

# New Insights Concerning the Glucose-dependent Insulin Secretagogue Action of Glucagon-like Peptide-1 in Pancreatic $\beta$ -Cells

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Original

## Abstract

The GLP-1 receptor is a Class B heptahelical G-protein-coupled receptor that stimulates cAMP production in pancreatic  $\beta$ -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  $\text{Ca}^{2+}$ -dependent exocytosis. Simultaneously, GLP-1 promotes  $\text{Ca}^{2+}$  influx and mobilizes an intracellular source of  $\text{Ca}^{2+}$ . GLP-1 sensitizes intracellular  $\text{Ca}^{2+}$  release channels (ryanodine and  $\text{IP}_3$  receptors) to stimulatory effects of  $\text{Ca}^{2+}$ , thereby promoting  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  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  $\text{K}^+$  channels (K-ATP), membrane depolarization, and influx of  $\text{Ca}^{2+}$  through voltage-dependent  $\text{Ca}^{2+}$  channels (VDCCs).  $\text{Ca}^{2+}$  influx stimulates exocytosis of secretory granules by promoting their fusion with the plasma membrane. Under conditions where  $\text{Ca}^{2+}$  release channels are sensitized by GLP-1,  $\text{Ca}^{2+}$  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  $\beta$ -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

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## 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  $\beta$ -cell. Binding of GLP-1 to its 62 kDa Class B heptahelical  $\text{G}_s$ -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 aug-

mented [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  $\beta$ -cells within the is-

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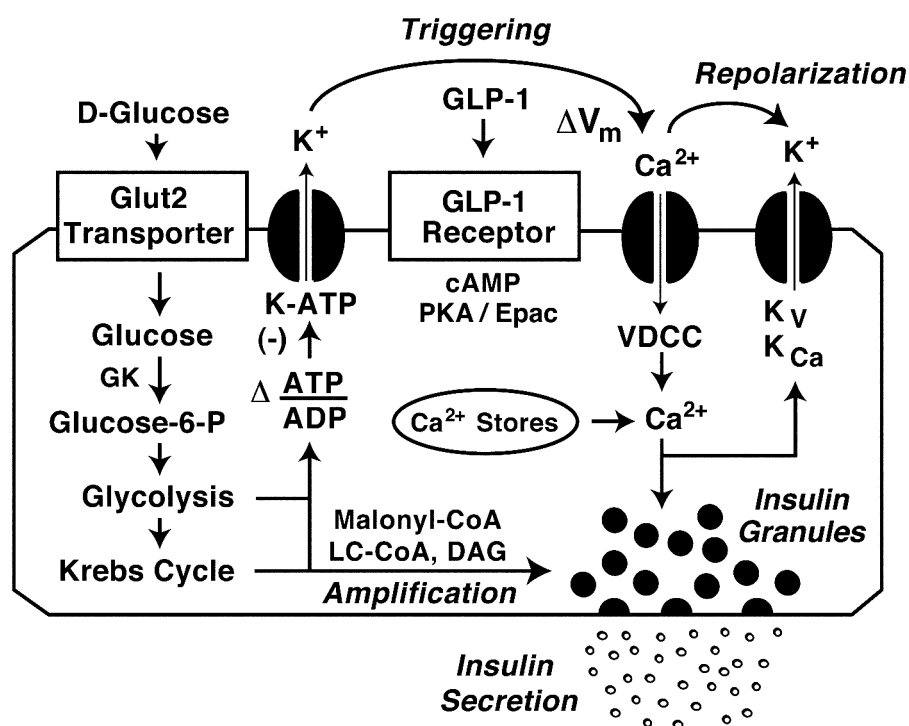


Fig. 1 The “triggering” and “amplification” pathways by which oxidative glucose metabolism stimulates exocytosis. Uptake of glucose is mediated by the type 2 facilitative glucose transporter (Glut2), and glucose is converted to glucose-6-phosphate (glucose-6-P) by glucokinase (GK). Glycolytic and mitochondrial respiration produces an increase in [ATP]/[ADP] concentration ratio that inhibits K-ATP channels. Membrane depolarization ( $\Delta V_m$ ), activates VDCCs, stimulates  $Ca^{2+}$  influx and initiates  $Ca^{2+}$ -dependent exocytosis (triggering pathway). The opening of voltage-dependent  $Ca^{2+}$  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  $\beta$ -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  $\beta$ -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- $\epsilon$ , 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 $\text{Ca}^{2+}$ Influx and Mobilizes an Intracellular Source of $\text{Ca}^{2+}$

GLP-1 exerts pronounced stimulatory effects on  $\beta$ -cell  $\text{Ca}^{2+}$  signaling [44–46], actions that underlie its ability to promote  $\text{Ca}^{2+}$ -dependent exocytosis of secretory granules. Exposure of  $\beta$ -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  $\text{Ca}^{2+}$  from intracellular  $\text{Ca}^{2+}$  stores [47,48], whereas the sustained increase results from influx of  $\text{Ca}^{2+}$  through VDCCs [44]. These effects of GLP-1 require  $\beta$ -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  $\text{Ca}^{2+}$  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  $\text{K}^+$  efflux through voltage-dependent delayed rectifier  $\text{K}^+$  channels ( $\text{K}_V$ ), thereby slowing action potential repolarization (Fig. 1). Inhibition of  $\text{K}_V$  channels by GLP-1 requires not only PKA-mediated phosphorylation, but also EGF-R transactivation with concomitant stimulation of PI-3K and PKC- $\zeta$  [50].

The mobilization of intracellular  $\text{Ca}^{2+}$  by GLP-1 is a process of  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release (CICR), and is initiated by the increase in  $[\text{Ca}^{2+}]_i$  that results from  $\text{Ca}^{2+}$  influx through VDCCs (Fig. 2). GLP-1 most likely acts via PKA and Epac to sensitize ryanodine receptor (RyR) and  $\text{IP}_3$  receptor ( $\text{IP}_3$ -R) intracellular  $\text{Ca}^{2+}$  release channels to stimulatory effects of  $\text{Ca}^{2+}$ , 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  $\text{Ca}^{2+}$  concentrations allows for the appearance of CICR (Fig. 2).

Interestingly, the source of  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  from both cellular compartments (Fig. 2). What remains to be determined is exactly how GLP-1 modifies the function of  $\text{Ca}^{2+}$  release channels. Precedent exists for stimulatory actions of cAMP at RyR and  $\text{IP}_3$ -R  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  influx through VDCCs is established to be a stimulus for insulin secretion, is also clear that exocytosis results from the release of  $\text{Ca}^{2+}$  from intracellular  $\text{Ca}^{2+}$  stores [56].  $\text{Ca}^{2+}$  influx stimulates CICR when  $\beta$ -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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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-

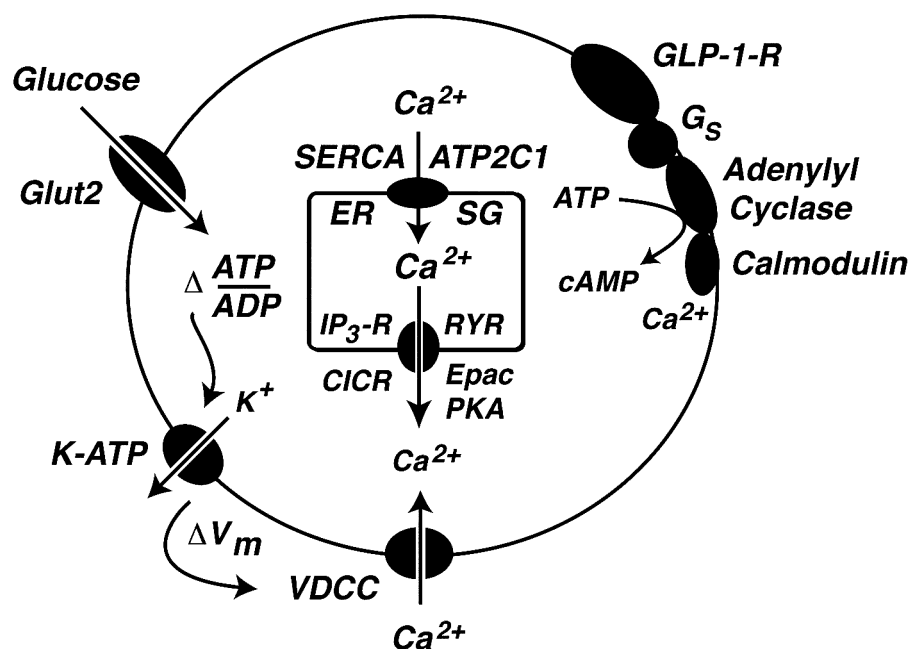
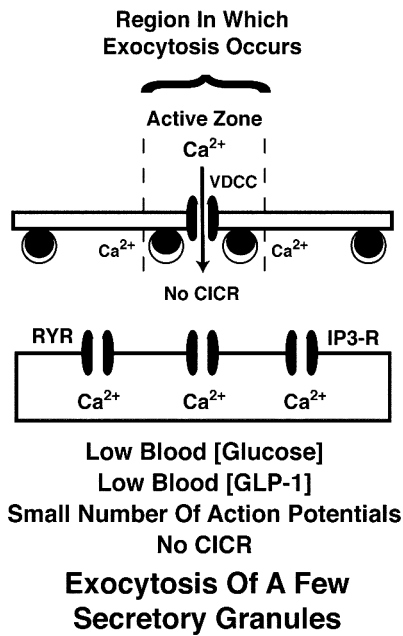
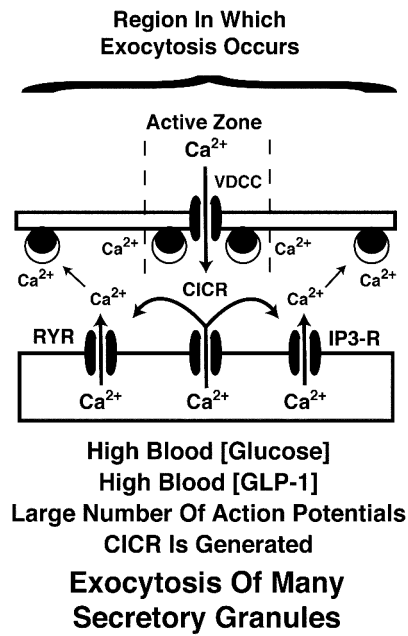


Fig. 2 GLP-1 interacts with glucose metabolism to mobilize an intracellular source of  $\text{Ca}^{2+}$ . Glucose-dependent closure of K-ATP channels produces membrane depolarization ( $\Delta V_m$ ) and influx of  $\text{Ca}^{2+}$  through VDCCs. GLP-1 sensitizes ryanodine receptor (RyR) and  $\text{IP}_3$  receptor ( $\text{IP}_3$ -R) intracellular  $\text{Ca}^{2+}$  release channels to stimulatory effects of  $\text{Ca}^{2+}$ , thereby allowing  $\text{Ca}^{2+}$  influx to initiate  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release (CICR) from  $\text{Ca}^{2+}$  stores located in the endoplasmic reticulum (ER) and secretory granules (SG). Uptake of  $\text{Ca}^{2+}$  into the ER and SG is mediated by the SERCA and ATP2C1  $\text{Ca}^{2+}$ -ATPases, respectively. RyR and  $\text{IP}_3$ -R  $\text{Ca}^{2+}$  release channels act as second messenger coincidence detectors because they open in response to a simultaneous increase in cAMP and  $\text{Ca}^{2+}$  concentrations.

## Fasting State



## Feeding State



**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  $\beta$ -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<sub>3</sub>-R Ca<sup>2+</sup> release channels are not sensitized, and no CICR is observed in response to Ca<sup>2+</sup> influx. Under these conditions, the increase in [Ca<sup>2+</sup>]<sub>i</sub> generated by influx of Ca<sup>2+</sup> 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<sup>2+</sup> release channels to stimulatory effects of Ca<sup>2+</sup>, thus allowing the appearance of CICR in response to Ca<sup>2+</sup> influx. CICR generates a global increase in [Ca<sup>2+</sup>]<sub>i</sub> and initiates the exocytosis of a large number of secretory granules located outside of the active zones.

sites for an effective  $\beta$ -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]<sub>m</sub>). These studies reveal a previously unrecognized ability of GLP-1 to increase [ATP]<sub>m</sub> 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]<sub>m</sub> is accompanied by CICR, and is not observed when intracellular Ca<sup>2+</sup> is buffered or when Ca<sup>2+</sup> stores are depleted [29].

These findings are of interest from the perspective of current concepts regarding “metabolic priming” in the  $\beta$ -cell. Metabolic priming is a facilitation of mitochondrial ATP production, and it is observed under experimental conditions that produce an increase in [Ca<sup>2+</sup>]<sub>i</sub> [59]. When  $\beta$ -cells are equilibrated in a low concentration of glucose, brief application of KCl produces Ca<sup>2+</sup> influx, an increase in [Ca<sup>2+</sup>]<sub>i</sub>, and a slight increase in [ATP]<sub>m</sub>. KCl “preconditioning” of this type produces metabolic priming since it allows for a larger increase in [ATP]<sub>m</sub> than normal when  $\beta$ -cells are subsequently exposed to a higher concentration of glucose [60]. These observations suggest that, as with KCl, GLP-1 might act via Ca<sup>2+</sup> 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<sup>2+</sup> might explain how a Ca<sup>2+</sup>-elevating hormone such as GLP-1 interacts with  $\beta$ -cell glucose metabolism to stimulate ATP production [61].

### GLP-1 Inhibits K-ATP Channel Function

K-ATP channel activity in  $\beta$ -cells is reduced under conditions that produce a simultaneous increase in intracellular cAMP and Ca<sup>2+</sup> 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<sup>2+</sup> 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  $\beta$ -cell function. By mobilizing Ca<sup>2+</sup> stores in a cAMP and Ca<sup>2+</sup>-dependent manner, GLP-1 may generate a cytosolic Ca<sup>2+</sup> 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<sup>2+</sup>-mobilizing transmitters that fail to stimulate cAMP production may also fail to influence K-ATP channel adenine nucleotide sensitivity; under



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