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Phospholipase C-ε links Epac2 activation to the potentiation of glucose-stimulated insulin secretion from mouse islets of Langerhans

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Abbreviations: Db-cAMP-AM, N^6,2'-O-dibutyryladenosine-3',5'-cyclic monophosphate acetoxymethyl ester; 6-Bnz-cAMP-AM, N^6-benzoyladenosine-3',5'-cyclic monophosphate acetoxymethyl ester: 8-pCPT-2'-O-Me-cAMP-AM, 8-(4-Chlorophenylthio)-2'-O-methyladenosine-3',5'cyclic monophosphate acetoxymethyl ester; CICR, Ca^{2+}-induced Ca^{2+} release; Epac2, type-2 isoform of the exchange protein directly activated by cAMP; ESCA-AM, Epac-selective cAMP analog acetoxymethyl ester; FRET, fluorescence resonance energy transfer; GLP-1, glucagon-like peptide-1; GSIS, glucose-stimulated insulin secretion; PLC-ε, phospholipase C-epsilon

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Glucose-stimulated insulin secretion (GSIS) from pancreatic β-cells is potentiated by cAMP-elevating agents, such as the incretin hormone glucagon-like peptide-1 (GLP-1) and cAMP exerts its insulin secretagogue action by activating both protein kinase A (PKA) and the cAMP-regulated guanine nucleotide exchange factor designated as Epac2. Although prior studies of mouse islets demonstrated that Epac2 acts via Rap1 GTPase to potentiate GSIS, it is not understood which downstream targets of Rap1 promote the exocytosis of insulin. Here, we measured insulin secretion stimulated by a cAMP analog that is a selective activator of Epac proteins in order to demonstrate that a Rap1-regulated phospholipase C-epsilon (PLC-ε) links Epac2 activation to the potentiation of GSIS. Our analysis demonstrates that the Epac activator 8-pCPT-2'-O-Me-cAMP-AM potentiates GSIS from the islets of wild-type (WT) mice, whereas it has a greatly reduced insulin secretagogue action in the islets of Epac2 (-/-) and PLC-ε (-/-) knockout (KO) mice. Importantly, the insulin secretagogue action of 8-pCPT-2'-O-Me-cAMP-AM in WT mouse islets cannot be explained by an unexpected action of this cAMP analog to activate PKA, as verified through the use of a FRET-based A-kinase activity reporter (AKAR3) that reports PKA activation. Since the KO of PLC-ε disrupts the ability of 8-pCPT-2'-O-Me-cAMP-AM to potentiate GSIS, while also disrupting its ability to stimulate an increase of β-cell $[Ca^{2+}]_i$, the available evidence indicates that it is a Rap1-regulated PLC-ε that links Epac2 activation to Ca^{2+}-dependent exocytosis of insulin.

Introduction

Epac2 is a cAMP-regulated guanine nucleotide-exchange factor that activates the monomeric GTPase known as Rap1 in order to control numerous physiological processes.[1] One ongoing controversy in endocrine cell physiology concerns the potential roles of Epac2 and Rap1 in the regulation of pancreatic insulin secretion, especially with regards to the abilities of cAMP-elevating agents to potentiate glucose-stimulated insulin secretion (GSIS) from β-cells located in the islets of Langerhans. Shibasaki and co-workers reported that Epac2 and Rap1 play essential roles in conferring stimulatory effects of cAMP on mouse islet insulin secretion,[2] whereas Hatakeyama and co-workers reported that Epac2 plays no such role, and that insulin secretion is instead under the control of the cAMP-binding protein designated as protein kinase A (PKA).[3-5] Other teams of investigators have provided an alternative viewpoint, one in which cAMP potentiates GSIS by simultaneously activating Epac2 and PKA.[6-10] This ongoing controversy is of considerable interest since the activation of Epac2 and/or PKA may explain the ability of an intestinally derived incretin

hormone (glucagon-like peptide-1; GLP-1) to stimulate pancreatic insulin secretion, and to lower levels of blood glucose, in patients diagnosed with type 2 diabetes mellitus.[11-16] In this addendum, we address this controversy while also discussing our recent study of Dzhura et al.[17] in which it was reported that the GLP-1 receptor agonist Exendin-4 mobilized Ca^{2+} in mouse β-cells by activating Epac2, Rap1 and a novel Rap-regulated, phosphoinositide-specific, phospholipase C-epsilon (PLC-ε). We also provide new information that the knockout (KO) of PLC-ε gene expression disrupts the ability of a selective Epac activator (8-pCPT-2'-O-Me-cAMP-AM) to potentiate GSIS from mouse islets. On the basis of such findings, we propose that there exists a previously unrecognized role for PLC-ε as a determinant of mouse islet insulin secretion that is under the control of Epac2.

Ca^{2+} Mobilizing Properties of the GLP-1 Receptor

The GLP-1 receptor is a Class II GTP-binding protein-coupled receptor that is expressed on pancreatic β-cells, and it is activated not only by the incretin hormone GLP-1, but also by the incretin mimetic Exendin-4. Incretin mimetics are agents that mimic the action of GLP-1 to potentiate GSIS from the pancreas, and as is the case for GLP-1, they have the ability to raise levels of cAMP and Ca^{2+} in the islet β-cells.[11-16] Evidence exists that cAMP production is functionally coupled to intracellular Ca^{2+} mobilization in β-cells, and it is increasingly apparent that under conditions in which β-cells are exposed to cAMP-elevating agents, Ca^{2+}-dependent exocytosis of insulin can be stimulated by Ca^{2+} released from intracellular Ca^{2+} stores.[15,18-32] Using methods that involve the ultraviolet light-catalyzed "uncaging" of Ca^{2+} in β-cells loaded with the photolabile Ca^{2+} chelator NP-EGTA, Dzhura et al. extended on the original findings of Kang and co-workers[28,29,33,34] to demonstrate that there is a mechanism of endoplasmic reticulum (ER) Ca^{2+}-induced Ca^{2+} release (CICR) that is facilitated by cAMP in β-cells.[17] Furthermore, it was demonstrated that this action of cAMP resulted from its ability to simultaneously activate PKA and Epac2.[17] Using mice in which there is a knockout (KO) of Epac2 or PLC-ε gene expression, Dzhura et al. then demonstrated that Epac2 signals through Rap1 to activate PLC-ε, and that subsequent Ca^{2+} mobilization involves both protein kinase C (PKC) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII).[17] Importantly, these new findings are in general agreement with the prior findings of Kang and co-workers in which it was demonstrated that the cAMP-elevating agent forskolin facilitated CICR in mouse β-cells, and that this action of forskolin was antagonized after pharmacological blockade of ER Ca^{2+} release channels that correspond to inositol trisphosphate receptors and ryanodine receptors.[34]

Insulin Secretagogue Properties of 8-pCPT-2'-O-Me-cAMP-AM

With this background information in mind, a question of importance concerns whether or not a β-cell Epac2, Rap1 and PLC-ε signal transduction "module" is coupled to insulin secretion. This might be expected given the fact that Ca^{2+}, PKC and CaMKII are all established to participate in insulin exocytosis.[35] Our recent work has addressed this issue using the same Epac2 and PLC-ε KO mice that were used in the study of Dzhura et al. while also relying on the use of 8-pCPT-2'-O-Me-cAMP-AM to achieve selective Epac2 activation in the absence of PKA activation.[36,37] Thus, to investigate the insulin secretagogue properties of 8-pCPT-2'-O-Me-cAMP-AM, our approach involved the use of static incubation assays in which the ability of this Epac-selective cAMP analog acetoxymethyl ester (ESCA-AM) to potentiate GSIS from isolated islets was measured.

Initially, we found that the KO of PLC-ε had a small (ca. 25%) but significant ($p < 0.05$) inhibitory effect on GSIS stimulated in the absence of added 8-pCPT-2'-O-Me-cAMP-AM. Thus, when the glucose concentration was stepped from 2.8 to 20 mM, the fold-stimulation of insulin secretion was 3.4-fold for the islets of PLC-ε (+/+) wild-type (WT) mice (**Fig. 1A**), whereas it was 2.6-fold for the islets of PLC-ε (-/-) KO mice (**Fig. 1B**). This secretory defect in the islets of PLC-ε (-/-) KO mice might be related to the fact that glucose metabolism has the ability to raise levels of cAMP at the cytosolic face of the β-cell plasma membrane.[10] In this scenario, insulin secretion stimulated by glucose would be supported by the cAMP-dependent activation of PLC-ε such that the KO of PLC-ε would partially disrupt GSIS. Future studies will assess this possibility be establishing whether glucose metabolism does in fact activate PLC-ε in the islets of WT mice.

Extending on this analysis, it was then demonstrated that 8-pCPT-2'-O-Me-cAMP-AM exerted a dose-dependent action to potentiate GSIS from the islets of PLC-ε (+/+) WT mice (**Fig. 1A**). Similarly, GSIS from PLC-ε (+/+) WT mouse islets was also potentiated by the cAMP analogs Db-cAMP-AM and 6-Bnz-cAMP-AM, both of which activate PKA in β-cells (**Fig. 1A**).[17,36] However, in the islets of PLC-ε (-/-) KO mice, the action of 8-pCPT-2'-O-Me-cAMP-AM to potentiate GSIS was significantly impaired, whereas the actions of Db-cAMP-AM and 6-Bnz-cAMP-AM were not significantly affected (**Fig. 1B**). Thus, the fold-potentiation of GSIS measured in response to 1 μM 8-pCPT-2'-O-Me-cAMP-AM was calculated to be 2.2-fold for PLC-ε (+/+) WT islets, and 1.3-fold for PLC-ε (-/-) KO islets, respectively ($p < 0.05$). Even more remarkable was the finding that in PLC-ε (-/-) KO mouse islets, a lower concentration (0.3 μM) of 8-pCPT-2'-O-Me-cAMP-AM was completely incapable of potentiating GSIS, whereas it was effective in the islets of PLC-ε (+/+) WT mice (**Fig. 1C**). These findings indicate that the ability of a selective Epac2 activator to potentiate GSIS is contingent on the intra-islet expression of PLC-ε.

We next sought to validate our prediction that the insulin secretagogue action of 8-pCPT-2'-O-Me-cAMP-AM was mediated by Epac2, acting in its role to promote the Rap1-dependent stimulation of PLC-ε. To this end, we first sought to assure ourselves that 8-pCPT-2'-O-Me-cAMP-AM did not unexpectedly activate PKA. Our approach was to perform live-cell imaging studies of single mouse β-cells virally transduced with AKAR3, a genetically encoded A-kinase activity reporter-3 (AKAR3).[38] This biosensor reports PKA

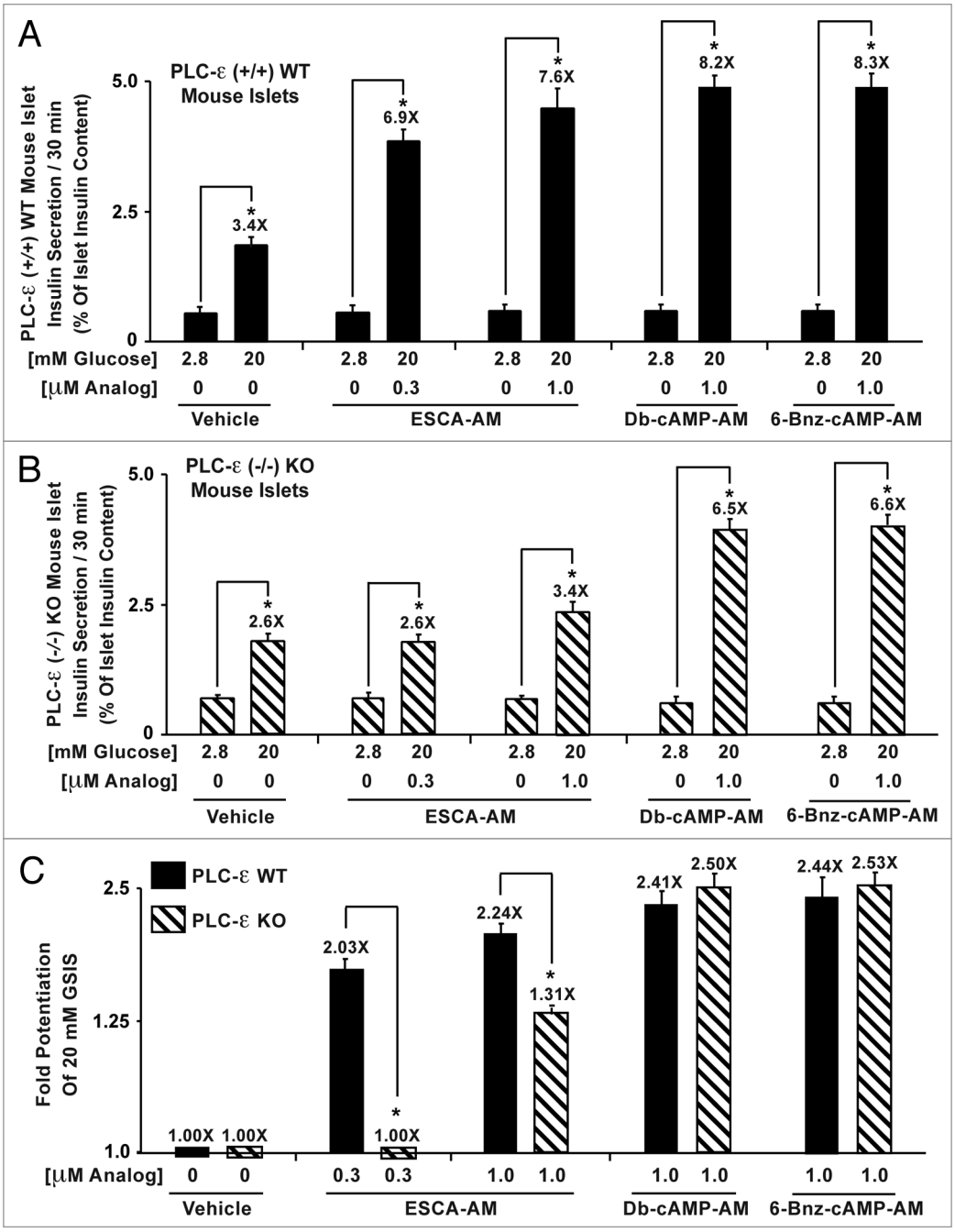

Figure 1. GSIS under the control of Epac2 is disrupted in the islets of PLC-ε KO mice. (A) 8-pCPT-2′-O-Me-cAMP-AM (abbreviated as ESCA-AM), Db-cAMP-AM and 6-Bnz-cAMP-AM exerted dose-dependent actions to potentiate GSIS in a static incubation assay using islets of PLC-ε (+/+) WT mice. In this assay, insulin secretion was measured during two consecutive 30 min intervals in which the KRBH contained either 2.8 mM glucose with no added cAMP analogs or 20 mM glucose with or without added cAMP analogs. Each histogram bar indicates the fractional insulin secretion in 30 min for 10 islets per well. (B) the action of 8-pCPT-2′-O-Me-cAMP-AM but not Db-cAMP-AM or 6-Bnz-cAMP-AM to potentiate GSIS was disrupted in the islets of PLC-ε (-/-) KO mice. (C) illustrated is a side-by-side comparison of the findings summarized in (A and B). Note that in PLC-ε (-/-) KO mouse islets, a low concentration of 8-pCPT-2′-O-Me-cAMP-AM (0.3 μM) failed to potentiate GSIS, whereas a higher concentration (1.0 μM) exerted a smaller effect as compared to its action in PLC-ε (+/+) WT mouse islets (1.31 vs. 2.24 fold potentiation of GSIS in KO vs. WT islets, respectively). Results are the means ± SEM of three independent experiments using three WT and three KO mice. *$p < 0.05$. Note that in (A and B), insulin secretion is expressed as the percentage of islet insulin content, as determined by ELISA of insulin present in the KRBH, or by ELISA of total extractable insulin in lysates of islets. On average, the total insulin content of PLC-ε (+/+) WT mouse islets was 640 ng/10 islets, and for PLC-ε (-/-) KO mouse islets it was 401 ng/10 islets. A full description of the PLC-ε KO mice and the static incubation assay used to measure insulin secretion is provided in prior publications.[9,17,36,39,59-61]

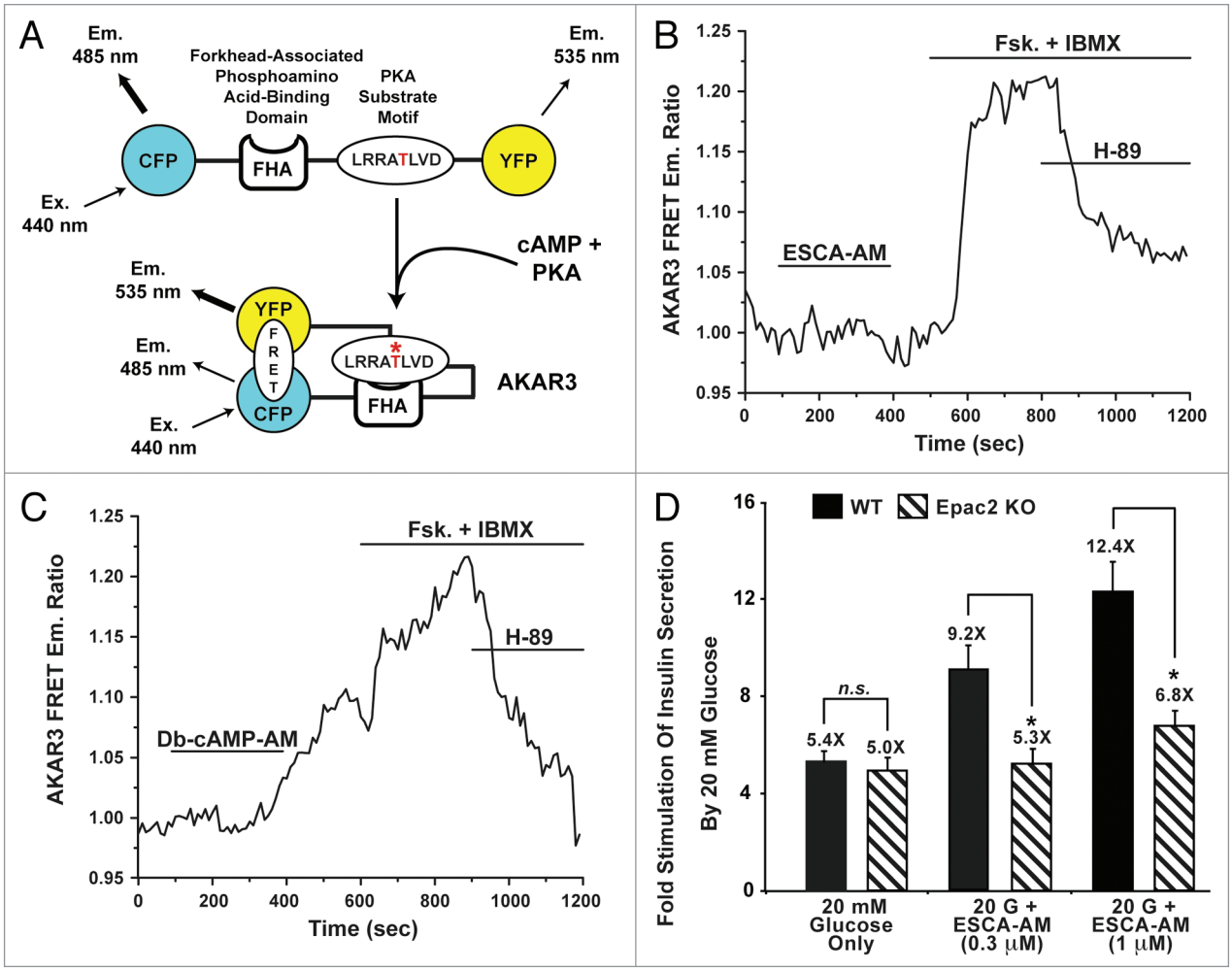

Figure 2. Epac2 but not PKA mediates the insulin secretagogue action of 8-pCPT-2′-O-Me-cAMP-AM. (A) structure of AKAR3 and its regulation by PKA. Phosphorylation of a threonine residue (red star) in the PKA substrate motif results in the binding of this motif to the forkhead-associated binding domain (FHA) to confer increased FRET between CFP and YFP. (B) 8-pCPT-2′-O-Me-cAMP-AM (ESCA-AM, application indicated by a horizontal bar) failed to activate AKAR3 in a mouse β-cell, whereas an increase of AKAR3 FRET emission ratio (Em. Ratio) signifying PKA activation was measured in response to combined application of forskolin (Fsk.) and IBMX. These actions of forskolin and IBMX were reversed by the PKA inhibitor H-89. (C) AKAR3 was activated by the PKA-selective cAMP analog Db-cAMP-AM, or by forskolin and IBMX, and these actions were reversed by H-89. (D) potentiation of GSIS by 8-pCPT-2′-O-Me-cAMP-AM, as measured in a static incubation assay, was nearly abolished in the islets of Epac2 KO mice. Results in (B and C) are representative of findings obtained in two independent experiments using one mouse/experiment in which each analog was tested using n = 6 β-cells/analog. The concentrations of test substances in (B and C) were: ESCA-AM, 1 μM; Db-cAMP-AM, 1 μM; Fsk., 2 μM; IBMX, 100 μM; H-89, 10 μM. Results for part D are the means ± SEM of three independent experiments using three mice. *$p < 0.05$. For a full description of the methods used for FRET-based assays of AKAR3, or for a description of how the Epac2 KO mice were generated, see prior publications.[9,17]

activity due to the fact that PKA-mediated phosphorylation of AKAR3 leads to increased intramolecular fluorescence resonance energy transfer (FRET) measurable as an increased 535/485 nm emission ratio (**Fig. 2A**). Live-cell imaging assays of single β-cells expressing AKAR3 established that 8-pCPT-2'-O-Me-cAMP-AM (1 μM) failed to activate PKA since it failed to increase FRET (**Fig. 2B**). However, the PKA activator Db-cAMP-AM (1 μM) did increase FRET (**Fig. 2C**). Furthermore, FRET was increased in response to combined administration of the cAMP-elevating agents forskolin (Fsk.) and isobutylmethylxanthine (IBMX), both of which served as positive controls for PKA activation (**Fig. 2B and C**; note that these effects were reversed by PKA inhibitor H-89). Thus, when tested at 1 μM, a concentration that potentiated GSIS from WT mouse islets, 8-pCPT-2'-O-Me-cAMP-AM failed to activate PKA in single mouse β-cells.

A second approach was then undertaken in which the action of 8-pCPT-2'-O-Me-cAMP-AM to potentiate GSIS was compared in the islets of Epac2 (+/+) WT vs. Epac2 (-/-) KO mice. It was established that the KO of Epac2 abrogated the action of 0.3 μM 8-pCPT-2'-O-Me-cAMP-AM to potentiate GSIS, whereas a residual action of 1.0 μM 8-pCPT-2'-O-Me-cAMP-AM was still measurable (**Fig. 2D**). Although not investigated, this residual action of 8-pCPT-2'-O-Me-cAMP-AM in the islets of Epac2 KO mice might be explained by its ability to activate Epac1. In fact, one

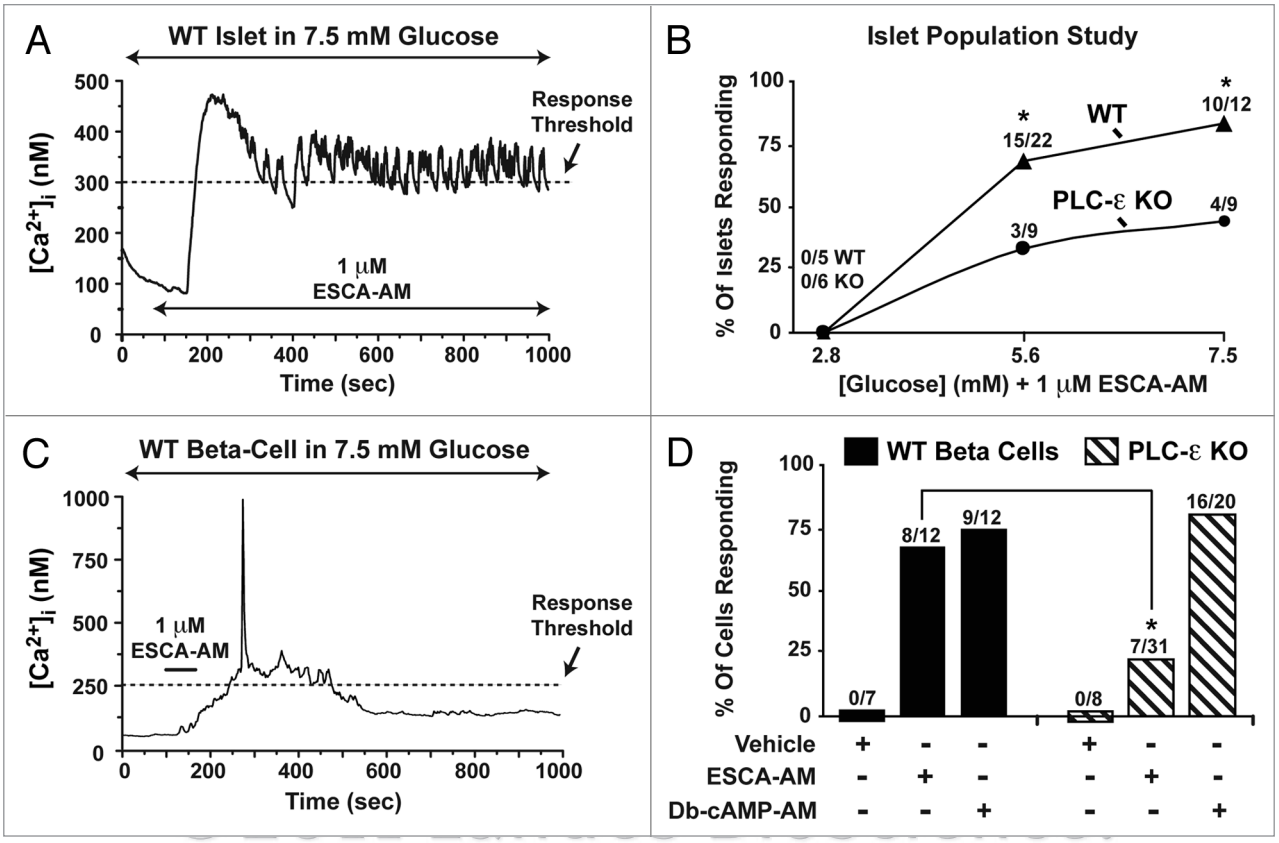

Figure 3. KO of PLC-ε disrupts Epac2-regulated Ca^{2+} in islets and β-cells. (A and B) 8-pCPT-2′-O-Me-cAMP-AM (ESCA-AM) exerted a glucose-dependent action to increase $[Ca^{2+}]_i$ in PLC-ε (+/+) WT mouse islets, and that this action was reduced in PLC-ε (-/-) KO mouse islets. (C and D) 8-pCPT-2′-O-Me-cAMP-AM increased $[Ca^{2+}]_i$ in single islet cells of PLC-ε (+/+) WT mice equilibrated in SES containing 7.5 mM glucose, and this effect was mimicked by Db-cAMP-AM (not shown). For such studies, β-cells were identified on the basis of their large diameter and granular morphology. Note that the action of 8-pCPT-2′-O-Me-cAMP-AM but not Db-cAMP-AM was reduced in the β-cells of PLC-ε (-/-) KO mice. For (B and D), the number of islets or β-cells that exhibited an increase of $[Ca^{2+}]_i$ equal to or greater than the response threshold (dashed lines in A and C) is indicated by the fractional numerator, whereas the denominator indicates the number of cells assayed. All cAMP analogs were tested at 1 μM. Results summarize three experiments for each assay using three WT or three KO mice. For a full description of the methods used for fura-2 based spectrofluorimetry, and for the methods used to apply test substances, see prior publications.[9,29,34]

prior report has documented the expression of Epac1 mRNA in mouse islets.[39]

Altered Ca^{2+} Handling in Islets and β-Cells of PLC-ε KO Mice

Since PLC-ε couples cAMP production to PIP_2 hydrolysis and the mobilization of intracellular Ca^{2+}, it could be that the insulin secretory defect we measured in the islets of PLC-ε KO mice (**Fig. 1**) resulted from the failure of 8-pCPT-2'-O-Me-cAMP-AM to increase $[Ca^{2+}]_i$ and to initiate insulin exocytosis. Although this is an attractive hypothesis, there exists only one published study examining cytosolic Ca^{2+} handling in the β-cells of PLC-ε (-/-) KO mice. In that study, 8-pCPT-2'-O-Me-cAMP-AM facilitated CICR in β-cells of PLC-ε (+/+) WT mice, and this action was abrogated in β-cells of PLC-ε (-/-) KO mice.[17] However, it is important to note that this action of 8-pCPT-2'-O-Me-cAMP-AM was evaluated under non-physiological conditions in which CICR was triggered by the UV flash photolysis-induced uncaging of Ca^{2+} in β-cells loaded with NP-EGTA. Furthermore, the experimental approach used in that prior study was biased towards an analysis of CICR since we find that the loading of β-cells with NP-EGTA (a Ca^{2+} buffer) prevents the previously reported action of 8-pCPT-2'-O-Me-cAMP-AM to inhibit ATP-sensitive K^+ channels (K_{ATP}), to depolarize β-cells, and to simulate Ca^{2+} influx through voltage-dependent Ca^{2+} channels.[40,41] Therefore, we used standard methods of fura-2 spectrofluorimetry without NP-EGTA loading in order to evaluate how 8-pCPT-2'-O-Me-cAMP-AM influenced the $[Ca^{2+}]_i$ in whole islets and single β-cells of WT or PLC-ε KO mice.

Assays of $[Ca^{2+}]_i$ were performed using fura-2 loaded islets equilibrated in a standard extracellular saline (SES) containing 7.5 mM glucose. For the islets of PLC-ε (+/+) WT mice, it was demonstrated that 8-pCPT-2'-O-Me-cAMP-AM (1 μM) stimulated a sustained increase of whole-islet $[Ca^{2+}]_i$ (**Fig. 3A**). This action of 8-pCPT-2'-O-Me-cAMP-AM was glucose-dependent since it was measurable under conditions in which the SES contained 5.6 or 7.5 mM glucose, but not 2.8 mM glucose (**Fig. 3B**). Furthermore, raising the concentration of glucose from 5.6 to 7.5 mM increased the likelihood that 8-pCPT-2'-O-Me-cAMP-AM

would produce an increase of $[Ca^{2+}]_i$ that exceeded an arbitrarily defined threshold value of 300 nM (**Fig. 3A and B**). Remarkably, in the islets of PLC-ε (-/-) KO mice equilibrated in SES containing 5.6 or 7.5 mM glucose, the action of 8-pCPT-2'-O-Me-cAMP-AM to increase $[Ca^{2+}]_i$ was reduced (**Fig. 3B**). This analysis was then expanded in order to demonstrate that under conditions in which the SES contained 7.5 mM glucose, 8-pCPT-2'-O-Me-cAMP-AM (1 μM) increased $[Ca^{2+}]_i$ in single β-cells of PLC-ε (+/+) WT mice (**Fig. 3C**). By performing population studies at the single-cell level, it was then possible to demonstrate that the action of 8-pCPT-2'-O-Me-cAMP-AM to increase $[Ca^{2+}]_i$ was strongly suppressed in the β-cells of PLC-ε (-/-) KO mice (**Fig. 3D**). Such studies also revealed that the PKA selective cAMP analog Db-cAMP-AM (1 μM) raised $[Ca^{2+}]_i$ in single β-cells of PLC-ε (+/+) WT mice, and that this action of Db-cAMP-AM was preserved in the β-cells of PLC-ε (-/-) KO mice (**Fig. 3D**). Thus, the KO of PLC-ε disrupted β-cell and whole-islet Ca^{2+} signaling that was under the control of Epac2, while leaving PKA-regulated Ca^{2+} signaling intact.

Discussion

The above-summarized findings concerning insulin secretion and Ca^{2+} handling are understandable in view of the capacity of Epac proteins to activate Rap1, thereby allowing the active GTP-bound form of Rap1 to stimulate PLC-ε.[42-44] Although PLC-ε is expressed in mouse and human islets,[17] the only published information linking PLC-ε activation to altered islet function is the finding that the KO of PLC-ε gene expression in mice disrupts the Ca^{2+} mobilizing action of 8-pCPT-2'-O-Me-cAMP-AM in β-cells of these mice.[17] Since this action of 8-pCPT-2'-O-Me-cAMP-AM is also disrupted in β-cells of Epac2 KO mice, and since 8-pCPT-2'-O-Me-cAMP-AM fails to mobilize Ca^{2+} in WT mouse β-cells transduced with a GTPase activating protein (RapGAP) that downregulates Rap1 activity,[17] there is good reason to believe that Epac2 and Rap1 do in fact control the activity of PLC-ε in β-cells. What has remained uncertain up to now is whether PLC-ε participates in the control of islet insulin secretion. Thus, the primary significance of the new studies reported here is that the intra-islet expression of PLC-ε is demonstrated to be a contributing factor in support of insulin secretion that is under the control of Epac2.

Surprisingly, we found that the ability of 8-pCPT-2'-O-Me-cAMP-AM to elevate $[Ca^{2+}]_i$ was only partially disrupted in the islets of PLC-ε KO mice (**Fig. 3B**), whereas the action of 8-pCPT-2'-O-Me-cAMP-AM to potentiate GSIS was nearly abrogated (**Fig. 1**). One possible explanation for these findings is that the secretory defect present in the islets of PLC-ε KO mice does not simply involve Ca^{2+}. For example, evidence exists that PLC-ε is coupled to the activation of PKC in β-cells.[17] Since PKC plays an especially important role as a determinant of insulin exocytosis,[45,46] the secretory defect present in the islets of PLC-ε KO mice might be explained by defective PKC activation in addition to an alteration of Ca^{2+} handling. Regardless of the exact mechanism underlying this secretory defect, it is important to emphasize that the defect does not appear to be one of a general nature. In fact, a full potentiation of GSIS was measured in the islets of PLC-ε KO mice treated with cAMP analogs that activate PKA (**Fig. 1A–C**). Furthermore, GSIS measured in the absence of cAMP analogs was diminished by only 25% in the islets of PLC-ε (-/-) KO mice (c.f., **Fig. 1A and B**). Such findings seem to indicate that the KO of PLC-ε does not disrupt the "late" steps of Ca^{2+}-dependent exocytosis in β-cells, a conclusion that will be tested in future studies using the voltage-clamp technique, or in ELISA assays of insulin secretion stimulated by depolarizing agents such as KCl.

Finally, it should be noted that it still remains to be determined whether or not the action of GLP-1 to potentiate GSIS is retained or disrupted in the islets of PLC-ε KO mice.[13,47] Since GLP-1 has the capacity to activate PKA, and since PKA activity potentiates GSIS by phosphorylating Snapin,[48] it seems likely that at least some insulin secretagogue actions of GLP-1 do not require the Epac2, Rap1 and PLC-ε signal transduction "module" we report to be expressed in β-cells. If so, it will be of special interest to identify which kinetic and/or mechanistic components of GSIS are differentially regulated by Epac2, PLC-ε and PKA. It should also be noted that in the islets of PLC-ε KO mice, we found that there was a ca. 33% reduction in islet insulin content as compared with islets of WT mice, an observation that is explained by the fact that the islets of adult PLC-ε KO mice are smaller in size by a factor of ca. one-third (see legend, **Fig. 1**). Thus, it may be that PLC-ε plays some role in the control of β-cell growth and/or differentiation. In fact, studies of other cell types have already established that multiple growth control signal transduction pathways converge to activate PLC-ε.[43] In this regard, it is especially interesting that the epidermal growth factor receptor (EGF-R) is coupled through Ras GTPases to the activation of PLC-ε.[42] Since EGF-R transactivation stimulated by GLP-1 occurs in β-cells,[49,50] the KO of PLC-ε might disrupt important growth-promoting actions of GLP-1 in the islets.

Conclusion

Although the new findings presented here provide evidence for an Epac2 and PLC-ε mediated action of 8-pCPT-2'-O-Me-cAMP-AM to stimulate insulin secretion, this may not be the sole mechanism by which Epac activators exert their secretagogue effects. For example, Epac2 may control insulin secretion in a PLC-ε independent manner by virtue of direct or indirect interactions of Epac2 with insulin granule or SNARE complex-associated proteins (**Fig. 4**). These proteins include Rim2, Piccolo, SNAP-25 and the sulfonylurea receptor-1 (SUR1) subunit of K_{ATP} channels.[51-55] Such interactions may allow Epac activators to facilitate exocytosis, possibly by increasing the size of a readily-releasable pool of secretory granules. Epac activators are also capable of increasing β-cell membrane excitability, an effect due to their inhibitory action at K_{ATP} channels.[40,41,56] In fact, Epac activators such as 8-pCPT-2'-O-Me-cAMP-AM depolarize human β-cells and raise levels of $[Ca^{2+}]_i$.[9] Thus, it is not surprising that we found that the KO of PLC-ε

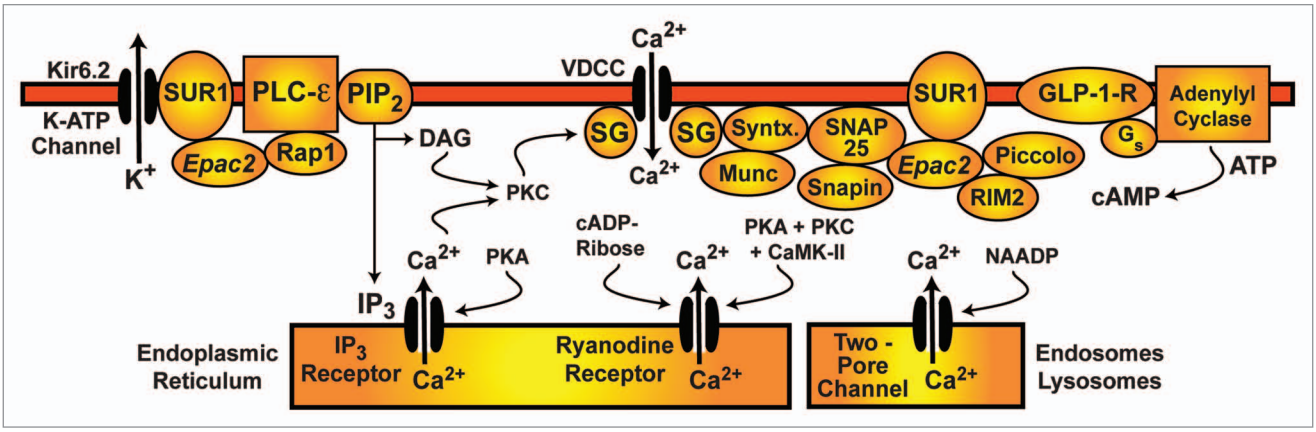

Figure 4. GSIS under the control of cAMP in β-cells. Binding of GLP-1 to the GLP-1 receptor (GLP-1R) leads to cAMP production and the activation of Epac2 and PKA. The SUR1 subunit of K_{ATP} channels recruits Epac2 to the plasma membrane where a signaling complex comprised of Epac2, Rap1 and PLC-ε is formed. Rap1 is activated by Epac2, and the activated form of Rap1 binds to and activates PLC-ε. PIP_2 hydrolysis catalyzed by PLC-ε leads to the generation of diacylglycerol (DAG) and inositol trisphosphate (IP_3). DAG and Ca^{2+} coordinately activate PKC, and PKC phosphorylates secretory granule-associated proteins, thereby increasing the likelihood that these granules will undergo exocytosis in response to Ca^{2+} influx through voltage-dependent Ca^{2+} channels (VDCCs). Exocytosis is also initiated in response to the release of Ca^{2+} from intracellular Ca^{2+} stores. Ca^{2+} within these stores is mobilized due to the opening of Ca^{2+} release channels that correspond to IP_3 receptors and ryanodine receptors located on the endoplasmic reticulum. Additional Ca^{2+} release results from the opening of two-pore Ca^{2+} channels that correspond to nicotinic acid adenine dinucleotide phosphate (NAADP) receptors located on endosomes or lysosomes. Ryanodine receptors release Ca^{2+} in a process of CICR that is facilitated by cADP-ribose, PKA and Ca^{2+}/calmodulin-dependent protein kinase-II (CaMK-II). Additional actions of PKA include its ability to enhance the opening of IP_3 receptors, and to directly enhance exocytosis by phosphorylating Snapin. Note that PLC-ε activity may result in a depletion of PIP_2 in the immediate vicinity of K_{ATP} channels. This would be expected to reduce the apparent affinity of K_{ATP} channels for ATP so that they will close more efficiently in response to β-cell glucose metabolism. Exocytosis is also stimulated due to the direct or indirect interactions of Epac2 with RIM2, Piccolo, SNAP-25, Munc13-1, Snapin and Syntaxin (Syntx). Evidence exists that all of these processes are under the control of cAMP and possibly GLP-1.

did not completely abolish the action of 8-pCPT-2'-O-Me-cAMP-AM to potentiate GSIS (**Fig. 1C**). It is also worth noting that mouse islets express Epac1, albeit at lower levels than Epac2,[39] and that the cAMP-binding domain of Epac1 incorporated within a FRET-based biosensor (Epac1-camps) is responsive to Exendin-4 in β-cells.[57] Thus, Epac1 signaling in the β-cells might explain our finding that the KO of Epac2 failed to completely abolish the action of 8-pCPT-2'-O-Me-cAMP-AM to potentiate GSIS (**Fig. 2D**). Finally, it should be emphasized that crosstalk exists between Epac2 and PKA in the control of insulin secretion. In fact, the secretagogue action of 8-pCPT-2'-O-Me-cAMP-AM in human islets is conditional on PKA activity that is permissive,[9] and that may facilitate a "post-priming" step in Ca^{2+}-dependent exocytosis.[58] In conclusion, the relative importance of Epac isoforms, Epac-interacting proteins, Epac effectors and PKA, to the cAMP-dependent control of pancreatic insulin secretion remains an outstanding issue.

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