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.

Key words

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 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 is-

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lets 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, Ca^{2+} 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 nucleototide-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 stim-

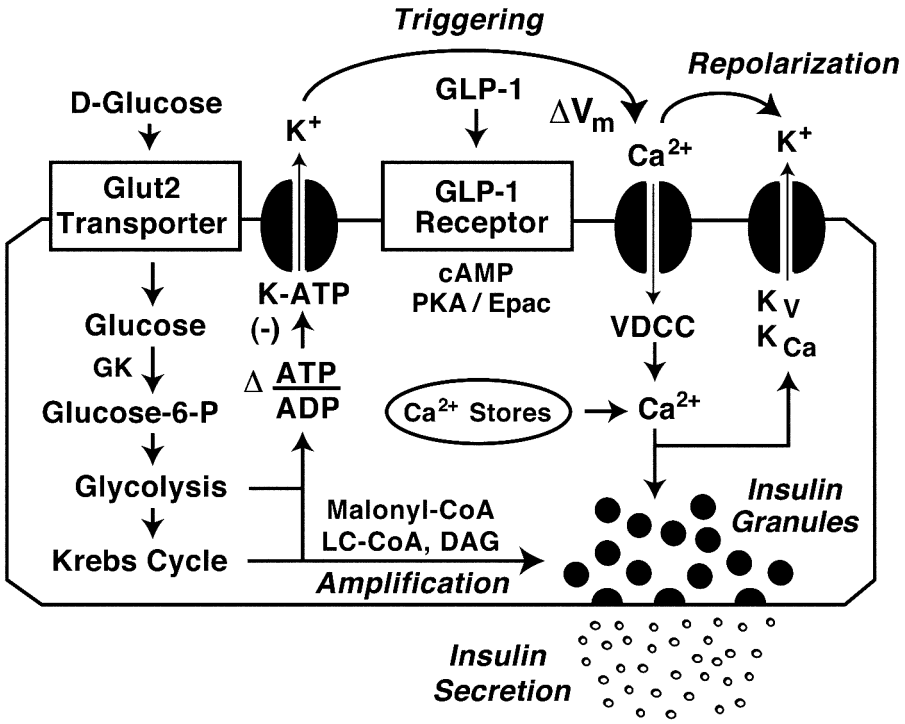

Fig. **1** The "triggering" and "amplification" pathways by which oxidative glucose metabolism stimulates exocytosis. Uptake of glucose is mediated by the type 2 facilitative glucose transporter (Glut2), and glucose is converted to glucose-6-phosphate (glucose-6-P) by glucokinase (GK). Glycolytic and 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 Ca^{2+}-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 Ca^{2+}-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.

ulates 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 $[Ca^{2+}]_i$ followed by a slowly developing and sustained increase [47]. The transient increase in $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$ that results from Ca^{2+} influx through VDCCs (Fig. **2**). GLP-1 most likely acts via PKA and Epac to sensitize ryanodine receptor (RYR) and IP_3 receptor (IP_3-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 RYR 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 RYR and IP_3-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 $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$. 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 prerequi-

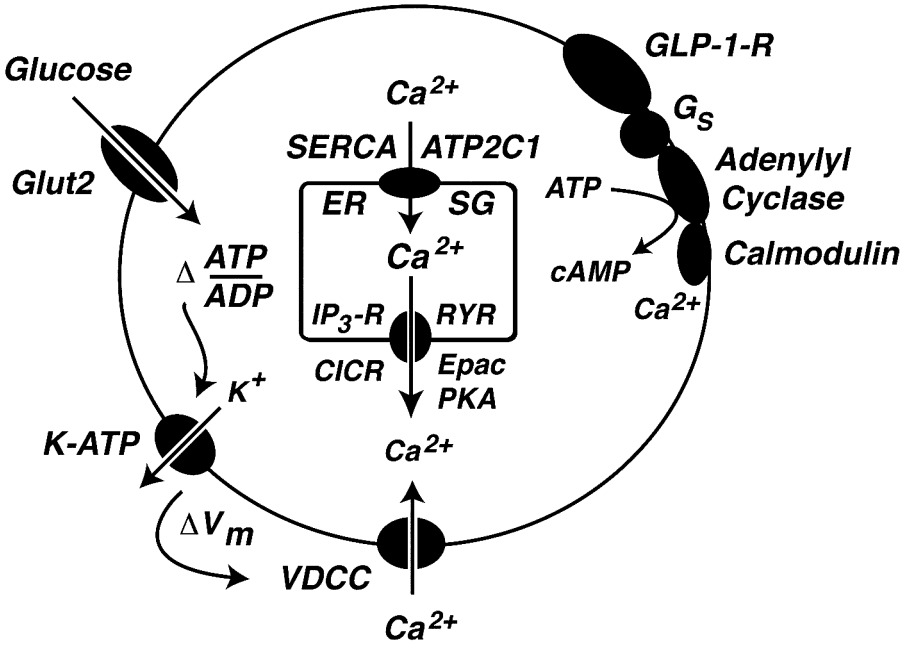

Fig. **2** GLP-1 interacts with glucose metabolism to mobilize an intracellular source of Ca^{2+}. Glucose-dependent closure of K-ATP channels produces membrane depolarization (ΔV_m) and influx of Ca^{2+} through VDCCs. GLP-1 sensitizes ryanodine receptor (RYR) and IP_3 receptor (IP_3-R) intracellular 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.

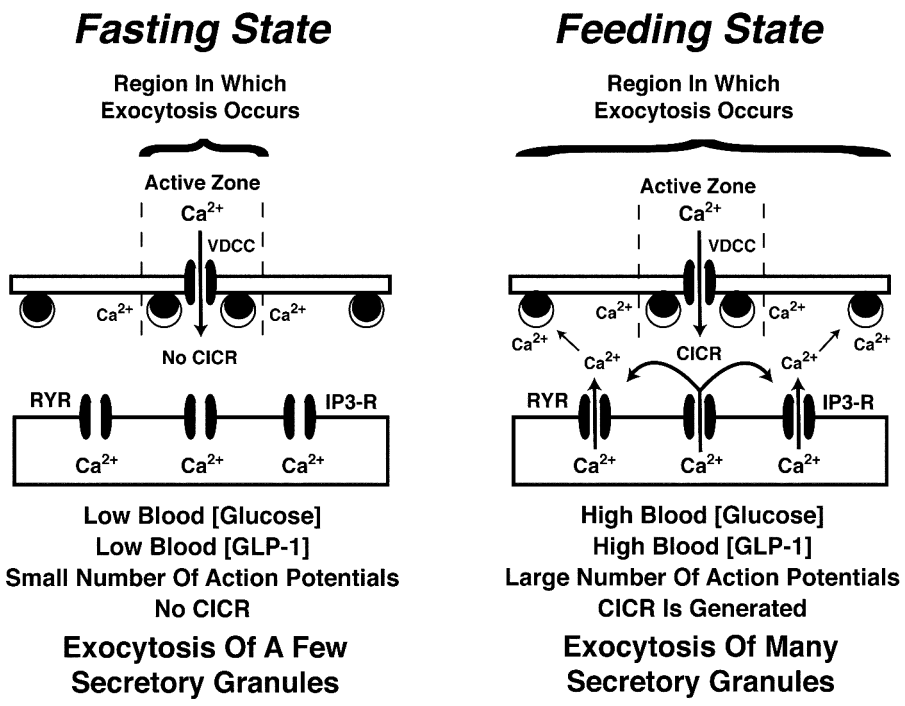

Fig. **3** Contrasting mechanisms of exocytosis that occur during the fasting and feeding states. In the fasting state, the concentration of blood glucose is low and β-cells generate action potentials infrequently. Exocytosis occurs at active zones where a small number of secretory granules are located in close proximity to VDCCs. Since the level of GLP-1 in the blood is not elevated during the fasting state, RYR and IP_3-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 $[Ca^{2+}]_i$ 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 $[Ca^{2+}]$ and initiates the exocytosis of a large number of secretory granules located outside of the active zones.

sites 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 ($[ATP]_m$). These studies reveal a previously unrecognized ability of GLP-1 to increase $[ATP]_m$ 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 $[ATP]_m$ 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 $[Ca^{2+}]_i$ [59]. When β-cells are equilibrated in a low concentration of glucose, brief application of KCl produces Ca^{2+} influx, an increase in $[Ca^{2+}]_i$, and a slight increase in $[ATP]_m$. KCl "preconditioning" of this type produces metabolic priming since it allows for a larger increase in $[ATP]_m$ 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 [ATP]/[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

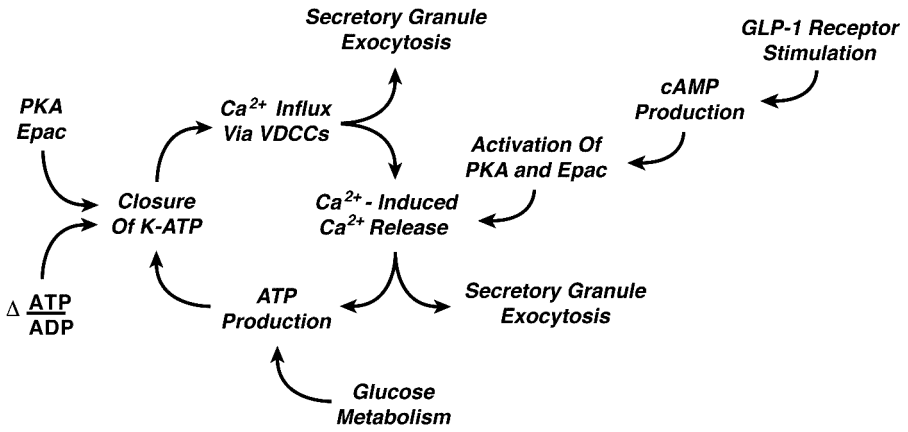

Fig. **4** "Feed-forward" hypothesis for regulated insulin secretion. GLP-1 activates PKA and Epac by stimulating cAMP production. cAMP-dependent mobilization of intracellular Ca^{2+} via CICR generates a Ca^{2+} signal that stimulates exocytosis and which also interacts with glucose metabolism to stimulate 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 [ATP]/[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.

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 $[ATP]_m$ and increases the cytosolic [ATP]/[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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