-R signaling for normal glucose tolerance [30]. However, the above study also showed that the intraperitoneal glucose tolerance test (ipGTT), which normally does not trigger the release of incretin hormones, also resulted in glucose intolerance in GLP1-R KO mice. This suggests that some constitutive activity of the GLP-1-R signaling pathway or the basal GLP-1 concentration (present also during ipGTT) importantly contribute to GSIS after a load of glucose only (without further change in GLP-1 levels). In sum, the role of GLP-1-R signaling in maintaining normal glucose tolerance in general and the differential roles of the Epac2A and PKA arms of this neurohormonal amplifying pathway in specific await further clarification.

Since the incretin effect in pancreatic beta cells is mediated via the neurohormonal amplifying pathway through PKA and Epac2A, the specific role of PKA in glucose homeostasis was assessed *in vivo* by disinhibiting PKA activity in a genetic mouse model [31]. A conditional homozygous ablation of the PKA regulatory subunit (Prkar1a KO) resulted in increased glucose tolerance with a concomitantly enhanced GSIS after ipGTT. The enhancing role of PKA was confirmed also in humans in the same study [31]. When crossing Prkar1a KO mice with Epac2A KO mice, the resultant Prkar1/Epac2A KO mice exhibited a reduction in GSIS compared with Prkar1a KOs [32]. This implicates that Epac2A expression is permissive and necessary for the maximum effect of GLP-1R stimulation on GSIS.

Regarding the role of Epac2, several studies demonstrated that Epac2A ablation failed to exert any influence either on glucose tolerance or on GSIS after ipGTT in mice [20,32,33]. Interestingly, Epac2A KOs exhibit early morphological (e.g., increased fat deposits) and biochemical (e.g., increased leptin and decreased adiponectin serum levels) signs of obesity. In combination with unperturbed glucose homeostasis, these results require further clarification of the role of Epac2A in GSIS. Application of GLP-1R activators in Epac2A KO mice revealed an impaired glucose tolerance (GT) and glucose stimulated insulin secretion (GSIS) during ipGTT [32], thus suggesting that Epac2A plays a crucial role in mediating the effects of incretins on GSIS rather than contributing to GSIS without additional stimulation by incretins. Surprisingly, the same study reported that during oGTT glucose tolerance was not perturbed in Epac2A KO mice [32]. It is possible that Epac2A plays a role in increasing GSIS at pharmacological levels of GLP-1-R stimulation, but not at more physiological concentrations of GLP-1 reached during an oGTT. Furthermore, Epac2A seems to be important also during the development of insulin resistance and in combination therapies for T2DM treatment involving incretins and SUs.

Taken together, PKA and Epac2A may play an important role in (i) normal glucose homeostasis, in (ii) incretin potentiation of GSIS, (iii) potentiation of GSIS in obesity, and (iv) potentiation of GSIS in combination therapy involving SUs and incretins. The molecular mechanisms behind this are still not fully elucidated and involve many steps in stimulus-secretion coupling (SSC), ranging from the regulation of ion channel activity and the triggering Ca^{2+} signal to the most distant step in SSC, exocytosis. Finally, the effect of cAMP on insulin secretion could at least partly be explained also by the impact on inter-cellular coupling. In the subsequent chapters of this review, we will focus on these intra- and inter-cellular mechanisms in more detail.

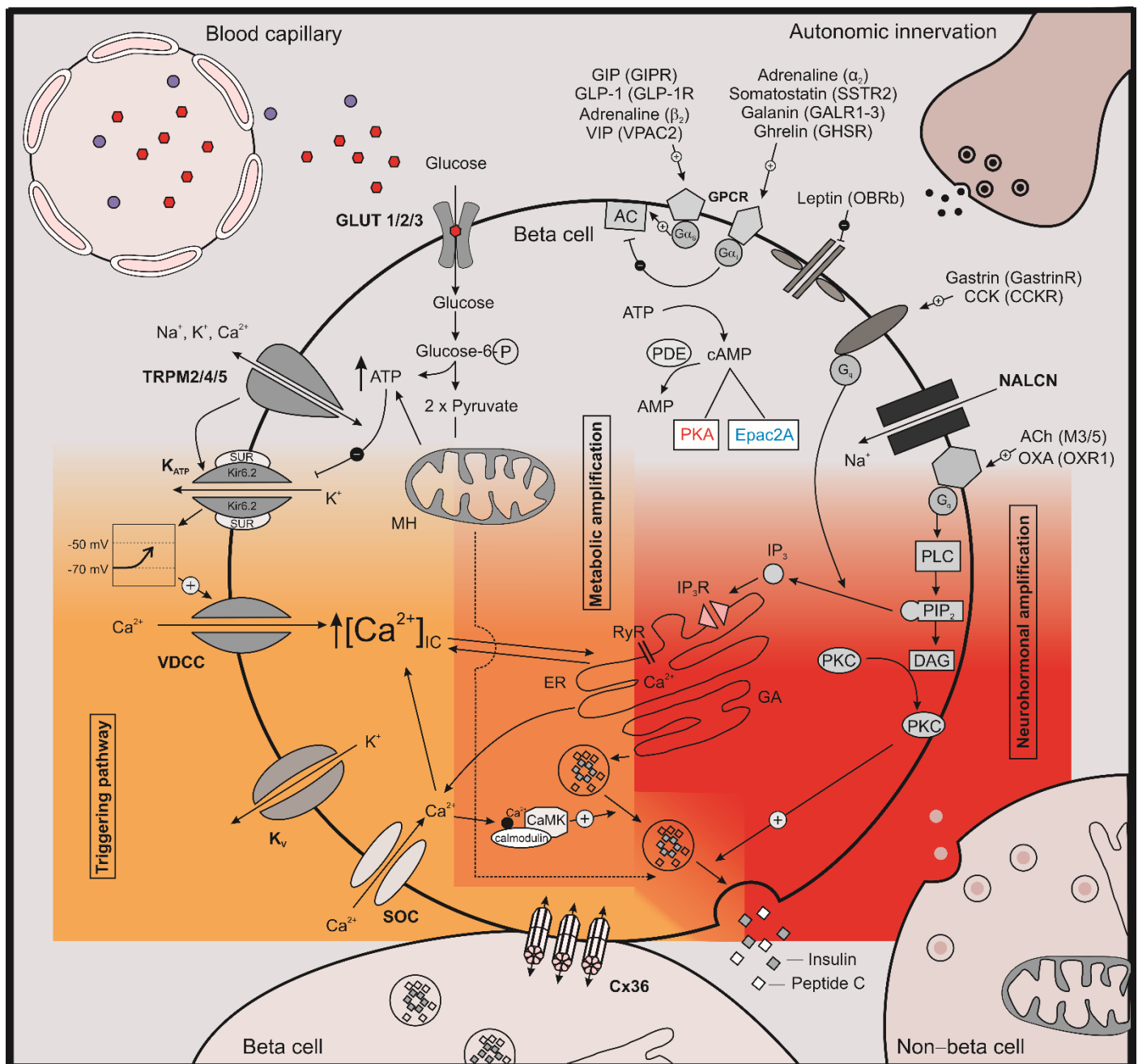


Figure 1: Stimulus-secretion coupling in beta cells. The three interconnected intracellular signaling pathways in pancreatic beta cells. The K_{ATP} dependent triggering pathways is indicated in dark yellow, the metabolic amplifying pathway in orange and the neurohormonal amplifying pathway in red. Parts of a non-beta cell and a neighboring Cx36-connected beta cell are shown on the bottom, blood capillary and autonomic innervation are shown on top. GLP-1 - glucagon-like peptide 1; GIP - glucose-dependent insulinotropic peptide; GIPR - glucose-dependent insulinotropic peptide receptor; GLP-1R - glucagon-like peptide 1 receptor; β_2 - beta-2 adrenergic receptor; α_2 - alpha-2 adrenergic receptor, VPAC2 - vasoactive intestinal peptide receptor 2; GALR1-3 - galanin receptor 1-3; SSTR2 - somatostatin receptor 2; GHSR - growth hormone secretagogue receptor; OBRb - long form of the leptin receptor; M3-5 - muscarinic acetylcholine receptor 3-5; OXA - orexin A; OX1 - Orexin-1 receptor; GPCR - G-protein-coupled receptor; cAMP - cyclic adenosine monophosphate; AMP - adenosine monophosphate, PDE - phosphodiesterase; PKA - protein kinase A; Epac2A - exchange protein directly activated by cAMP; TRPM - transient receptor potential melastatin; K_{ATP} - ATP dependent potassium channel; Kir6.2 - major subunit of the ATP-dependent K^+ channel; SUR - sulfonylurea receptor; VDCC - voltage-dependent calcium channel; K_v - voltage-dependent potassium channels; SOC - Store-operated channel; MH - mitochondrion; AC - adenylyl cyclase, ER - endoplasmic reticulum; VIP - vasoactive intestinal polypeptide; ACh - acetylcholine, PLC - phospholipase C; PIP_2 - phosphatidylinositol 4,5-bisphosphate; IP_3 - inositol trisphosphate; IP_3R - inositol trisphosphate receptor, DAG - diacylglycerol; PKC - protein kinase C; GA - Golgi apparatus; K^+ - potassium ions; Na^+ - sodium ions; Ca^{2+} - calcium ions; RyR - ryanodine receptors; Cx36 - connexin-36; CAMK - Ca^{2+} /calmodulin-dependent protein kinase, Glucose-6P -

glucose-6 phosphate; $[Ca^{2+}]_{IC}$ – intracellular calcium concentration; G_q – Gq protein alpha subunit; G_s – Gs alpha subunit; G_i – Gi protein alpha subunit; NALCN – sodium leak channel; CCK – cholecystokinin; CCKR – cholecystokinin receptor;

2. The role of cAMP on stimulus secretion coupling

2.1 The effect of cAMP on ion channels

Ni et al. have found that oscillations in PKA activity directly mirror the oscillations in cAMP levels [34]. The cAMP-mediated potentiation of insulin secretion is further regulated by (i) activators of AC, (ii) inhibitors of phosphodiesterase (PDE), which degrades cAMP and, (iii) is stimulated by high $[Ca^{2+}]_{IC}$ [35].

Since AC is a transmembrane protein, the highest cAMP concentration can be expected to be at the submembrane compartment, which is of great relevance for the regulation of ion channels and for the process of exocytosis [36].

2.1.1. K_{ATP} channels

K_{ATP} channels belong to a family of inward-rectifying K⁺ channels [37]. They are composed of 4 potassium-selective pore-forming Kir6.2 subunits which have binding sites for ATP, and 4 regulatory SUR1 subunits with binding sites for MgADP, enabling them to be controlled by changes in $[ATP]_{IC}$ and $[ATP]_{IC}/[ADP]_{IC}$ [38,39].

Light et al. have demonstrated that PKA regulates K_{ATP} channels in an ADP-dependent manner [40]. When glucose concentration is high, the cytoplasmic ADP levels are relatively low (ATP production is high), and PKA inhibits K_{ATP} channels, while at elevated ADP levels (low glucose) it increases their activity. As proposed by some authors, this regulation is governed by a molecular mechanism which includes ADP-sensitive binding of PKA to the Kir6.2 subunit at serine residue S372 or to the SUR1 subunits of K_{ATP} channels at serine residues S1571 (human only) and S1448 [40,41].

Inhibition of K_{ATP} channels can also be mediated by Epac2A [42]. This is of great interest since it has been reported that SUs can directly activate Epac2A and that activation of Epac2A signaling is actually required for SU-induced insulin secretion [19-21].

2.1.2. K_v channels

In human beta cells, the voltage-dependent K⁺ current is mediated by delayed rectifying K⁺ (K_v) channels and big K⁺ conductance (BK) channels [43] which are involved in the repolarization phase of action potentials [44]. Among the K_v channels, K_v2.1 channels are an important component of the delayed rectifying current identified in mammalian beta cells [45,46]. However, there are substantial differences in ion channel subtypes among species that shape the action potentials [47]. In mice, the most widespread channels are the K_v2.1 [43], whereas in men single cell transcriptome profiling has revealed that the expression of KCNB2 gene, which encodes K_v2.2, is nearly 10-fold higher than the expression of KCNB1 encoding K_v2.1, suggesting a dominant role of K_v2.2. in human beta cells [48]. Together, K_v2.1 and K_v2.2 channels account for 65–80 % of potassium currents in human beta cells and their inhibition with various inhibitors increases beta cell activity [44,49].

Recent studies have reported that K_v channels are inhibited by cAMP, which results in prolongation of action potential duration, subsequently elevating intracellular Ca²⁺ induced by activation of cAMP signaling [50], and that MgATP plays a pivotal role in maintaining the activity of K_v2.1 channels [51]. An electrophysiological study of isolated rat beta cells reported that K⁺ currents through K_v channels are regulated by cAMP/PKA and not by the PKC pathway [52]. Additionally, the effect of KMUP-1, a xanthine derivative which increases cellular cyclic nucleotides and activates K⁺ channels in high glucose, was

reversed by the PKA inhibitor H-89, suggesting that KMUP-1 might be a promising pharmacological substance for treating insulin resistance [52].

Another mechanism regulating K_v channels has been described to work through incretin stimulation of PKA. Kim et al. have reported that the incretin hormone GIP suppressed ionic current through $K_v1.4$ channels by activating PKA which phosphorylates the C-terminal domain of $K_v1.4$ channel and mediates rapid phosphorylation-dependent endocytosis of $K_v1.4$. This mechanism highlights an important novel role for GIP in regulating surface expression of $K_v1.4$ channels and modulation of potassium currents [53].

Many other delayed rectifying K_v channels are present in beta cells and may have roles other than mediating the K^+ efflux. Specifically, $K_v2.1$ channels were reported to directly facilitate insulin secretion [54] through interaction with Syntaxin-1A and SNAP-25 in the SNARE complex of the exocytotic machinery [55,56].

2.1.3. TRP channels

Auxiliary depolarizing or “leak” currents are mainly carried by influx of Na^+ ions through transient receptor potential (TRP) channels, of which beta cells express many subtypes. Some of them are also permeable to Ca^{2+} (e.g. TRPA1, TRPC1, TRPC4, TRPM2, TRPM3, TRPM5, TRPV1, TRPV2, and TRPV4). Each TRP channel consists of 4 subunits which may not be of the same type, giving rise to a variety of ion channels that are regulated through various mechanisms [57]. For many TRP channels, their main roles are known, but not for all of them and even less is known about the mechanisms that regulate their gating, selectivity, and interactions. Only those with known regulatory mechanism in beta cells will be briefly presented here.

TRPV2 channels are constitutively active in beta cells, and if stimulated by high glucose, insulin, or insulin-like growth factors, they translocate to plasma membrane, resulting in Ca^{2+} influx and increased insulin secretion [58]. Furthermore, they are unique in their being translocated from cytoplasm to the plasma membrane when stimulated by high glucose or insulin [58]. They may contribute substantially to the depolarizing “leak” current in beta-cells. To our knowledge, there are no data regarding the influence of PKA or Epac2A on these channels.

TRPM2 channels are expressed on cell membranes of rodent and human beta cells as well as in many cell lines and are involved in insulin secretion in response to glucose stimulation [59,60]. Their most potent activator is cyclic ADP-ribose (cADPR) [61], but the channel opening also requires binding of Ca^{2+} to the transmembrane domains [62,63]. Other activators include nitric oxide, H_2O_2 , free radicals, and β -NAD $^+$. TRPM2 was shown to activate with nanomolar concentrations of GLP-1 through the cAMP-Epac2A pathway [60,64,65] but the involvement of cADPR and a glucose stimulus are needed [66]. In contrast, inhibition of cAMP signaling was achieved by low concentration of adrenaline which activates α_2A adrenoceptors [67], by nanomolar ghrelin concentrations [68], and by low pH [69]. Yoshida et al have shown that activation of nonselective cation currents through TRPM2 channels by glucose and GLP-1 facilitates membrane depolarization and elevates cAMP concentration which activates the Epac2A-mediated pathway. In addition, nonselective cation currents were attenuated in TRPM2-deficient mice and were not activated in the presence of PKA activators, supporting the stimulatory role of Epac2A and not PKA in the regulation of TRPM2 [65].

Rodent and human beta cells possess various splicing isoforms of TRPM3 channels with different permeabilities to Na^+ , Ca^{2+} and Zn^{2+} ions [70]. They are activated by binding of pregnenolone sulphate at the extracellular side of the channels causing inward cationic current which depolarizes beta cells, increases $[Ca^{2+}]_i$ and stimulates insulin secretion [71]. At the same time, there is an increase in expression of the transcription factor Egr-1 which leads to increased insulin gene transcription via binding to the regulatory region of Pdx-1 gene [72]. In T2DM patients, TRPM3 channels are significantly downregulated

corroborating the diminished insulin secretion [48]. Positive regulation of TRPM3 is also achieved by phosphatidylinositol 4,5-bisphosphate (PIP2) [73,74]. To our knowledge, no data exist regarding the influence of PKA or Epac2A on these channels.

TRPM4 is a nonselective monovalent cation channel and is regulated by voltage [75]. It is activated in a dose-dependent manner by increasing Ca^{2+} concentrations [76], which results in a depolarizing inward current usually carried by Na^+ ions [77]. Using calcium imaging with dual excitation fluorometric imaging system, Marigo et al. have shown that inhibition of TRPM4 significantly decreased the magnitude of Ca^{2+} signals generated by agonists in comparison to control, and that the decrease in the $[\text{Ca}^{2+}]_{\text{ic}}$ also resulted in reduced insulin secretion [76]. Furthermore, the response of beta cells to TRPM4 activation was biphasic, the first phase being due to localized activation of TRPM4 within the plasma membrane and the second phase being due to the recruitment of vesicles with TRPM4 channels to the plasma membrane [77]. The sensitivity of TRPM4 channels to Ca^{2+} was shown to be regulated by protein kinase C and calmodulin [78], while the most important regulator was proposed to be phosphatidylinositol 4,5-bisphosphate (PIP2) which sensitizes the channel to $[\text{Ca}^{2+}]_{\text{ic}}$, and whose depletion desensitizes the channel [79,80]. When stimulated by glucose, TRPM4 channels experience a dual effect of the elevated glucose metabolism. On the one hand, cytoplasmic $\text{MgATP}/\text{MgADP}$ ratio increases, which results in PIP2 increase in the plasma membrane [81], which might be a potential mechanism of TRPM4 sensitization by glucose, while on the other hand glucose increases the cytoplasmic ATP concentration, which inhibits TRPM4 channels [82]. Recently, Shigeto et al. showed that the incretin hormone GLP-1 exerts its depolarizing effect on beta cells by activating phospholipase C (PLC) pathway which has mobilized intracellular Ca^{2+} from thapsigargin-sensitive Ca^{2+} stores, thereby increasing $[\text{Ca}^{2+}]_{\text{ic}}$ and resulting in activation of TRPM4 and TRPM5 channels. Concordantly, GLP-1 effects were negligible in islets from C57BL6/N *Trpm4* and *Trpm5* KO mice. Using PKA inhibitors, this GLP-1 stimulated insulin secretion was shown to occur without detectable increases in intracellular cAMP and PKA activity and was therefore suggested to be PKA-independent [83,84].

TRPM5 channels were found in rodent beta cells and insulinoma cell lines but were almost absent in one study of human beta cells [85]. In mice, TRPM5 channels are involved in mediating glucose-induced oscillations of membrane potential and $[\text{Ca}^{2+}]_{\text{ic}}$ and consistent with this, *Trpm5*^{-/-} mice have reduced insulin secretion and impaired glucose tolerance [86]. TRPM5 are more sensitive to $[\text{Ca}^{2+}]_{\text{ic}}$ than TRPM4 (EC50 values of $0.70 \pm 0.1 \mu\text{M}$ and $20.2 \pm 4.0 \mu\text{M}$, respectively), however they are insensitive to inhibition with ATP at concentrations up to 1 mM [82]. Like TRPM4, it was shown that GLP-1-stimulated insulin secretion is coupled to TRPM5 activation via the PKC pathway [83] producing PIP2 which concentration-dependently affects TRPM5 [74]. In diabetic mice models, chronic hyperinsulinemia strongly reduced *Trpm5* mRNA levels and thus downregulated the expression of TRPM5 receptors, an effect that was successfully reversed by leptin treatment [87].

2.2. The effect of cAMP on $[\text{Ca}^{2+}]_{\text{ic}}$

The effect of GLP-1 on $[\text{Ca}^{2+}]_{\text{ic}}$ was recorded in rodent as well as human beta cells [88-91]. In low glucose, which does not increase $[\text{Ca}^{2+}]_{\text{ic}}$ *per se*, cAMP has almost no effect on insulin secretion [13,14] and cAMP-elevating agents are unable to raise $[\text{Ca}^{2+}]_{\text{ic}}$ in the absence of stimulatory glucose concentration [66,92,93]. This indicates that the amplifying effect of GLP-1 requires interaction with the triggering $[\text{Ca}^{2+}]_{\text{ic}}$ signal. GLP-1 increases voltage-dependent Ca^{2+} currents by increasing the activity of L-type VDCCs and inhibition of said channels inhibits $[\text{Ca}^{2+}]_{\text{ic}}$ rise in response to GLP-1 or forskolin [94-97]. This is due to a leftward shift in the voltage-dependent activation, likely via a PKA-mediated phosphorylation of channels, as well as a rightward shift in voltage-dependent steady-state inactivation, meaning that fewer channels are inactivated at a given membrane potential [98,99].

Of the two L-VDCC subtypes expressed in beta cells, both can mediate the GLP-1-induced potentiation of glucose-induced insulin secretion, however Cav1.3 seems to be preferentially coupled to GLP-1 receptor activation. This signaling pathway depends on intact intracellular stores, PKA and PKC activation [100]. While both Epac2 and PKA are important in regulating the activity of Cav1.2, Cav1.3 activation is mainly a PKA-dependent process, in which Epac2 has a secondary role [100,101].

The effect of cAMP on beta cell Ca^{2+} signaling is not restricted to the voltage-dependent Ca^{2+} influx, but also involves a PKA-dependent and PKA-independent Ca^{2+} mobilization from internal stores [97,102-104]. Following beta cell depolarization and activation of VDCCs, Ca^{2+} entering cytoplasm triggers additional release of Ca^{2+} from intracellular compartments [105]. This process, known as Ca^{2+} -induced Ca^{2+} -release (CICR), was previously believed to be of minor physiological importance in pancreatic beta cells, since emptying of the endoplasmic reticulum by thapsigargin failed to reduce insulin secretion [106]. This seems to be true only in glucose-stimulated conditions, while exendin-4, a high affinity GLP-1 agonist, facilitates CICR [107]. Moreover, latest results suggest that even under glucose stimulation intracellular Ca^{2+} stores play a crucial role in shaping beta cell $[\text{Ca}^{2+}]_{\text{ic}}$ responses [108].

Since exendin-4 failed to induce $[\text{Ca}^{2+}]_{\text{ic}}$ increase before uncaging of Ca^{2+} by UV flash, the effect of exendin-4 in beta cell is not mediated through membrane depolarization and subsequent opening of VDCCs [107], but by sensitizing the intracellular Ca^{2+} release mechanism to the stimulatory effect of an increase in $[\text{Ca}^{2+}]_{\text{ic}}$, thereby allowing CICR to be triggered by uncaging of Ca^{2+} [104].

The exact molecular mechanism by which GLP-1 and its analogues modify Ca^{2+} is not clear, but the effect of Epac2 is probably mediated through the Rap protein and PLC- ϵ , since downregulation of Rap activity, as well as knock-out of PLC- ϵ , failed to facilitate CICR [107,109].

Several conflicting results were published regarding the role of PKA and Epac2 in the process of neurohormonal amplification by incretins. Facilitation of CICR after exendin-4 stimulation was diminished, but not abrogated in EPAC2 KO islets, and PKA inhibition failed to (completely) prevent incretin-induced CICR in control islets [66,107,110,111]. While some studies show both PKA and Epac2 selective agonists can induce a rapid and sustained Ca^{2+} rise [66] or trigger CICR [104], other have only recorded this response after PKA activation, but not after application of Epac2 activators [112]. Taken together, this indicates that incretins act both through PKA-dependent and PKA-independent pathways. Since inhibition of PKA nearly abolished the potentiating effect of Epac2 on glucose-induced insulin secretion, despite failing to prevent the Ca^{2+} response, it would seem that PKA has a permissive role operative further downstream in the SSC cascade [97].

Since at least *in vitro*, half maximal activation of PKA and Epac2 occur at different cAMP concentrations, the quantity of cAMP could be another way how Epac2- and PKA-specific effect are mediated [113]. To complicate matters even further, contemporary research with advancements in technology [114] keeps identifying additional aspects of what was believed to be a well-understood signaling mechanism, e.g., Holz et al. recently showed that the individual subunit isoforms belonging to PKA can act independently from the accepted canonical signaling pathway, elucidating another layer of complexity to the mechanism of cAMP signaling [115].

Whether CICR is mediated via inositol trisphosphate receptors (IP₃R) or ryanodine receptors (RyR) is still debatable. In beta cells, functional receptors of both types are present, with IP₃R type 3 and RyR type 2 being the most abundantly expressed [116,117]. Some argue that PKA-mediated CICR via IP₃ is the major mechanism by which cAMP amplifies insulin release [112]. Others, however, have reported that the Ca^{2+} rise in response to Epac2-selective agonists is not accompanied by a detectable increase in IP₃ and that pre-treatment of cells with ryanodine effectively blocks the Ca^{2+} response to incretins [91,117]. Also, the IP₃R inhibitor xestospongin C did not affect the GLP-1 induced Ca^{2+} responses [42,66].

While opinions differ regarding the type of receptor, it is generally accepted that the mobilization of intracellular stores induced by GLP-1 is cAMP-dependent [95] and acts via receptor sensitization [112]. Recent research shows that both types of receptors are important, at least in glucose-induced Ca^{2+} signaling [108].

Most studies recognize the endoplasmic reticulum as the principal intracellular Ca^{2+} source, since depletion of the ER by thapsigargin, a potent inhibitor of sarco/endoplasmic reticulum Ca^{2+} -ATPases (SERCA), typically deems beta cells unresponsive to GLP-1, as well as PKA and Epac2-selective agonists [107,117,118]. However, mobilizing Ca^{2+} from ER stores is not the only possible way to increase $[\text{Ca}^{2+}]_c$. There is a report indicating that Epac activators might signal through nicotinic acid adenine dinucleotide phosphate (NAADP) receptors, known also as two-pore Ca^{2+} channels to mobilize Ca^{2+} from non-ER compartments. These acidic stores are presumed to be responsible for the fast, transient phase of Ca^{2+} response [66]. However, other studies have found these non-ER stores to be less likely to play a role in GLP-1 induced CICR [107].

2.2.1. $[\text{Ca}^{2+}]_c$ oscillations and other types of responses

A detailed characterization of changes in $[\text{Ca}^{2+}]_c$ dynamics during metabolic amplification by cAMP is lacking in published literature. The qualitative and quantitative diversity of results arise from the variety of methods and protocols used to investigate Ca^{2+} responses in pancreatic beta cells.

While both slow and fast $[\text{Ca}^{2+}]_c$ oscillations rely on periodic entry of Ca^{2+} , the fast pattern also seems to depend on mobilization of intracellular Ca^{2+} stores and agents that increase cAMP promote the appearance of regular fast $[\text{Ca}^{2+}]_c$ oscillations [108,119].

Older studies, where GLP-1 was applied in pulses, ranging from 30 s [96] to a few minutes, typically report an a rapid $[\text{Ca}^{2+}]_c$ peak, occasionally followed by a smaller, sustained $[\text{Ca}^{2+}]_c$ elevation [89,96]. Holz et al. described two main temporal components of the GLP-1-induced $[\text{Ca}^{2+}]_c$ dynamics and proposed a mechanism behind them. One is transient, on the time scale of seconds, the other sustained, measured in minutes. While the latter is believed to reflect an increased flux of Ca^{2+} ions through plasma membrane, the first is believed to be due to CICR, activated by a Ca^{2+} influx through L-type VDCCs. These fast Ca^{2+} transients were recorded in beta cells voltage-clamped at 50 mV, a membrane potential above the resting potential to allow GLP-1 to promote the opening of VDCCs and the rise in Ca^{2+} was not associated with a change in membrane current. However, the fast transients were blocked by nimodipine, exposure to a Ca^{2+} -free medium, hypoglycemic glucose concentrations, thapsigargin, and ryanodine, all supporting the model where GLP-1 facilitates Ca^{2+} influx and RYR-mediated CICR to amplify insulin granule exocytosis [96]. Contrary to this, Kim et al showed that only the late/sustained phase of Ca^{2+} signal is sensitive to thapsigargin and ryanodine, while the initial, transient part is thapsigargin-resistant and is believed to represent Ca^{2+} release from acidic stores [66].

When $[\text{Ca}^{2+}]_c$ oscillations are recorded in response to glucose stimulation, addition of GLP-1 or cAMP agonists typically increases their frequency [90,120-122]. A more substantial rise in $[\text{cAMP}]_c$, achieved by simultaneous activation of adenylyl cyclase and inhibition of phosphodiesterase (IBMX) leads to a sustained, non-oscillatory increase in $[\text{Ca}^{2+}]_c$ [110], which is sometimes preceded by an escalation in oscillatory frequency [90]. This could indicate that both cAMP concentration and dynamics are relevant for the oscillatory character of Ca^{2+} responses, especially since GLP-1 also induces cAMP oscillations and, at least under glucose stimulation, both types of oscillations seem to be well synchronized [123]. In some older studies, the addition of GLP-1 during glucose stimulation of mouse islets caused additional $[\text{Ca}^{2+}]_c$ elevation with attenuation or abolition of Ca^{2+} oscillations [111], or either lengthening of the oscillations or a sustained peak [124]. These results, as well as the ones above only describing transient and/or sustained elevation could be explained by technical limitations in measuring $[\text{Ca}^{2+}]_c$.

It is worth emphasizing that while the changes in $[\text{Ca}^{2+}]_c$ are minor, the amount of insulin secreted in response to cAMP elevation is much greater, indicating that the rise in

$[Ca^{2+}]_i$ is just one and probably not the most quantitatively important cAMP-sensitive step in the SSC cascade [90].

2.3. *The effect of cAMP on exocytosis*

Distally to the triggering increase in $[Ca^{2+}]_i$ in the SSC cascade, cAMP also directly affects exocytosis [102,125]. TIRF microscopy revealed that cAMP in the presence of glucose but not by itself enhances the frequency of fusion events of insulin granules during both phases of insulin secretion [19]. This action seems to consist of both a PKA-dependent and a PKA-independent mechanism. The latter, involving activation of Rap1 by Epac2A is essential primarily in the first phase of insulin secretion, not by triggering exocytosis *per se*, but by facilitating the recruitment of the granules to the plasma membrane [19]. A recent study on Epac2 KO mice showed an attenuation of the first phase of insulin secretion, while the following second phase involved PKA signaling [19]. Some other studies reported the involvement of Epac2A also in the second phase of insulin secretion [19,97,126]. cAMP elevation was shown to stimulate Epac2 clustering at the site of docked granules, facilitating granule priming and exocytosis [127]. Epac2/Rap signaling augments insulin secretion by increasing the size of the ready releasable pool of granules (RRP) and by recruiting insulin granules to the plasma membrane [19]. These effects are not all Rap mediated, since Epac2A also interacts with Piccolo, Rim2 α and Rab3, all required for cAMP-regulated insulin granule exocytosis [128]. Epac2, by complexing with the Rab3-interacting molecule Rim2, facilitates docking and priming of secretory granules [129]. Furthermore, granule acidification, a process important in granule priming, is influenced by Epac2 as well, as it regulates Cl⁻ influx [130]. Epac2 also facilitates exocytosis of already docked and primed granules [130,131], at least partially through interactions with the t-SNARE component SNAP-25 via the cAMP-Epac2\Rim2 pathway, which is essential for exocytosis [132]. Epac2 may also regulate exocytosis as a part of the Rim2, Munc13-1, SNARE protein, syntaxin complex that results in Munc13-1 mediated unfolding of syntaxin. This complex has been shown to be under the control of PKA as well [133]. PKA-dependent mechanism is also involved in exocytosis by sensitizing the secretory machinery to Ca^{2+} [134], increasing the number of highly Ca^{2+} -sensitive pool of granules [135,136], and increasing the mobility and replenishment of the readily-releasable pool of granules and is therefore mainly responsible for the second phase of insulin secretion [131]. Along with the above mentioned syntaxin, SNAP-25 [132] and Snapin [31] appear to be exocytotic machinery proteins under dual control of both the PKA-dependent and the PKA-independent pathways. PKA-dependent phosphorylation of Snapin thus appears to play a role in the merger of both cAMP stimulated pathways as well as in facilitation of exocytotic protein interactions and subsequent GSIS [31].

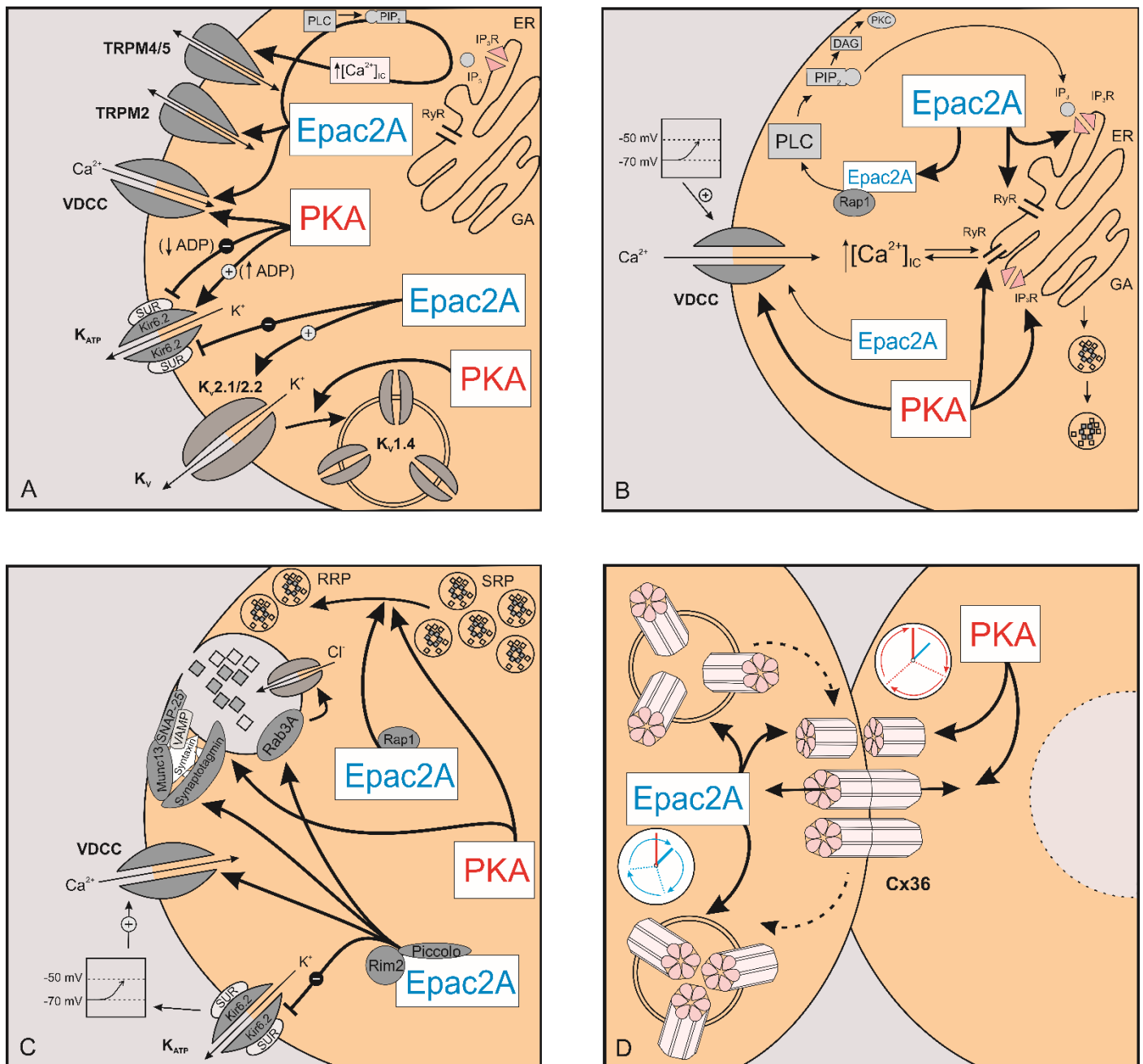


Figure 2: The role of PKA and Epac2A in stimulus-secretion and intercellular coupling The effect of PKA and Epac2A on ion channels (**A**), $[Ca^{2+}]_i$ (**B**), exocytosis (**C**) and intercellular coupling (**D**). PKA – protein kinase A; Epac2A – exchange protein directly activated by cAMP; TRPM2 – transient receptor potential melastatin 2; TRPM4/5 – transient receptor potential melastatin 4/5; ADP – enosine diphosphate; K_{ATP} – ATP dependent potassium channel; Kir6.2 – major subunit of the ATP-depdent K^+ channel; SUR – sulfonylurea receptor; VDCC – voltage-dependent calcium channel; K_v – voltage-dependent potassium channels; ER – endoplasmic reticulum; PLC – phospholipase C; PIP_2 – phosphatidylinositol 4,5-bisphosphate; IP_3 – inositol trisphosphate; IP_3R – inositol trisphosphate receptor, DAG – diacylglycerol; PKC – protein kinase C; GA – Golgi apparatus; K^+ – potassium ions; Na^+ – sodium ions; Ca^{2+} – calcium ions; Cl^- – chloride ions; RyR – ryanodine receptor; Cx36 – connexin-36; $[Ca^{2+}]_i$ – intracellular calcium concentration; Rim2 – Rab GTPase interacting molecule; Rpa1 – Ras-related protein 1; Munc13 – mammalian uncoordinated-13; VAMP – vesicle-associated membrane protein; Rab3A – Ras-related protein Rab-3A; SNAP-25 – synaptosomal-associated protein 25; RRP – ready releasable pool; SRP – slowly releasable pool

3. The role of cAMP in intercellular coupling

The pancreatic islets of Langerhans are multicellular micro-organs within which communication among a variety of cells with unique functions must occur to ensure a proper control of metabolic homeostasis. The beta cells are the most abundant cell type within the islets, which along with glucagon-producing α -cells, somatostatin-producing δ -cells, PP cells, and a minority of other cell types constitute the endocrine component of the pancreas [137]. Islet cells communicate via direct electrical coupling through gap-junctions as well as by paracrine, autocrine and juxtacrine signalling [138-141]. For beta cells, electrical coupling established through gap junctions composed of connexin36 (Cx36) is particularly important, as it provides the necessary and the most important substrate for coordinated responses of the beta cell population, a prerequisite for the well-regulated secretion of insulin [142-144]. Specifically, gap junctions are a type of specialized membrane contacts that enable direct communication by allowing small molecules to pass directly into the cytoplasm of adjacent cells, giving rise to propagating intercellular depolarization and $[Ca^{2+}]_{IC}$ waves [47,142,145-152] that underlie complex functional connectivity in islets [153-158]. Noteworthy, disruptions of gap-junctional communication were found to abolish coherent beta cell activity and lead to impaired plasma insulin oscillations and to glucose intolerance [159,160], as observed in numerous models of obesity and diabetes mellitus [143,144,155,161,162]. Moreover, prolonged exposure to high concentrations of glucose and fatty acids, as expected in diabetes, were found to downregulate Cx36 expression and disrupt the coherent patterns of intercellular synchronization [163,164], whereby some pharmacological agents seem to be able to at least partly repair the defective signaling pattern [155]. Gap junction coupling was also reported to be disrupted by pro-inflammatory cytokines, which also contribute to the decline in islet function during the pathogenesis of diabetes [165]. Along these lines, Cx36 gap junction coupling and its modulation are increasingly recognized as vital components in normal islet function [166-168] and potentially viable targets to help restore insulin secretion in diabetes [169-171].

How the neurohormonal signaling pathways influence gap-junctional communication and how they relate to the collective activity of beta cell networks is incompletely understood. First clues that cAMP affects cell-to-cell communication came from electrophysiological studies. Increasing intracellular cAMP concentration with forskolin in microdissected mouse islets enhanced conduction between couples of impaired beta cells by 24 % [172]. In contrast to microdissection, in isolated islets forskolin incubation failed to change the distribution of gap junction conductance of beta cells [173]. Finally, a third possible outcome of forskolin application on GJs was reported in insulin-secreting cell lines where application of forskolin decreased Cx36 expression [163]. Despite these contradictory reports, only a few studies attempted to decipher the mechanism underlying the modulatory effect of cAMP on gap junctions. A chronic hyperlipidemia-induced down-regulation of Cx36 was reported to be associated with an overexpression of the inducible cAMP early repressor ICER-1 γ [174,175], thereby implying a direct impact of the cAMP/PKA pathway on the gap junctional coupling. Moreover, additional evidence from other tissues, such as retinal AII amacrine cells or neurons of the inferior olive, corroborated the inhibitory role of PKA on Cx36 function [176-178]. Conversely, in rat myocardial cells, cAMP was found to enhance Cx43-mediated gap junctional coupling through both PKA and Epac pathways [179,180], thereby substantiating further the complex and multifaceted role the incretin pathways play in multicellular systems.

Within the islets, a next line of evidence on the involvement of neurohormonal signaling pathways in cell-to-cell communication came from mice with ablation of Cx36. The logic behind this approach is that the incretin pathway (via PKA or Epac2A) increases insulin secretion, and if this effect is modulated in Cx36 KO mice, this would correlate the effect of either PKA or Epac2 to a changes in gap junctional conduction. Along this line, the PKA specific agonist 6-Bnz-cAMP, but not the Epac2A specific agonist 8-Me-2-O-pCPT-cAMP, produced a significant elevation of insulin secretion from Cx36 KO isolated islets compared to islets with intact intercellular connectivity [181]. This suggested that the PKA arm of the incretin pathway acts via a change in gap junction conductance, an observation that was also noticed in MIN-6 cell lines, but with a contradictory effect of

increasing insulin secretion when PKA was activated [182]. To complicate things even further, the modulatory effect of cAMP on gap junctions was studied only at low glucose (2 mM) [181]. The authors proposed that cAMP functions to suppress insulin release only at basal blood glucose levels and that this mechanism may serve as an alternative mechanism to the more commonly accepted path in which hyperpolarizing currents through gap junctions from less activated beta cells inhibit the more active beta cells. Furthermore, in human donor islets, by using a different methodological approach, an effect of cAMP on intercellular coupling was demonstrated by pharmacologically blocking gap junctions using 18- α -glycyrrhetic acid that resulted in desynchronization of the calcium response to GLP-1 and decreased insulin secretion [155]. Surprisingly, inhibiting gap junctional communication did not decrease insulin secretion in response to stimulation by 11 mM glucose only, i.e., without GLP-1, pointing to a possible non-gap junctional action of gap junction blockers on the incretin signaling pathway. Moreover, it has been shown that human islets respond to secretagogues in a highly coordinated manner guided by a network of interlinked cells and lipotoxicity was shown to impair these responses. Notably, the deleterious effects of chronically elevated free fatty acid levels on the islet dynamics underlying insulin release were argued to be brought about through PKA- and cAMP-dependent inhibition of intercellular communication via gap junctions [155].

Very recently, Farnsworth et al. [167] examined the respective roles of PKA and Epac2 in beta cell-to-cell coupling very explicitly. In their study, in islets with decreased Cx36 coupling due to the action of pro-inflammatory cytokines, Exendin-4, which increases cAMP, was found to overcome cytokine-induced dysfunction of islets and restore the coupling. To determine to what extent different arms of the neurohormonal signaling pathways contribute to the protection of the gap junction coupling, selective inhibitors and activators of the cAMP-activated PKA and cAMP-dependent Epac2A pathways were utilized. It turned out that both pathways play a role in mediating cAMP regulation of Cx36 in islets, but in a different manner. PKA did not alter coupling in healthy islets but improved it significantly under the stress of acute short-term cytokine treatment. Because Cx36 levels at the cell membrane were not altered in this case, this implies that PKA regulates Cx36 coupling via a fast mechanism, such as Cx36 phosphorylation and channel gating. Moreover, Epac2A was also found to play a role in mediating Cx36 gap junction coupling and protect against cytokine-induced decreases in Cx36 gap junction coupling. However, in contrast to PKA, a significant contribution was noticed only under long-term treatment, thereby suggesting that Epac2A regulates Cx36 coupling via a slower mechanisms, such as trafficking, assembly, or turnover of Cx36 channels. Further investigations will be needed to assess these ideas and the underlying mechanisms in more detail, but in general they go well in hand with what was reported in other systems [144]. We represent these concepts in Fig. 2D, where the impact of PKA and Epac2A is illustrated. While PKA regulates Cx36 coupling via gating of gap junction channel conductance, Epac2A operates on slower scales by regulating Cx36 trafficking, gap junction assembly, or Cx36 endocytosis in beta cells [167].

Finally, in our very recent study we investigated explicitly how cAMP-mediated amplification affected the collective beta cell activity within the islets [183]. Our results have shown that the activation of cAMP signaling did not only profoundly increase beta cell activity but also enhanced synchronicity and the coordination of intercellular signals. To provide a clear demonstration of this behavior, we present in Fig. 3 an exemplary recording of an islet that was stimulated with 9 mM glucose and subsequently with 10 μ M forskolin. The increase in cytosolic cAMP caused by forskolin evoked a more than a two-fold increase in the oscillation frequency, a slight decrease in the duration of oscillations, and an increase in the level of synchronization, reflected by the average correlation coefficient. The raster plots in Figs. 3E and F indicate that after the application of forskolin, the multicellular dynamics is dominated by global and well-aligned Ca^{2+} waves, whilst under stimulation with glucose only, the activity patterns are more erratic. To assess the collective activity in further detail, we constructed functional connectivity networks [158], separately for the stimulation with glucose only and glucose with additional forskolin.

Clearly, in the latter case, the network is characterized by more functional connections resulting in a more integral network, which indicates a higher degree of synchronization between beta cells. This is well in agreement with our previous results, where in control experiments with prolonged glucose stimulation only, synchronized behavior diminished with time, but the activation of cAMP prevented this effect and enhanced the degree of beta cell synchronicity [183]. Most importantly, performing the same set of experiments with Epac2A KO mice, stimulation with forskolin restored the functional network integrity only partly. This implies that both arms of the neurohormonal amplifying pathway play a role by shaping the collective beta cell activity within the islets. Notably, this interpretation also goes well in hand with the recent study by Farnsworth et al. [167], where both PKA- and Epac2A-dependent pathways were shown to influence the gap-junctional coupling between beta cells. However, PKA was suggested to regulate Cx36 coupling through a fast mechanism, such as channel gating, while Epac2A controls slower mechanisms of regulation, such as Cx36 turnover in the plasma membrane.

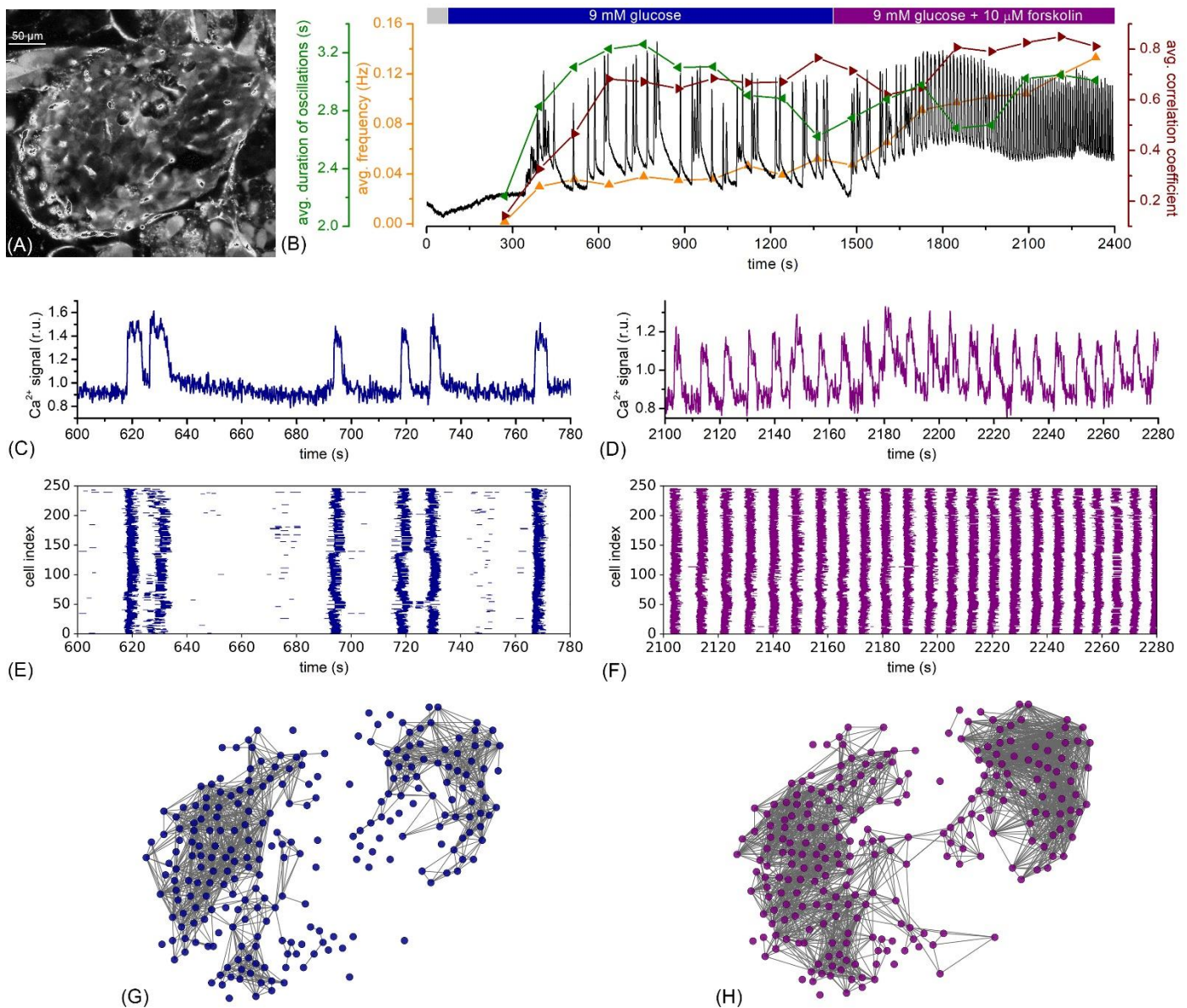


Figure 3: Multicellular beta cell activity under stimulatory glucose levels and the additional activation of the neurohormonal amplifying pathways by forskolin. (A) Confocal image of an islet of Langerhans within the tissue slice with well stained cells using fluorescent calcium dye Calbryte 520AM; (B) Average Ca^{2+} signal calculated on the basis of all beta cells in the islet (black line), and the temporal evolution of the frequency (orange), duration of oscillations (green), and average correlation coefficient (red). For the calculation of signaling parameters, we used a sliding

window of 3 minutes and a window overlap of 1 minute. The stimulation protocol is visualized on the top of the panel, whereby the grey rectangle signifies substimulatory 6 mM glucose concentration; (C-H) Recorded traces from an exemplary cell (C,D), raster plots of binarized Ca^{2+} activity of all selected cells in the islet (E,F), and the corresponding functional beta cell networks (G,H) under stimulation with 9 mM glucose (C,E,G) and with 9 mM glucose + 10 μM forskolin (D,F,H). The multicellular Ca^{2+} activity of beta cells was captured by means of confocal laser scanning microscopy in an acute pancreatic tissue slice (see Refs. [149,184] for methodological details).

4. The role of cAMP during the development of insulin resistance

In insulin resistance, compensatory beta cell adaptation ensures increased insulin secretion, which is usually able to sustain normoglycemia for long periods of time [185,186]. However, when beta cell compensation fails to meet increasing insulin demands imposed by insulin resistance, glucose intolerance first becomes apparent as mild and later overt hyperglycemia that characterizes a full-blown type 2 diabetes mellitus [187]. Several high-quality studies have established that the elevated plasma insulin levels in complete compensation (hyperinsulinemia and normoglycemia) or in partially compensated prediabetic state (hyperinsulinemia and mild hyperglycemia) are related with morphological adaptation in the form of an increased beta cell mass [188]. Importantly, the evidence is not unanimous, and some reports have reported only moderate [189] or no increases in beta cell mass in insulin resistance compared with controls [190]. It has been demonstrated that GLP-1 enhances beta cell survival by activating beta cell proliferation and differentiation and inhibiting apoptosis, but it remains to be clarified whether this is a PKA- or Epac2A-mediated effect [191]. Importantly, later during worsening of insulin resistance and glucose intolerance, beta cell mass is typically decreased [192]. In a diet-induced mouse model of diabetes supplemented with streptozotocin (STZ) administration, chronic GLP-1R stimulation partially rescued the decrease in beta cell mass [193], confirming the role of cAMP in morphological adaptation even in the setting of decompensated diabetes.

In stark contrast, comparatively few studies have addressed the role of beta cell functional adaptation in the compensatory response to insulin resistance and thus changes in beta cell stimulus-secretion coupling and intercellular coupling remain poorly characterized in obesity [194-196]. Additionally, most data about beta cell functional adaptation come from isolated beta cells [195,196], which are unable to fully capture the normal physiological scenario compared with isolated islets [197,198] or tissue slices [149,150]. In a genetic model of obesity (*ob/ob*) characterized by hyperinsulinemia and moderate hyperglycemia, beta cells displayed (i) enhanced mitochondrial function, (ii) increased firing of action potentials and longer bursts and interburst intervals upon stimulation with 11 mM glucose, (iii) and more depolarized values of membrane potential at burst onset, action potential foot and peak. Moreover, islets from these mice exhibited (iv) more sensitive and enhanced glucose-induced $[\text{Ca}^{2+}]_{\text{ic}}$ changes at normally sub-stimulatory or low stimulatory glucose concentrations and (v) a preponderance of slow $[\text{Ca}^{2+}]_{\text{ic}}$ oscillations compared with fast oscillations that prevail in control mice. Furthermore, both (vi) insulin secretion and (vii) exocytosis were increased and (viii) beta cell coupling was reduced [164]. Similarly, in high fat diet (HFD)-induced insulin resistant mice that are hyperinsulinemic and normoglycemic, (i) elevated insulin gene expression and content were found, together with (ii) an augmented GSIS, and (iii) a higher amplitude of action potentials. Moreover, (iv) glucose-induced $[\text{Ca}^{2+}]_{\text{ic}}$ changes in isolated cells and islets were enhanced and (v) there was a higher proportion of beta cells that reacted to stimulation by glucose compared with controls. Finally, (vi) exocytotic responses were enhanced in insulin resistant mice compared with controls [199]. Similarly to the decrease in beta cell mass, beta cell function starts to deteriorate once overt type 2 diabetes mellitus develops. Beta cell decompensation encompasses oxidative and endoplasmic reticulum (ER) stress, dedifferentiation, and lower expression of GLUT2, glucokinase, as well as anomalies in ER Ca^{2+} mobilization. Additionally, beta cell connectivity has been shown to deteriorate during progression of type 2 diabetes and this might be one of the earliest defects [144,187,200]. These alterations are believed to be a consequence of chronic hyperglycemia, hyperlipidemia and a proinflammatory metabolic state [155,157,165,169,201].

Limited data are available to explain which aspects of beta cell functional adaptation depend on the GLP-1 receptor signaling cascade, cAMP and specifically on PKA and Epac2A. Epac2A seems to play a role in potentiation of GSIS under conditions of increased demand for insulin, i.e., in insulin resistance, such as during obesity [202]. Studies employing HFD in the Epac2A KO mice to induce insulin resistance implicated that Epac2A ablation results in increased body weight, impaired glucose tolerance [32,33] and decreased GSIS during 4 weeks of a HFD diet intervention compared with control mice on the same diet [32]. Surprisingly, another study reported an increase in GSIS during 8 weeks of HFD diet in Epac2A KO mice compared with control mice on the same diet [33]. A mechanistic explanation for how Epac2A could bring about an improvement in GSIS during an ipGTT in insulin resistance is not available at the moment, but it was suggested that under these conditions, glucose-induced production of cAMP and Epac2A activation might become operative [203]. However, experimental verification is missing, as well as an explanation for the differential effect on GSIS in Epac2A KO mice on HFD at 4 and 8 weeks. To complicate matters even further, it was demonstrated that the Epac2-dependent GSIS potentiation also involves the brain-islet axis. The neuropeptide Orexin A, secreted from the lateral hypothalamic area, potentiated GSIS *in vitro*, and this effect was blocked in Epac2-deficient mice [204]. Paradoxically, the plasma levels of OXA were decreased in rodent models of T2DM [205,206] and in obese women [207]. To the best of our knowledge, a single study addressed the role of PKA in the setting of insulin resistance. Disruption of the protein PKA inhibitor beta (PKIB), an effective inhibitor of PKA activity, improved glucose sensitivity and GSIS in a 20-week HFD-induced mouse model [208], suggesting a PKA-potentiating effect on GSIS. To our knowledge, there are no data on the roles of Epac2A and PKA in changes of $[Ca^{2+}]_i$ oscillations, sensitivity of the exocytotic machinery, or intercellular coupling during diet-induced T2DM. A single study demonstrated that in human islets cAMP stimulates synchronous $[Ca^{2+}]_i$ increases that augment insulin secretion and that the signals become asynchronous with an accompanying reduction in insulin secretion in lipotoxic conditions [155].

Finally, a role of Epac2A was implicated in hypersecretion of insulin observed in combination therapies for T2DM treatment [209]. Coadministration of liraglutide (a GLP-1 R agonist) and glimepiride (sulfonylurea) resulted in an increased blood glucose during oGTT in Epac2A KO mice on HFD. These results indicate that the glucose-lowering effect of the combination of liraglutide and glimepiride is diminished in Epac2A KO mice. Thus, Epac2A seems to play a role in insulin secretion induced by the combination of an incretin and sulfonylurea, especially in a model of diet-induced obesity, and may provide an explanation for the sulfonylurea-dependent difference in the incidence rate of hypoglycemia observed in combination therapies.

5. Conclusions

Concentrating on a possible role of cAMP in general and its two main signaling arms in specific, our journey led us from the most proximal membrane potential step in the stimulus-secretion coupling cascade, via the central $[Ca^{2+}]_i$ changes, to the most distal event, exocytosis. We also addressed the aspect of intercellular coupling and the relevance *in vivo*. It is quite self-evident that our knowledge about the role of cAMP is limited and biased due to the influence of historically and currently available electro- and optophysiological tools, pharmacological agents, development of specific animal models, and access to human tissue, among many other factors. Since a deeper insight into the normal intra- and intercellular coupling in beta cells and the role played by cAMP is central for a better understanding of the natural history of diabetes mellitus, prevention, for finding new targets and developing new pharmacological approaches, we firmly believe that future studies will help push the frontiers of knowledge and truly hope that our review has helped identify some most needed next steps.

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