Molecular Basis of cAMP Signaling in Pancreatic β Cells

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George G. Holz, Oleg G. Chepurny, Colin A. Leech, Woo-Jin Song, and Mehboob A. Hussain

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G.G. Holz (✉)
Departments of Medicine and Pharmacology, SUNY Upstate Medical University, Syracuse, NY, USA
e-mail: holzg@upstate.edu

O.G. Chepurny • C.A. Leech
Department of Medicine, SUNY Upstate Medical University, Syracuse, NY, USA
e-mail: chepurno@upstate.edu; leechc@upstate.edu

W.-J. Song
Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD, USA
e-mail: wsong10@jhmi.edu; jinysong@yahoo.com

M.A. Hussain
Departments of Pediatrics, Medicine, and Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD, USA
e-mail: mhussai4@jhmi.edu

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Abstract

Recent advances in conditional gene targeting and cyclic nucleotide research further our understanding of how the incretin hormone GLP-1 exerts a therapeutically important action to restore pancreatic insulin secretion in patients with type 2 diabetes mellitus (T2DM). These studies demonstrate that the pancreatic β-cell GLP-1 receptor has the capacity to signal through two distinct branches of the adenosine 3′,5′-cyclic monophosphate (cAMP) signal transduction network; one branch activates protein kinase A (PKA), and the second engages a cAMP-regulated guanine nucleotide exchange factor designated as Epac2. Under normal dietary conditions, specific activation of the cAMP-PKA branch in mice dramatically augments glucose-stimulated insulin secretion (GSIS). However, under conditions of diet-induced insulin resistance, cAMP-Epac2 signaling in the control of GSIS becomes prominent. This chapter provides an update on GLP-1 receptor signaling in the islets of Langerhans, with special emphasis on key molecular events that confer "plasticity" in the β-cell cAMP signal transduction network. The reader is reminded that an excellent review of β-cell cAMP signaling can also be found in the prior first edition of this book.

Keywords

Cyclic AMP • Protein kinase A • Epac2 • GLP-1 • Diabetes

Introduction

The cytosolic second messenger cAMP is a key activating signaling molecule supporting insulin exocytosis from pancreatic β-cells located in the islets of Langerhans (Holz 2004a; Leech et al. 2010a; Tengholm 2012). cAMP exerts its insulin secretagogue actions by binding to and activating either protein kinase A (PKA, a serine/threonine protein kinase) or Epac2 (a guanine nucleotide exchange factor which in turn activates Rap1 GTPase). cAMP modulates insulin exocytosis so that it potentiates glucose-stimulated insulin secretion (GSIS) from the β-cells (Holz and Habener 1992). As illustrated in Figs. 1 and 2, the downstream targets of PKA, Epac2, and glucose that are relevant to insulin secretion include proteins that control β-cell membrane excitability (ATP-sensitive K^+ channels, K_{ATP}), Ca^{2+} influx (voltage-dependent Ca^{2+} channels, VDCCs; nonselective cation channels, NSCCs), intracellular Ca^{2+} mobilization (IP_3 receptors, IP_3R; ryanodine receptors, RYR), as well as secretory granule and SNARE complex-associated proteins that promote Ca^{2+}-dependent exocytosis of insulin (syntaxin, SNAP-25, VAMP2, RIM2, Piccolo, Munc13-1) (Seino and Shibasaki 2005; Holz et al. 2006; Kwan and Gaisano 2007; Seino et al. 2009; Vikman et al. 2009; Leech et al. 2011; Song et al. 2011, 2013; Hussain et al. 2012; Kasai et al. 2012).

cAMP biosynthesis in β-cells is catalyzed by transmembrane adenylyl cyclases (TMACs) that use ATP as a substrate in order to generate cAMP, and it is

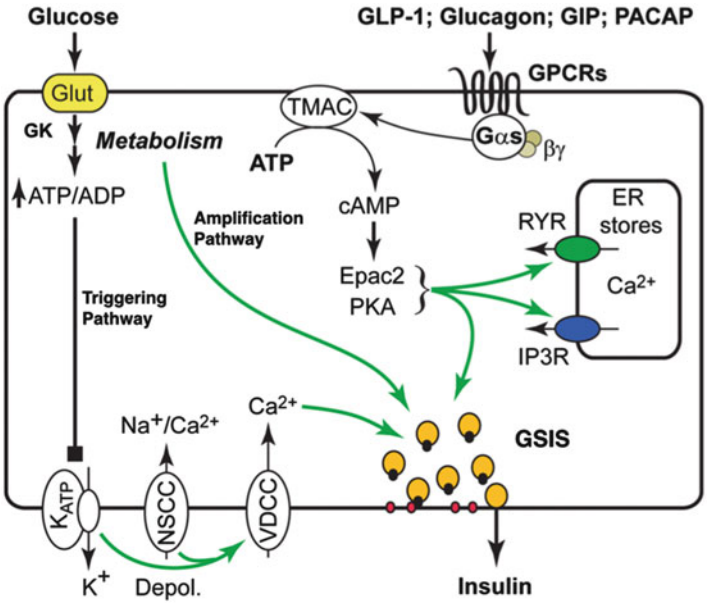

Fig. 1 β-cell GPCR activation by GLP-1, glucagon, GIP, or PACAP results in cAMP production catalyzed by TMACs. cAMP activates Epac2 and PKA in order to potentiate glucose-stimulated insulin secretion (*GSIS*). Glucose sensing by the β-cell requires glucose uptake mediated by glucose transporters (*Glut*), whereas cytosolic glucokinase (*GK*) acts as the rate-limiting enzyme for oxidative glucose metabolism. A triggering pathway for GSIS involves K_{ATP} channel closure, membrane depolarization (*Depol.*), and Ca^{2+} influx that occurs in response to the increase of cytosolic ATP/ADP concentration ratio that glucose metabolism produces. Ca^{2+} triggers exocytosis of insulin, and this action of Ca^{2+} is enhanced by PKA. Activation of Epac2 facilitates glucose-dependent closure of K_{ATP} channels, thereby sensitizing β-cells to the stimulatory effect of glucose. Thus, GLP-1 is a β-cell glucose sensitizer. Note that the Ca^{2+} signal important to exocytosis is generated by Ca^{2+} entry through VDCCs or by the mobilization of Ca^{2+} from intracellular Ca^{2+} stores. Intracellular Ca^{2+} release channels (*IP_3R*, *RYR*) located on the ER are targets of PKA and Epac2, thereby allowing cAMP to facilitate glucose-dependent release of Ca^{2+} from the ER. Nonselective Ca^{2+} channels (*NSCC*) activated in response to ER Ca^{2+} mobilization generate a depolarizing inward Na^+/Ca^{2+} current in order to increase β-cell excitability. Resultant action potential generation leads to additional Ca^{2+} influx and insulin exocytosis. These established mechanisms of "triggered" insulin secretion are reinforced by a K_{ATP} channel-independent amplification pathway. Although less well understood, it couples glucose metabolism to the recruitment of secretory granules to the plasma membrane where they undergo exocytosis in response to Ca^{2+}

terminated by cyclic nucleotide phosphodiesterases (PDEs) that hydrolyze cAMP to 5′-AMP (Furman et al. 2010). Since cAMP-elevating agents have little or no insulin secretagogue action in the absence of glucose, and since insulin secretion can be stimulated by glucose in the absence of cAMP-elevating agents, it is generally accepted that the primary stimulus for insulin secretion is glucose, whereas cAMP acts to potentiate GSIS from β-cells (Henquin 2000).

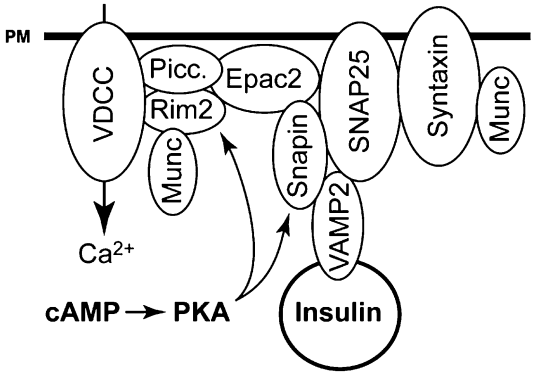

Fig. 2 SNARE complex and secretory granule-associated proteins interact in order to mediate the action of cAMP to potentiate GSIS. These proteins include syntaxin and SNAP-25 located on the plasma membrane (*PM*) and VAMP2 located on the secretory granules. High concentrations of glucose promote the interaction of SNAP-25 with VAMP2, and this interaction is enhanced under conditions in which PKA and Epac2 are activated. Snapin is a substrate for PKA, and its phosphorylation on Ser-50 facilitates its interactions with SNAP-25 and Epac2. Direct binding of cAMP to Epac2 promotes its interaction with SNAP-25. When both PKA and Epac2 are activated, SNARE complex assembly is enhanced so that insulin exocytosis may occur in response to depolarization-induced entry of Ca^{2+} through VDCCs. Rim2 (Rab3-interacting molecule2), Piccolo (a Ca^{2+} sensor), and Munc (a PKA substrate) are Epac2-interacting proteins that also participate in the cAMP-dependent control of insulin secretion

Pharmacological agents that increase levels of β-cell cAMP in order to potentiate GSIS include stimulators of TMAC activity (forskolin, cholera toxin, pertussis toxin) or inhibitors of PDE activity (IBMX) (Holz et al. 2000; Pyne and Furman 2003). The incretin hormone glucagon-like peptide-1 (GLP-1) acting at the β-cell GLP-1 receptor (GLP-1R) stimulates TMACs in order to potentiate GSIS (Thorens 1992; Mojsov et al. 1987; Orskov et al. 1988; Gromada et al. 1998b; Holz 2004b), whereas neurotransmitters such as galanin and norepinephrine inhibit TMACs to inhibit insulin secretion (Sharp 1996; Straub and Sharp 2012). The hormone leptin acting via the β-cell Ob-Rb receptor stimulates PDE isoform 3B (PDE3B) in order to inhibit insulin secretion (Zhao et al. 1998; Emilsson et al. 1997; Kieffer et al. 1997; Kulkarni et al. 1997), and a targeted knockout (KO) of Ob-Rb in β-cells of mice leads to a marked enhancement of GSIS (Morioka et al. 2012).

Class II GTP-binding protein-coupled receptors (GPCRs) expressed on β-cells are coupled to cAMP production (Winzell and Ahrén 2007; Ahrén 2009; Couvineau and Laburthe 2012), and they bind GLP-1, glucagon, glucose-dependent insulinotropic peptide (GIP), and pituitary adenylyl cyclase-activating polypeptide (PACAP). The Gila monster lizard *Heloderma* is the source of GLP-1R agonist exendin-4, and its fragment exendin-(9–39) is a GLP-1R antagonist that inhibits cAMP production and insulin secretion (De Leon et al. 2008). Unexpectedly, Class II GPCRs are structurally related to CIRL (the Ca^{2+}-independent receptor for α-latrotoxin), whereas GLP-1 shares structural homology with α-latrotoxin, a venom derived from the black widow spider *Latrodectus*. These findings have

prompted efforts to develop chimeric peptides that are comprised of amino acid sequences found in both GLP-1 and α-latrotoxin. For example, human islet insulin secretion is stimulated by one such peptide designated as black widow GLP-1 (Holz and Habener 1998).

GPR119 is a Class I GPCR that mediates stimulatory effects of 2-oleoyl glycerol, lysophosphatidylcholine, and fatty acid amides (e.g., oleoylethanolamide; OEA) on cAMP production and β-cell insulin secretion (Soga et al. 2005; Overton et al. 2008; Chu et al. 2007; Hansen et al. 2011). Synthetic small molecules that activate GPR119 (e.g., AR231453) are orally administrable and are currently under investigation for use in the treatment of T2DM (Jones et al. 2009; Shah and Kowalski 2010; Hansen et al. 2012). The potential usefulness of GPR119 agonists for this purpose is emphasized by the fact that they also stimulate intestinal GLP-1 release (Chu et al. 2008; Lan et al. 2009, 2012; Hansen et al. 2011).

Drug discovery efforts have yielded β-cell cAMP-elevating GLP-1R agonists such as exenatide and liraglutide that mimic the action of GLP-1 to lower levels of blood glucose in patients with T2DM (Gutniak et al. 1992; Nathan et al. 1992; Drucker and Nauck 2006; Lovshin and Drucker 2009). In contrast to endogenous GLP-1, these GLP-1R agonists are resistant to degradation by dipeptidyl peptidase-IV (DPP-IV) (Kieffer et al. 1995). Thus, they have an extended duration of action when administered by subcutaneous injection.

Orally administrable DPP-IV inhibitors such as sitagliptin and vildagliptin also exert insulin secretagogue and blood glucose-lowering actions in patients with T2DM (Drucker and Nauck 2006; Karagiannis et al. 2012). These agents delay metabolic degradation of GLP-1, thereby enabling endogenously secreted GLP-1 to more effectively raise levels of cAMP in β-cells (Dalle et al. 2013). Current drug discovery efforts seek to broaden the base of GLP-1R-targeted therapeutics by developing an orally administrable form of GLP-1 that is a conjugate of vitamin B_{12} (Clardy-James et al. 2013). B_{12}-GLP-1 exploits the vitamin B_{12} uptake system that utilizes intrinsic factor (IF) in order to achieve intestinal absorption of the conjugate.

Non-peptide orally administrable GLP-1R agonists may also broaden the incretin-based therapeutic armamentarium. This approach might yield GLP-1R agonists that allosterically activate the receptor by binding to sites on the receptor that are not identical to sites that bind GLP-1 (Koole et al. 2010). Further, such small molecules may be designed to activate the receptor in a manner that "biases" its signal transduction properties. Thus, GLP-1R agonists might be tailored to selectively activate either the cAMP signaling mechanism or growth factor signaling mechanisms important to β-cell function (Koole et al. 2010).

The safety profile of GLP-1R agonists and DPP-IV inhibitors is recently questioned in reports that link their use in humans to an increased incidence of pancreatitis, exocrine pancreas dysplasia, and islet α-cell hyperplasia (Butler et al. 2013; Singh et al. 2013). These findings need to be substantiated, but it is of interest that prior in vivo studies of rodents or in vitro studies of human islets demonstrate that GLP-1R agonists enhance β-cell neogenesis, proliferation, and survival (Xu et al. 1999; Tourrel et al. 2001; Li et al. 2003; Farilla et al. 2003).

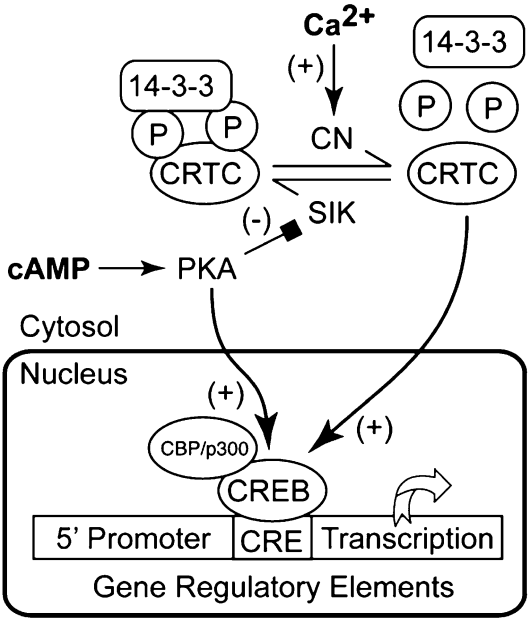

Fig. 3 cAMP-stimulated gene expression in β-cells results from PKA holoenzyme activation with consequent translocation of PKA catalytic subunits to the nucleus where PKA phosphorylates CREB on Ser-133. Histone acetyltransferases p300 and CBP are transcriptional co-activators that enhance binding of Ser-133-CREB to cAMP response elements (*CRE*) located in 5′ gene promoter sequences. Ser-133-CREB binding to CREs is also enhanced by cAMP-regulated transcriptional co-activators (*CRTC*). At low glucose and in the absence of cAMP-elevating agents, CRTC is phosphorylated by salt-inducible kinase (*SIK*) to promote its association with 14-3-3 proteins, thereby sequestering CRTC in the cytoplasm. High glucose stimulates an increase of $[Ca^{2+}]_i$ that activates the phosphatase calcineurin (*CN*) in order to dephosphorylate CRTC, whereas cAMP-elevating agents act via PKA to inhibit SIK activity in order to slow phosphorylation of CRTC. The net effect is that dephosphorylated CRTC dissociates from 14-3-3 proteins so that it may translocate to the nucleus, bind Ser-133-CREB, and co-activate transcription. Note that CRTC is a cAMP and Ca^{2+} coincidence detector important to β-cell gene expression

In rodents, GLP-1R agonists produce an increase of β-cell mass, but it is not clear if such a potentially beneficial effect occurs in patients with T2DM (Friedrichsen et al. 2006; Song et al. 2008; Lavine and Attie 2010; Tschen et al. 2011).

When considering how GLP-1R agonists act as β-cell trophic factors, there is evidence for an insulinotropic action at the transcriptional level that is either PKA dependent (Drucker et al. 1987) or PKA independent (Skoglund et al. 2000; Chepurny et al. 2002). The PKA-dependent action of GLP-1R agonists is mediated by cAMP response elements (CREs) located in the human insulin gene (Hay et al. 2005). As illustrated in Fig. 3, CREs bind the cAMP response element-binding protein (CREB), a basic region leucine zipper transcription factor (bZIP) that is regulated by PKA and co-activators p300 and CRTC in β-cells (Altarejos and Montminy 2011; Dalle et al. 2011b). These CREs also bind bZIPs that mediate

PKA-independent actions of GLP-1R agonists, and in this regard the insulinotropic action of GLP-1 is sensitive to Ro 318220, a serine/threonine protein kinase inhibitor that inhibits MAPK-activated kinases (RSKs) and mitogen/stress-activated kinases (MSK) that serve as CREB kinases (Chepurny et al. 2002). Transcriptional activation of insulin gene expression by GLP-1 is also accompanied by GLP-1-stimulated translational biosynthesis of proinsulin (Fehmann and Habener 1992).

PKA-mediated induction of insulin receptor substrate-2 (IRS-2) expression promotes β-cell growth in response to GLP-1 (Jhala et al. 2003; Park et al. 2006), and studies of β-cell lines or neonatal β-cells indicate that PKA also mediates transcriptional induction of cyclin D1 by GLP-1 in order to stimulate proliferation (Kim et al. 2006; Friedrichsen et al. 2006). Furthermore, a proliferative action of GLP-1 results from PKA-mediated phosphorylation of β-catenin, thereby indicating that the β-cell cAMP-PKA signaling branch exhibits signal transduction cross talk with a noncanonical Wnt signaling pathway that uses the transcription factor TCF7L2 to control gene expression (Liu and Habener 2008). PKA also mediates the action of GLP-1 to promote nuclear localization of transcription factor PDX-1, thereby enhancing the differentiated state of β-cells (Wang et al. 2001). SAD-A kinase is reported to be under the control of PKA in order for cAMP to stimulate insulin secretion (Nie et al. 2013).

A surprising finding is that a truncated GLP-1 designated as GLP-1(28–36) amide stimulates cAMP production in β-cells, thereby activating the β-catenin/TCF7L2 signaling pathway (Shao et al. 2013). Furthermore, GLP-1(28–36)amide protects against β-cell glucotoxicity by improving mitochondrial function (Liu et al. 2012). GLP-1(28–36)amide is a cell-penetrating peptide that does not exert its effects by binding to the GLP-1R, but instead acts intracellularly. Thus, it is not clear how GLP-1(28–36) amide stimulates cAMP production.

Since there is evidence that the β-cell GLP-1R signals through cAMP sensor Epac2, the possibility exists that this cAMP-regulated guanine nucleotide exchange factor participates not only in the control of insulin secretion but also β-cell growth. However, recent studies demonstrate that β-cell mass is preserved in mice with a whole-body knockout (KO) of Epac2 gene expression (Song et al. 2013). Still, additional findings demonstrate a role for Epac2 in the protection of β-cells from cytotoxicity induced by reactive oxygen species (ROS) (Mukai et al. 2011). Redox control in β-cells is under the control of thioredoxin (TxN), and TxNIPs are thioredoxin-interacting proteins that downregulate the ROS buffering capacity of thioredoxin. Thus, it is significant that GLP-1 acts via Epac2 to suppress TxNIP expression in β-cells (Shao et al. 2010).

cAMP-independent actions of GLP-1 exist, and they are also of significance when considering how GLP-1 maintains β-cells in a healthy state (Holz and Chepurny 2005). Such actions include the ability of GLP-1R agonists to counteract endoplasmic reticulum stress (Yusta et al. 2006) and to signal via the GLP-1R through β-arrestin (Sonoda et al. 2008; Dalle et al. 2011a) and epidermal growth factor (EGF) receptor transactivation (Buteau et al. 2003) in order to downregulate the activities of proapoptotic protein Bad (Quoyer et al. 2010), the SirT1

deacetylase (Bastien-Dionne et al. 2011), and transcription factor FoxO1 (Buteau et al. 2006). GLP-1 also upregulates the activities of c-Src kinase (Talbot et al. 2012), phosphatidylinositol 3-kinase (PI-3-kinase) (Buteau et al. 1999), protein kinase B (PKB) (Wang et al. 2004), protein kinase c-ζ (PKCζ) (Buteau et al. 2001), and extracellular signal-regulated protein kinases (ERK1/2) (Arnette et al. 2003). As alluded to above, it may be possible to develop allosteric GLP-1R agonists with biased signaling properties that preferentially activate these various signaling pathways.

In Vivo Actions Of cAMP-Elevating Agents In Humans

GLP-1 and GIP are released from enteroendocrine L-cells and K-cells, respectively (Kieffer and Habener 1999; Baggio and Drucker 2007; Holst 2007; McIntosh et al. 2010). These cells are located in the intestinal wall where they act as nutrient sensors such that nutrient ingestion stimulates the release of GLP-1 and GIP into the systemic circulation. During the postprandial increase of blood glucose concentration, released GLP-1 and GIP potentiate GSIS from β-cells. Thus, GLP-1 and GIP mediate the "incretin effect" whereby gut-derived signals synergize with intestinally absorbed glucose to potentiate insulin secretion (Creutzfeldt 2005). In patients with T2DM that undergo Roux-en-Y gastric bypass surgery (RYGB), an improvement of β-cell function and glucose tolerance is observed, and these beneficial effects are related to an exaggerated release of GLP-1 from L-cells (Jorgensen et al. 2013).

It is especially interesting that T2DM can be treated with GLP-1R agonists, whereas GIP receptor agonists are ineffective (Nauck et al. 1993). Why this is the case is not clear, but it is possible that in T2DM, β-cell GIP receptor expression is reduced (Lynn et al. 2001). Alternatively, the action of GIP at the β-cell may require a cofactor that is absent in T2DM. For example, xenin-25, a peptide co-secreted with GIP from K-cells, activates local enteric nervous system reflexes that enhance β-cell GIP sensitivity in healthy individuals but not in patients with T2DM (Wice et al. 2010, 2012). Dysfunctional xenin-25 action could therefore explain why GIP is not an insulin secretagogue in T2DM.

GLP-1 receptors are expressed not only on β-cells but also on vagal sensory nerve endings that innervate the intestinal wall where L-cells are located (Ahrén 2000). Thus, locally secreted GLP-1 may act via vagal-vagal reflex pathways in which afferent sensory neuron activity is transmitted to the central nervous system, with consequent efferent activity transmitted to islets by the parasympathetic autonomic nervous system (Burcelin 2010; Hayes 2012). Parasympathetic ganglia neurons release the neurotransmitter PACAP in order to stimulate cAMP production in β-cells (Ahrén 2008), so it is possible that intestinally released GLP-1 acts indirectly via neuronally released PACAP to stimulate insulin secretion. Since GLP-1 has a short half-life in the systemic circulation (<5 min in humans), and since it is secreted in close proximity to vagal sensory nerve endings located in the wall of the intestine, a circumstance may exist in which the indirect action of GLP-1 mediated by the GLP-1R on the vagus nerve overshadows the direct action of

circulating GLP-1 at the GLP-1R on β-cells. However, a different situation exists when considering the actions of DPP-IV-resistant GLP-1R agonists since these peptides have an extended duration of action in the circulation (>30 min). Studies of mice that express the GLP-1R only in the pancreas demonstrate that a direct action of GLP-1R agonists at the β-cell GLP-1R is sufficient to potentiate GSIS and to improve glucoregulation in the absence of vagal neuron stimulation (Lamont et al. 2012). Thus, it seems likely that the β-cell GLP-1R mediates a direct insulin secretagogue action of DPP-IV-resistant GLP-1R agonists in humans.

In Vitro Evidence That Glucose Metabolism Stimulates cAMP Production

Surprisingly, cAMP production is stimulated by β-cell glucose metabolism (Landa et al. 2005; Dyachok et al. 2006, 2008; Kim et al. 2008a; Idevall-Hagren et al. 2010; Tian et al. 2011), and in the 1970s it was proposed that cAMP mediates the action of glucose to stimulate insulin secretion (Charles et al. 1975). Such an effect of glucose might be a consequence of its ability to stimulate Ca^{2+} influx and to raise levels of cytosolic Ca^{2+}, thereby stimulating TMACs that are under the control of Ca^{2+}/calmodulin (Ca^{2+}/CaM) (Delmiere et al. 2003; Roger et al. 2011). Alternatively, glucose metabolism might be coupled to HCO_3^- production that activates a soluble adenylyl cyclase (sAC) in β-cells (Ramos et al. 2008; Zippen et al. 2013).

Glucose metabolism provides ATP for TMAC-catalyzed cAMP production in β-cells. Levels of ATP at low concentrations of glucose are limiting for cAMP production such that an elevation of glucose concentration leads to increased ATP availability (Takahashi et al. 1999; Kasai et al. 2002). cAMP activates PKA, and PKA-mediated phosphorylation facilitates Ca^{2+}-dependent exocytosis of insulin (Thams et al. 2005; Hatakeyama et al. 2006, 2007). As illustrated in Fig. 4, cAMP generated by glucose metabolism also activates Epac2 (Idevall-Hagren et al. 2013), but it is uncertain if glucose and GLP-1 activate identical pools of PKA and Epac2. Finally, WFS1, an endoplasmic reticulum protein, supports glucose-stimulated TMAC activity in an as-yet-to-be determined manner (Fonseca et al. 2012).

Mathematical models predict how cytosolic levels of cAMP and Ca^{2+} oscillate under conditions in which β-cells are exposed to glucose and GLP-1 (Fridlyand et al. 2007; Ni et al. 2011; Takeda et al. 2011). In the absence of GLP-1, glucose metabolism has a modest stimulatory effect on cAMP production due to the fact that it provides substrate ATP, while also providing a cytosolic Ca^{2+} signal that stimulates Ca^{2+}/CaM-regulated TMACs. Simultaneously, Ca^{2+}-regulated PDEs are activated in order to lower levels of cAMP. Under these conditions, oscillations of cAMP and Ca^{2+} occur, and these oscillations are anti-phasic such that high levels of cAMP coincide with low levels of Ca^{2+} (Landa et al. 2005). An important prediction of these mathematical models is that exposure of β-cells to GLP-1 in the presence of glucose results in a reversal of the oscillatory activity such that high levels of cAMP coincide with high levels of Ca^{2+}. This reversal is explained by the fact that TMAC activity is also stimulated by Gs proteins linked to GLP-1

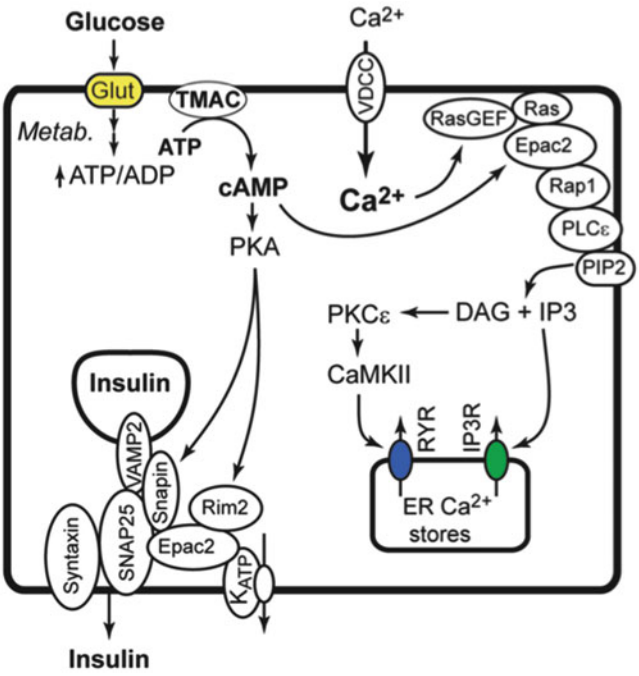

Fig. 4 Glucose metabolism provides ATP for TMAC-catalyzed cAMP production, PKA activation, and Epac2A activation. Glucose metabolism also stimulates an increase of $[Ca^{2+}]_i$ that activates guanine nucleotide exchange factors for Ras GTPase (*Ras-GEF*). Activated Ras-GTP binds to the Ras-association domain of Epac2 and recruits it to the plasma membrane where it activates Rap1 GTPase. Activated Rap1-GTP then binds the Rap-association domain of PLCϵ in order to stimulate its intrinsic catalytic activity, thereby initiating PIP_2 hydrolysis with consequent production of DAG and IP_3. DAG activates protein kinase C-ε (*PKCε*) in order to activate CaM-KII which then phosphorylates and activates RYR located on the ER. Simultaneously, IP_3 activates IP_3R on the ER, and Ca^{2+} released from the ER acts to promote additional Ca^{2+}-induced Ca^{2+} release from the ER. Ca^{2+} released in this manner acts as a direct trigger for insulin secretion under conditions in which PKA activity sensitizes the release mechanism to Ca^{2+}

receptors. Thus, TMACs act as molecular coincidence detectors for Ca^{2+}/CaM and Gs in order to generate synchronous inphase oscillations of cAMP and Ca^{2+} that are of importance to insulin secretion from β-cells (Holz et al. 2008b).

Insulin Exocytosis Is Stimulated Directly By cAMP: The Role Of PKA

An established literature documents the role of cAMP as a stimulator of insulin secretion, as measured in studies of isolated islets (Prentki and Matschinski 1987; Howell et al. 1994), or in live-cell imaging and patch clamp-based assays of exocytosis

occurring in single β-cells (Seino et al. 2009; Kasai et al. 2010; Dolenšek et al. 2011). The action of cAMP occurs at "late" or "distal" steps of β-cell stimulus-secretion coupling in which cAMP has a direct action to enhance secretory granule exocytosis (Ämmälä et al. 1993; Gillis and Misler 1993; Barnett et al. 1994). As illustrated in Figs. 1, 2, and 4, this action of cAMP is both PKA dependent and PKA independent (Renstrom et al. 1997), and evidence exists that the SNARE complex-associated protein snapin mediates the PKA-dependent component (Song et al. 2011), whereas Epac2 mediates the PKA-independent component (Ozaki et al. 2000; Eliasson et al. 2003). It is presently unclear whether compartmentalized cAMP signaling results in a situation in which certain Class II GPCRs preferentially couple to either the PKA-dependent or PKA-independent branches of this cAMP signaling network.

PKA-mediated phosphorylation has diverse stimulatory effects on insulin exocytosis. In one model illustrated in Fig. 5, ATP-dependent "priming" of secretory granules located within a readily releasable pool (RRP) renders them competent to undergo exocytosis. PKA then acts at a postpriming step to enhance their Ca^{2+}-dependent fusion with the plasma membrane (Takahashi et al. 1999). Although the identity of the postpriming substrate protein phosphorylated by PKA remains to be determined, this PKA activity is stimulated by glucose metabolism and is permissive for exocytosis (Hatakeyama et al. 2006, 2007). In fact, the ability of selective Epac activator 8-pCPT-2′-*O*-Me-cAMP-AM to potentiate GSIS from human islets requires concomitant permissive PKA activity (Chepurny et al. 2010).

PKA activity also renders secretory granules within the RRP more sensitive to the stimulatory action of Ca^{2+} so that they have an increased probability to undergo exocytosis in response to Ca^{2+} (Skelin and Rupnik 2011). This action of PKA is complemented by its ability to recruit a reserve pool of secretory granules from the cytoplasm to the plasma membrane so that the RRP may be refilled under conditions of sustained exocytosis (Renstrom et al. 1997). Simultaneously, PKA activity increases the number of highly Ca^{2+}-sensitive secretory granules, some of which are located outside of the RRP (Wan et al. 2004; Yang and Gillis 2004).

Another model seeks to explain how GLP-1 potentiates GSIS in a Ca^{2+}-dependent manner (Kang et al. 2003; Holz 2004b). In the absence of GLP-1, glucose metabolism stimulates the exocytosis of secretory granules located within "active zones" where microdomains of high cytosolic [Ca^{2+}] form at VDCCs. When β-cells are exposed to GLP-1, PKA activity sensitizes secretory granules to the action of Ca^{2+}, thereby ensuring that exocytosis will also occur at regions of the plasma membrane located outside of active zones. This Ca^{2+} sensitization allows a new larger source of granules to undergo exocytosis. For example, PKA activity enables additional secretory granules to undergo exocytosis in response to Ca^{2+} mobilized via a mechanism of Ca^{2+}-induced Ca^{2+} release (CICR) (Holz et al. 1999; Kang and Holz 2003).

Conceivably, all of the above-summarized processes act in concert to enable GLP-1 to potentiate GSIS. However, much of what we know concerning PKA signaling in the β-cell is based on studies using cAMP analogs in order to selectively activate PKA. New studies reveal the dangers of such an approach since Epac2 can be activated by 6-Bn-cAMP-AM, an *N*6-Benzyladenine-substituted

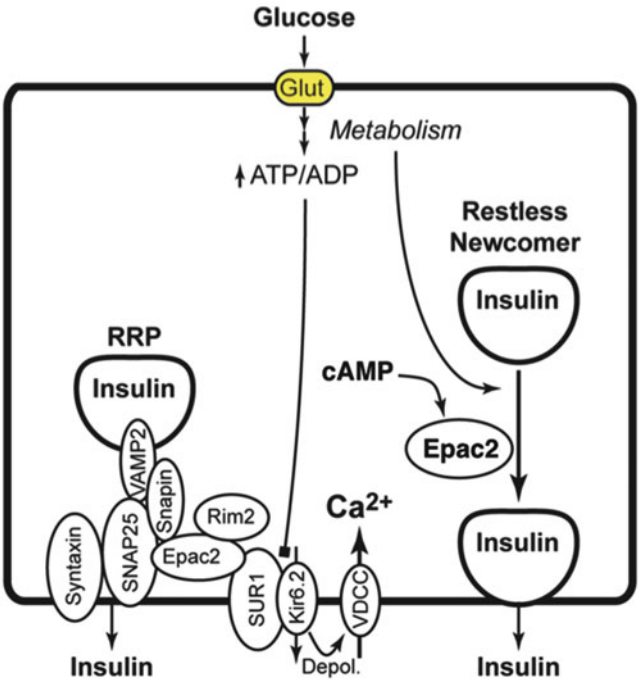

Fig. 5 Electrophysiological studies of β-cells define a readily releasable pool (*RRP*) of secretory granules that are prepositioned at the plasma membrane in order to undergo exocytosis in response to Ca^{2+} (*left side* of illustration). In marked contrast, imaging studies using total internal reflection microscopy (*TIRF*) of secretory granule trafficking indicate that secretory granules located in the cytoplasm move to the plasma membrane and immediately undergo exocytosis in response to Ca^{2+}. These secretory granules are designated as "restless newcomers," and they do not require propositioning at the plasma membrane (*right side* of illustration). This mechanism of restless newcomer exocytosis is dually regulated by glucose metabolism and cAMP, and it plays a prominent role in first phase GSIS. Studies with Epac2 KO mice demonstrate that expression of Epac2 is necessary in order for cAMP to potentiate first phase restless newcomer exocytosis. Glucose metabolism may not only provide a Ca^{2+} signal for exocytosis, but it may also induce remodeling of a cortical actin barrier so that secretory granules within the cytoplasm may transit to the plasma membrane

cAMP analog that was considered to be PKA selective (G. G. Holz, unpublished studies). As summarized below, studies using a molecular approach involving gene targeting provide new evidence for a role of PKA in the control of GSIS.

Insulin Exocytosis Is Stimulated Directly By cAMP: The Role Of Epac2

Epac2 participates in the direct control of insulin exocytosis by cAMP, and this action of Epac2 may also mediate the action of GLP-1 to potentiate GSIS (Kashima et al. 2001). Live-cell imaging studies of single β-cells provide key insights into

how these effects are achieved. By imaging the movement of β-cell secretory granules in response to glucose, it is possible to demonstrate that secretory granules fuse with the plasma membrane quickly (first phase) or with a delay (second phase). Under these conditions, cAMP potentiates first phase exocytosis in an Epac2-mediated manner (Shibasaki et al. 2007). This action of Epac2 correlates with its binding to SNARE protein SNAP-25 (Vikman et al. 2009) and SNARE complex-associated proteins Rim2 and Piccolo (Ozaki et al. 2000; Fujimoto et al. 2002; Shibasaki et al. 2004). It also correlates with Epac2-mediated phosphorylation of a microtubule-associated protein (syntabulin) that influences secretory granule trafficking (Ying et al. 2012). However, Epac2 is primarily an activator of Rap1 GTPase, so it is not yet clear how these signaling events lead to a potentiation of first phase exocytosis.

Epac2 also mediates cAMP-dependent acidification of β-cell secretory granules, thereby rendering them competent to undergo fast exocytosis in response to Ca^{2+} influx through VDCCs (Eliasson et al. 2003). This action of Epac2 is specific for an immediately releasable pool (IRP) of secretory granules that undergo exocytosis during first phase GSIS. Mechanistically, the activation of Epac2 promotes granule acidification by establishing a Cl^- concentration gradient that enables entry of protons across the secretory granule membrane. Surprisingly, a KO of the SUR1 subunit of K_{ATP} channels disrupts this action of Epac2. Since SUR1 is present in the secretory granule membrane where Cl^- channels are present (Geng et al. 2003), it could be that Epac2 and SUR1 mediate an action of cAMP to control secretory granule Cl^- channel function.

There is also evidence for cAMP-dependent stimulation of Cl^- channel activity in the plasma membrane of β-cells (Kinard and Satin 1995). Opening of these Cl^- channels generates β-cell depolarization due to the fact that the reversal potential for the corresponding Cl^- current is −34 mV (Kinard and Satin 1995). This Cl^- current is activated not only by cAMP but also by the sulfonylurea glyburide. Thus, it could be that Cl^- channels present in the β-cell plasma membrane, as well as in the secretory granule membrane, are under the control of SUR1 serving in its role as an Epac2-interacting protein (Shibasaki et al. 2004). Still, it remains to be determined if and how the guanine nucleotide exchange factor activity of Epac2 leads to Rap1-dependent opening of Cl^- channels.

When considering how Rap1 might mediate a direct action of Epac2 to control exocytosis, it is significant that there is expression of a Rap1-regulated phospholipase C-ε (PLCε) in mouse β-cells (Dzhura et al. 2010). PLCϵ contains a Rap1-association domain, thereby allowing cAMP to act via Epac2 and Rap1 to stimulate its catalytic activity. PLCϵ catalyzes hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2), and it links cAMP signaling to diacylglycerol (DAG) production and PKC activation (Smrcka et al. 2012). DAG formed in the plasma membrane may then bind SNARE complex-associated proteins such as Munc13-1 in order to facilitate exocytosis (Betz et al. 1998). Simultaneously, activated PKC recruited to the plasma membrane may phosphorylate SNAP-25 in order to facilitate exocytosis (Yang et al. 2007). In this regard, it is noteworthy that sulfonylureas directly stimulate β-cell exocytosis in a PKC-dependent manner (Eliasson et al. 1996).

Since sulfonylureas are reported to directly activate Epac2 (Zhang et al. 2009), an unexpected situation may exist in which Epac2, Rap1, and PLCϵ mediate a direct stimulatory action of sulfonylureas at β-cell secretory granules.

Contrasting Roles Of PKA And Epac2 In The Control Of GSIS

Glucose metabolism in β-cells is coupled to K_{ATP} channel closure, with resultant depolarization-induced entry of Ca^{2+} through VDCCs (Henquin 2000). In healthy β-cells this Ca^{2+} entry produces an increase of $[Ca^{2+}]_i$ that stimulates insulin exocytosis, and one study using genetically engineered mice in which there is a KO of PKA regulatory subunit 1a (PKArs1a) demonstrates that enhanced PKA activity potentiates GSIS (Song et al. 2011).

Epac2 also plays a role in the control of insulin secretion. New findings demonstrate that in healthy mice fed with a normal diet, a whole-body KO of Epac2 does not disrupt GSIS, but it does impair the action of GLP-1R agonist exendin-4 to potentiate first phase GSIS, both in vitro and in vivo (Song et al. 2013). Remarkably, a different situation exists when mice are fed with a high-fat diet that induces insulin resistance. In these unhealthy mice, Epac2 ablation disrupts insulin secretion in response to glucose alone (Song et al. 2013). Thus, a role for Epac2 in the control of GSIS is measurable in a rodent model of obesity-related T2DM.

In human T2DM an unhealthy situation also exists in which there is reduced coupling of β-cell glucose metabolism to K_{ATP} channel closure so that glucose fails to fully generate the Ca^{2+} signal that triggers insulin exocytosis (Doliba et al. 2012a). This pathology might be explained by aberrant glucose sensing by β-cell glucokinase (Doliba et al. 2012b), or by defects of mitochondrial metabolism (Wiederkehr and Wollheim 2008; Mulder and Ling 2009; Patti and Corvera 2010). The pathology might also be explained by a reduced capacity of K_{ATP} channels to close in response to the increase of cytosolic ATP/ADP concentration ratio that glucose metabolism produces. With these points in mind, we propose that Epac2 activation corrects for metabolic defects in T2DM, thereby restoring the Ca^{2+} signal that triggers insulin exocytosis. Thus, we predict that in T2DM, Epac2 participates in the restoration of GSIS by GLP-1R agonists.

Restoration Of GSIS In T2DM: A Role For Ca^{2+} Influx

A loss of first phase GSIS is one of the earliest indicators of β-cell dysfunction in a prediabetic patient (Brunzell et al. 1976). However, first phase GSIS is quickly restored during intravenous infusion of GLP-1 receptor agonist exenatide to these patients (Fehse et al. 2005). Such observations indicate that β-cells of early-stage T2DM patients have sufficient quantities of insulin available for exocytosis, yet first phase GSIS is somehow disturbed. Importantly, the secretory defect occurring in β-cells of T2DM patients might not be generalized since adequate quantities of

insulin are secreted in response to sulfonylureas (Seino et al. 2011). Sulfonylureas inhibit β-cell K_{ATP} channels to produce Ca^{2+} influx, so it might be that the fundamental mechanisms of Ca^{2+}-dependent exocytosis are not disrupted in β-cells of T2DM patients. These observations lead us to hypothesize that in T2DM, the coupling of β-cell glucose metabolism to K_{ATP} channel closure is reduced so that glucose fails to generate the necessary Ca^{2+} signal that initiates insulin secretion. When GLP-1R agonists are administered, the coupling of glucose metabolism to K_{ATP} channel closure is restored so that Ca^{2+}-dependent exocytosis of insulin may occur.

Restoration of K_{ATP} channel closure by GLP-1 is measurable under experimental conditions in which β-cells are initially exposed to a glucose-free solution that depletes intracellular ATP (Holz et al. 1993). Under these conditions, transient reintroduction of glucose weakly inhibits K_{ATP} channel activity, and this action of glucose is greatly potentiated by GLP-1, thereby generating "bursts" of action potentials (Holz et al. 1993). Such a restorative action of GLP-1 might reflect its ability to stimulate β-cell glucose metabolism. Alternatively, it might reflect an ability of GLP-1 to alter the adenine nucleotide sensitivity of K_{ATP} channels so that these channels will close more efficiently in response to an increase of cytosolic ATP/ADP concentration ratio that glucose metabolism produces (Tarasov et al. 2013).

Studies of mice lacking SUR1 and Kir6.2 subunits of K_{ATP} channels provide evidence for a K_{ATP} channel-dependent action of GLP-1 to stimulate insulin secretion. In these SUR1 and Kir6.2 KO mice, GLP-1 potentiation of GSIS is absent (Nakazaki et al. 2002; Shiota et al. 2002) or reduced (Miki et al. 2005). Furthermore, in mice harboring a tyrosine-to-stop codon (Y12STOP) mutation in the gene coding for Kir6.2, K_{ATP} channel expression and GLP-1-stimulated insulin secretion are absent (Hugill et al. 2010). Important findings are also provided by a study of patients with neonatal diabetes mellitus (NDM) owing to gain-of-function mutations (C435R; R1380) in the gene coding for SUR1 (Bourron et al. 2012). These mutations lead to overactive K_{ATP} channels and a consequent reduction of GSIS. Remarkably, the administration of a GLP-1R agonist restores insulin secretion in these patients.

PKA and Epac2 mediate the action of GLP-1 to close K_{ATP} channels such that PKA reduces the stimulatory action of Mg-ADP at SUR1 (Light et al. 2002), whereas Epac2 enhances the inhibitory action of ATP at Kir6.2 (Kang et al. 2008). The net effect is that GLP-1 produces a left shift in the dose-response relationship describing how an increase of cytosolic ATP/ADP concentration ratio (x-axis) inhibits K_{ATP} channel activity (y-axis). This mechanism of K_{ATP} channel modulation underlies the ability of GLP-1 to act as a β-cell glucose sensitizer so that it can facilitate glucose metabolism-dependent depolarization of β-cells (Holz et al. 1993). Numerous studies of human, rat, and mouse β-cells demonstrate that the glucose-dependent depolarizing action of GLP-1 in β-cells is reproduced by cAMP-elevating agents such as forskolin, IBMX, and glucagon, or by membrane-permeant cAMP analogs (Henquin and Meissner 1983; Henquin et al. 1983; Henquin and Meissner 1984a, b; Eddlestone et al. 1985; Barnett et al. 1994; He et al. 1998; Gromada et al. 1998a; Fernandez and Valdeolmillos 1999;

Suga et al. 2000; Ding et al. 2001; McQuaid et al. 2006; Kang et al. 2006, 2008; Chepurny et al. 2010; Leech et al. 2010b, 2011).

Under conditions of K_{ATP} channel closure in which β-cell depolarization initiates bursts of action potentials, there also exists an effect of GLP-1 to inhibit the delayed rectifier voltage-dependent K^+ current (MacDonald et al. 2003). This action of GLP-1 in β-cells is mediated by the PKA signaling pathway in conjunction with epidermal growth factor transactivation signaling that stimulates PI-3K and PKCζ activities (MacDonald et al. 2003). By inhibiting the voltage-dependent K^+ current (K_v), GLP-1 prolongs the action potential duration, thereby enhancing Ca^{2+} influx through VDCCs (Yada et al. 1993).

Nonselective cation channels (NSCCs) activated by GLP-1 in β-cells provide a depolarizing inward Na^+ current that is also important to action potential generation (Holz et al. 1995; Leech and Habener 1997). These channels are dually stimulated by cAMP and Ca^{2+}, and they appear to be a subtype of Ca^{2+} -activated NSCC, although their molecular identities remain to be ascertained. New data indicate that β-cell NSCCs are activated by Epac2 (Yoshida et al. 2012; Jarrard et al. 2013). Therefore, Epac2 might mediate a stimulatory action of GLP-1 at these channels in order to promote Ca^{2+} influx.

GLP-1 might also promote Ca^{2+} influx by upregulating β-cell glucose metabolism that closes K_{ATP} channels. For example, GLP-1 is reported to signal through cAMP and Epac2 to increase β-cell glucokinase (GK) activity (Ding et al. 2011; Park et al. 2012). Since GK activity constitutes the rate-limiting step in β-cell glucose sensing, any Epac2-mediated action of GLP-1 at GK is expected to be of major physiological significance. Potentially just as important is one report that GLP-1 stimulates mitochondrial ATP production in a β-cell line (MIN6 cells) (Tsuboi et al. 2003). However, studies using human and rodent islets dispute all of these findings (Peyot et al. 2009; Doliba et al. 2012a; Song et al. 2013), leaving it unclear whether GLP-1 does in fact stimulate β-cell glucose metabolism.

Restoration Of GSIS In T2DM: A Role For Ca^{2+} Mobilization

GLP-1 and various cAMP-elevating agents such as forskolin and PACAP mobilize an intracellular source of Ca^{2+} in β-cells (Leech et al. 2011). Thus, a Ca^{2+} mobilizing action of GLP-1 is expected to become important under conditions of T2DM in which the ability of glucose metabolism to stimulate β-cell Ca^{2+} influx is impaired. Furthermore, since β-cell mitochondrial ATP production is stimulated by Ca^{2+} released from endoplasmic reticulum (ER) Ca^{2+} stores (Tsuboi et al. 2003), the ER Ca^{2+} mobilizing action of GLP-1 might lead to a restoration of ATP production in β-cells of patients with T2DM. Therefore, it is of interest to summarize what is known concerning how GLP-1 acts via cAMP, PKA, and Epac2 to mobilize Ca^{2+} in β-cells.

As illustrated in Fig. 1, the Ca^{2+} mobilizing action of GLP-1 is explained by PKA-mediated phosphorylation of IP_3 receptor (IP_3R) and ryanodine receptor (RYR) intracellular Ca^{2+} release channels located on the ER (Holz et al. 1999;

Dyachok and Gylfe 2004; Islam et al. 1998). When considering the IP_3R, the second messenger IP_3 acts as a co-agonist with Ca^{2+} to gate the opening of IP_3R, and this process is facilitated by GLP-1 in a PKA-dependent manner. Similarly, GLP-1 sensitizes RYR to the stimulatory action of Ca^{2+} in order to facilitate Ca^{2+}-induced Ca^{2+} release (CICR) from the ER. When β-cells are exposed only to glucose, resultant Ca^{2+} influx has a limited ability to promote Ca^{2+} release from the ER. However, ER Ca^{2+} release is more efficiently triggered under conditions in which β-cells are simultaneously exposed to glucose and GLP-1. These findings lead us to propose that in T2DM, there is weak Ca^{2+} influx initiated by unhealthy β-cell glucose sensing and that GLP-1 compensates for this defect by facilitating ER Ca^{2+} release, thereby restoring a cytosolic Ca^{2+} signal important to GSIS.

As illustrated in Fig. 4, an Epac2-mediated action of GLP-1 complements these PKA-dependent mechanisms of Ca^{2+} mobilization (Kang et al. 2001, 2003, 2005). It mobilizes Ca^{2+} from the ER of β-cells, and it results from Epac2-dependent activation of a Rap1-regulated PLCϵ (Dzhura et al. 2010). Thus, PLCϵ links GLP-1R-stimulated cAMP production to PIP_2 hydrolysis with resultant IP_3 production, IP_3R activation, and ER Ca^{2+} mobilization. Simultaneously, DAG production and PKC activation initiate a signaling cascade that culminates with Ca^{2+}/calmodulin-dependent protein kinase-II (CaM-KII)-catalyzed phosphorylation of RYR in order to facilitate CICR (Dzhura et al. 2010). Remarkably, this Epac2-mediated action of GLP-1 to control RYR is similar to that which is described for ventricular cardiomyocytes in which RYR is under the control of β_1-adrenergic receptors (Oestreich et al. 2007, 2009). Just as intriguing, GLP-1 acts via Epac2 and PIP_2 hydrolysis in order to stimulate atrial natriuretic peptide (ANP) release from atrial cardiomyocytes (Kim et al. 2013). Thus, it appears that an evolutionarily conserved cAMP signaling "module" comprised of Epac2, Rap1, and PLCϵ controls CICR in β-cells and cardiomyocytes, while also promoting Ca^{2+}-dependent exocytosis of secretory granules in β-cells that contain insulin and in cardiomyocytes that contain ANP.

Less well understood is the action of GLP-1 to stimulate cyclic ADP-ribose (cADP-R) and nicotinic acid adenine dinucleotide phosphate (NAADP) production in order to mobilize Ca^{2+} from the ER, endosomes, and lysosomes of β-cells (Kim et al. 2008b). Evidently, cADP-R promotes RYR-mediated CICR, whereas NAADP acts directly at 2-pore Ca^{2+} release channels (TPCs). The NAADP receptor antagonist Ned-19 reduces GSIS from mouse islets, thereby demonstrating a clear functional link between intracellular Ca^{2+} mobilization and insulin exocytosis (Naylor et al. 2009).

When considering how cAMP-dependent intracellular Ca^{2+} mobilization influences insulin secretion, there is reason to believe that Ca^{2+} released in this manner promotes the activation of NSCCs located in the plasma membrane. Since NSCCs generate a depolarizing inward Na^+ current, their activation increases β-cell excitability in order to generate bursts of action potentials, especially under conditions of high membrane resistance in which K_{ATP} channels are closed (Cha et al. 2011). The ensuing increase of $[Ca^{2+}]_i$ is then reversed by cAMP-stimulated reuptake of Ca^{2+} into the ER (Yaekura and Yada 1998). Although ER Ca^{2+} depletion that

accompanies ER Ca^{2+} release is expected to activate store-operated Ca^{2+} channels in the plasma membrane, the existence of a cAMP-regulated store-operated Ca^{2+} current (SOC) in β-cells is questioned since cAMP fails to promote association of ER Ca^{2+} sensor Stim1 with the pore-forming subunit Orai1 of store-operated Ca^{2+} channels located in the plasma membrane (Tian et al. 2012).

As illustrated in Fig. 4, remarkable findings exist concerning PLCϵ KO mice. First, the Ca^{2+} mobilizing action of selective Epac activator 8-pCPT-2′-*O*-Me-cAMP-AM is nearly abolished in PLCϵ KO mice (Dzhura et al. 2010). Second, islets of PLCϵ KO mice are smaller in diameter and contain less insulin than control wild-type (WT) mice (Dzhura et al. 2011), a finding that is consistent with the established role of PLCϵ in growth control processes in other cell types (Smrcka et al. 2012). Especially interesting are findings that 8-pCPT-2′-*O*-Me-cAMP-AM stimulates β-cell PIP_2 hydrolysis (Leech et al. 2010b; Kumar et al. 2012) but that it has a reduced capacity to potentiate GSIS from islets of PLCϵ KO mice (Dzhura et al. 2011). Since the Ca^{2+} mobilizing action of 8-pCPT-2′-*O*-Me-cAMP-AM in WT mouse β-cells is disrupted by a Rap-GAP that inactivates Rap1 (Dzhura et al. 2010), it is clear that PLCϵ is a downstream target of Epac2 and Rap1 for cAMP-dependent control of insulin secretion (Shibasaki et al. 2007; Kelly et al. 2008).

In Vivo Studies Of The cAMP: PKA Signaling Branch In β-Cells

Defined genetic mouse models allow detailed in vivo analyses of the GLP-1 signaling pathways in β-cells. Specifically, the cAMP-PKA and cAMP-EPAC2A signaling branches within β-cells can be individually investigated using these models. Here, we adopt a standard classification scheme for naming the multiple isoforms of PKA regulatory subunits (Taylor et al. 2008), and we also adopt terminology in which Epac2A (i.e., full-length Epac2) is the predominant isoform of Epac2 expressed in β-cells (Niimura et al. 2009).

The PKA holoenzyme consists of the catalytic subunit (PKAcs) bound to four different regulatory subunits (PKARs 1a, 1b, 2a, and 2b). Among these, PKARs1a (prkar1a) is highly expressed in pancreatic islets (Petyuk et al. 2008). To investigate the cAMP-PKA signaling branch in β-cells, it is possible to use a mouse model specifically lacking pancreatic prkar1a (Δprkar1a) (Song et al. 2011) by interbreeding PDX1-CRE deleter mice (Gu et al. 2002) with prkar1a floxed mice (Kirschner et al. 2005). As expected, Δprkar1a islets do not contain prkar1a, whereas PKAcs activity is increased, as reflected by increased phosphorylation of PKAcs target CREB (Song et al. 2011). Thus, Δprkar1a mice exhibit PKA activity that is constitutively elevated in their islets. The islet and β-cell mass of Δprkar1a and control wild-type (WT) littermates are similar, indicating that constitutively increased islet PKA activity does not increase β-cell proliferation in vivo (Song et al. 2011). Furthermore, the proliferation marker Ki67 is also similar in Δprkar1a and WT littermates. Based on these observations, it appears that increased β-cell proliferation is not achieved after selective activation of the

cAMP-PKA signaling branch. Such findings are remarkable in view of the fact that β-cell proliferation is stimulated in WT mice during pharmacologic activation of the GLP-1R with exendin-4 (Song et al. 2008).

When examined at baseline fasting conditions, Δprkar1a mice do not show any abnormalities in glucose homeostasis (Song et al. 2011). Baseline glucose and insulin levels are similar to those in control littermates. However, Δprkar1a mice exhibit augmented insulin secretion, as measured in an intraperitoneal glucose tolerance test (ipGTT). GSIS is prompt and serum insulin concentrations after acute administration of glucose are eight- to tenfold higher than in littermate controls. These findings obtained with Δprkar1a mice are similar to findings obtained using mice that are engineered to allow inducible expression of a constitutively active PKAcs transgene specifically in β-cells (Kaihara et al. 2013). In these studies of transgenic mice, PKAcs activity can be induced in adult mice, which are then evaluated in an ipGTT at different glucose doses. As is the case for Δprkar1a mice, these transgenic mice with constitutively increased PKAcs activity show augmented GSIS at every glucose dose administered (Kaihara et al. 2013).

Collectively, these observations obtained with two mouse models show that specific upregulation of cAMP-PKA signaling – as found during pharmacologic GLP-1R stimulation – (a) retains β-cell glucose responsiveness and (b) allows insulin secretion to be shut off at glucose levels below physiologic fasting glycemia, and (c) at glucose levels above physiologic fasting levels, insulin secretion is dramatically augmented. Given that a whole-body KO of Epac2 does not disrupt GSIS in healthy mice (Song et al. 2013; see below), these findings suggest that in healthy β-cells, the cAMP-PKA signaling branch can in fact mediate the potentiation of endogenous incretin action. However, one caveat to this interpretation is that mouse models of constitutive PKA activity do not necessarily recapitulate compartmentalized cAMP signaling that is expected to occur in β-cells after pharmacologic GLP-1R agonist stimulation (Holz et al. 2008b). Furthermore, since these engineered mice have chronically elevated PKA activity, enhanced GSIS could reflect alterations of β-cell gene expression that are secondary to CREB activation (Dalle et al. 2011b).

It remains unclear how the cAMP-PKA signaling branch modulates glucose-stimulated Ca^{2+} handling under conditions of constitutive PKA activity. Based on findings obtained in single cell assays of β-cell depolarization, PKA should sensitize β-cells to the stimulatory action of glucose (Holz et al. 1993). Thus, the consequences of increased PKA activity need to be studied over a full range of glucose concentrations. With this limitation in mind, healthy β-cells with inducible and cell-specific transgenic PKAcs overexpression do not show any appreciable change in Ca^{2+} dynamics in response to a high (i.e., saturating) concentration of glucose (Kaihara et al. 2013). In contrast, islets from Δprkar1a mice show increased Ca^{2+} dynamics after glucose stimulation (Song et al. 2013). These divergent findings obtained using different mouse models may be explained by different experimental approaches such as nonidentical means of activating PKA, different glucose concentrations tested, and differences in the outcomes of single β-cell vs. whole islet measurements.

An important aspect of compartmentalized cAMP signaling in the β-cell is that PKA-anchoring proteins (A kinase-anchoring proteins; AKAPs) bind PKA regulatory subunits in order to control and define the subcellular location of PKAcs function/activity (Welch et al. 2010). It may be concluded that subcellular anchoring of PKA is required in order for GLP-1 to stimulate insulin secretion (Lester et al. 1997; Fraser et al. 1998). Thus, pharmacologic disruption of PKA anchoring impairs cAMP-dependent potentiation of GSIS (Lester et al. 1997). Still, it should be noted that AKAPs also anchor Epac proteins within defined subcellular compartments (Hong et al. 2008; Nijholt et al. 2008). Furthermore, AKAPs can anchor protein phosphatase 2B (PP2B), PKC, and PDEs (Scott and Santana 2010). It may be concluded that the potential exists for highly coordinate β-cell cAMP signaling involving PKA, Epac2, PP2B, PKC, and PDEs.

Global disruption of AKAP150 gene expression in mice impairs the ability of these mice to respond to a glucose challenge with insulin secretion while also inhibiting the action of cAMP to potentiate GSIS (Hinke et al. 2012). Furthermore, the lack of AKAP150 impairs the functionality of L-type Ca^{2+} channels and Ca^{2+} handling in the β-cell (Hinke et al. 2012). Surprisingly, in the absence of AKAP150 there is increased insulin sensitivity, thereby improving glucose tolerance in these AKAP150 KO mice (Hinke et al. 2012). Equally surprising are findings obtained using AKAP150 knock-in mice that harbor mutations in binding motifs of AKAP150 that normally permit it to interact with PKA regulatory subunits or PP2B. These studies demonstrate that a disruption of the PP2B-binding site, but not the PKA-binding site, replicates the metabolic phenotype of the whole-body AKAP150 KO (Hinke et al. 2012). This finding confirms the importance of AKAP150 in β-cell function, albeit surprisingly pointing toward a central role for the anchoring of PP2B by AKAP150. In this regard, it is noteworthy that GSIS is accompanied by PP2B-catalyzed dephosphorylation of kinesin heavy chain (Donelan et al. 2002), a component of the microtubule-associated motor protein kinesin that plays a role in the transport of secretory granules to the plasma membrane.

MyRIP (myosin and rab-interacting protein) is an AKAP that anchors PKA to the exocyst complex, an assembly of proteins that mediates secretory granule trafficking and targeting to the plasma membrane. In rat INS-1 insulin-secreting cells, an siRNA-mediated knockdown of MyRIP disrupts exocytosis in response to glucose and forskolin, and in these cells, MyRIP interacts with the Sec6 and Sec8 components of the exocyst complex (Goehring et al. 2007). Studies further indicate a reciprocal interplay between cAMP-PKA and MyRIP. While MyRIP anchors PKA to the exocyst complex, MyRIP is also phosphorylated in response to cAMP-PKA. Phospho-MyRIP in turn associates with Myosin Va (MyoVa), a motor protein involved in the transport of secretory granules (Brozzi et al. 2012). Phosphorylation of MyRIP also leads to increased phosphorylation of the MyoVa docking-receptor Rph-3A. Collectively, these data indicate that when cAMP levels are elevated, MyRIP forms a functional protein complex with MyoVa on secretory granules in order to promote secretory granule transport. Furthermore, MyRIP facilitates PKA-mediated phosphorylation of secretory granule-associated proteins to enhance exocytosis (Brozzi et al. 2012).

The SNARE complex-associated protein snapin is an established target of PKA in neurons (Chheda et al. 2001), and snapin is expressed at high levels in β-cells, where PKA activation induces its phosphorylation at serine residue 50 (Song et al. 2011). As illustrated in Fig. 2, snapin phosphorylation facilitates interactions between the vesicle-associated SNARE protein (v-SNARE; VAMP2) and the target cell surface-associated protein (t-SNARE; SNAP-25) (Chheda et al. 2001; Song et al. 2011). Interestingly, SNAP-25 also interacts with Epac2A in β-cells (Vikman et al. 2009; Song et al. 2011). These findings suggest a scenario in which multiple cAMP-dependent signaling pathways converge to assemble a complex in which each participating protein concentrates its functional role at the site of imminent exocytosis.

Another interesting aspect of snapin biology is that when mice are rendered glucose intolerant after receiving a lipid-enriched (high-fat) diet (60 % calories from saturated fats), snapin is hyperglycosylated with *N*-acetyl-glucosamine at amino acid residue serine 50 (O-GlcNac). Activation of GLP-1R signaling reverses snapin-*O*-GlcNacylation at serine 50 and favors S50 phosphorylation, thereby enabling snapin to associate with SNAP-25 and Epac2A (Song et al. 2011). This finding provides a unifying molecular mechanism for β-cell dysfunction, which occurs at the level of exocytosis and is rapidly and effectively reversed by pharmacologic GLP-1R agonists.

In Vivo Studies Of The cAMP: Epac2A Signaling Branch In β-Cells

The discovery of cAMP-regulated guanine nucleotide exchange factors designated as cAMP-GEF-I and cAMP-GEF-II (now known as Epac1 and Epac2) by two independent groups (de Rooij et al. 1998; Kawasaki et al. 1998) provides an explanation for PKA-independent control of insulin secretion by cAMP (Renstrom et al. 1997; Kashima et al. 2001; Nakazaki et al. 2002; Eliasson et al. 2003; Hashiguchi et al. 2006; Kwan et al. 2007; Kelley et al. 2009; Vikman et al. 2009; Chepurny et al. 2010; Idevall-Hagren et al. 2010; Dzhura et al. 2011). Consistent with the expression of Epac2 in β-cells (Leech et al. 2000; Ozaki et al. 2000), there exist PKA-independent stimulatory actions of cAMP to raise levels of Ca^{2+} in β-cells (Bode et al. 1999; Kang et al. 2001). As illustrated in Fig. 3, the PKA-independent action of cAMP to potentiate GSIS is mediated by Epac2 and its partner Rap1.

Epac2A, the full-length form of Epac2, is the predominant isoform of Epac expressed in β-cells (Niimura et al. 2009). An assessment of the role of Epac2A in the control of GSIS can be achieved using recently developed tools including membrane-permeable Epac-selective cAMP analogs (ESCAS) that activate Epac proteins but not PKA when used at low concentrations (Vliem et al. 2008; Chepurny et al. 2009), specific small molecular Epac2 inhibitors (Tsalkova et al. 2012; Chen et al. 2013), and whole-body Epac2A KO mice (Shibasaki et al. 2007; Dzhura et al. 2010), double Epac1 and Epac2 KO mice (Yang et al. 2012), as well as floxed Epac2A mice for the cell type-specific KO of Epac1 or Epac2 (Pereira et al. 2013). Thus, there exist new strategies with which to

assess the importance of Epac2 to the control of GSIS. Initial findings indicate that β-cell mass is preserved in whole-body Epac2 KO mice, whereas a defect of glucoregulation is measurable when these mice are fed with a high-fat diet (Song et al. 2013).

The generation of a mouse model with a whole-body KO of Epac2A gene expression greatly advances our understanding of how Epac2A influences insulin secretion (Song et al. 2013). β-cells and islets from Epac2A KO mice exhibit smaller elevations of cytosolic Ca^{2+} concentration in response to GLP-1R agonist exendin-4 (Dzhura et al. 2010; Song et al. 2013), and this impairment correlates with a reduced potentiation of first phase GSIS by exendin-4 in vitro (Song et al. 2013). Furthermore, in vivo assays demonstrate a reduced insulin secretagogue action of exendin-4, as measured in Epac2A KO mice following intraperitoneal administration of both glucose and exendin-4 (Song et al. 2013). Thus, Epac2A mediates, at least in part, GLP-1R agonist action to potentiate GSIS.

Epac2A interacts with secretory granule and SNARE complex-associated proteins that are important to insulin secretion (Seino et al. 2009). Since these interactions are absent in Epac2A KO mice, the reduced insulin secretagogue action of exendin-4 in Epac2A KO mice may be explained, at least in part, by the failure of cAMP to directly stimulate secretory granule exocytosis in β-cells. As illustrated in Fig. 5, imaging studies with β-cells of Epac2 KO mice indicate that Epac2 mediates cAMP-dependent potentiation of a novel mechanism of exocytosis in which secretory granules located in the cytoplasm transit to the plasma membrane where they undergo immediate release, a process of Ca^{2+}-dependent exocytosis designated as "restless newcomer" exocytosis (Shibasaki et al. 2007). Although the molecular basis for restless newcomer exocytosis is not known, it could be that this mechanism of exocytosis requires direct interactions of Epac2 with secretory granule or SNARE complex-associated proteins. Thus, the reduced capacity of exendin-4 to potentiate first phase GSIS in islets of Epac2 KO mice may be explained not only by defective Ca^{2+} handling in the β-cell but also by the failure of cAMP to directly promote restless newcomer exocytosis.

The understanding of Epac2A function in β-cells broadened dramatically when the Seino laboratory identified Epac2A as a direct cellular target of the sulfonylurea class of blood glucose-lowering agents (Zhang et al. 2009). While Epac2A KO mice exhibit normal oral and intraperitoneal glucose tolerance at baseline, they remarkably exhibit a reduced response to sulfonylureas (Zhang et al. 2009). Thus, the sulfonylureas are proposed to function by two distinct mechanisms: (a) they bind to SUR1 in order to close β-cell K_{ATP} channels and to promote Ca^{2+}-dependent insulin secretion independently of glucose metabolism, and (b) they bind to Epac2A in order to directly potentiate GSIS.

As illustrated in Fig. 4, under conditions in which β-cells are exposed to a low concentration of glucose, sulfonylureas directly inhibit K_{ATP} channels in order to generate a Ca^{2+} signal that stimulates exocytosis of secretory granules prepositioned at the plasma membrane where they are "docked" and "primed." When β-cells are exposed to a stimulatory concentration of glucose, it could be that sulfonylureas also act via Epac2A to enhance glucose-dependent restless newcomer exocytosis. These

considerations are of therapeutic significance in view of the finding that gliclazide is unique among sulfonylureas in that it does not activate Epac2A, but binds only to SUR1 to close K_{ATP} channels (Zhang et al. 2009). What remains to be determined is exactly how sulfonylureas activate Epac2A. Since Epac2-dependent Rap1 activation by sulfonylureas is not measurable in a solution assay using recombinant Epac2A and Rap1 (Tsalkova et al. 2011; Rehmann 2012), it could be that sulfonylureas act indirectly to activate Epac2 in living cells, as might be expected since high concentrations of sulfonylureas elevate levels of cAMP by inhibiting PDEs in islets (Goldfine et al. 1971). However, PDE inhibition may not be a factor since imaging studies of living cells indicate that sulfonylureas activate Epac2A but not Epac1 (Herbst et al. 2011).

Studies with Epac2A KO mice clarify distinctions between the functional roles of Epac2A and PKA in β-cells (Song et al. 2013). Epac2A KO mice, as compared to WT littermates, exhibit impaired adaptation of insulin secretion in response to insulin resistance induced by a short-term (1 month) high-fat content diet (60 % calories from saturated fats). In addition, when the cAMP-PKA branch is disinhibited in Δprkar1a mice, the additional absence of Epac2A blunts the augmented GSIS, which is seen in Δprkar1a.

Epac2A KO mice fed with a normal diet show reduced responsiveness to GLP-1R activation by exendin-4 in an in vivo assay of insulin secretion, and this is also the case for in vitro studies examining insulin secretion from isolated islets of Epac2A KO mice (Song et al. 2013). Remarkably, Epac2A KO mice also show reduced potentiation of GSIS in response to pharmacologic activation of GPR40, a GPCR for long-chain fatty acids (Song et al. 2013). This finding is unexpected because GPR40 activation potentiates GSIS in a cAMP-independent manner, one involving PLCβ, PIP_2 hydrolysis, and Ca^{2+} mobilization (Mancini and Poitout 2013). Clearly, a better understanding of GPR40 is warranted in view of the fact that GPR40 agonists are in early phases of clinical use for the treatment of T2DM (Mancini and Poitout 2013).

In summary, Epac2A appears to be a key molecule that is required for the β-cell to respond functionally to increased insulin resistance (as found after high-fat diet) as well as to a multitude of β-cell-targeted secretagogues (GLP-1R agonists, sulfonylureas, GPR40 activators). Thus, Epac2A selective activators may constitute a new class of blood glucose-lowering agents for pharmacological intervention in the treatment of T2DM. Future studies will also be required to examine the role of Epac2A in the pathogenesis of β-cell dysfunction in T2DM and/or the metabolic syndrome. Future studies using mouse models with cell- and tissue-specific Epac2A ablation will also be necessary to discriminate any metabolic effects of Epac2A in non-β-cells.

Conclusion

Plasticity in the β-cell cAMP signaling network is increasingly viewed as an adaptive response to metabolic demands imposed by changes in nutritional status, or in response to pathophysiological processes such as insulin resistance and

glucolipotoxicity (Hinke et al. 2004). The short-term outcomes of altered cAMP signaling include a restoration of Ca^{2+} handling and secretory granule exocytosis in experimental models of T2DM. These changes induced by cAMP occur within minutes, and they result from PKA-mediated phosphorylation of snapin accompanied by Epac2-mediated activation of Rap1 and PLCϵ. Less well understood are long-term changes of β-cell function in response to cAMP. These changes can occur on a time scale of hours, days, weeks, or months and are explained by changes in gene expression for key transcription factors, enzymes of glucose metabolism, and mediators of insulin exocytosis. Whereas PKA predominates as a stimulus for insulin secretion in healthy β-cells, a role for Epac2 in the control of GSIS is revealed under conditions of a high-fat diet (HFD).

The HFD mouse model of T2DM is characterized by compensatory islet hyperplasia with increased β-cell mass and intact β-cell glucose sensitivity, but exaggerated insulin secretion that counters peripheral insulin resistance (Winzell and Ahrén 2004). With continued administration of the HFD, there is reduced β-cell mass, diminished β-cell glucose sensitivity, and a loss of GSIS. GLP-1R agonists correct for these defects, either by preserving β-cell mass or by restoring β-cell glucose sensitivity. Compensatory processes induced by the HFD lead to a situation in which Epac2 becomes of critical importance to GSIS, even in the absence of administered GLP-1R agonists (Song et al. 2013). Why this is the case is not clear, but it might indicate that under conditions of the HFD, glucose metabolism is coupled to cAMP production and Epac2 activation in order to stimulate insulin secretion.

Equally intriguing is the finding that GLP-1R expression is upregulated in islets of mice fed with the HFD (Ahlkvist et al. 2013) and that Epac2 expression is stimulated after treatment of T2DM donor human islets with GLP-1R agonist exendin-4 (Lupi et al. 2008). Since insulin resistance is characteristic of both T2DM and the HFD mouse model, it is possible that trophic factors such as betatrophin released from the liver circulate in response to diminished insulin action (Yi et al. 2013) in order to control the expression and/or function of the GLP-1R and Epac2 in β-cells. Alternatively, the HFD might induce epigenetic control of cAMP signaling, as recently demonstrated for Epac2 (Lee et al. 2012).

Based on studies of rodents, it is possible that in human T2DM, there is an uncoupling of β-cell glucose metabolism to cAMP production (Abdel-Halim et al. 1996; Dachicourt et al. 1996). However, in the Goto-Kakazaki (GK) rat model of T2DM, a secretory defect exists in which GSIS is downregulated despite the fact that glucose-dependent cAMP production is elevated (Dolz et al. 2011). Treatment of GK islets with GLP-1 produces an exaggerated stimulation of cAMP production, thereby restoring GSIS (Dolz et al. 2011). Since Epac2 is less sensitive to cAMP in comparison with PKA (Holz et al. 2008a), it could be that Epac2 is recruited by GLP-1 into the β-cell cAMP signaling network in order to achieve this restoration of GSIS. Importantly, such an Epac2-mediated action of GLP-1 would be conditional on basal PKA activity that supports exocytosis (Chepurny et al. 2010). Thus, a new paradigm may be evident in which β-cell stimulus-secretion coupling under the control of glucose and cAMP exhibits plasticity such

that the relative importance of PKA and Epac2 to GSIS is determined by nutritional and metabolic status. The challenge now is to relate these findings concerning mice or rats to our understanding of human T2DM, while also seeking to identify new strategies with which to manipulate the β-cell cAMP signaling network.

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Cross-References

▶ ATP-Sensitive Potassium Channels in Health and Disease
▶ Calcium Signaling in the Islets
▶ Electrical, Calcium, and Metabolic Oscillations in Pancreatic Islets
▶ Electrophysiology of Islet Cells
▶ Exocytosis in Islet β-Cells

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