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New Insights Concerning the Glucose-dependent Insulin Secretagogue Action of Glucagon-like Peptide-1 in Pancreatic β -Cells

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Abstract

The GLP-1 receptor is a Class B heptahelical G-protein-coupled receptor that stimulates cAMP production in pancreatic β -cells. GLP-1 utilizes this receptor to activate two distinct classes of cAMP-binding proteins: protein kinase A (PKA) and the Epac family of cAMP-regulated guanine nucleotide exchange factors (cAMPGEFs). Actions of GLP-1 mediated by PKA and Epac include the recruitment and priming of secretory granules, thereby increasing the number of granules available for Ca^{2+} -dependent exocytosis. Simultaneously, GLP-1 promotes Ca^{2+} influx and mobilizes an intracellular source of Ca^{2+} . GLP-1 sensitizes intracellular Ca^{2+} release channels (ryanodine and IP_3 receptors) to stimulatory effects of Ca^{2+} , thereby promoting Ca^{2+} -induced Ca^{2+} release (CICR). In the model presented here, CICR activates mitochondrial dehydrogenases, thereby upregulating glucose-dependent production of ATP. The resultant increase in cytosolic [ATP]/[ADP] concentration ratio leads to closure of ATP-sensitive K^+ channels (K-ATP), membrane depolarization, and influx of Ca^{2+} through voltage-dependent Ca^{2+} channels (VDCCs). Ca^{2+} influx stimulates exocytosis of secretory granules by promoting their fusion with the plasma membrane. Under conditions where Ca^{2+} release channels are sensitized by GLP-1, Ca^{2+} influx also stimulates CICR, generating an additional round of ATP production and K-ATP channel closure. In the absence of glucose, no “fuel” is available to support ATP production, and GLP-1 fails to stimulate insulin secretion. This new “feed-forward” hypothesis of β -cell stimulus-secretion coupling may provide a mechanistic explanation as to how GLP-1 exerts a beneficial blood glucose-lowering effect in type 2 diabetic subjects.

Keywords

Glucose; GLP-1; cAMP; PKA; Epac; Insulin secretion

Introduction

Glucagon-like peptide-1-(7–36)-amide (GLP-1) is a blood glucose-lowering hormone that activates a surprisingly diverse array of signaling pathways in the pancreatic β -cell. Binding of GLP-1 to its 62 kDa Class B heptahelical G_s -protein-coupled receptor activates adenylyl cyclase, stimulates cAMP production, and potentiates glucose-dependent insulin secretion from the pancreas [1,2]. First-phase and second-phase insulin secretion are enhanced, and pulsatile insulin secretion in humans is augmented [3,4]. These immediate effects of GLP-1

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are complemented by its delayed insulinotropic action in stimulating insulin gene transcription and upregulating translational biosynthesis of preproinsulin [5,6]. By serving as an intermediary linking intestinal nutrient absorption to pancreatic insulin secretion, GLP-1 fulfills its physiological role as an incretin hormone within the enteroinsular axis [7,8].

Simultaneously, GLP-1 increases pancreatic insulin secretory capacity by stimulating the formation of new β -cells within the islets of Langerhans. Activation of GLP-1 receptors accelerates the conversion of pancreatic ductal stem cells to new β -cells while exerting a major proliferative effect in stimulating mitosis in existing β -cells [9,10]. These neogenic and proliferative actions of GLP-1 are complemented by its ability to protect against β -cell death [11–14]. It may be concluded that in addition to its insulinotropic properties, GLP-1 acts as a β -cell growth factor.

Growth factor-like actions of GLP-1 result from its ability to stimulate mitogen-activated protein kinases (p38 MAPK, ERK1/2), src kinase (pp60^{src}), phosphatidylinositol 3-kinase (PI-3K), atypical protein kinase C- ζ (PKC- ζ), and protein kinase B (PKB, *Akt*) [15,16]. GLP-1 also transactivates the epidermal growth factor receptor (EGF-R) [16], upregulates the expression of insulin receptor substrate 2 (IRS-2) [17], and interacts with insulin or insulin-like growth factor 1 (IGF-1) signaling pathways that control β -cell function [18]. By influencing the function of key transcription factors (CREB; PDX-1), GLP-1 stimulates coordinate gene expression, thereby maintaining β -cells in a fully differentiated state [19–21].

This constellation of unique insulinotropic and growth factor-like signaling properties has prompted interest in the use of GLP-1 and its synthetic peptide analogs (Exenatide, NN2211, CJC-1131) as novel blood glucose lowering agents for treatment of type 2 diabetes mellitus [22]. When administered to type 2 diabetic subjects, GLP-1 lowers the concentration of fasting blood glucose, restores the missing first phase component of glucose-dependent insulin secretion, and delays the postprandial hyperglycemic excursion. Since GLP-1 is rapidly degraded by dipeptidyl peptidase IV (DPP-IV), the antidiabetogenic properties of endogenously secreted GLP-1 are enhanced by DPP-IV inhibitors (LAF237, MK-0431, NN7201) [23].

Summarized here are new findings that shed light on how GLP-1 exerts its stimulatory effect on pancreatic insulin secretion. Presented is a new “feed-forward” hypothesis for stimulus-secretion coupling that seeks to explain how GLP-1 interacts with oxidative glucose metabolism to regulate mitochondrial ATP production, ion channel function, Ca²⁺ signaling, and exocytosis in the β -cell. For additional discussion of GLP-1 and the β -cell signaling pathways it regulates, the reader is referred to a prior review of this subject matter [24].

GLP-1 as an Insulin Secretagogue Hormone

It is now established that the insulin secretagogue action of GLP-1 is dependent on exposure of β -cells to concentrations of glucose that are themselves stimulatory for insulin secretion. This interaction is understandable if GLP-1 acts as a modulator of the β -cell glucose signaling system. Prior studies have demonstrated that GLP-1 increases the efficacy (maximal effect) and potency (threshold concentration) of glucose as a stimulus for insulin secretion [25]. As GLP-1 fails to stimulate insulin secretion in the absence of glucose, the available evidence indicates that it acts as a β -cell “glucose sensitizer.” This action may explain how GLP-1 restores glucose-dependent insulin secretion in metabolically compromised β -cells, a phenomenon referred to as the induction of “glucose competence” [26,27].

When administered to human subjects, GLP-1 only stimulates insulin secretion when the concentration of blood glucose is above fasting levels. Since the concentration of blood glucose falls in response to administered GLP-1, the insulin secretagogue action of GLP-1 is self-terminating. Therefore, unlike insulin, GLP-1 possesses a natural safeguard and is less likely to induce hypoglycemia.

To understand why the insulin secretagogue action of GLP-1 is glucose-dependent, it is first necessary to consider exactly how metabolism of glucose influences β -cell function (Fig. 1). The ability of β -cells to sense extracellular glucose requires uptake of the sugar via a type 2 facilitative glucose transporter (Glut2). Glucokinase, a type IV hexokinase that is rate-limiting for glucose sensing, converts glucose to glucose-6-phosphate, which is then metabolized via glycolysis to generate pyruvate. Oxidation of pyruvate by mitochondrial pyruvate dehydrogenase generates acetyl-CoA which is utilized in the Krebs cycle to generate hydrogen atoms for respiratory chain electron transport, oxidative phosphorylation, and synthesis of ATP. The ensuing increase in cytosolic [ATP]/[ADP] concentration ratio inhibits efflux of K^+ through plasma membrane ATP-sensitive K^+ channels (K-ATP). The resulting membrane depolarization activates voltage-dependent Ca^{2+} channels (VDCCs), thereby stimulating Ca^{2+} influx, an increase in $[Ca^{2+}]_i$, and exocytosis of secretory granules.

This sequence of metabolic and ionic events constitutes a “triggering” pathway for insulin secretion (Fig. 1) [28]. What is remarkable is that GLP-1 acts as a modulator of this triggering pathway. GLP-1 interacts with glucose metabolism to promote mitochondrial ATP production [29], thereby increasing the cytosolic [ATP]/[ADP] concentration ratio. GLP-1 also modifies the adenine nucleotide-sensitivity of K-ATP channels, reducing their sensitivity to ADP while increasing their sensitivity to ATP [30,31]. The net effect is glucose-dependent K-ATP channel closure, membrane depolarization and Ca^{2+} influx.

The importance of K-ATP channels as targets of GLP-1 action has been emphasized by recent reports demonstrating that the insulin secretagogue action of GLP-1 is diminished in sulfonylurea receptor 1 (SUR1) “knock out” (KO) mice that lack K-ATP channels [32,33]. SUR1 is a subunit of the K-ATP channel, and it mediates inhibitory effects of GLP-1 and glucose metabolism on channel function. An alternative interpretation of this KO phenotype is that SUR1 may also regulate the ATP-dependent priming of secretory granules, a step that renders them release competent [34]. Since SUR1 KO mice have a reduced number of primed granules available for exocytosis, this alternative interpretation predicts that these mice should exhibit a generalized secretory defect not necessarily related to the inhibition of K-ATP channels. However, the finding that β -cells derived from SUR1 KO exhibit a robust secretory response to administered acetylcholine runs against this alternative interpretation [33,35].

There is also an “amplification” pathway for glucose-dependent insulin secretion (Fig. 1) [28]. The amplification pathway increases the effectiveness of Ca^{2+} as a stimulus for exocytosis, and may also be responsible for the recruitment of secretory granules to the plasma membrane. Because the amplification pathway stimulates exocytosis in a Ca^{2+} -dependent manner, this mechanism requires an increase in $[Ca^{2+}]_i$ generated by the triggering pathway. For this reason, outdated terminology equating amplification to a “K-ATP-independent” mechanism of exocytosis should be abandoned. Instead, available evidence indicates that the amplification pathway facilitates exocytosis stimulated by the triggering pathway.

An explanation for how GLP-1 might upregulate the function of the amplification pathway is provided by the “malonyl Co-A hypothesis” of glucose-dependent insulin secretion [36]. Carboxylation of pyruvate by pyruvate carboxylase allows β -cell glucose metabolism to

generate citrate that is exported out of the mitochondria for ultimate conversion to malonyl-CoA. Since malonyl-CoA inhibits mitochondrial oxidation of free fatty acids (FFAs), its synthesis links glucose metabolism to increased levels of cytosolic FFAs. Increased availability of FFAs favors the synthesis of long-chain fatty acyl-CoA esters (LC-CoA) and diacylglycerol (DAG). Both lipid metabolites are proposed to exert stimulatory effects on insulin secretion by virtue of their ability to promote acylation (LC-CoA) and protein kinase C mediated phosphorylation (DAG) of secretory granule-associated proteins. The net effect is an “amplification” of Ca^{2+} -dependent exocytosis. Since GLP-1 acts via PKA to stimulate lipolysis and to liberate FFAs [37], it may also stimulate glucose-dependent production of LC-CoA and/or DAG, thereby favoring amplification.

PKA and Epac-mediated Signaling Properties of the GLP-1 Receptor

Although GLP-1 activates multiple signaling pathways in the β -cell, all available evidence indicates that the second messenger, cAMP, serves as the primary effector by which GLP-1 exerts its insulin secretagogue action [24]. New findings demonstrate that GLP-1 utilizes cAMP to activate not only protein kinase A (PKA), but also the Epac family (Epac1, Epac2) of cAMP-regulated guanine nucleotide exchange factors (cAMPGEFs) [38]. cAMPGEFs are cAMP-binding proteins that couple cAMP production to the activation of low molecular-weight G-proteins of the Rap family (Rap1, Rap2). Potential downstream effectors of the activated cAMPGEF/Rap signaling complex include ERK1/2 MAPK, phospholipase C- ϵ , and PKB [38].

One PKA-mediated action of GLP-1 is to inhibit K-ATP channel function via phosphorylation of the channel's SUR1 subunit [30]. This action of GLP-1 upregulates the triggering pathway and may be of particular importance for the appearance of first-phase glucose-dependent insulin secretion in type 2 diabetic subjects. It is also apparent that GLP-1 utilizes PKA to recruit secretory granules from a reserve pool to a readily releasable pool [34,39]. This action of GLP-1 supports sustained exocytosis, and may allow for the appearance of second-phase insulin secretion in type 2 diabetic subjects. The PKA-mediated recruitment of secretory granules is complemented by a “post priming” action of PKA to facilitate Ca^{2+} -dependent fusion of secretory granules with the plasma membrane [40]. This action of PKA may upregulate the triggering and amplification pathways simultaneously.

Actions of GLP-1 mediated by cAMPGEFs include its ability to stimulate ATP-dependent priming of secretory granules, thereby rendering them release-competent [34]. This action of GLP-1 may result from interactions of Epac2 with insulin granule-associated proteins (Rim2, Piccolo) [41,42] or with SUR1 [43]. Since direct protein-protein interactions of Epac2 and SUR1 are demonstrable [43], it seems likely that cAMPGEFs may also confer cAMP-dependent inhibition of K-ATP channel activity, possibly by increasing the channel's sensitivity to ATP [31]. In summary, some actions of GLP-1 are Epac-mediated and may be independent of Rap G-proteins.

GLP-1 Stimulates Ca^{2+} Influx and Mobilizes an Intracellular Source of Ca^{2+}

GLP-1 exerts pronounced stimulatory effects on β -cell Ca^{2+} signaling [44–46], actions that underlie its ability to promote Ca^{2+} -dependent exocytosis of secretory granules. Exposure of β -cells to GLP-1 stimulates a fast transient increase in $[\text{Ca}^{2+}]_i$ followed by a slowly developing and sustained increase [47]. The transient increase in $[\text{Ca}^{2+}]_i$ results from cAMP-dependent release of Ca^{2+} from intracellular Ca^{2+} stores [47,48], whereas the sustained increase results from influx of Ca^{2+} through VDCCs [44]. These effects of GLP-1 require β -cell glucose metabolism and result from simultaneous activation of PKA [44] and Epac [49].

Although GLP-1 exerts a small direct stimulatory action at VDCCs [39], the primary impetus for Ca^{2+} influx through these channels is the membrane depolarization that results from interactions of GLP-1 and glucose metabolism to inhibit K-ATP channel function (Fig. 1). This action of GLP-1 is complemented by its ability to inhibit K^+ efflux through voltage-dependent delayed rectifier K^+ channels (K_V), thereby slowing action potential repolarization (Fig. 1). Inhibition of K_V channels by GLP-1 requires not only PKA-mediated phosphorylation, but also EGF-R transactivation with concomitant stimulation of PI-3K and PKC- ζ [50].

The mobilization of intracellular Ca^{2+} by GLP-1 is a process of Ca^{2+} -induced Ca^{2+} release (CICR), and is initiated by the increase in $[\text{Ca}^{2+}]_i$ that results from Ca^{2+} influx through VDCCs (Fig. 2). GLP-1 most likely acts via PKA and Epac to sensitize ryanodine receptor (RYP) and IP_3 receptor ($\text{IP}_3\text{-R}$) intracellular Ca^{2+} release channels to stimulatory effects of Ca^{2+} , thereby gating the channels from a closed to open conformation [48,49,51–53]. A novel form of second messenger coincidence detection may exist in which a simultaneous increase in intracellular cAMP and Ca^{2+} concentrations allows for the appearance of CICR (Fig. 2).

Interestingly, the source of Ca^{2+} mobilized via CICR may reside not only in the endoplasmic reticulum (ER) [47,49], but also within the secretory granules (SG) [54]. Because RYP is expressed on the ER and SG membranes [55], CICR is expected to release Ca^{2+} from both cellular compartments (Fig. 2). What remains to be determined is exactly how GLP-1 modifies the function of Ca^{2+} release channels. Precedent exists for stimulatory actions of cAMP at RYP and $\text{IP}_3\text{-R}$ Ca^{2+} release channels, actions attributable to PKA-mediated phosphorylation [47,48,52,53]. Such an effect might be complemented by Epac-mediated actions of cAMP, either through direct interactions of Epac with the channels, or via PKA-independent phosphorylation of the channels [49,51].

Although Ca^{2+} influx through VDCCs is established to be a stimulus for insulin secretion, is also clear that exocytosis results from the release of Ca^{2+} from intracellular Ca^{2+} stores [56]. Ca^{2+} influx stimulates CICR when β -cells are exposed to glucose in the presence of GLP-1. Under these conditions, CICR generates a global increase in $[\text{Ca}^{2+}]_i$ that stimulates the exocytosis of a large number of secretory granules located at a considerable distance from VDCCs (Fig. 3). In the absence of GLP-1, glucose-dependent Ca^{2+} influx fails to stimulate CICR, so only a small number of secretory granules are released at “active zones” where the opening of VDCCs generates microdomains of elevated $[\text{Ca}^{2+}]_i$. These observations indicate that the spatial distribution of intracellular Ca^{2+} dictates the pattern of exocytosis observed during the feeding and fasting states (Fig. 3) [56].

GLP-1 Stimulates Mitochondrial ATP Production

Whereas the insulin secretagogue action of GLP-1 is blocked by mannoheptulose [57], a glycolysis inhibitor, it is supported by succinic acid dimethyl ester [58], a non-glucidic nutrient that becomes available for mitochondrial metabolism once deesterified. It may be concluded that events intimately associated with glycolytic and mitochondrial metabolism are necessary prerequisites for an effective β -cell secretory response to GLP-1. Confirmation that this is the case has been provided by new studies examining the stimulatory effect of GLP-1 on mitochondrial ATP concentration ($[\text{ATP}]_m$). These studies reveal a previously unrecognized ability of GLP-1 to increase $[\text{ATP}]_m$ in MIN6 insulin-secreting cells [29]. This action of GLP-1 requires exposure of cells to glucose, as expected if GLP-1 stimulates glucose-dependent mitochondrial ATP production. The stimulatory effect of GLP-1 on $[\text{ATP}]_m$ is accompanied by CICR, and is not observed when intracellular Ca^{2+} is buffered or when Ca^{2+} stores are depleted [29].

These findings are of interest from the perspective of current concepts regarding “metabolic priming” in the β -cell. Metabolic priming is a facilitation of mitochondrial ATP production, and it is observed under experimental conditions that produce an increase in $[\text{Ca}^{2+}]_i$ [59]. When β -cells are equilibrated in a low concentration of glucose, brief application of KCl produces Ca^{2+} influx, an increase in $[\text{Ca}^{2+}]_i$, and a slight increase in $[\text{ATP}]_m$. KCl “preconditioning” of this type produces metabolic priming since it allows for a larger increase in $[\text{ATP}]_m$ than normal when β -cells are subsequently exposed to a higher concentration of glucose [60]. These observations suggest that, as with KCl, GLP-1 might act via Ca^{2+} to prime a key step of mitochondrial metabolism important in glucose-dependent ATP production. Indeed, a stimulation of Krebs cycle and/or NADH shuttle-linked mitochondrial dehydrogenases by Ca^{2+} might explain how a Ca^{2+} -elevating hormone such as GLP-1 interacts with β -cell glucose metabolism to stimulate ATP production [61].

GLP-1 Inhibits K-ATP Channel Function

K-ATP channel activity in β -cells is reduced under conditions that produce a simultaneous increase in intracellular cAMP and Ca^{2+} concentrations [62]. Inhibition of K-ATP channels by cAMP-elevating agents is associated with increased levels of reduced pyridine nucleotides, as measured by the determination of NAD(P)H autofluorescence [63]. Reduced pyridine nucleotides accumulate as a consequence of oxidative glucose metabolism, suggesting a previously unrecognized interaction of cAMP and Ca^{2+} to stimulate mitochondrial ATP production and to increase the cytosolic $[\text{ATP}]/[\text{ADP}]$ concentration ratio while inhibiting K-ATP channel function. Taken together, such findings provide additional evidence for the existence of a novel form of second messenger coincidence detection critical to β -cell function. By mobilizing Ca^{2+} stores in a cAMP and Ca^{2+} -dependent manner, GLP-1 may generate a cytosolic Ca^{2+} signal (CICR) that is a stimulus for glucose-dependent ATP production and K-ATP channel inhibition.

Stimulatory effects of GLP-1 on mitochondrial ATP production are accompanied by alterations in K-ATP channel adenine nucleotide sensitivity. GLP-1 decreases the channel's sensitivity to ADP, thereby inhibiting channel function [30]. This action of GLP-1 is cAMP-dependent and is mediated by PKA. In contrast, GLP-1 acts independently of PKA to increase the channel's sensitivity to ATP, thereby closing the channel [31]. Although not yet confirmed, this PKA-independent action of GLP-1 might reflect its ability to activate Epac [38]. Such alterations of adenine nucleotide sensitivity are likely to play a major role in determining the effectiveness of mitochondrial ATP production as an inhibitor of K-ATP channel function. For example, Ca^{2+} -mobilizing transmitters that fail to stimulate cAMP production may also fail to influence K-ATP channel adenine nucleotide sensitivity; under these conditions, their stimulatory effect on mitochondrial ATP production may not be accompanied by a detectable decrease in K-ATP channel activity.

Conclusion: A “Feed-forward Hypothesis” for Regulated Insulin Secretion

Fig. 4 shows a model that seeks to explain how GLP-1 interacts with β -cell glucose metabolism to stimulate insulin secretion. The model predicts that metabolic priming within mitochondria plays a key role in allowing GLP-1 to upregulate glucose-dependent ATP production. Under conditions where intracellular Ca^{2+} release channels are sensitized by GLP-1, Ca^{2+} influx triggers CICR, generating a Ca^{2+} signal that stimulates the activity of mitochondrial dehydrogenases. This increases $[\text{ATP}]_m$ and increases the cytosolic $[\text{ATP}]/[\text{ADP}]$ concentration ratio. K-ATP channels close, the membrane is depolarized, and VDCCs are activated. Ca^{2+} influx through VDCCs stimulates exocytosis, while stimulating another round of CICR and ATP production. This sequence of events constitutes a self-perpetuating “feed-forward” mechanism that is responsible for the glucose-dependent

closure of K-ATP channels. Note that this action of GLP-1 is complemented by its ability to modulate the adenine nucleotide-sensitivity of K-ATP channels, thereby favoring their closure. Also note that the ability of GLP-1 to activate this feed-forward mechanism is dependent on the presence of glucose since glucose serves as the “fuel” for mitochondrial ATP production. This model agrees with the known ability of cAMP-elevating agents to inhibit single K-ATP channel activity, reduce the membrane conductance, and depolarize β -cells [27,64,65]. The model may also explain earlier reports that activators of cAMP signaling depolarize the silent phase of glucose-induced “bursts” of action potentials in intact islets [66–68]. Depolarization of this type augments oscillatory electrical activity that drives Ca^{2+} influx and which initiates pulsatile secretion of insulin.

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Fig. 1. The “triggering” and “amplification” pathways by which oxidative glucose metabolism stimulates exocytosis. Uptake of glucose is mediated by the type 2 facilitative glucose transporter (Glut2), and glucose is converted to glucose-6-phosphate (glucose-6-P) by glucokinase (GK). Glycolytic and mitochondrial respiration produces an increase in [ATP]/[ADP] concentration ratio that inhibits K-ATP channels. Membrane depolarization (ΔV_m), activates VDCCs, stimulates Ca^{2+} influx and initiates Ca^{2+} -dependent exocytosis (triggering pathway). The opening of voltage-dependent K⁺ channels (K_v) and Ca²⁺-activated K⁺ channels (K_{Ca}) terminates Ca^{2+} influx by repolarizing the membrane. Metabolism of glucose also generates coupling factors (malonyl CoA, LC-CoA, DAG) that facilitate Ca²⁺-dependent exocytosis (amplification pathway). GLP-1 potentiates glucose-dependent insulin secretion by virtue of its ability to stimulate cAMP production. The action of cAMP is mediated by PKA and Epac.

Fig. 2.

GLP-1 interacts with glucose metabolism to mobilize an intracellular source of Ca^{2+} . Glucose-dependent closure of K-ATP channels produces membrane depolarization (ΔV_m) and influx of Ca^{2+} through VDCCs. GLP-1 sensitizes ryanodine receptor (RYR) and IP_3 receptor (IP_3 -R) intracellular Ca^{2+} release channels to stimulatory effects of Ca^{2+} , thereby allowing Ca^{2+} influx to initiate Ca^{2+} -induced Ca^{2+} release (CICR) from Ca^{2+} stores located in the endoplasmic reticulum (ER) and secretory granules (SG). Uptake of Ca^{2+} into the ER and SG is mediated by the SERCA and ATP2C1 Ca^{2+} -ATPases, respectively. RYR and IP_3 -R Ca^{2+} release channels act as second messenger coincidence detectors because they open in response to a simultaneous increase in cAMP and Ca^{2+} concentrations.

Fasting State

Region In Which
Exocytosis Occurs

Low Blood [Glucose]

Low Blood [GLP-1]

Small Number Of Action Potentials

No CICR

**Exocytosis Of A Few
Secretory Granules**

Feeding State

Region In Which
Exocytosis Occurs

High Blood [Glucose]

High Blood [GLP-1]

Large Number Of Action Potentials

CICR Is Generated

**Exocytosis Of Many
Secretory Granules**

Fig. 3.

Contrasting mechanisms of exocytosis that occur during the fasting and feeding states. In the fasting state, the concentration of blood glucose is low and β -cells generate action potentials infrequently. Exocytosis occurs at active zones where a small number of secretory granules are located in close proximity to VDCC s. Since the level of GLP-1 in the blood is not elevated during the fasting state, RyR and $\text{IP}_3\text{-R}$ Ca^{2+} release channels are not sensitized, and no CICR is observed in response to Ca^{2+} influx. Under these conditions, the increase in $[\text{Ca}^{2+}]_i$ generated by influx of Ca^{2+} is spatially restricted and is unable to stimulate the release of secretory granules located outside of the active zones. Glucose and GLP-1 concentrations in the blood are elevated during the feeding state. Glucose and GLP-1 act synergistically to close K-ATP channels and to generate numerous action potentials. Simultaneously, GLP-1 sensitizes Ca^{2+} release channels to stimulatory effects of Ca^{2+} , thus allowing the appearance of CICR in response to Ca^{2+} influx. CICR generates a global increase in $[\text{Ca}^{2+}]_i$ and initiates the exocytosis of a large number of secretory granules located outside of the active zones.

"Feed-Forward Hypothesis" For Regulated Insulin Secretion

Fig. 4.

"Feed-forward" hypothesis for regulated insulin secretion. GLP-1 activates PKA and Epac by stimulating cAMP production. cAMP-dependent mobilization of intracellular Ca^{2+} via CICR generates a Ca^{2+} signal that stimulates exocytosis and which also interacts with glucose metabolism to stimulate mitochondrial ATP production. These mitochondria are strategically located at the plasma membrane in close proximity to K-ATP channels. Increased mitochondrial ATP production generates an increase in cytosolic $[\text{ATP}]/[\text{ADP}]$ concentration ratio, thus inhibiting K-ATP channels. Simultaneously, GLP-1 acts via PKA and Epac to decrease the sensitivity of K-ATP channels to ADP while increasing their sensitivity to ATP. Closure of K-ATP channels produces membrane depolarization and influx of Ca^{2+} through VDCCs. Entry of Ca^{2+} is a stimulus for exocytosis and it also initiates additional CICR because intracellular Ca^{2+} release channels are sensitized by GLP-1. A feed forward mechanism exists because CICR stimulates an additional round of ATP production, K-ATP channel closure, and Ca^{2+} -dependent exocytosis. Note that the ability of GLP-1 to stimulate insulin secretion is dependent on the presence of glucose because glucose serves as the "fuel" for mitochondrial ATP production.