

## Strathprints Institutional Repository

Furman, Brian L. and Ong, W.K. and Pyne, Nigel J. (2010) *Cyclic AMP signalling in pancreatic islets*. Advances in Experimental Medicine and Biology, 654. pp. 281-304. ISSN 0065-2598

Strathprints is designed to allow users to access the research output of the University of Strathclyde. Copyright © and Moral Rights for the papers on this site are retained by the individual authors and/or other copyright owners. You may not engage in further distribution of the material for any profitmaking activities or any commercial gain. You may freely distribute both the url (<http://strathprints.strath.ac.uk/>) and the content of this paper for research or study, educational, or not-for-profit purposes without prior permission or charge.

Any correspondence concerning this service should be sent to Strathprints administrator: <mailto:strathprints@strath.ac.uk>

## Chapter 13

# Cyclic AMP Signalling in Pancreatic Islets

Brian Furman, Wee Kiat Ong, and Nigel Pyne

**Abstract** Cyclic 3'5'AMP (cAMP) is an important physiological amplifier of glucose-induced insulin secretion by the pancreatic islet  $\beta$ -cell, where it is formed by the activity of adenylyl cyclases, which are stimulated by glucose, through elevation in intracellular calcium concentrations, and by the incretin hormones (GLP-1 and GIP). cAMP is rapidly degraded in the pancreatic islet  $\beta$ -cell by various cyclic nucleotide phosphodiesterase (PDE) enzymes. Many steps involved in glucose-induced insulin secretion are modulated by cAMP, which is also important in regulating pancreatic islet  $\beta$ -cell differentiation, growth and survival. This chapter discusses the formation, destruction and actions of cAMP in the islets with particular emphasis on the  $\beta$ -cell.

**Keywords** Cyclic AMP · Adenylyl cyclase · Phosphodiesterase · Insulin secretion · Protein kinase A · Epac · GLP-1

## 13.1 Introduction

Interest in the role of cyclic 3'5' AMP (cAMP) in regulating insulin secretion dates back more than 40 years, since Turtle and Kipnis [1] showed increases in cAMP in isolated islets in response to glucagon. Increases in islet  $\beta$ -cell cyclic AMP occur in response to nutrients, especially glucose. Glucose has been widely shown to increase intracellular levels of cAMP in islets and various insulin-secreting cell lines [2–6]. Although cyclic AMP does not appear to be essential for glucose-induced insulin secretion [3, 7–9], it is established as an important intracellular amplifier of this process [10–12]. Several hormones exert their effects on insulin secretion through increased  $\beta$ -cell cAMP levels. These include glucose-dependent

---

B. Furman (✉)

Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow G4ONR, Scotland, UK

insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) which are collectively referred to as the incretins, and which are also secreted in response to nutrients [13–16]. GLP-1 and GIP serve to augment meal-related insulin secretion [17]. Their physiological importance is evident from observations that mice lacking receptors for both incretin hormones show marked glucose intolerance and impairment of insulin secretion [18]. This chapter focuses largely on cAMP in the  $\beta$ -cell. Much less is known about the role of cAMP in other islet cells, although there is some information on this in relation to glucagon and somatostatin secretion/synthesis and these aspects will be addressed briefly at the end of the chapter.

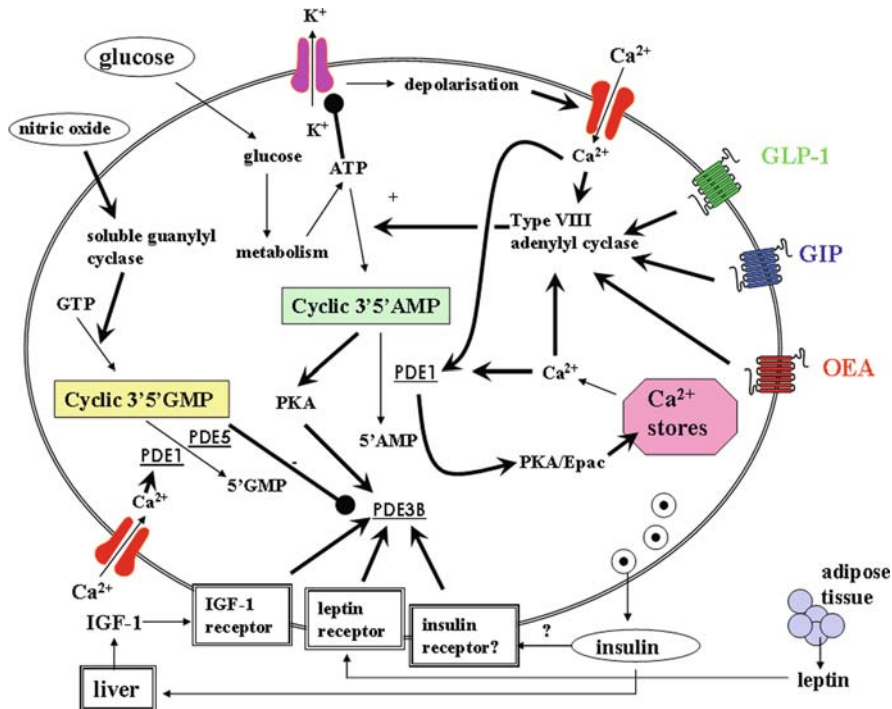
## 13.2 Control of cAMP Levels in the $\beta$ -Cell

The level of cyclic AMP in the  $\beta$ -cell depends on the balance between its formation through the activity of adenylyl cyclases (ACs) and its destruction by cyclic nucleotide phosphodiesterases (CN-PDEs). This is summarized in Fig. 13.1 and discussed below.

### 13.2.1 Formation of Cyclic AMP in the $\beta$ -Cell

Glucose-induced elevations in intracellular cAMP are probably secondary to changes in the concentration of calcium, which is itself elevated as a result of a number of mechanisms but primarily by  $\text{Ca}^{2+}$  influx through voltage-sensitive  $\text{Ca}^{2+}$  channels in response to membrane depolarization, following closure of ATP-sensitive potassium channels. Hormone-induced formation of cAMP results from stimulation of seven transmembrane G-protein-coupled receptors (GPCRs), leading to activation of the  $G_s$  protein and dissociation of the  $G\alpha\beta\gamma$  heterotrimeric complex and sequential activation of adenylyl cyclases [19]. The  $\beta$ -cell expresses several GPCRs coupled to  $G_s$ , stimulation of which leads to elevation in the  $\beta$ -cell level of cAMP. These include receptors for GLP-1, GIP, PACAP as well as the receptor GPR119 (see below). On the other hand, reductions in cAMP occur in response to several agents that activate GPCRs coupled to  $G_i$ , for example adrenaline [20],  $\text{PGE}_2$  [21] and NPY ( $Y_1$ ) [22]. There is also evidence for the role of the pertussis toxin-insensitive G-protein  $G_z$  in the reduction of cAMP and inhibition of insulin secretion in response to prostaglandin  $E^1$  [23].

GLP-1, through stimulation of its Class II GPCR, activates AC with consequent production of intracellular cAMP [24, 25]. Oxyntomodulin, which like GLP-1, is derived from the proglucagon gene, also binds to the GLP-1 receptor, increases cAMP levels and stimulates insulin secretion [26]. There is also evidence for coupling to  $G_i/G_o$ , and, in various, non- $\beta$ -cell systems to other G-proteins ( $G_q/11\alpha$ ), although the physiological significance of this remains to be established. Sonoda et al. [27] identified an unusual role for  $\beta$ -arrestin-1 in coupling the GLP-1 receptor to



**Fig. 13.1** Summary of the mechanisms for the formation and destruction of cAMP in the pancreatic islet  $\beta$ -cell. Glucose is transported into the  $\beta$ -cell using GLUT2 and is then metabolized generating ATP. This results in closure of the  $K_{ATP}$  channel, membrane depolarization and calcium influx through voltage-sensitive calcium channels. Calcium is also mobilized from intracellular stores by  $Ca^{2+}$  (calcium-induced calcium release – not shown). The increased cytosolic-free  $Ca^{2+}$  triggers exocytosis. These processes are amplified through increases in cAMP effected both through activation of adenylyl cyclases by glucose itself (through calcium-activated adenylyl cyclase – type VIII- AC VIII) and by the incretin hormones GLP-1 and GIP, acting through G-protein-coupled receptors in the  $\beta$ -cell membrane. Endogenous agonists for the G-protein-coupled receptor GPR119 include oleoylethanolamide (OEA). Activation of GLP-1 receptors acts synergistically with glucose in activating AC VIII and also activates other adenylyl cyclases, including soluble adenylyl cyclase (not shown). Activation of adenylyl cyclases increases the formation of cAMP which activates PKA and Epac which mediate the actions of cAMP in the cell. PKA/Epac facilitates calcium-induced calcium release which in turn may also activate AC VIII. The destruction of cAMP is effected through various phosphodiesterases (PDEs).  $Ca^{2+}$  activates PDE1 whereas PKA activates PDE3B, which is also activated by other signals generated through the IGF-1 and leptin receptors, as well as, possibly, the insulin receptor. On the other hand, PDE3B may be inhibited by increases in cGMP, allowing cross-talk between cGMP and cAMP signalling. Roles for other PDEs (PDE4, 8B and 10A) have been proposed (modified from [54])

adenylyl cyclase in INS-1 cells, thereby increasing cAMP and stimulating insulin secretion.

GIP produces its biological effects by interacting with its Class II G-protein-coupled receptor coupled to the production of cyclic AMP [28–30]. The pancreatic

islet  $\beta$ -cell GIP receptor is down-regulated by exposure to high concentrations of glucose, which prevents the GIP-induced elevation in intracellular cAMP [31]. This is hypothesized to explain the lack of response of diabetic patients to the peptide.

PACAP is expressed in nerve fibres and the pancreatic islets and is a potent stimulator of insulin secretion [32, 33] through activation of adenylyl cyclase [34]. There are several receptors for PACAP, with the PAC1 receptor (PAC1-R) and VPAC2 receptor (VPAC2-R) thought to be the most important in relation to insulin secretion [35].

GPR119 is a Class I GPCR, the expression of which is restricted largely to pancreatic islets, although lesser amounts of message are detected in the human gastrointestinal tract and in the rodent brain [36–38]. The potential endogenous ligands for this receptor so far identified are oleoyl lysophosphatidylcholine and oleylethanolamide, although there is as yet no evidence that they are available in sufficient concentrations in the blood to stimulate the  $\beta$ -cell GPR119 receptor in vivo. The receptor is coupled through  $G_s$  to adenylyl cyclase, and its activation produces an increase in cAMP and stimulation of insulin secretion.

### 13.2.1.1 Adenylyl Cyclases in the Pancreatic Islet $\beta$ -Cell

There are at least nine different membrane-bound isoforms of AC, described as AC I–AC IX and expressed in mammalian cells [39, 40]. An additional, soluble form is also expressed in certain mammalian cells [41]. RT-PCR studies, as well as immunohistochemical staining, using rat and human islets, rat  $\beta$ -cells, and clonal  $\beta$ -cell lines have shown expression of AC II [42] and III, IV, V, VI, VII and VIII [5, 43–45]. All isoforms of adenylyl cyclase, apart from ACIX, are activated by the diterpene forskolin, which produces marked increases in cAMP in numerous cell types [46, 47]. There are three calcium-activated ACs (AC1, ACIII and ACVIII), and the presence of calcium–calmodulin-activated ACVIII probably explains activation of cyclic AMP formation in response to glucose, which rapidly elevates  $[Ca^{2+}]_i$ . This AC isoform is synergistically activated by both  $G_s\alpha$  and calcium/calmodulin [48]. Thus, the combination of glucose and GLP-1 increases cAMP accumulation in rat isolated primary  $\beta$ -cells or clonal  $\beta$ -cell lines more markedly than either alone, the effect being reduced if calcium entry through voltage-sensitive L-type channels is prevented using verapamil [45]. The expression of type VI (but not types II, III or V) adenylyl cyclase was increased along with the expression of the GLP-1 receptor rat pups fed a high-carbohydrate diet for 12 days [42]. These findings provide some circumstantial evidence that the type VI adenylyl cyclase may be associated with GLP-1 signalling. More recently, a role for soluble AC was proposed to explain the different kinetics of cAMP formation in response to glucose and GLP-1 in INS-1E cells. GLP-1 produced a rapid increase as a result of activation of transmembrane AC, whereas the increase in cAMP in response to glucose was delayed and was attributed to activation of the calcium, bicarbonate and ATP-sensitive soluble AC [6].

Paradoxically, acetylcholine, which increases insulin secretion through stimulation of muscarinic receptors coupled to phospholipase C/protein kinase C pathways,

also activated adenylyl cyclases and elevated cAMP content in islets from GK-diabetic rats [49]. The insulin secretory response to acetylcholine in these islets was blocked by inhibitors of adenylyl cyclase or PKA inhibitors. The abnormal nature of the islet in these rats may somehow has facilitated cross-talk resulting in activation of a calcium-sensitive adenylyl cyclase, or a PKC-sensitive adenylyl cyclase, e.g. ACII [40], in response to acetylcholine.

### ***13.2.2 Destruction of cAMP in the Pancreatic Islet $\beta$ -Cell -Cyclic Nucleotide Phosphodiesterases***

Cyclic nucleotide phosphodiesterases (CN-PDEs) provide the only known means for the rapid inactivation of the cyclic nucleotides cAMP and cGMP in most cells. There are now known to be at least 100 PDE enzymes derived from 11 known gene families (PDE1-11). The enzymes show differences in their tissue distribution, substrate selectivities (cGMP vs cAMP), kinetics, regulation, and susceptibility to pharmacological inhibition. There are several excellent reviews [50–53], and the properties of those PDE enzymes present in pancreatic islets have been reviewed elsewhere [54, 55]. The key observations are summarized in this chapter, together with more recent findings.

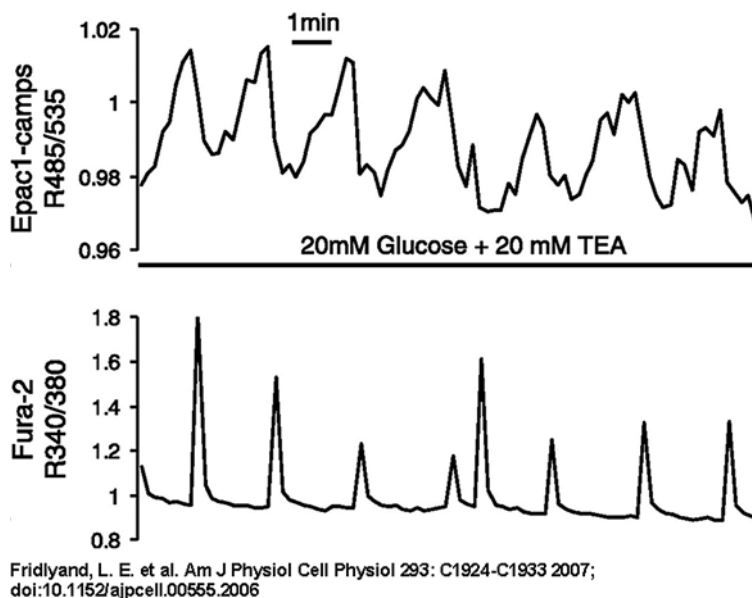
Several PDE isoforms, including PDE1 [56–61], PDE3B [59–67], PDE4 [59, 60, 64] and PDE8B [68], contribute to the total  $\beta$ -cell PDE activity, and several of these isoforms regulate glucose-induced insulin secretion and other cAMP-mediated  $\beta$ -cell functions in islets and in cell lines [see 54, 55 for references]. There is much evidence from RT-PCR, immunostaining, siRNA and biochemical and functional studies using selective inhibitors that PDE3B plays a key role in both islets and insulin-secreting cell lines in terms of regulating insulin secretion [54, 55, 61, 63–66]. Additional evidence for the role of PDE3B in regulating  $\beta$ -cell cAMP and insulin secretion was obtained by over-expressing PDE3B in the INS-1  $\beta$ -cell line and in islets and by using transgenic animals over-expressing PDE3B in the  $\beta$ -cell. These in vitro and in vivo studies clearly showed that glucose-induced, as well as GLP-1-induced, insulin secretion was impaired by PDE3B over-expression. Interestingly, both endogenous and over-expressed PDE3B was found to be located in insulin granules and the plasma membrane [67]. In vitro, the over-expression of PDE3B markedly reduced cAMP-induced exocytosis and animals over-expressing PDE3B in islets showed markedly impaired glucose tolerance [65–67]. In addition, activation of PDE3B appears to mediate the effect of IGF-1 [63] and leptin [69] in inhibiting insulin secretion.

The role of cGMP in regulating insulin secretion is not established, but several studies have shown that nitric oxide, acting through a soluble guanylyl cyclase and GMP formation, augments insulin secretion through several mechanisms shared with cAMP (see Section 13.3.1) [70–73]. These observations might be explained by cGMP-dependent inhibition of PDE3B and concomitant increases in [cAMP]<sub>i</sub>.

Although evidence for the importance of PDE3B is widely supported there is also evidence, but no consensus, for roles for other PDEs. Roles for PDE1C and PDE4 have been suggested on the basis of the use of either selective inhibitors [59, 64] or siRNA [64]. Depletion of PDE8B using siRNA produced a marked enhancement of glucose-induced insulin secretion from INS-1E cells [64, 68] and rat islets [68]. A role for PDE10A has been proposed and selective inhibitors have been patented for the treatment of diabetes [74], but there is no consensus on the expression of this PDE in the  $\beta$ -cell, and in one study [64] selective knockdown of PDE10A failed to modify glucose-induced insulin secretion in INS-1 cells.

### 13.2.3 Dynamics of cAMP Formation and Destruction

Real-time measurements of changes in cAMP in  $\beta$ -cells or islets have been hugely facilitated by the development of new technologies, particularly the development of genetically encoded fluorescence resonance energy transfer (FRET)-based biosensors and the associated imaging techniques. These have either been transiently transfected into  $\beta$ -cell lines or primary  $\beta$ -cells [5, 75–78] or been incorporated in vivo by generating a transgenic mouse expressing a pancreatic  $\beta$ -cell-targeted cAMP reporter which was inducible in response to tetracycline [4]. In MIN6  $\beta$ -cells, the use of the biosynthetic FRET-based cAMP sensor Epac1-camps, together with FURA-2 to detect  $[Ca^{2+}]_i$ , showed a close coupling of changes in cAMP and  $[Ca^{2+}]_i$  [5]. Exendin-4 and forskolin induced pronounced FRET signals. Formation of cAMP in response to these agents was preceded by increases in  $[Ca^{2+}]_i$  and was dependent upon extracellular calcium. Moreover, increases in  $[Ca^{2+}]_i$  evoked by other agents (carbachol,  $K^+$ , and tolbutamide) also stimulated cAMP formation. Simultaneous imaging of  $[Ca^{2+}]_i$  and cAMP during glucose stimulation (in the presence of TEA) revealed a tight coupling between oscillations in  $[Ca^{2+}]_i$  and cAMP with peak cAMP concentrations being seen at the nadir of  $[Ca^{2+}]_i$ . The data are consistent with the possibility that  $Ca^{2+}$ -activated adenylyl cyclases (AC VIII or AC III) and PDEs (PDE1C?) contribute to the oscillatory changes in cAMP seen in these studies. How this concept fits with the widely accepted role of PDE3B in regulating the cAMP pool relevant to insulin secretion (Section 13.2.2) remains to be determined. Other experimental studies (Fig. 13.2) and mathematical modelling have supported these ideas [75]. Imaging of the islets from transgenic mice expressing a  $\beta$ -cell-targeted reporter showed a rapid, biphasic and concentration-dependent (5.5–35 mM) increase in cAMP in response to glucose. This preceded increases in  $[Ca^{2+}]_i$  and was independent of extracellular  $[Ca^{2+}]$  [4]. In INS-1 cells, GLP-1 produced marked oscillations in cAMP at low concentrations (0.3–1 nM) with higher concentrations (10 nM) producing more sustained elevations [77]. GLP-1 also produced marked  $Ca^{2+}$  spiking, which rapidly followed the increases in cAMP. This pattern of changes in cAMP and  $Ca^{2+}$  was mimicked by application of short pulses of the non-selective PDE inhibitor, IBMX. The rapidity of the cAMP-induced  $Ca^{2+}$  signal suggests a close proximity of the cAMP to the sites of calcium entry/release



#### AJP - Cell Physiology

**Fig. 13.2**  $\text{Ca}^{2+}$  and cAMP oscillations in glucose-stimulated MIN6 cells. Simultaneous imaging of cytosolic cAMP concentration ( $[\text{cAMP}]_i$ ; *top trace*,  $R_{485/535}$ ) and cytosolic  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ; *bottom trace*,  $R_{340/380}$ ) in a single MIN6 cell stimulated with 20 mM glucose and 20 mM tetraethylammonium chloride (TEA). Note that second messenger oscillations were out of phase, with each  $[\text{Ca}^{2+}]_i$  spike coupled to a rapid and transient reduction in  $[\text{cAMP}]_i$ . (Reproduced from Fridlyand LE, Harbeck MC, Roe MW, Philipson LH. Regulation of cAMP dynamics by  $\text{Ca}^{2+}$  and G protein-coupled receptors in the pancreatic beta-cell: a computational approach. *Am J Physiol Cell Physiol* 293: C1924–33, 2007 [75] with permission)

(see next section). On the other hand, translocation of the catalytic subunit of PKA to the nucleus occurred relatively slowly and only in response to sustained increases in cAMP. Glucose also induced oscillations of intracellular cAMP levels in MIN6 and mouse primary  $\beta$ -cells. These oscillations correlated with pulsatile insulin secretion and both cAMP oscillations and pulsatile insulin release were reduced by inhibiting adenylyl cyclases [78]. Forskolin, glucagon and IBMX all augmented the frequency of glucose-induced oscillations in  $[\text{Ca}^{2+}]_i$  in mouse pancreatic islets [79]

#### 13.2.4 Intracellular Compartmentalization of cAMP Formation, Action and Degradation

It is now established that intracellular cAMP is not uniformly distributed in the cell and exists in different cellular locations to fulfil different functions. Local generation,



hydrolysis and activity of cAMP are ensured by spatial distribution into compartments, or signalling complexes, of adenylyl cyclases, PDEs and effector proteins, as well as phosphatases that terminate the activity of various kinases (e.g. 80, 81). This spatial anchoring of signalling complexes is effected by a family of A-kinase anchoring proteins (AKAPs). Recent work has suggested the importance of AKAPs in the insulin-secreting  $\beta$ -cell. Peptides that competitively inhibit the interaction between the regulatory subunit of PKA and the AKAP inhibited GLP-1-induced insulin secretion from rat islets without modifying its ability to elevate intracellular cAMP [9]. Expression of this inhibitory peptide in the clonal rat  $\beta$ -cell line, RINm5F, resulted in a redistribution of the PKA regulatory subunit and inhibited elevations in  $[Ca^{2+}]_i$  and insulin secretion in response to a cAMP analogue. Expression of an AKAP (AKAP18) in clonal insulin-secreting cells (RINm5f) augmented GLP-1-induced insulin release, whereas expression of a mutant form in these cells was inhibitory [82]. These findings were supported by others [83] who used a cell-permeable peptide (TAT-AKAPis) to competitively inhibit PKA–AKAP interactions in INS-1 cells. This peptide disrupted PKA–AKAP interactions and inhibited both glucagon-induced augmentation of insulin secretion and phosphorylation of p44/p42 MAPKs and cAMP response element binding protein. While relatively little is known about the role of phosphatases in terminating phosphorylation-mediated actions of cAMP in the pancreatic islet  $\beta$ -cell [84], there is evidence that the AKAP AKAP79 (the human homologue of AKAP150) is important in targeting the serine–threonine phosphatase PP2B to PKA-sensitive target proteins [85].

### 13.3 Functions of Cyclic AMP in the Pancreatic Islet $\beta$ -Cell

cAMP modulates a number of  $\beta$ -cell functions including insulin secretion, insulin synthesis,  $\beta$ -cell replication, and  $\beta$ -cell apoptosis. Actions of cAMP in general are mediated by at least two distinct mechanisms. The first of these is through protein kinase A (PKA)-mediated phosphorylation [86]. However, a second, and PKA-independent, effect of cAMP on insulin secretion [87–88] is mediated by the cyclic AMP-binding proteins known either as cAMP-regulated guanine nucleotide exchange factors (GEFs) or as exchange proteins activated by cAMP (Epacs) which target the small G-protein Rap1 [86]. Interestingly, most of the  $\beta$ -cell Rap1, at least in MIN6 cells, appears to be co-localized with insulin secretory granules [89]. When activated by cAMP, Epac, which exists as two isoforms (Epac1 and Epac2) exchanges GDP for GTP and activates downstream signalling. The pancreatic islet  $\beta$ -cell expresses both Epac1 and Epac2 [90]. Antisense oligodeoxynucleotides against Epac reduced the effect of a permeant cAMP analogue in augmenting glucose-induced insulin secretion in pancreatic islets [91]. Studies using selective inhibitors/activators of PKA, selective activators of Epac or the use of dominant-negative forms of Epac are revealing the roles of Epacs vs PKA in the  $\beta$ -cell. Novel cAMP analogues, such as 8-(4-chlorophenylthio)-2'-*O*-methyladenosine-3'-5'-cyclic monophosphate (8-pCPT-2'-*O*-Me-cAMP), and its

much more cell-permeant acetoxy methyl ester [92] activate Epac but not PKA, having a 100-fold lower affinity for PKA relative to Epac [86]. Similarly, cAMP analogues such as N6-Bnz-cAMP selectively activate PKA relative to Epac. Both Epac and PKA mediate the effects of cAMP on insulin secretion. However, at least in INS-1 cells, PKA-mediated effects account for the greater proportion of cAMP effects [92]. There is evidence for interaction between PKA-mediated and Epac-mediated effects in augmenting insulin secretion in native  $\beta$ -cells [93]. Some of the reported discrepancies may be explained by the poor cell permeability of some Epac-selective cAMP analogues [92].

The cyclic AMP-mediated effects of GIP and GLP-1 on insulin secretion involve both PKA [24] and PKA-independent actions. The latter are probably mediated through Epac, as evidenced by the comparative effects of the PKA inhibitor H89 and antisense oligodeoxynucleotides (ODNs) against Epac in reducing incretin-augmented insulin secretion [91, 94]. Interestingly, Epac-dependent effects of cAMP on insulin release are impaired in islets from mice lacking the SUR subunit of the  $K_{ATP}$  channel [94, 95].

### 13.3.1 Insulin Secretion

Malaisse's group was the first to systematically examine the actions of cAMP on insulin secretion [96, 97]. Elevations in cAMP in the  $\beta$ -cell augment glucose-induced insulin secretion at several sites in the secretory pathway.

#### 13.3.1.1 Effects on the $\beta$ -Cell ATP-Sensitive Potassium Channel

The  $\beta$ -cell ATP-sensitive potassium channel ( $K_{ATP}$  channel) plays a fundamental role in glucose-induced insulin secretion. Elevation of cAMP in the  $\beta$ -cell using GLP-1, forskolin, or the non-selective PDE inhibitor IBMX inhibits the  $\beta$ -cell  $K_{ATP}$  channel promoting depolarization of the cell [98–103]. This effect was reported to be mediated via PKA in INS-1 cells [101] through phosphorylation of the SUR1 subunit. On the other hand, Epac was found to inhibit this channel in both human  $\beta$ -cells and INS-1 cells, producing a leftward shift in the ATP-concentration–effect curve [102, 103]. The same study [103] suggested a PKA-mediated *activation* of the ATP-sensitive K channel.

#### 13.3.1.2 Voltage-Sensitive Potassium Channels

Activation of voltage-sensitive potassium channels contribute to a restoration of the  $\beta$ -cell membrane potential and a termination of insulin secretion. GIP, acting through a PKA-dependent mechanism, reduced K currents through voltage-sensitive potassium channels in HEK cells transfected with the GIP receptor and Kv1.4 channels, as well as in human islets and INS-1 cells [104]. GLP-1 and the GLP-1 mimetic exendin-4 also inhibited voltage-dependent K currents effects again being PKA dependent as evidenced by the preventative effects of PKA inhibition [105, 106]

### 13.3.1.3 Elevations in Intracellular Calcium $[Ca^{2+}]_i$

Increases in  $[Ca^{2+}]_i$  can be effected through two main mechanisms, namely influx through voltage-sensitive  $Ca^{2+}$  channels and mobilization of  $Ca^{2+}$  from intracellular stores and cAMP influences both these mechanisms in the  $\beta$ -cell.

#### Voltage-Sensitive $Ca^{2+}$ Channels

Entry of  $Ca^{2+}$  through L-type voltage-sensitive calcium channels in response to membrane depolarization is an important trigger for exocytosis. Agents elevating cAMP as well as cAMP itself augment the opening of channel and increase calcium influx [99, 107–109] through PKA-dependent mechanisms. This is consistent with observations that forskolin and IBMX were shown to produce phosphorylation of the cardiac-type  $\alpha 1$  subunit of the voltage-sensitive calcium channel in a mouse  $\beta$ -cell line  $\beta TC3$  [110].

#### Mobilization of $Ca^{2+}$ from Intracellular Stores

##### *Calcium-Induced Calcium Release*

In addition to facilitating calcium entry, agents that elevate  $\beta$ -cell cAMP also promote calcium-induced  $Ca^{2+}$  release [111–116]. For example, the uncaging of calcium from a membrane-permeable caged calcium (NP EGTA) produced a large, transient increase in  $[Ca^{2+}]_i$  but only in the presence of the GLP-1 mimetic exendin 4 or the adenylyl cyclase activator forskolin. This could be replicated by non-selective cAMP analogues or those that selectively activated either PKA or Epac. The effects of exendin-4 were relatively insensitive to the PKA inhibitor H89 but were inhibited by expression of a dominant-negative Epac2 [116], suggesting an important role of Epac2 in the sensitizing effect of cAMP on calcium-induced  $Ca^{2+}$  release. The importance of non-PKA-dependent effects of GLP-1 in elevating  $[Ca^{2+}]_i$  was also reported previously [117].

The mechanism whereby cAMP promotes calcium-induced  $Ca^{2+}$  release may be through activation of the ryanodine channel in the ER [93, 112, 113] and/or through phosphorylation of the  $IP_3$  receptor [118]. The interaction of cAMP, via PKA, with  $IP_3$  receptors is supported by the finding that 2-aminoethoxydiphenyl borate, a cell-permeable  $IP_3$ -receptor antagonist, blocked the PKA-mediated cAMP amplification of calcium-induced  $Ca^{2+}$  release [119].

##### *Generation of $Ca^{2+}$ -Mobilizing Second Messengers*

GLP-1 was shown to increase intracellular production of nicotinic acid adenine dinucleotide phosphate (NAADP) and cyclic ADP-ribose (ADPR) through cAMP mechanisms mediated by both PKA and Epac [120]. The production of the second messengers, cyclic ADPR and NAADP, is catalyzed by ADPR cyclases. Both mobilize  $Ca^{2+}$  from intracellular stores and NAADP stimulates insulin secretion. The

relative role of cyclic ADPR and NAADP in producing cAMP-mediated increases in  $[Ca^{2+}]_i$  remain to be determined.

#### 13.3.1.4 Direct Effect on Exocytosis

Ammala et al. [107] and Gillis and Misler [121] were the first to demonstrate that cAMP produced direct effects on exocytosis. This effect was suggested to represent the most important effect of cAMP on insulin release [107]. Both GIP and GLP-1 promote PKA-dependent and PKA-independent exocytosis, independently of changes in calcium entry [87, 99, 122]. Moreover, photo release of caged cAMP produces a marked increase in granule exocytosis that is independent of changes in  $[Ca^{2+}]_i$  [87, 99, 123, 124]. GLP-1 and cAMP augmented depolarization-induced exocytosis, and the effects of cAMP were mediated through both PKA-dependent and PKA-independent, Epac-mediated effects [95]. cAMP also enhanced exocytosis in single INS-1 cells, the effect being augmented by inhibition of PDE3 [65]. In permeabilized rat islets cAMP enhanced calcium-induced insulin secretion, independently of changes in  $[Ca^{2+}]_i$ ; this effect was largely dependent on Epac as it was mimicked by an Epac-selective, but not by a PKA selective, cAMP analogue and was unaffected by a PKA inhibitor [125]. Use of two-photon extracellular polar tracer (TEP) imaging and electron microscopy showed different roles of PKA or Epac in the enhancement by cAMP of calcium-evoked exocytosis of small compared with large, secretory vesicles [124]. Effects of cAMP on large vesicle exocytosis appeared to be PKA dependent, whereas effects on small vesicles were mediated via Epac.

There are different pools of insulin secretory granules in the  $\beta$ -cell. The first phase of glucose-induced insulin secretion is due to the release of granules docked at the membrane in a readily releasable pool and the second phase is dependent on the mobilization of granules to refill this readily releasable pool. The effects of cAMP, which augments both first and second phases of insulin secretion, are at least partly attributable to an expansion and refilling of the readily releasable pool [126–128]. Knockout of Epac2 specifically blocks the first phase of glucose-induced granule–plasma membrane fusions, suggesting the importance of cAMP signalling through Epac2 in this phase [89]. This supports earlier findings that the augmentation by cAMP of short depolarizations was Epac dependent, whereas the effect on longer depolarizations was largely PKA dependent and was more sensitive to cAMP [95]. The second phase of exocytosis appears to be mediated via both PKA and Epac [95, 127, 128], although a PKA dependency of the first phase of glucose-induced exocytosis has also been reported [123].

#### 13.3.1.5 Activation of Protein Kinase C

Protein kinase C (PKC) is another second messenger contributing to the regulation of insulin secretion, and one study suggests that PKC may mediate some of the insulin secretory effects of agents that elevate cAMP. Thus, GLP-1 was shown to activate the translocation of PKC $\alpha$  and PKC $\epsilon$  in INS-1 cells and its effects are

mimicked by forskolin. This activation was  $\text{Ca}^{2+}$  dependent, and it was hypothesized that it was effected through mobilization of  $\text{Ca}^{2+}$  as a result, for example, of PKA sensitization of the  $\text{IP}_3$  channel and consequent  $\text{Ca}^{2+}$ -mediated activation of phospholipase C [129].

### 13.4 Role of cAMP in Insulin Synthesis and in $\beta$ -Cell Differentiation, Proliferation, and Survival

The incretin GLP-1, acting to an important extent through cAMP effector mechanisms, increases insulin synthesis, promotes  $\beta$ -cell proliferation and inhibits  $\beta$ -cell apoptosis [25], although there is evidence for cAMP-independent effects [130]. Indeed much of the evidence for the importance of cAMP in these processes is derived from studies using GLP-1 and exendin-4. The finding that mice with a  $\beta$ -cell-specific deficiency in the  $\alpha$  subunit of  $G_s$  showed reduced  $\beta$ -cell mass, reduced islet content of insulin, reduced  $\beta$ -cell proliferation, and increased  $\beta$ -cell apoptosis, and marked hyperglycaemia suggests the fundamental importance of responsiveness to incretin hormones [131] in  $\beta$ -cell homeostasis.

Glucose-mediated increases in insulin synthesis involve the phosphorylation of the transcription factor pancreatic duodenal homeobox-1 (PDX-1) and its translocation to the nucleus [132]. There is strong evidence for the importance of cAMP, acting through PKA-dependent mechanisms, in mediating the ability of GLP-1 to increase  $\beta$ -cell levels of PDX-1, stimulate its translocation to the nucleus and consequently activate the insulin gene promoter [133]. PDX-1 expression is itself required for the generation of cAMP in response to exendin-4 through controlling the expression of the GLP-1 receptor and the  $G_s$  protein  $\alpha$  subunit [134].

CREB (cAMP response element binding protein) is the key transcriptional activator that mediates the effects of cAMP on gene regulation and its effects in regulating islet  $\beta$ -cell proliferation and survival. cAMP, through a PKA-dependent mechanism, and glucose act synergistically to regulate CREB activation in MIN6 or INS-1 cells [135, 136]. This appears to involve cAMP/PKA and glucose-induced modulation of the phosphorylation status of TORC2, a key co-activator of CREB, and the stimulation of its translocation to the nucleus [135, 136].

#### 13.4.1 Immediate Early Response Genes

Cyclic AMP appears to mediate the effects of glucose in stimulating the  $\beta$ -cell expression of immediate early response genes such as *c-myc* [137] and *c-fos* [138], which probably play an important role in the effects of glucose in regulating the gene expression of metabolic enzymes, cell growth, and apoptosis. In Min6 insulin-secreting cells Glauser et al. [139] identified 592 targets and 1278 immediate early genes responding to co-stimulation with glucose and cAMP (chlorophenylthio-cAMP, a cell-permeant cAMP analogue) and suggested an important role for the transcription factor AP-1. Indeed, the AP-1-regulated gene sulfiredoxin was

identified among the targets that were sequentially induced in primary cells from rat islets. In the same context, cAMP also amplifies the effect of glucose in stimulating the MAPK/ERK pathway [6, 140–142]. The augmentation of glucose-induced activation of ERK in response to GLP-1 required both influx of  $\text{Ca}^{2+}$  through voltage-dependent calcium channels and was PKA dependent [143] and GIP activates this kinase pathway through cyclic AMP and PKA [144].

### ***13.4.2 Protection Against $\beta$ -Cell Apoptosis and Stimulation of $\beta$ -Cell Proliferation***

There is abundant evidence for suppression of  $\beta$ -cell apoptosis by agents that elevate cAMP, including GLP-1, GIP, exendin-4, ghrelin and obestatin [135, 145–151]. This appears to be PKA mediated [148, 149]. Paradoxically, some  $\beta$ -cell lines were made more susceptible to apoptosis following exposure to dibutyryl cyclic AMP [152] or the cyclic AMP-elevating agent forskolin [153]. The anti-apoptotic effects of cAMP are mediated, in part, by increased expression of the anti-apoptotic proteins Bcl-2 and Bcl-xL [135, 146], and are PKA dependent [135, 146, 151]. The anti-apoptotic effects also involve caspase inhibition [147]. Inhibition of cytokine-mediated nitric oxide production by  $\beta$ -cells [154] may also be implicated.

In addition to preventing apoptosis of  $\beta$ -cells, the incretin hormones and other agents elevating cAMP promote  $\beta$ -cell proliferation through PKA-dependent mechanisms [134, 155, 156]. This effect appears to involve expression of cyclin D1 [155, 157] and cyclin A2 [134]. In this context, there may be an interaction of cAMP with Wnt signalling, which plays an important role in  $\beta$ -cell proliferation and survival with upregulation of cyclins D1 and D2 [158]. Thus, GLP-1 and exendin-4 activated Wnt signalling in INS-1 cells and in isolated islets [159]. Exendin-induced  $\beta$ -cell proliferation was inhibited by blocking  $\beta$ -catenin or the transcription factor TCF7L2, critical mediators of Wnt signalling [159].

An additional mechanism whereby cAMP modulates  $\beta$ -cell proliferation may be through regulation of the CREB antagonists cAMP response element modulator CREM- $\alpha$  and ICER1 and the dual specificity phosphatase DUSP14, a negative regulator of the MAPK/ERK1/2 pathway. Thus, genes for these proteins were rapidly and strongly upregulated by GLP-1 in a  $\beta$ -cell line and in rat primary  $\beta$ -cells, an effect that was mimicked by forskolin and blocked by the PKA inhibitor H89 but not by an Epac inhibitor. shRNA-mediated knockdown of CREM- $\alpha$  or DUSP14, or expression of a dominant-negative DUSP14, augmented GLP-1-induced  $\beta$ -cell proliferation [156].

## **13.5 Possible Roles of cAMP in Other Islet Cell Types**

Relatively little is known about the role of cAMP in other islet cells, although there is some information on its role in the glucagon-secreting and somatostatin-secreting

cells. Forskolin was shown to stimulate glucagon secretion from rat islets [160]. GLP-1 (and GIP) augmented depolarization-evoked exocytosis from rat  $\alpha$ -cells; this effect was accompanied by elevations in intracellular cAMP, increases in  $\text{Ca}^{2+}$  currents and was mediated by PKA [161]. Exposure of an  $\alpha$ -cell line (INR1-G9) expressing recombinant GLP-1 receptors to GLP-1 increased the formation of cAMP and elevated free cytosolic  $[\text{Ca}^{2+}]$  [162]. In the same cell line, an Epac-selective cAMP analogue stimulated the expression of the glucagon gene promoter and stimulated glucagon production, although not glucagon secretion [163]. Moreover, a dominant-negative Epac-2 attenuated forskolin-stimulated expression of the glucagon gene promoter in the INR1-G9 cells [163]. While these data indicate a stimulatory effect of GLP-1 on glucagon synthesis and secretion, GLP-1 is known to inhibit glucagon secretion, an action likely to contribute to its therapeutic effect in the treatment of diabetes [164]. The inhibition of glucagon secretion by GLP-1 is thus likely to be mediated by a paracrine action in the islets, for example, through stimulation of somatostatin secretion, which markedly inhibits glucagon release [165]. In this context, GLP-1, oxyntomodulin and glucagon were shown to potently stimulate somatostatin secretion from somatostatin-secreting cell lines (RIN T3; RIN 1048-38) and to stimulate the accumulation of cAMP [166, 167]. Increases in cAMP levels in response to forskolin, theophylline or dibutyryl cAMP were shown to be associated with increased somatostatin release from isolated islets [168].

Glucagon itself stimulates glucagon release by activating glucagon, rather than GLP-1, receptors, through cAMP-dependent mechanisms involving both PKA and Epac [169].

Adrenaline, or isoprenaline, acting through  $\beta$ -adrenoceptors, augmented depolarization-evoked glucagon secretion from rat primary  $\alpha$ -cells [170]. This effect was mimicked by forskolin and was PKA dependent. As in the  $\beta$ -cell the PKA-dependent effects appear to involve more than one mechanism, including increased  $\text{Ca}^{2+}$  entry and augmentation of the effects of  $\text{Ca}^{2+}$ . Photo release of caged cAMP increased exocytosis even when intracellular  $[\text{Ca}^{2+}]$  was clamped [170]. These data were supported by observations using mouse primary  $\alpha$ -cells, in which adrenaline-induced increases in  $\alpha$ -cell  $[\text{Ca}^{2+}]_i$  were mediated, in part, by elevations in cAMP and activation of PKA [171].

## 13.6 Conclusion

cAMP is clearly an important mediator/modulator of many  $\beta$ -cell functions from hormone secretion to proliferation, survival and synthetic functions and is also likely to be important in other islet cell types. Further work will elucidate the precise mechanisms whereby PKA and Epac, the known mediators of the effects of cAMP, exert their effects on these cellular processes. Novel ways of targeting cAMP mechanisms through small molecules, rather than peptides, may open up new treatments for diabetes mellitus. Small molecules targeting the GRP119 receptor are under

development [37]. A number of non-peptide agents that act both as direct agonists and allosteric modulators of the GLP-1 receptor are also being examined [172].

## References

1. Turtle J, Kipnis D. An adrenergic receptor mechanism for the control of cyclic 3',5' adenosine monophosphate synthesis in tissues. *Biochem Biophys Res Commun* 1967;28:797–802.
2. Charles M, Fanska R, Schmid F, Forsham P, Grodsky G. Adenosine 3',5'-monophosphate in pancreatic islets: glucose-induced insulin release. *Science* 1973;179:569–571.
3. Grill V, Cerasi E. Activation by glucose of adenyl cyclase in pancreatic islets of the rat. *FEBS Lett* 1973;33:311–4.
4. Kim J, Roberts C, Berg S, Caicedo A, Roper S, Chaudhari N. Imaging cyclic AMP changes in pancreatic islets of transgenic reporter mice. *PLoS ONE* 2008;3:e2127.
5. Landa LJ, Harbeck M, Kaihara K, Chepurny O, Kitiphongspattana K, Graf O, Nikolaev V, Lohse M, Holz G, Roe M. Interplay of  $\text{Ca}^{2+}$  and cAMP signaling in the insulin-secreting MIN6 beta-cell line. *J Biol Chem* 2005;280:31294–302.
6. Ramos L, Zippin J, Kamenetsky M, Buck J, Levin L. Glucose and GLP-1 stimulate cAMP production via distinct adenylyl cyclases in INS-1E insulinoma cells. *J Gen Physiol* 2008;132:329–38.
7. Sharp G. The adenylate cyclase-cyclic AMP system in islets of Langerhans and its role in the control of insulin release. *Diabetologia* 1979;16:287–96.
8. Persaud S, Jones P, Howell S. Glucose-stimulated insulin secretion is not dependent on activation of protein kinase A. *Biochem Biophys Res Commun* 1990;173:833–9.
9. Lester L, Langeberg L, Scott J. Anchoring of protein kinase A facilitates hormone-mediated insulin secretion. *Proc Natl Acad Sci U S A* 1997;94:14942–7.
10. Holz G, Habener J. Signal transduction crosstalk in the endocrine system: pancreatic beta-cells and the glucose competence concept. *Trends Biochem Sci* 1992;17:388–93.
11. Howell S, Jones P, Persaud S. Regulation of insulin secretion: the role of second messengers. *Diabetologia* 1994;37 Suppl 2:S30–5.
12. Braun M, Ramracheya R, Johnson P, Rorsman P. Exocytotic properties of human pancreatic beta-cells. *Ann N Y Acad Sci* 2009;1152:187–93.
13. MacIntosh C, Horowitz M, Verhagen M, Smout A, Wishart J, Morris H, Goble E, Morley J, Chapman I. Effect of small intestinal nutrient infusion on appetite, gastrointestinal hormone release, and gastric myoelectrical activity in young and older men. *Am J Gastroenterol* 2001;96:997–1007.
14. Brubaker P, Anini Y. Direct and indirect mechanisms regulating secretion of glucagon-like peptide-1 and glucagon-like peptide-2. *Can J Physiol Pharmacol* 2003;81:1005–12.
15. Feinle C, Chapman I, Wishart J, Horowitz M. Plasma glucagon-like peptide-1 (GLP-1) responses to duodenal fat and glucose infusions in lean and obese men. *Peptides* 2002;23:1491–95.
16. Wolfe M, Zhao K, Glazier K, Jarboe L, Tseng C. Regulation of glucose-dependent insulinotropic polypeptide release by protein in the rat. *Am J Physiol Gastrointest Liver Physiol* 2000;279:G561–6.
17. Thorens B. Expression cloning of the pancreatic beta cell receptor for the gluco-incretin hormone glucagon-like peptide 1. *Proc Natl Acad Sci U S A* 1992;89:8641–5.
18. Preitner F, Ibberson M, Franklin I, Binnert C, Pende M, Gjiniocvi A, Hansotia T, Drucker D, Wollheim C, Burcelin R, Thorens B. Gluco-incretins control insulin secretion at multiple levels as revealed in mice lacking GLP-1 and GIP receptors. *J Clin Invest* 2004;113:635–45.
19. Selbie L, Hill S. G protein-coupled-receptor cross-talk: the fine-tuning of multiple receptor-signalling pathways. *Trends Pharmacol Sci* 1998;19:87–93.



20. Yamazaki S, Katada T, Ui M. Alpha 2-adrenergic inhibition of insulin secretion via interference with cyclic AMP generation in rat pancreatic islets. *Mol Pharmacol* 1982;21:648–53.
21. Robertson R, Tsai P, Little S, Zhang H, Walseth T. Receptor-mediated adenylate cyclase-coupled mechanism for PGE<sub>2</sub> inhibition of insulin secretion in HIT cells. *Diabetes* 1987;36:1047–53.
22. Morgan D, Kulkarni R, Hurley J, Wang Z, Wang R, Ghatei M, Karlens A, Bloom S, Smith D. Inhibition of glucose stimulated insulin secretion by neuropeptide Y is mediated via the Y1 receptor and inhibition of adenylyl cyclase in RIN 5AH rat insulinoma cells. *Diabetologia* 1998;41:1482–91.
23. Kimple M, Nixon A, Kelly P, Bailey C, Young K, Fields T, Casey P. A role for G<sub>z</sub> in pancreatic islet  $\beta$ -cell biology. *J Biol Chem* 2005;280:31708–13.
24. Drucker D, Philippe J, Mojsov S, Chick W, Habener J. Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. *Proc Natl Acad Sci U S A* 1987;84:3434–8.
25. Doyle M, Egan J. Mechanisms of action of glucagon-like peptide 1 in the pancreas. *Pharmacol Ther* 2007;113:546–93.
26. Maida A, Lovshin J, Baggio L, Drucker D. The glucagon-like peptide-1 receptor agonist oxyntomodulin enhances beta-cell function but does not inhibit gastric emptying in mice. *Endocrinology* 2008;149:5670–8.
27. Sonoda N, Imamura T, Yoshizaki T, Babendure J, Lu J, Olefsky J. Beta-Arrestin-1 mediates glucagon-like peptide-1 signaling to insulin secretion in cultured pancreatic beta cells. *Proc Natl Acad Sci U S A* 2008;105:6614–9.
28. Amiranoff B, Vauclin-Jacques N, Laburthe M. Functional GIP receptors in a hamster pancreatic beta cell line, In 111: specific binding and biological effects. *Biochem Biophys Res Commun* 1984;123:671–6.
29. Siegel E, Creutzfeldt W. Stimulation of insulin release in isolated rat islets by GIP in physiological concentrations and its relation to islet cyclic AMP content. *Diabetologia* 1985;28:857–61.
30. Wheeler M, Gelling R, McIntosh C, Georgiou J, Brown J, Pederson R. Functional expression of the rat pancreatic islet glucose-dependent insulinotropic polypeptide receptor: ligand binding and intracellular signaling properties. *Endocrinology* 1995;136:4629–9.
31. Zhou J, Livak M, Bernier M, Muller D, Carlson O, Elahi D, Maudsley S, Egan J. Ubiquitination is involved in glucose-mediated downregulation of GIP receptors in islets. *Am J Physiol Endocrinol Metab* 2007;293:E538–47.
32. Yada T, Sakurada M, Ihida K, Nakata M, Murata F, Arimura A, Kikuchi M. Pituitary adenylate cyclase activating polypeptide is an extraordinarily potent intra-pancreatic regulator of insulin secretion from islet beta-cells. *J Biol Chem* 1994;269:1290–3.
33. Ahrén B. Role of pituitary adenylate cyclase-activating polypeptide in the pancreatic endocrine system. *Ann N Y Acad Sci.* 2008;1144:28–35.
34. Borboni P, Porzio O, Pierucci D, Cicconi S, Magnaterra R, Federici M, Sesti G, Lauro D, D'Agata V, Cavallaro S, Marlier L. Molecular and functional characterization of pituitary adenylate cyclase-activating polypeptide (PACAP-38)/vasoactive intestinal polypeptide receptors in pancreatic beta-cells and effects of PACAP-38 on components of the insulin secretory system. *Endocrinology* 1999;140:5530–7.
35. Yamada S, Komatsu M, Sato Y, Yamauchi K, Kojima I, Aizawa T, Hashizume K. Time-dependent stimulation of insulin exocytosis by 3',5'-cyclic adenosine monophosphate in the rat islet beta-cell. *Endocrinology* 2002;143:4203–9.
36. Soga T, Ohishi T, Matsui T, Saito T, Matsumoto M, Takasaki J, Matsumoto S, Kamohara M, Hiyama H, Yoshida S, Momose K, Ueda Y, Matsushima H, Kobori M, Furuichi K. Lysophosphatidylcholine enhances glucose-dependent insulin secretion via an orphan G-protein-coupled receptor. *Biochem Biophys Res Commun* 2005;326:744–51.
37. Overton H, Babbs A, Doel S, Fyfe M, Gardner L, Griffin G, Jackson H, Procter M, Rasamison C, Tang-Christensen M, Widdowson P, Williams G, Reynet C. Deorphanization

- of a G protein-coupled receptor for oleylethanolamide and its use in the discovery of small-molecule hypophagic agents. *Cell Metab* 2006;3:167–75.
38. Chu Z, Jones R, He H, Carroll C, Gutierrez V, Lucman A, Moloney M, Gao H, Mondala H, Bagnol D, Unett D, Liang Y, Demarest K, Semple G, Behan D, Leonard J. A role for beta-cell-expressed G protein-coupled receptor 119 in glycemic control by enhancing glucose-dependent insulin release. *Endocrinology* 2007;148:2601–9.
39. Hanoune J, Defer N. Regulation and role of adenylyl cyclase isoforms. *Annu Rev Pharmacol* 2001;Toxicol.;41:145–74.
40. Willoughby D, Cooper D. Organization and  $\text{Ca}^{2+}$  regulation of adenylyl cyclases in cAMP microdomains. *Physiol Rev* 2007;87:965–1010.
41. Kamenetsky M, Middelhaufe S, Bank E, Levin L, Buck J, Steegborn C. Molecular details of cAMP generation in mammalian cells: a tale of two systems. *J Mol Biol* 2006;362:623–39.
42. Srinivasan M, Aalinkeel R, Song F, Lee B, Laychock S, Patel M. Adaptive changes in insulin secretion by islets from neonatal rats raised on a high-carbohydrate formula. *Am J Physiol Endocrinol Metab* 2000;279:E1347–57.
43. Leech C, Castonguay M, Habener J. Expression of adenylyl cyclase subtypes in pancreatic beta-cells. *Biochem Biophys Res Commun* 1999;254:703–6.
44. Guenifi A, Portela-Gomes G, Grimelius L, Efendić S, Abdel-Halim S. Adenylyl cyclase isoform expression in non-diabetic and diabetic Goto-Kakizaki (GK) rat pancreas. Evidence for distinct overexpression of type-8 adenylyl cyclase in diabetic GK rat islets. *Histochem Cell Biol* 2000;113:81–9.
45. Delmeire D, Flamez D, Hinke S, Cali J, Pipeleers D, Schuit F. Type VIII adenylyl cyclase in rat beta cells: coincidence signal detector/generator for glucose and GLP-1. *Diabetologia* 2003;46:1383–93.
46. Seamon K, Daly J. Forskolin: its biological and chemical properties. *Adv Cyclic Nucleotide Protein Phosphorylation Res* 1986;20:1–150.
47. Insel P, Ostrom R. Forskolin as a tool for examining adenylyl cyclase expression, regulation, and G protein signaling. *Cell Mol Neurobiol* 2003;23:305–14.
48. Cali J, Zwaagstra J, Mons N, Cooper D, Krupinski J. Type VIII adenylyl cyclase. A  $\text{Ca}^{2+}$ /calmodulin-stimulated enzyme expressed in discrete regions of rat brain. *J Biol Chem* 1994;269:12190–5.
49. Dolz M, Bailbé D, Giroix M, Calderari S, Gangnerau M, Serradas P, Rickenbach K, Irminger J, Portha B. Restitution of defective glucose-stimulated insulin secretion in diabetic GK rat by acetylcholine uncovers paradoxical stimulatory effect of beta-cell muscarinic receptor activation on cAMP production. *Diabetes* 2005;54:3229–37.
50. Perry M, Higgs G. Chemotherapeutic potential of phosphodiesterase inhibitors. *Curr Opin Chem Biol* 1998;2:472–81.
51. Soderling S, Beavo J. Regulation of cAMP and cGMP signaling. new phosphodiesterases and new functions. *Curr Opin Cell Biol* 2000;12:174–9.
52. Mehats C, Andersen C, Filopanti M, Jin S, Conti M. Cyclic nucleotide phosphodiesterases and their role in endocrine cell signaling. *Trends Endocrinol Metab* 2002;13:29–35.
53. Conti M, Beavo J. Biochemistry and physiology of cyclic nucleotide phosphodiesterases: essential components in cyclic nucleotide signaling. *Annu Rev Biochem* 2007;76:481–511.
54. Pyne N, Furman B. Cyclic nucleotide phosphodiesterases in pancreatic islets. *Diabetologia* 2003;46:1179–89.
55. Furman B, Pyne N, Flatt P, O'Harte F. Targeting beta-cell cyclic 3'5' adenosine monophosphate for the development of novel drugs for treating type 2 diabetes mellitus. A review. *J Pharm Pharmacol* 2004;56:1477–92.
56. Sugden M, Ashcroft S. Cyclic nucleotide phosphodiesterase of rat pancreatic islets. Effects of  $\text{Ca}^{2+}$ , calmodulin and trifluoperazine. *Biochem J* 1981;197:459–64.
57. Capito K, Hedeskov C, Thams P. Cyclic AMP phosphodiesterase activity in mouse pancreatic islets. Effects of calmodulin and phospholipids. *Acta Endocrinol (Copenh)* 1986;111:533–38.

58. Lipson L, Oldham S. The role of calmodulin in insulin secretion: the presence of a calmodulin-stimulatable phosphodiesterase in pancreatic islets of normal and pregnant rats. *Life Sci* 1983;32:775–80.
59. Han P, Werber J, Surana M, Fleischer N, Michaeli T. The calcium/calmodulin-dependent phosphodiesterase PDE1C down-regulates glucose-induced insulin secretion. *J Biol Chem* 1999;274:22337–44.
60. Ahmad M, Flatt P, Furman B, Pyne N. The role of the cyclic GMP-inhibited cyclic AMP-specific phosphodiesterase (PDE3) in regulating clonal BRIN-BD11 insulin secreting cell survival. *Cell Signal* 2000;12:541–48.
61. Shafiee-Nick R, Pyne N, Furman B. Effects of type-selective phosphodiesterase inhibitors on glucose-induced insulin secretion and islet phosphodiesterase activity. *Br J Pharmacol* 1995;115:1486–92.
62. Parker J, VanVolkenburg M, Ketchum R, Brayman K, Andrews K. Cyclic AMP phosphodiesterases of human and rat islets of Langerhans: contributions of types III and IV to the modulation of insulin secretion. *Biochem Biophys Res Commun* 1995;217:916–23.
63. Zhao A, Zhao H, Teague J, Fujimoto W, Beavo J. Attenuation of insulin secretion by insulin-like growth factor 1 is mediated through activation of phosphodiesterase 3B. *Proc Natl Acad Sci U S A* 1997;94:3223–28.
64. Waddleton D, Wu W, Feng Y, Thompson C, Wu M, Zhou Y, Howard A, Thornberry N, Li J, Mancini J. Phosphodiesterase 3 and 4 comprise the major cAMP metabolizing enzymes responsible for insulin secretion in INS-1 (832/13) cells and rat islets. *Biochem Pharmacol* 2008;76:884–93.
65. Härndahl L, Jing X, Ivarsson R, Degerman E, Åhrén B, Manganiello V, Renström E, Holst L. Important role of phosphodiesterase 3B for the stimulatory action of cAMP on pancreatic beta-cell exocytosis and release of insulin. *J Biol Chem* 2002;277:37446–55.
66. Härndahl L, Wierup N, Enerbäck S, Mulder H, Manganiello V, Sundler F, Degerman E, Åhrén B, Holst L. Beta-cell-targeted overexpression of phosphodiesterase 3B in mice causes impaired insulin secretion, glucose intolerance, and deranged islet morphology. *J Biol Chem* 2004;279:15214–22.
67. Walz H, Härndahl L, Wierup N, Zmuda-Trzebiatowska E, Svennelid F, Manganiello V, Ploug T, Sundler F, Degerman E, Åhrén B, Holst L. Early and rapid development of insulin resistance, islet dysfunction and glucose intolerance after high-fat feeding in mice overexpressing phosphodiesterase 3B. *J Endocrinol* 2006;189:629–41.
68. Dov A, Abramovitch E, Warwar N, Neshet R. Diminished phosphodiesterase-8B potentiates biphasic insulin response to glucose. *Endocrinology* 2008;149:741–8.
69. Zhao A, Bornfeldt K, Beavo J. Leptin inhibits insulin secretion by activation of phosphodiesterase 3B. *J Clin Invest* 1998;102:869–73.
70. Grapenhiesser E, Gylfe E, Dansk H, Hellman B. Nitric oxide induces synchronous  $\text{Ca}^{2+}$  transients in pancreatic beta cells lacking contact. *Pancreas* 2001;23:387–92.
71. Smukler S, Tang L, Wheeler M, Salapatek A. Exogenous nitric oxide and endogenous glucose-stimulated beta-cell nitric oxide augment insulin release. *Diabetes* 2002;51:3450–60.
72. Kaneko Y, Ishikawa T, Amano S, Nakayama K. Dual effect of nitric oxide on cytosolic  $\text{Ca}^{2+}$  concentration and insulin secretion in rat pancreatic beta-cells. *Am J Physiol Cell Physiol* 2003;284:C1215–22.
73. Sunouchi T, Suzuki K, Nakayama K, Ishikawa T. Dual effect of nitric oxide on ATP-sensitive  $\text{K}^+$  channels in rat pancreatic beta cells. *Pflügers Arch* 2008;456:573–9.
74. Cantin L, Magnuson S, Gunn D, Barucci N, Breuhaas M, Bullock W, Burke J, Claus T, Daly M, Decarr L, Gore-Willse A, Hoover-Litty H, Kumarasinghe E, Li Y, Liang S, Livingston J, Lowinger T, Macdougall M, Ogutu H, Olague A, Ott-Morgan R, Schoenleber R, Tersteegen A, Wickens P, Zhang Z, Zhu J, Zhu L, Sweet L. PDE-10A inhibitors as insulin secretagogues. *Bioorg Med Chem Lett* 2007;17:2869–73.
75. Fridlyand LE, Harbeck MC, Roe MW, Philipson LH. Regulation of cAMP dynamics by  $\text{Ca}^{2+}$  and G protein-coupled receptors in the pancreatic  $\beta$ -cell. a computational approach. *Am J Physiol Cell Physiol*. 2007;293:C1924–33.

76. Dyachok O, Isakov Y, S  getorp J, Tengholm A. Oscillations of cyclic AMP in hormone-stimulated insulin-secreting beta-cells. *Nature* 2006;439:349–52.
77. Dyachok O, S  getorp J, Isakov Y, Tengholm A. cAMP oscillations restrict protein kinase A redistribution in insulin-secreting cells. *Biochem Soc Trans* 2006;34:498–501.
78. Dyachok O, Idevall-Hagren O, S  getorp J, Tian G, Wuttke A, Arri  umerlou C, Akusj  arvi G, Gylfe E, Tengholm A. Glucose-induced cyclic AMP oscillations regulate pulsatile insulin secretion. *Cell Metab* 2008;8:26–37.
79. Baltrusch S, Lenzen S. Regulation of  $[Ca^{2+}]_i$  oscillations in mouse pancreatic islets by adrenergic agonists. *Biochem Biophys Res Commun* 2007;363:1038–43.
80. J  rnaess E, Task  n K. Spatiotemporal control of cAMP signalling processes by anchored signalling complexes. *Biochem Soc Trans* 2007;35:931–37.
81. Dodge-Kafka K, Kapiloff M. The mAKAP signaling complex: integration of cAMP, calcium, and MAP kinase signaling pathways. *Eur J Cell Biol* 2006;85:593–602.
82. Fraser I, Tavalin S, Lester L, Langeberg L, Westphal A, Dean R, Marri  on N, Scott J. A novel lipid-anchored A-kinase Anchoring Protein facilitates cAMP-responsive membrane events. *EMBO J* 1998;17:2261–72.
83. Faruque O, Le-Nguyen D, Lajoix A, Vives E, Petit P, Bataille D, Hani e-H. Cell-permeable peptide-based disruption of endogenous PKA-AKAP complexes: a tool for studying the molecular roles of AKAP-mediated PKA subcellular anchoring. *Am J Physiol Cell Physiol* 2009;296:C306–16.
84. Jones PM, Persaud SJ. Protein kinases, protein phosphorylation, and the regulation of insulin secretion from pancreatic  $\beta$ -cells. *Endocr Rev*. 1998;429–461.
85. Lester LB, Faux MC, Nauert JB, Scott JD. Targeted protein kinase A and PP-2B regulate insulin secretion through reversible phosphorylation. *Endocrinology*. 2001;142(3):1218–27.
86. Kopperud R, Krakstad C, Selheim F, D  skeland S. cAMP effector mechanisms. Novel twists for an 'old' signaling system. *FEBS Lett* 2003;546:121–6.
87. Renstr  m E, Eliasson L, Rorsman P. Protein kinase A-dependent and independent stimulation of exocytosis by cAMP in mouse pancreatic B-cells. *J Physiol* 1997;502:105–18.
88. Seino S, Shibasaki T. PKA-dependent and PKA-independent pathways for cAMP-regulated exocytosis. *Physiol Rev* 2005;85:1303–42.
89. Shibasaki T, Takahashi H, Miki T, Sunaga Y, Matsumura K, Yamanaka M, Zhang C, Tamamoto A, Satoh T, Miyazaki J, Seino S. Essential role of Epac2/Rap1 signaling in regulation of insulin granule dynamics by cAMP. *Proc Natl Acad Sci U S A*; 2007;104:19333–193.
90. Holz G. Epac: A new cAMP-binding protein in support of glucagon-like peptide-1 receptor-mediated signal transduction in the pancreatic beta-cell. *Diabetes* 2004;53:5–13.
91. Kashima Y, Miki T, Shibasaki T, Ozaki N, Miyazaki M, Yano H, Seino S. Critical role of cAMP-GEFII—Rim2 complex in incretin-potentiated insulin secretion. *J Biol Chem* 2001;276:46046–53.
92. Chepurny O, Leech C, Kelley G, Dzhura I, Dzhura E, Li X, Rindler M, Schwede F, Genieser H, Holz G. Enhanced Rap1 activation and insulin secretagogue properties of an acetoxymethyl ester of an Epac-selective cyclic AMP analog in rat INS-1 cells: Studies with 8-pCPT-2'-O-Me-cAMP-AM. *J Biol Chem*, 2009.
93. Liu G, Jacobo S, Hilliard N, Hockerman G. Differential modulation of Cav1.2 and Cav1.3-mediated glucose-stimulated insulin secretion by cAMP in INS-1 cells: distinct roles for exchange protein directly activated by cAMP 2 (Epac2) and protein kinase A. *J Pharmacol Exp Ther* 2006;318:152–60.
94. Nakazaki M, Crane A, Hu M, Seghers V, Ullrich S, Aguilar-Bryan L, Bryan J. cAMP-activated protein kinase-independent potentiation of insulin secretion by cAMP is impaired in SUR1 null islets. *Diabetes* 2002;51:3440–9.
95. Eliasson L, Ma X, Renstr  m E, Barg S, Berggren P, Galvanovskis J, Gromada J, Jing X, Lundquist I, Salehi A, Sewing S, Rorsman P. SUR1 regulates PKA-independent cAMP-induced granule priming in mouse pancreatic B-cells. *J Gen Physiol* 2003;121:181–97.
96. Malaisse W, Malaisse-Lagae F, Mayhew D. A possible role for the adenyl cyclase system in insulin secretion. *J Clin Invest* 1967;46:1724–34.

97. Brisson G, Malaisse-Lagae F, Malaisse W. The stimulus-secretion coupling of glucose-induced insulin release. VII. A proposed site of action for adenosine-3',5'-cyclic monophosphate. *J Clin Invest* 1972;51:232–41.
98. Holz G, Kühtreiber W, Habener J. Pancreatic beta-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1(7-37). *Nature* 1993;361:362–5.
99. Gromada J, Bokvist K, Ding W, Holst J, Nielsen J, Rorsman P. Glucagon-like peptide 1 (7-36) amide stimulates exocytosis in human pancreatic beta-cells by both proximal and distal regulatory steps in stimulus-secretion coupling. *Diabetes* 1998;47:57–65.
100. He L, Mears D, Atwater I, Kitasato H. Glucagon induces suppression of ATP-sensitive K<sup>+</sup> channel activity through a Ca<sup>2+</sup>/calmodulin-dependent pathway in mouse pancreatic  $\beta$ -cells. *J Membr Biol* 1998;166:237–44.
101. Light P, Manning Fox J, Riedel M, Wheeler M. Glucagon-like peptide-1 inhibits pancreatic ATP-sensitive potassium channels via a protein kinase A- and ADP-dependent mechanism. *Mol Endocrinol* 2002;16:2135–44.
102. Kang G, Chepurny O, Malester B, Rindler M, Rehmann H, Bos J, Schwede F, Coetzee W, Holz G. cAMP sensor Epac as a determinant of ATP-sensitive potassium channel activity in human pancreatic  $\beta$  cells and rat INS-1 cells. *J Physiol* 2006;573:595–609.
103. Kang G, Leech C, Chepurny O, Coetzee W, Holz G. Role of the cAMP sensor Epac as a determinant of KATP channel ATP sensitivity in human pancreatic  $\beta$ -cells and rat INS-1 cells. *J Physiol* 2008;586:1307–19.
104. Kim S, Choi W, Han J, Warnock G, Fedida D, McIntosh C. A novel mechanism for the suppression of a voltage-gated potassium channel by glucose-dependent insulinotropic polypeptide: protein kinase A-dependent endocytosis. *J Biol Chem* 2005;280:28692–700.
105. MacDonald P, Salapatek A, Wheeler M. Glucagon-like peptide-1 receptor activation antagonizes voltage-dependent repolarizing K<sup>+</sup> currents in  $\beta$ -cells: a possible glucose-dependent insulinotropic mechanism. *Diabetes* 51 Suppl 2002;3:S443–47.
106. MacDonald P, Wang X, Xia F, El-Kholy W, Targonsky E, Tsushima R, Wheeler M. Antagonism of rat  $\beta$ -cell voltage-dependent K<sup>+</sup> currents by exendin 4 requires dual activation of the cAMP/protein kinase A and phosphatidylinositol 3-kinase signaling pathways. *J Biol Chem* 2003;278:52446–53.
107. Ammälä C, Ashcroft F, Rorsman P. Calcium-independent potentiation of insulin release by cyclic AMP in single beta-cells. *Nature* 1993;363:356–58.
108. Kanno T, Suga S, Wu J, Kimura M, Wakui M. Intracellular cAMP potentiates voltage-dependent activation of L-type Ca<sup>2+</sup> channels in rat islet beta-cells. *Pflugers Arch* 1998;435:578–80.
109. Suga S, Kanno T, Nakano K, Takeo T, Dobashi Y, Wakui M. GLP-I (7-36) amide augments Ba<sup>2+</sup> current through L-type Ca<sup>2+</sup> channel of rat pancreatic  $\beta$ -cell in a cAMP-dependent manner. *Diabetes* 1997;46:1755–60.
110. Leiser M, Fleischer N. cAMP-dependent phosphorylation of the cardiac-type  $\alpha$ 1 subunit of the voltage-dependent Ca<sup>2+</sup> channel in a murine pancreatic  $\beta$ -cell line. *Diabetes* 1996;45:1412–8.
111. Gromada J, Dissing S, Bokvist K, Renström E, Frøkjær-Jensen J, Wulff B, Rorsman P. Glucagon-like peptide I increases cytoplasmic calcium in insulin-secreting beta TC3-cells by enhancement of intracellular calcium mobilization. *Diabetes* 1995;44:767–74.
112. Islam M, Leibiger I, Leibiger B, Rossi D, Sorrentino V, Ekström T, Westerblad H, Andrade F, Berggren P. In situ activation of the type 2 ryanodine receptor in pancreatic  $\beta$  cells requires cAMP-dependent phosphorylation. *Proc Natl Acad Sci U S A* 1998;95:6145–50.
113. Holz G, Leech C, Heller R, Castonguay M, Habener J. cAMP-dependent mobilization of intracellular Ca<sup>2+</sup> stores by activation of ryanodine receptors in pancreatic beta-cells. A Ca<sup>2+</sup> signaling system stimulated by the insulinotropic hormone glucagon-like peptide-1-(7-37). *J Biol Chem* 1999;274:14147–56.
114. Kang G, Chepurny O, Holz G. cAMP-regulated guanine nucleotide exchange factor II (Epac2) mediates Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release in INS-1 pancreatic  $\beta$ -cells. *J Physiol* 2001;536:375–85.

115. Kang G, Joseph J, Chepurny O, Monaco M, Wheeler M, Bos J, Schwede F, Genieser H, Holz G. Epac-selective cAMP analog 8-pCPT-2'-O-Me-cAMP as a stimulus for  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release and exocytosis in pancreatic  $\beta$ -cells. *J Biol Chem* 2003;278:8279–85.
116. Kang G, Chepurny O, Rindler M, Collis L, Chepurny Z, Li W, Harbeck M, Roe M, Holz G. A cAMP and  $\text{Ca}^{2+}$  coincidence detector in support of  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release in mouse pancreatic  $\beta$  cells. *J Physiol* 2005;566:173–88.
117. Bode H, Moormann B, Dabew R, Göke B. Glucagon-like peptide 1 elevates cytosolic calcium in pancreatic beta-cells independently of protein kinase A. *Endocrinology* 1999;140:3919–27.
118. Tsuboi T, da Silva Xavier G, Holz G, Jouaville L, Thomas A, Rutter G. Glucagon-like peptide-1 mobilizes intracellular  $\text{Ca}^{2+}$  and stimulates mitochondrial ATP synthesis in pancreatic MIN6  $\beta$ -cells. *Biochem J* 2003;369:287–99.
119. Dyachok O, Gylfe E.  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release via inositol 1,4,5-trisphosphate receptors is amplified by protein kinase A and triggers exocytosis in pancreatic  $\beta$ -cells. *J Biol Chem* 2004;279:45455–61.
120. Kim BJ, Park KH, Yim CY, Takasawa S, Okamoto H, Im MJ, Kim UH. Generation of nicotinic acid adenine dinucleotide phosphate and cyclic ADP-ribose by glucagon-like peptide-1 evokes  $\text{Ca}^{2+}$  signal that is essential for insulin secretion in mouse pancreatic islets. *Diabetes* 2008;57:868–78.
121. Gillis K, Mislis S. Enhancers of cytosolic cAMP augment depolarization-induced exocytosis from pancreatic B-cells: evidence for effects distal to  $\text{Ca}^{2+}$  entry. *Pflugers Arch* 1993;424:195–7.
122. Ding W, Gromada J. Protein kinase A-dependent stimulation of exocytosis in mouse pancreatic beta-cells by glucose-dependent insulintropic polypeptide. *Diabetes* 1997;46:615–21.
123. Hatakeyama H, Kishimoto T, Nemoto T, Kasai H, Takahashi N. Rapid glucose sensing by protein kinase A for insulin exocytosis in mouse pancreatic islets. *J Physiol* 2006;570:271–82.
124. Hatakeyama H, Takahashi N, Kishimoto T, Nemoto T, Kasai H. Two cAMP-dependent pathways differentially regulate exocytosis of large dense-core and small vesicles in mouse  $\beta$ -cells. *J Physiol* 2007;582:1087–98.
125. Hashiguchi H, Nakazaki M, Koriyama N, Fukudome M, Aso K, Tei C. Cyclic AMP/cAMP-GEF pathway amplifies insulin exocytosis induced by  $\text{Ca}^{2+}$  and ATP in rat islet beta-cells. *Diabetes Metab Res Rev* 2006;22:64–71.
126. Kwan E, Gaisano H. Glucagon-like peptide 1 regulates sequential and compound exocytosis in pancreatic islet  $\beta$ -cells. *Diabetes* 2005;54:2734–43.
127. Kwan E, Xie L, Sheu L, Ohtsuka T, Gaisano H. Interaction between Munc13-1 and RIM is critical for glucagon-like peptide-1 mediated rescue of exocytotic defects in Munc13-1 deficient pancreatic beta-cells. *Diabetes* 2007;56:2579–88.
128. Kwan E, Gao X, Leung Y, Gaisano H. Activation of exchange protein directly activated by cyclic adenosine monophosphate and protein kinase A regulate common and distinct steps in promoting plasma membrane exocytic and granule-to-granule fusions in rat islet beta cells. *Pancreas* 2007;35:e45–54.
129. Suzuki Y, Zhang H, Saito N, Kojima I, Urano T, Mogami H. Glucagon-like peptide 1 activates protein kinase C through  $\text{Ca}^{2+}$ -dependent activation of phospholipase C in insulin-secreting cells. *J Biol Chem* 2006;281:28499–507.
130. Chepurny O, Hussain M, Holz G. Exendin-4 as a stimulator of rat insulin I gene promoter activity via bZIP/CRE interactions sensitive to serine/threonine protein kinase inhibitor Ro 31-8220. *Endocrinology* 2002;143:2303–13.
131. Xie T, Chen M, Zhang Q, Ma Z, Weinstein L.  $\beta$ -cell-specific deficiency of the stimulatory G protein  $\alpha$ -subunit  $\text{G}_{\alpha}$  leads to reduced  $\beta$ -cell mass and insulin-deficient diabetes. *Proc Natl Acad Sci U S A*. 2007;104:19601–6.
132. Elrick L, Docherty K. Phosphorylation-dependent nucleocytoplasmic shuttling of pancreatic duodenal homeobox-1. *Diabetes* 2001;50:2244–52.

133. Wang X, Zhou J, Doyle M, Egan J. Glucagon-like peptide-1 causes pancreatic duodenal homeobox-1 protein translocation from the cytoplasm to the nucleus of pancreatic  $\beta$ -cells by a cyclic adenosine monophosphate/protein kinase A-dependent mechanism. *Endocrinology* 2001;142:1820–27.
134. Song W, Schreiber W, Zhong E, Liu F, Kornfeld B, Wondisford F, Hussain M. Exendin-4 stimulation of cyclin A2 in  $\beta$ -cell proliferation. *Diabetes* 2008;57:2371–81.
135. Kim S, Nian C, Widenmaier S, McIntosh C. Glucose-dependent insulinotropic polypeptide-mediated up-regulation of beta-cell antiapoptotic Bcl-2 gene expression is coordinated by cyclic AMP (cAMP) response element binding protein (CREB) and cAMP-responsive CREB coactivator 2. *Mol Cell Biol* 2008;28:1644–56.
136. Jansson D, Ng A, Fu A, Depatie C, Al Azzabi M, Screaton R. Glucose controls CREB activity in islet cells via regulated phosphorylation of TORC2. *Proc Natl Acad Sci U S A* 2008;105:10161–66.
137. Jonas J, Laybutt D, Steil G, Trivedi N, Pertusa J, Van de Castele M, Weir G, Henquin J. High glucose stimulates early response gene c-Myc expression in rat pancreatic beta cells. *J Biol Chem* 2001;276:35375–81.
138. Susini S, Roche E, Prentki M, Schlegel W. Glucose and glucoincretin peptides synergize to induce c-fos, c-jun, junB, zif-268, and nur-77 gene expression in pancreatic beta(INS-1) cells. *FASEB J* 1998;12:1173–82.
139. Glauser D, Brun T, Gauthier B, Schlegel W. Transcriptional response of pancreatic beta cells to metabolic stimulation: large scale identification of immediate-early and secondary response genes. *BMC Mol Biol* 2007;8:54.
140. Frödin M, Sekine N, Roche E, Filloux C, Prentki M, Wollheim C, Van Obberghen E. Glucose, other secretagogues, and nerve growth factor stimulate mitogen-activated protein kinase in the insulin-secreting beta-cell line, INS-1. *J Biol Chem* 1995;270:7882–89.
141. Benes C, Roisin M, Van Tan H, Creuzet C, Miyazaki J, Fagard R. Rapid activation and nuclear translocation of mitogen-activated protein kinases in response to physiological concentration of glucose in the MIN6 pancreatic beta cell line. *J Biol Chem* 1998;273:15507–13.
142. Benes C, Poitout V, Marie J, Martin-Perez J, Roisin M, Fagard R. Mode of regulation of the extracellular signal-regulated kinases in the pancreatic beta-cell line MIN6 and their implication in the regulation of insulin gene transcription. *Biochem J* 1999;340 (Pt 1): 219–25.
143. Gomez E, Pritchard C, Herbert T. cAMP-dependent protein kinase and  $\text{Ca}^{2+}$  influx through L-type voltage-gated calcium channels mediate Raf-independent activation of extracellular regulated kinase in response to glucagon-like peptide-1 in pancreatic  $\beta$ -cells. *J Biol Chem* 2002;277:48146–51.
144. Ehses J, Pelech S, Pederson R, McIntosh C. Glucose-dependent insulinotropic polypeptide activates the Raf-Mek1/2-ERK1/2 module via a cyclic AMP/cAMP-dependent protein kinase/Rap1-mediated pathway. *J Biol Chem* 2002;277:37088–97.
145. Drucker D. Glucagon-like peptides: regulators of cell proliferation, differentiation, and apoptosis. *Mol Endocrinol* 2003;17:161–71.
146. Hui H, Nourparvar A, Zhao X, Perfetti R. Glucagon-like peptide-1 inhibits apoptosis of insulin-secreting cells via a cyclic 5'-adenosine monophosphate-dependent protein kinase A- and a phosphatidylinositol 3-kinase-dependent pathway. *Endocrinology* 2003;144:1444–55.
147. Ehses J, Casilla V, Doty T, Pospisilik J, Winter K, Demuth H, Pederson R, McIntosh C. Glucose-dependent insulinotropic polypeptide promotes beta-(INS-1) cell survival via cyclic adenosine monophosphate-mediated caspase-3 inhibition and regulation of p38 mitogen-activated protein kinase. *Endocrinology* 2003;144:4433–45.
148. Ranta F, Avram D, Berchtold S, Düfer M, Drews G, Lang F, Ullrich S. Dexamethasone induces cell death in insulin-secreting cells, an effect reversed by exendin-4. *Diabetes* 2006;55:1380–90.
149. Granata R, Settanni F, Biancone L, Trovato L, Nano R, Bertuzzi F, Destefanis S, Annunziata M, Martinetti M, Catapano F, Ghè C, Isgaard J, Papotti M, Ghigo E, Muccioli G. Acylated

- and unacylated ghrelin promote proliferation and inhibit apoptosis of pancreatic beta-cells and human islets: involvement of 3',5'-cyclic adenosine monophosphate/protein kinase A, extracellular signal-regulated kinase 1/2, and phosphatidylinositol 3-Kinase/Akt signaling. *Endocrinology* 2007;148:512–29.
150. Granata R, Settanni F, Gallo D, Trovato L, Biancone L, Cantaluppi V, Nano R, Annunziata M, Campiglia P, Arnoletti E, Ghè C, Volante M, Papotti M, Muccioli G, Ghigo E. Obestatin promotes survival of pancreatic  $\beta$ -cells and human islets and induces expression of genes involved in the regulation of  $\beta$ -cell mass and function. *Diabetes* 2008;57:967–79.
151. Ferdaoussi M, Abdelli S, Yang J, Cornu M, Niederhauser G, Favre D, Widmann C, Regazzi R, Thorens B, Waeber G, Abderrahmani A. Exendin-4 protects  $\beta$ -cells from interleukin-1 beta-induced apoptosis by interfering with the c-Jun NH2-terminal kinase pathway. *Diabetes* 2008;57:1205–15.
152. Loweth A, Williams G, Scarpello J, Morgan N. Heterotrimeric G-proteins are implicated in the regulation of apoptosis in pancreatic  $\beta$ -cells. *Exp Cell Res* 1996;229:69–76.
153. Ahmad M, Abdel-Wahab YH, Tate R, Flatt PR, Pyne NJ, Furman BL. Effect of type-selective inhibitors on cyclic nucleotide phosphodiesterase activity and insulin secretion in the clonal insulin secreting cell line BRIN-BD11. *Br J Pharmacol*. 2000;129:1228–34.
154. Andersen H, Mauricio D, Karlsen A, Mandrup-Poulsen T, Nielsen J, Nerup J. Interleukin-1  $\beta$ -induced nitric oxide production from isolated rat islets is modulated by D-glucose and 3-isobutyl-1-methyl xanthine. *Eur J Endocrinol* 1996;134:251–9.
155. Friedrichsen B, Neubauer N, Lee Y, Gram V, Blume N, Petersen J, Nielsen J, Møldrup A. Stimulation of pancreatic  $\beta$ -cell replication by incretins involves transcriptional induction of cyclin D1 via multiple signalling pathways. *J Endocrinol* 2006;188:481–92.
156. Klinger S, Poussin C, Debril M, Dolci W, Halban P, Thorens B. Increasing GLP-1-induced  $\beta$ -cell proliferation by silencing the negative regulators of signaling cAMP response element modulator-alpha and DUSP14. *Diabetes* 2008;57:584–93.
157. Kim M, Kang J, Park Y, Ryu G, Ko S, Jeong I, Koh K, Rhie D, Yoon S, Hahn S, Kim M, Jo Y. Exendin-4 induction of cyclin D1 expression in INS-1  $\beta$ -cells: involvement of cAMP-responsive element. *J Endocrinol* 2006;188:623–33.
158. Welters H, Kulkarni R. Wnt signaling: relevance to  $\beta$ -cell biology and diabetes. *Trends Endocrinol Metab*. 2008;349–55.
159. Liu Z, Habener J. Glucagon-like peptide-1 activation of TCF7L2-dependent Wnt signaling enhances pancreatic  $\beta$  cell proliferation. *J Biol Chem* 2008;283:8723–35.
160. Hii C, Howell S. Role of second messengers in the regulation of glucagon secretion from isolated rat islets of Langerhans. *Mol Cell Endocrinol* 1987;50:37–44.
161. Ding W, Renström E, Rorsman P, Buschard K, Gromada J. Glucagon-like peptide I and glucose-dependent insulintropic polypeptide stimulate  $\text{Ca}^{2+}$ -induced secretion in rat  $\alpha$ -cells by a protein kinase A-mediated mechanism. *Diabetes* 1997;46:792–800.
162. Dillon J, Lu M, Bowen S, Homan L. The recombinant rat glucagon-like peptide-1 receptor, expressed in an  $\alpha$ -cell line, is coupled to adenylyl cyclase activation and intracellular calcium release. *Exp Clin Endocrinol Diabetes* 2005;113:182–9.
163. Islam D, Zhang N, Wang P, Li H, Brubaker P, Gaisano H, Wang Q, Jin T. Epac is involved in cAMP-stimulated proglucagon expression and hormone production but not hormone secretion in pancreatic  $\alpha$ - and intestinal L-cell lines. *Am J Physiol Endocrinol Metab* 2009;296:E174–81.
164. Dunning B, Foley J, Ahren B. Alpha cell function in health and disease: influence of glucagon-like peptide-1. *Diabetologia* 2005;48:1700–13.
165. Gromada J, Høy M, Buschard K, Salehi A, Rorsman P. Somatostatin inhibits exocytosis in rat pancreatic  $\alpha$ -cells by G(i2)-dependent activation of calcineurin and depriming of secretory granules. *J Physiol* 2001;535:519–32.
166. Gros L, Thorens B, Bataille D, Kervran A. Glucagon-like peptide-1-(7-36) amide, oxyntomodulin, and glucagon interact with a common receptor in a somatostatin-secreting cell line. *Endocrinology*. 1993;133:631–8.



167. Fehmann H, Janssen M, Göke B. Interaction of glucagon-like peptide-I (GLP-I) and galanin in insulin (beta TC-1)- and somatostatin (RIN T3)-secreting cells and evidence that both peptides have no receptors on glucagon (INR1G9)-secreting cells. *Acta Diabetol* 1995;32:176–81.
168. Patel Y, Papachristou D, Zingg H, Farkas E. Regulation of islet somatostatin secretion and gene expression: selective effects of adenosine 3',5'-monophosphate and phorbol esters in normal islets of Langerhans and in a somatostatin-producing rat islet clonal cell line 1027 B2. *Endocrinology* 1991;128:1754–62.
169. Ma X, Zhang Y, Gromada J, Sewing S, Berggren P, Buschard K, Salehi A, Vikman J, Rorsman P, Eliasson L. Glucagon stimulates exocytosis in mouse and rat pancreatic  $\alpha$ -cells by binding to glucagon receptors. *Mol Endocrinol* 2005;19:198–12.
170. Gromada J, Bokvist K, Ding WG, Barg S, Buschard K, Renström E, Rorsman P. Adrenaline stimulates glucagon secretion in pancreatic A-cells by increasing the  $\text{Ca}^{2+}$  current and the number of granules close to the L-type  $\text{Ca}^{2+}$  channels. *J Gen Physiol* 1997;110:217–28.
171. Vieira E, Liu Y, Gylfe E. Involvement of  $\alpha 1$  and beta-adrenoceptors in adrenaline stimulation of the glucagon-secreting mouse alpha-cell. *Naunyn Schmiedeberg's Arch Pharmacol* 2004;369:179–83.
172. Knudsen L, Kiel D, Teng M, Behrens C, Bhumralkar D, Kodra J, Holst J, Jeppesen C, Johnson M, de Jong J, Jorgensen A, Kercher T, Kostrowicki J, Madsen P, Olesen P, Petersen J, Poulsen F, Sidelmann U, Sturis J, Truesdale L, May J, Lau J. Small-molecule agonists for the glucagon-like peptide 1 receptor. *Proc Natl Acad Sci U S A* 104: 2007;937–42.