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New insights concerning the molecular basis for defective glucoregulation in soluble adenylyl cyclase knockout mice

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Abstract

Recently published findings indicate that a knockout (KO) of soluble adenylyl cyclase (sAC, also known as AC-10) gene expression in mice leads to defective glucoregulation that is characterized by reduced pancreatic insulin secretion and reduced intraperitoneal glucose tolerance. Summarized here are current concepts regarding the molecular basis for this phenotype, with special emphasis on the potential role of sAC as a determinant of glucose-stimulated insulin secretion. Highlighted is new evidence that in pancreatic beta cells, oxidative glucose metabolism stimulates mitochondrial CO_2 production that in turn generates bicarbonate ion (HCO_3^-). Since HCO_3^- binds to and directly stimulates the activity of sAC, we propose that glucose-stimulated cAMP production in beta cells is mediated not simply by transmembrane adenylyl cyclases (TMACs), but also by sAC. Based on evidence that sAC is expressed in mitochondria, there exists the possibility that beta-cell glucose metabolism is linked to mitochondrial cAMP production with consequent facilitation of oxidative phosphorylation. Since sAC is also expressed in the cytoplasm, sAC catalyzed cAMP production may activate cAMP sensors such as PKA and Epac2 to control ion channel function, intracellular Ca^{2+} handling, and Ca^{2+}-dependent exocytosis. Thus, we propose that the existence of sAC in beta cells provides a new and unexpected explanation for previously reported actions of glucose metabolism to stimulate cAMP production. It seems possible that alterations of sAC activity might be of importance when evaluating new strategies for the treatment of type 2 diabetes (T2DM), or when evaluating why glucose metabolism fails to stimulate insulin secretion in patients diagnosed with T2DM.

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AUTHOR CONTRIBUTIONS

G.G.H. wrote the manuscript. O.G.C. and C.A.L. edited the manuscript. G.G.H. created Figure 1. C.A.L created Figure 2.

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Keywords

Soluble adenylyl cyclase; bicarbonate ion; cAMP; glucose; insulin secretion

1. Introduction

Pancreatic beta cells secrete insulin in response to the increase of blood glucose concentration that occurs after a meal [1]. Released insulin acts at insulin receptors on liver, fat, and muscle to promote uptake of glucose from the blood, while also suppressing hepatic release of glucose into the circulation [2]. As levels of blood glucose drop in response to insulin, this glucose-stimulated insulin secretion (GSIS) is terminated so that circulating levels of insulin will return to levels that are characteristic of the fasting state. When glucose fails to stimulate insulin secretion, or when tissues with insulin receptors become resistant to insulin, there can exist chronic hyperglycemia, as is the case for individuals with type 2 diabetes mellitus (T2DM) [3]. Since a loss of GSIS can occur prior to the development of insulin resistance in T2DM [4], there is at the present time great interest to understand the molecular defects of GSIS that occur in the context of T2DM.

2. Pancreatic beta-cell stimulus-secretion coupling

When considering how glucose stimulates insulin secretion from pancreatic beta cells, attention has focused on the “triggering” and “amplification” mechanisms of beta-cell stimulus-secretion coupling (Fig. 1) [5,6]. These two interdependent mechanisms of GSIS require beta-cell glucose uptake that is mediated by a facilitative glucose transporter (Glut1 for humans, Glut2 for rodents) [7,8]. However, the rate-limiting step in glucose sensing is governed by glucokinase (GK) [9], a type IV hexokinase that catalyzes conversion of glucose to glucose-6-phosphate (G6P) [10]. Oxidative glycolytic and mitochondrial metabolism of G6P stimulates an increase of the cytosolic ATP/ADP concentration ratio [11], and this key metabolic signal is directly responsible for the closure of ATP-sensitive K^+ channels (K-ATP), depolarization, and Ca^{2+} influx through voltage-dependent Ca^{2+} channels (VDCCs) [11].

Glucose-stimulated Ca^{2+} influx through VDCCs constitutes a “triggering” mechanism for the Ca^{2+}-dependent exocytosis of dense core secretory granules containing insulin (Fig. 1). More specifically, this triggering mechanism targets two distinct pools of secretory granules that undergo Ca^{2+}-dependent fusion with the plasma membrane. These pools are a readily releasable pool (RRP) and a reserve pool (RP) of secretory granules. The RRP and RP are distinguishable because exocytosis of the RRP is not constrained by a cytoskeletal cortical actin barrier, whereas exocytosis of the RP can only occur after glucose-induced remodeling of the cortical actin barrier [12,13]. Therefore, the RRP of secretory granules located at or near the plasma membrane undergoes exocytosis quickly in order to generate an immediate 1^{st} phase of GSIS, whereas the RP of secretory granules located deeper within the cytoplasm undergoes exocytosis with a delay in order to generate a 2^{nd} phase of GSIS. Importantly, neither 1^{st} nor 2^{nd} phase GSIS can occur in the absence of the Ca^{2+} signal that triggers exocytosis.

The “amplification” mechanism of GSIS allows glucose metabolism to increase the efficiency with which Ca^{2+} triggers exocytosis of secretory granules located in the RRP and the RP (Fig. 1) [5,6]. Although glucose metabolism promotes remodeling of the cortical actin barrier to enhance secretory granule transit to the plasma membrane [12,13], this remodeling may not explain how glucose metabolically amplifies insulin secretion [14–16]. Instead, glycolytic and/or mitochondrial metabolites may be of primary importance [5,6]. As discussed below, the second messenger adenosine-3′,5′-cyclic monophosphate (cAMP) is another potential coupling factor linking glucose metabolism to amplification. It is generated in response to glucose metabolism, and its synthesis is catalyzed by transmembrane adenylyl cyclases (TMACs) and a soluble adenylyl cyclase (sAC) expressed in beta cells.

When considering the relative importance of “triggering” and “amplification” to the control of insulin secretion by cAMP, there is evidence that in healthy beta cells, the amplification mechanism predominates. Thus, in healthy beta cells, glucose metabolism is capable of generating the cytosolic Ca^{2+} signal that triggers exocytosis. Under such conditions, cAMP amplifies soluble NSF attachment protein receptor (SNARE) complex-mediated actions of Ca^{2+} to trigger the release of a relatively small number of secretory granules located within the RRP [17]. Simultaneously, cAMP amplifies the Ca^{2+}-dependent exocytosis of a much larger pool of secretory granules located in the RP [17]. These amplifying actions of cAMP are detectable using methods of live-cell imaging in which it is demonstrated that cAMP enhances the translocation of so-called “restless newcomer” secretory granules from the cytoplasm to the plasma membrane [18]. Restless newcomer secretory granules undergo immediate fusion with the plasma membrane, and their exocytosis is a major factor that underlies the ability of cAMP to potentiate 1^{st} and 2^{nd} phase GSIS.

A different scenario exists when one considers how cAMP stimulates insulin secretion in patients with T2DM. In certain forms of this disorder, beta-cell glucose sensing is defective, possibly as a consequence of altered glycolytic and/or mitochondrial metabolism [19,20]. Under such conditions, glucose metabolism fails to produce the increase of cytosolic ATP/ADP concentration ratio that normally leads to K-ATP channel closure and depolarization-induced Ca^{2+} influx that triggers insulin exocytosis (Fig. 1). Remarkably, beta-cell glucose sensing is restored after the administration of glucagon-like peptide-1 (GLP-1), a cAMP-elevating agent that facilitates glucose-dependent ATP production, while also raising the $[Ca^{2+}]_i$ and potentiating GSIS [21,22]. For GLP-1, Holz *et al.* define this restoration of glucose sensing as the induction of beta-cell “glucose competence” [23–25].

Pictured are metabolic, ionic, and GPCR signaling pathways in a pancreatic beta-cell that mediate stimulatory actions of glucose and glucagon-like peptide-1 (GLP-1) on insulin secretion. A readily releasable pool (RRP) of secretory granules is positioned immediately beneath the plasma membrane, whereas a reserve pool (RP) of secretory granules is located deeper in the cytoplasm behind the cortical actin barrier (wavy line). The triggering pathway of GSIS links glucose metabolism to depolarization-induced Ca^{2+} influx that triggers exocytosis of the RRP and RP. The amplification pathway allows metabolic coupling factors to increase the efficiency of Ca^{2+} as a stimulus for exocytosis. These actions of glucose are reinforced by GLP-1 acting at its GPCR to stimulate cAMP production with consequent

activation of the cAMP sensors PKA and Epac2. Abbreviations: K_v, K_{Ca}, voltage and Ca^{2+} dependent K^+ channels; ΔV_m, depolarization; PLCε, phospholipase C-epsilon.

3. Defective insulin secretion in T2DM

GSIS can be evaluated *in vivo* using a protocol in which glucose is administered by intravenous infusion. When the glucose concentration is stepped from a low to a high level, the 1st and 2nd phase kinetic components of GSIS are measurable in healthy individuals, whereas there is a characteristic loss of 1st and 2nd phase GSIS in T2DM [4]. One way to explain this loss of GSIS is that in T2DM there are reduced numbers of beta cells [27]. Alternatively, there might be a molecular defect of exocytosis that prevents GSIS [28,29]. Differentiating between these two possibilities is not possible *in vivo*, but the results of post-mortem investigations indicate that in T2DM, the loss of GSIS is not explained simply by a decrease in the total number of beta-cells in the islets [4]. Thus, when considering a molecular defect of GSIS as a potential contributing factor to T2DM, it is surprising that the process of exocytosis involving fusion of secretory granules with the plasma membrane is reported not to be disturbed in beta cells from donors with T2DM [30]. Such findings suggest that in certain forms of T2DM, beta-cell stimulus-secretion coupling is disrupted at an early step of glucose sensing [31]. For example, there might be defective coupling of glucose metabolism to K-ATP channel closure and Ca^{2+} influx that triggers exocytosis. If so, it is understandable that T2DM patients can be treated with sulfonylureas (*e.g.*, tolbutamide) that directly inhibit K-ATP channels, or with GLP-1 receptor agonists that stimulate cAMP production in order to restore glucose sensing and K-ATP channel closure. In this regard, there is great interest concerning the identification of beta-cell cAMP-elevating agents that will restore defective GSIS, while also lowering levels of blood glucose in patients with T2DM [34–38]. Ideally, these cAMP-elevating agents will not stimulate excessive insulin secretion, and will not induce hypoglycemia, as can be the case for sulfonylureas [33].

4. sAC is a bicarbonate-stimulated adenylyl cyclase potentially important to GSIS

Here we advance the hypothesis that soluble adenylyl cyclase (sAC) might constitute a new molecular target for pharmacological intervention in the treatment of T2DM. More specifically, we propose that in T2DM, the coupling of glucose metabolism to cAMP production is diminished, and that activators of sAC will restore levels of cAMP so that defective GSIS is repaired. Our hypothesis is based on the finding that sAC is a HCO_3^- and Ca^{2+} stimulated adenylyl cyclase that also serves as a physiological ATP sensor [39–46]. Our hypothesis is also consistent with the established fact that oxidative metabolism of glucose generates CO_2 and ATP while also promoting an increase of $[Ca^{2+}]_i$ in beta cells [47]. In our model (Fig. 2), CO_2 generated by glucose metabolism will be converted by carbonic anhydrase (CA) to carbonic acid (H_2CO_3) that will dissociate at physiological pH to generate HCO_3^-. Bicarbonate ion generated in this manner will stimulate sAC to generate cAMP that in turn potentiates GSIS.

What evidence is there in support of this hypothesis? One finding of potential significance is that GSIS is reduced when rat islets are equilibrated in saline to which no HCO_3^- is added [48,49]. This experimental finding obtained in the 1970's is understandable since the balance of CA-catalyzed HCO_3^- production is influenced by HCO_3^- transporters that are responsive to intracellular and extracellular HCO_3^-. Potentially, live-cell imaging techniques using cAMP biosensors such as Epac1-camps can be employed in order to determine if sAC-catalyzed cAMP production is sensitive to alterations of extracellular HCO_3^- concentration. In this regard, it might be possible to generate recombinant fusion proteins of sAC and Epac1-camps so that levels of cAMP can be monitored directly at the site of sAC-catalyzed cAMP production.

It is interesting to note that the CA inhibitor acetazolamide suppresses GSIS from rat islets [50,51]. Potentially, this action of acetazolamide is a consequence of its ability to block HCO_3^- production that normally couples sAC activation to the stimulation of insulin secretion (Fig. 2). Alternatively, acetazolamide might act non-specifically as a Ca^{2+} channel blocker to suppress Ca^{2+} influx that triggers insulin secretion. In fact, acetazolamide is reported to reduce glucose-stimulated Ca^{2+} influx in rat islets [51]. Unfortunately, interpretation of these findings is complicated by the lack of electrophysiological data with which to evaluate potential Ca^{2+} channel blocking actions of acetazolamide. Still, it should be noted that prior patch clamp studies demonstrate an ability of cAMP to facilitate glucose-dependent Ca^{2+} influx in beta cells [52–55]. Thus, it could be that acetazolamide indirectly inhibits Ca^{2+} influx by interfering with sAC-catalyzed cAMP production. Appropriate electrophysiological studies are needed to test whether beta-cell Ca^{2+} channel activity is directly or indirectly inhibited by acetazolamide.

Since beta cells express mitochondrial carbonic anhydrase V (CA-V) [50], and since sAC activity in mitochondria facilitates oxidative phosphorylation in some cell types [56–58], it could be that sAC elevates levels of cAMP in beta-cell mitochondria, thereby allowing oxidative glucose metabolism to more efficiently stimulate insulin secretion (Fig. 2). This concept is consistent with the established importance of mitochondrial metabolism to GSIS [59]. Conceivably, prior studies of islets underestimated the effectiveness with which glucose stimulates cAMP production since measurements of cAMP content in whole islet lysates do not reflect the true concentration of cAMP in mitochondria. Live-cell imaging studies using cAMP reporters targeted to the mitochondria should resolve these issues.

If mitochondrial sAC activity is of importance to GSIS, a pharmacological inhibitor of sAC should uncouple glucose metabolism from cAMP production and insulin secretion. Unfortunately, the most commonly used sAC inhibitor (KH7) is now recognized to exert non-specific inhibitory effects on glucose metabolism [60] and mitochondrial respiration [61]. Such non-specific effects of KH7 render it difficult to interpret prior reports that KH7 blocks cAMP production and insulin secretion in response to glucose [44,62]. Established alternatives to the use of KH7 include catechol ester-mediated inhibition of sAC catalytic activity [62], or siRNA-mediated knockdown of sAC expression [62]. Still, neither of these two alternative approaches allows selective targeting of mitochondrial sAC in the absence of any effect at cytosolic sAC. The availability of more specific inhibitors would be useful in order to test for a potential functional interaction of mitochondrial sAC and TMACs. For

example, if mitochondrial sAC activation upregulates glucose-dependent mitochondrial ATP production, the resultant depolarization-induced Ca^{2+} influx might have the capacity to activate the type 8 TMAC (AC-8) that is under the control of Ca^{2+}/calmodulin in rodent beta cells [63–65].

Since sAC expression is not restricted to mitochondria [56], it is easy to imagine a scenario in which glucose-stimulated mitochondrial CO_2 production generates HCO_3^- that stimulates cytosolic sAC to activate cAMP-dependent protein kinase A (PKA) located in the vicinity of secretory granules. If so, this signaling cascade might explain prior reports that PKA mediates the action of glucose to stimulate insulin secretion [66], possibly by promoting phosphorylation of Snapin [67], by increasing the Ca^{2+} sensitivity of exocytosis [68], or by activating SAD-A kinase (SAD-AK) to stimulate cytoskeletal remodeling that enables secretory granule exocytosis during 2nd phase GSIS [69,70]. Since sAC and PKA are both found within nuclei of cells [56,71], sAC might also mediate stimulatory effects of glucose to elevate nuclear levels of cAMP, and to activate beta-cell gene transcription that is PKA and CREB regulated [71].

Interestingly, sAC-stimulated cAMP production also has the capacity to activate Rap1 GTPase in PC12 cells [72]. Rap1 is the immediate downstream effector of the cAMP-regulated guanine nucleotide exchange factors Epac1 and Epac2 [73,74], and Rap1 links cAMP production to the PKA-independent stimulation of insulin secretion [1,14,75–83]. Thus, it is remarkable that a knockout (KO) of Epac1 gene expression leads to defective GSIS and a loss of glucoregulation in mice [84]. Furthermore, a KO of Epac2 gene expression in mice leads to a failure of glucose to stimulate insulin secretion under pathophysiological conditions of diet-induced insulin resistance [85,86]. Thus, there is good reason to believe that Epac proteins mediate stimulatory effects of glucose on insulin secretion, and that sAC might participate in this process by raising levels of cAMP. These possibilities are testable using newly described synthetic small molecules that selectively inhibit Epac proteins [87–89].

To understand the potential roles of sAC and Epac2 in the control of GSIS, it is important to note that when beta cells are stimulated with glucose, Epac2 is recruited to the plasma membrane by activated Ras GTPase [90]. This recruitment is mediated by binding of Ras to the Ras-association (RA) domain of Epac2, and it requires that Epac2 be activated by cAMP [90]. Remarkably, recruitment of Epac2 to active Ras is also Ca^{2+}-dependent [90]. This finding is understandable if glucose metabolism stimulates Ca^{2+} influx that activates a Ca^{2+}-sensitive and Ras-specific guanine nucleotide exchange factor such as Ras-GRF1 [91]. Using TIRF microscopy to monitor the inner surface of the plasma membrane, Idevall-Hagren *et al.* find that glucose induces oscillations of cAMP and Ca^{2+} that are synchronized with Ras and Rap1 activation, and that correlate with reversible translocation of Epac2 to the plasma membrane [90]. Although these oscillations are blocked by an inhibitor of TMACs [90], it is possible that glucose metabolism activates mitochondrial sAC to generate cAMP and Ca^{2+} signals that support this oscillatory activity. For example, if sAC-catalyzed cAMP production in mitochondria facilitates glucose-dependent oxidative phosphorylation, the resultant increase of ATP/ADP concentration ratio will provide the metabolic signal for K-ATP channel closure and depolarization-induced Ca^{2+} influx. This Ca^{2+} signal will activate

Ras-GRF1 to sequentially activate Ras, while also acting at the AC-8 TMAC to further stimulate cAMP production. The net effect will be periodic recruitment of Epac2 to the plasma membrane, and this periodicity will be dictated by oscillations of $[Ca^{2+}]_i$ that are initiated by oxidative glucose metabolism under the control of sAC.

When evaluating the above-summarized beta-cell cAMP signaling mechanisms controlling insulin secretion, it is important to note that permissive PKA activity supports GSIS from rodent and human islets, even in the absence of any added cAMP-elevating agent [66,92]. Such findings indicate that it will be important in future studies to ascertain whether the permissive PKA activity that supports GSIS is generated by sAC and/or TMACs. Just as important, it will be of interest to determine if sAC or TMAC activity is altered in donor islets of T2DM patients. Prior studies using rat models of T2DM indicate that islets of these rats have defective coupling of glucose metabolism to cAMP production [93,94].

Glucose metabolism raises levels of HCO_3^-, Ca^{2+}, and ATP in pancreatic beta cells to stimulate sAC-catalyzed production of cAMP in the mitochondria and in the cytoplasm. cAMP in the mitochondria enhances oxidative phosphorylation that acts as a stimulus for insulin secretion. cAMP in the cytoplasm activates PKA to promote phosphorylation of substrate proteins (*e.g.*, Snapin) that regulate secretory granule exocytosis. Cytoplasmic cAMP also activates an Epac, Rap1, and PLCε signal transduction “module” that mobilizes Ca^{2+} from endoplasmic reticulum (ER) Ca^{2+} stores at which IP_3 receptors (IP_3R) and ryanodine receptors (RYR) are located [102,136–138]. In human beta cells, L-type VDCCs participate in action potential generation, whereas P/Q-type VDCCs participate in insulin exocytosis. Additional abbreviations: CICR, Ca^{2+}-induced Ca^{2+} release; DAG, diacylglycerol; GLUT, glucose transporter; GPCR, G protein-coupled receptor; Kir6.2, pore-forming subunit of K-ATP channels; PKC, protein kinase C; SAD-AK, synapses of amphids defective kinase-A; SUR1, sulfonylurea receptor-1 subunit of K-ATP channels.

5. Investigation of sAC activity using an insulin-secreting cell line

Ramos *et al.* report that a step-wise increase of glucose concentration from 2.5 to 16 mM leads to cAMP production in rat INS-1E insulin-secreting cells [62]. The response appears slowly (peak at 15 min) and is measurable under conditions in which cells are treated with the cyclic nucleotide phosphodiesterase (PDE) inhibitor IBMX. Despite interpretive problems associated with the lack of specificity of sAC inhibitor KH7 (see above) Ramos *et al.* also find that the cAMP-elevating action of glucose is suppressed by KH7, whereas a P-site inhibitor of TMACs (ddAdo) is ineffective. In contrast, the action of GLP-1 to raise levels of cAMP is unaffected by KH7 but is blocked by ddAdo. Immunoprecipitation, western blot, and immunocytochemical analyses also demonstrate expression of an *ca.* 50 kDa isoform of sAC in these INS-1E cells. Knockdown of sAC expression using sAC-specific siRNA reduces levels of sAC protein while also reducing the action of glucose to stimulate cAMP production [44,62]. Interestingly, agents that interfere with depolarization-induced Ca^{2+} influx (diazoxide, verapamil) or that buffer intracellular Ca^{2+} (EGTA-AM) eliminate the action of glucose to raise levels of cAMP [62]. Collectively, these findings indicate that for INS1-E cells, the binding of Ca^{2+} to a TMAC (*i.e.*, AC-8) or sAC is of significance to glucose-stimulated cAMP production. However, it is not yet possible to

decipher which of these two adenylyl cyclases plays a dominant role in the overall process of glucose-stimulated cAMP production. Finally, the utility of INS-1E cells for studies of this type is further emphasized by the finding that KH7, but not the TMAC inhibitor ddAdo, uncouples glucose metabolism from cAMP-dependent activation of ERK1/2 mitogen-activated protein kinases [62]. Moreover, the sAC inhibitors KH7 and catechol estrogen block glucose-stimulated insulin secretion from INS-1E cells [44].

6. Defective glucoregulation in sAC knockout mice

Using a new strain of sAC-C1 mice in which there is a global KO of sAC gene expression, Zippin *et al.* report that sAC participates in the *in vivo* control of blood glucose homeostasis [44]. This finding is established by comparing glucoregulation in homozygous sAC-C1 −/− KO mice as compared with heterozygous sAC-C1 +/− mice containing a single wild-type sAC allele. Thus, in an intraperitoneal (*i.p.*) glucose tolerance (IPGT) test that reveals direct stimulatory effects of glucose at the endocrine pancreas, levels of blood glucose rise to higher levels after administration of glucose to sAC −/− KO mice. Furthermore, *i.p.* administration of glucose to sAC −/− KO mice stimulates only a small increase of plasma insulin in comparison with sAC +/− mice. Such findings indicate that sAC expression is necessary in order for glucose tolerance and insulin secretion to be homeostatically regulated under conditions of *i.p.* glucose administration.

Zippin *et al.* also report remarkable findings concerning assays of 1st and 2nd phase GSIS using isolated islets of sAC-C1 KO mice [44]. By comparing insulin secretion from islets of sAC +/+ wild-type mice or sAC −/− KO mice, Zippin *et. al.* demonstrate that for the sAC KO, there is a reduction of both 1st and 2nd phase GSIS, as measured when the glucose concentration is stepped from 5 mM to 30 mM. Although a detailed analysis of islet insulin content and beta-cell mass has yet to be provided, histology demonstrates that the homozygous sAC-C1 KO mice have normal pancreatic morphology, normal islet structure, and normal islet size [44]. However, one important caveat to the interpretation of these studies is that the KO of sAC gene expression is not restricted to beta cells in sAC-C1 KO mice. Therefore, it will be important to assess how glucoregulation is altered in beta-cell specific sAC KO mice.

7. Is there a role for sAC in metabolic processes of beta-cell compensation?

When considering future avenues of research regarding sAC, it will be especially important to assess whether sAC participates in processes of beta-cell compensation that serve to maintain GSIS under conditions of metabolic stress. For example, it could be that the beta-cell cAMP signaling “network” exhibits plasticity under conditions of metabolic stress, and that sAC-stimulated cAMP production explains enhanced GSIS that serves to counteract insulin resistance induced by metabolic stress. A hint that this might be the case is provided by the study of Song *et al.* in which metabolic stress induced by a high fat diet (HFD) reveals a prominent role for Epac2 in the control of GSIS [85]. The HFD induces insulin resistance, and under these conditions, mouse beta cells undergo functional compensation to upregulate GSIS, whereas this compensation is lost in beta cells of Epac2 KO mice [85].

Based on the findings of Song *et al.*, HFD-induced beta-cell compensation in mice appears to involve an increased capacity of glucose metabolism to signal through Epac2 to increase levels of cytosolic Ca^{2+} [85]. Importantly, this compensation is measureable in assays of GSIS using isolated islets, and it occurs in the absence of an added GPCR agonist [85]. Since Epac2 activation in normal wild-type beta cells promotes Ca^{2+} influx while also mobilizing an intracellular source of Ca^{2+} [95–102], the coupling of sAC activity to Epac2 activation and Ca^{2+} handling seems like a fruitful area for future investigation. Potentially, sAC-C1 KO mice can be evaluated in order to determine if processes of beta-cell compensation important to GSIS are disrupted under conditions of the HFD. Since cAMP-elevating agents stimulate beta-cell proliferation [103–105], while protecting against beta-cell death [106], sAC activity might also maintain beta-cell mass under conditions of metabolic stress.

8. Regulation of beta-cell cAMP production by GPCRs and TMACs

sAC-catalyzed cAMP production in response to glucose is complemented by TMAC-catalyzed cAMP production that is initiated by agonist binding to G protein-coupled receptors (GPCRs). The GPCRs that stimulate cAMP production in beta cells include members of the Family B GPCRs that specifically bind glucagon, GLP-1, GIP, and PACAP [34,35,107]. Traditionally, it is thought that these GPCRs mediate the intra-islet (glucagon), hormonal (GLP-1, GIP) and neural (PACAP) stimulation of insulin secretion. However, recent findings indicate that GLP-1 released from intestinal L-cells also activates vagal-vagal reflexes that stimulate insulin secretion [35,108]. For the treatment of T2DM, synthetic GLP-1 receptor agonists exist, and they include exenatide and liraglutide [35].

Signal transduction crosstalk of sAC and TMACs might exist in beta cells because sAC-catalyzed cAMP production in mitochondria is predicted to enhance the action of glucose to promote depolarization-induced Ca^{2+} influx, thereby allowing Ca^{2+} to directly stimulate TMAC activity. Multiple isoforms of TMACs are expressed in beta cells [63], and in this regard AC-8 is especially important for rodent beta cells due to the fact that it serves as a molecular coincidence detector for the cAMP-dependent control of GSIS [64,65]. Coincidence detection at AC-8 occurs because the activity of AC-8 is stimulated by heterotrimeric G_s proteins and also by Ca^{2+}/CaM that binds directly to AC-8 [109,110]. For example, we propose that binding of GLP-1 to its GPCR stimulates AC-8, and that this effect is reinforced by glucose metabolism that is under the control of sAC and that elevates levels of Ca^{2+} in order to further stimulate AC-8. Synergistic activation of AC-8 is achieved when GLP-1 is paired with glucose, and under these conditions oscillations of cAMP and Ca^{2+} are generated so that GSIS is powerfully stimulated [111–116].

An additional noteworthy finding is the recent report of Hodson *et al.* that human islet GSIS is conditional on expression of the TMAC isoform 5 (AC-5) in beta cells [117]. In studies of human islets subjected to an shRNA-mediated knockdown of AC-5, it is possible to demonstrate that reduced AC-5 expression leads to a modest but significant reduction of GSIS under conditions in which the glucose concentration is stepped from 3 to 11 mM. In contrast, a knockdown of AC-5 does not alter the action of GLP-1 to stimulate insulin secretion under conditions in which the glucose concentration is fixed at 11 mM. By

imaging the fluorescent cAMP reporter Epac2-camps, or the fluorescent Ca^{2+} reporter fluo-2, in single human islets or single human beta cells, it is also possible to demonstrate that glucose is a less effective stimulus for cAMP production and Ca^{2+} influx in islets subjected to the knockdown of AC-5. Such findings are surprising in view of the fact that AC-5 was previously considered to be an adenylyl cyclase that is inhibited by Ca^{2+}. However, Hodson *et al.* point out that AC-5 is inhibited by concentrations of Ca^{2+} that are in excess of that normally found in the cytosol [117]. Taken as a whole, such findings concerning AC-5 are consistent with the prior finding of Chepurny *et al.* demonstrating a role for permissive PKA activity in support of GSIS [92], as well as the findings of Hatakeyama, Takahashi, Kasai, and co-workers who propose that beta-cell glucose metabolism is tightly linked to TMAC-stimulated cAMP production [66,118,119]. One particularly interesting outgrowth of this line of investigation is the apparent role of AC-5 as a signal transducer linking human beta-cell glucose metabolism to depolarization-induced Ca^{2+} influx [117]. Although it remains to be demonstrated, cAMP generated in response to glucose metabolism might be linked to Epac2 activation with consequent K-ATP channel closure that generates beta-cell depolarization [95,96].

9. Conclusion

Over forty years have elapsed since the first reports documenting an ability of glucose to raise levels of cAMP in the islets of Langerhans [120–131]. With time, these studies were discounted due to the relatively small change of cAMP concentration induced by glucose. Since such prior studies used biochemical assays to detect levels of cAMP in whole islets, we propose that these studies need to be reinterpreted because the methods that were used cannot detect compartmentalized cAMP production that is potentially sAC-mediated. Importantly, earlier studies found that the time course of 1^{st} phase GSIS matched that of the glucose-stimulated increase of cAMP concentration in whole islets [121,126,127]. In retrospect, this temporal coincidence is understandable if 1^{st} phase GSIS results from exocytosis that is at least in part cAMP-stimulated [14]. In this regard, we wish to emphasize that cAMP is not the only stimulus for GSIS, but that additional metabolic coupling factors derived from glucose metabolism also participate. However, since genetically-encoded cAMP biosensors are able to detect glucose-stimulated changes of cAMP concentration in single beta-cells and whole islets [90,112,113,132,133), there exists a new appreciation for actions of glucose to stimulate insulin secretion in a cAMP-mediated manner [134]. In fact, a capacity of cAMP to directly stimulate beta-cell exocytosis is convincingly documented in the report of Ammala *et al.* published in 1993 [135], at which time the functional significance of sAC was not yet appreciated. However, since the cloning of sAC from a rat testis cDNA library in the late 1990's [136], the significance of sAC to cAMP signaling in many cell types is increasingly apparent. With the availability of sAC KO mice in which there is defective glucoregulation accompanied by reduced GSIS [44], it is anticipated that the full significance of sAC to beta-cell biology, and possibly T2DM, will become readily apparent.

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Abbreviations

AC-5/8	adenylyl cyclase isoforms 5 or 8
ddAdo	2′,5′-dideoxyadenosine
CA	carbonic anhydrase
cAMP	adenosine-3′,5′-cyclic monophosphate
CREB	cAMP response element-binding protein
Epac1/2	exchange proteins activated by cyclic AMP isoforms 1/2
GIP	glucose-dependent insulinotropic polypeptide
GK	glucokinase
GLP-1	glucagon-like peptide-1
Glut1/2	glucose transporter isoforms 1/2
GPCR	G protein-coupled receptor
GSIS	glucose-stimulated insulin secretion
HFD	high fat diet
K-ATP	ATP-sensitive K^+ channel
KO	knockout
Kir6.2	inward rectifier pore-forming subunit
K_v	voltage-dependent K^+ channel
K_{Ca}	calcium-activated K^+ channel
PACAP	pituitary adenylyl cyclase-activating polypeptide
PDE	cyclic nucleotide phosphodiesterase
PKA	protein kinase A
PLCε	phospholipase C-epsilon
RA	Ras-association domain
RP	reserve pool
RRP	readily releasable pool
sAC	soluble adenylyl cyclase
SAD-AK	SAD-A kinase
SUR1	sulfonylurea receptor type1

T2DM	type 2 diabetes mellitus
TMAC	transmembrane adenylyl cyclase
VDCC	voltage-dependent Ca^{2+} channel

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Highlights

- sAC KO mice have reduced glucose tolerance and defective insulin secretion.
- sAC may link glucose metabolism to cAMP production in pancreatic beta-cells.
- cAMP generated by sAC may facilitate glucose-stimulated insulin secretion.

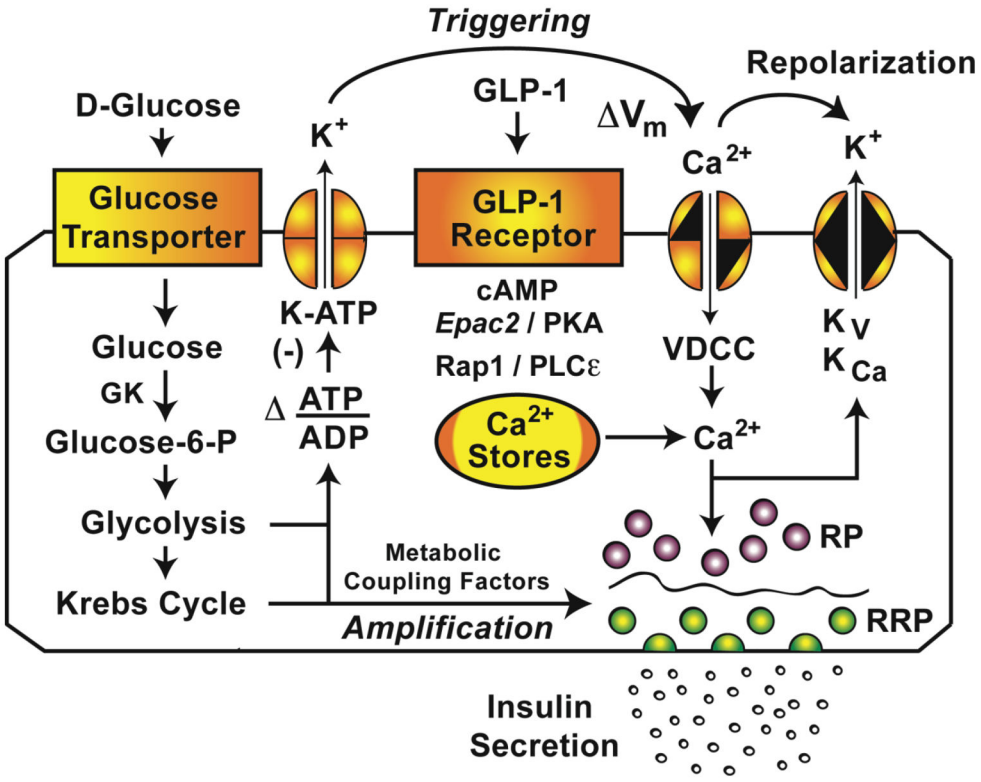

Figure 1.
Triggering and amplification pathways of GSIS.

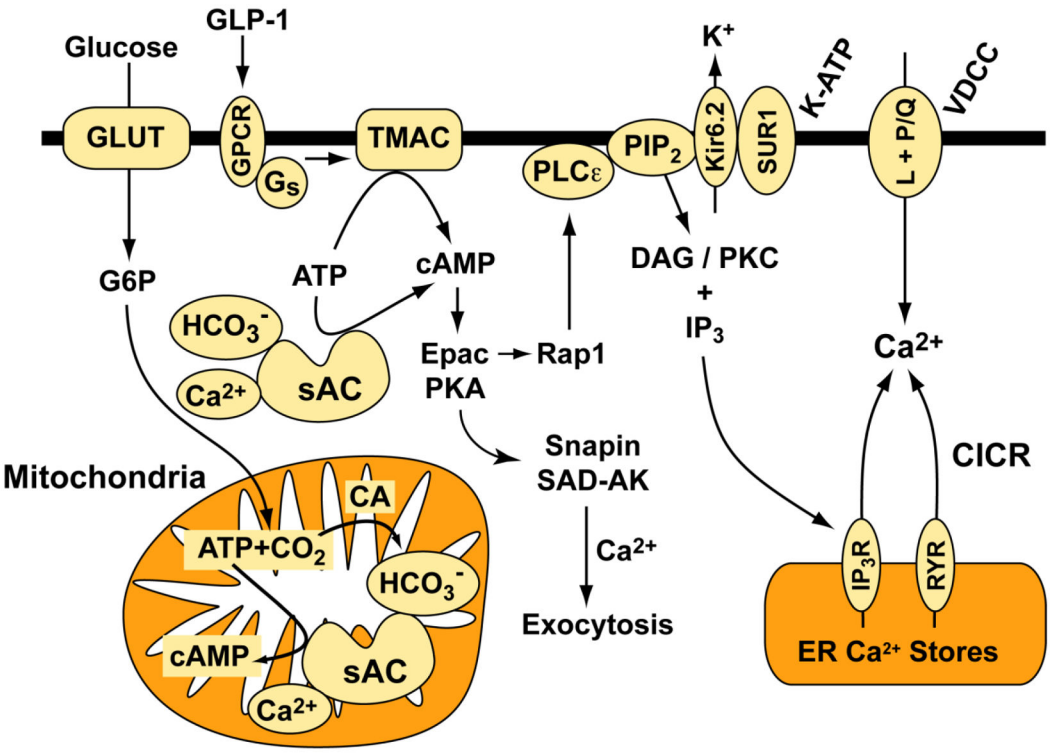

Figure 2.
Hypothetical role of sAC in GSIS.