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 β-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 β-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 β-cell differentiation, growth and survival. This chapter discusses the formation, destruction and actions of cAMP in the islets with particular emphasis on the β-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 β-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 β-cell cAMP levels. These include glucose-dependent

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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 β-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 β-Cell

The level of cyclic AMP in the β-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 β-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 Ca^{2+} influx through voltage-sensitive 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 β-cell expresses several GPCRs coupled to G_s, stimulation of which leads to elevation in the β-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], 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-β-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 β-arrestin-1 in coupling the GLP-1 receptor to

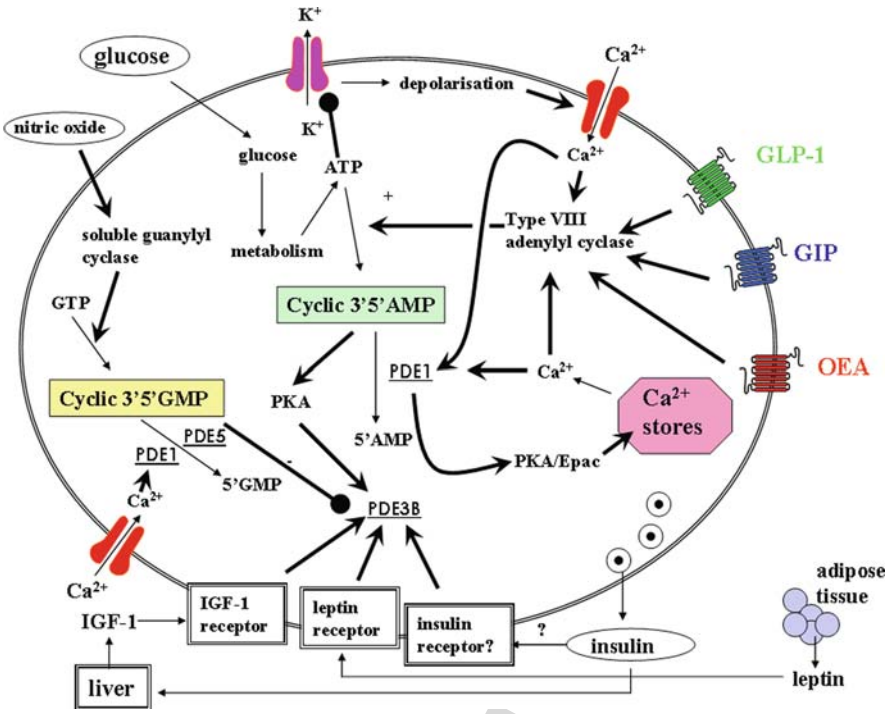

Fig. 13.1 Summary of the mechanisms for the formation and destruction of cAMP in the pancreatic islet β-cell. Glucose is transported into the β-cell using GLUT2 and is then metabolized generating ATP. This results in closure of the K_{ATP} channel, membrane depolarization and calcium influx through voltage-sensitive 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 β-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 β-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 oleoylethanolamide, although there is as yet no evidence that they are available in sufficient concentrations in the blood to stimulate the β-cell GRP119 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 β-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 β-cells, and clonal β-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 β-cells or clonal β-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 β-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 β-cell PDE activity, and several of these isoforms regulate glucose-induced insulin secretion and other cAMP-mediated β-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 β-cell cAMP and insulin secretion was obtained by over-expressing PDE3B in the INS-1 β-cell line and in islets and by using transgenic animals over-expressing PDE3B in the β-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 $[cAMP]_i$.

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 β-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 β-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 β-cell lines or primary β-cells [5, 75–78] or been incorporated in vivo by generating a transgenic mouse expressing a pancreatic β-cell-targeted cAMP reporter which was inducible in response to tetracycline [4]. In MIN6 β-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 β-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

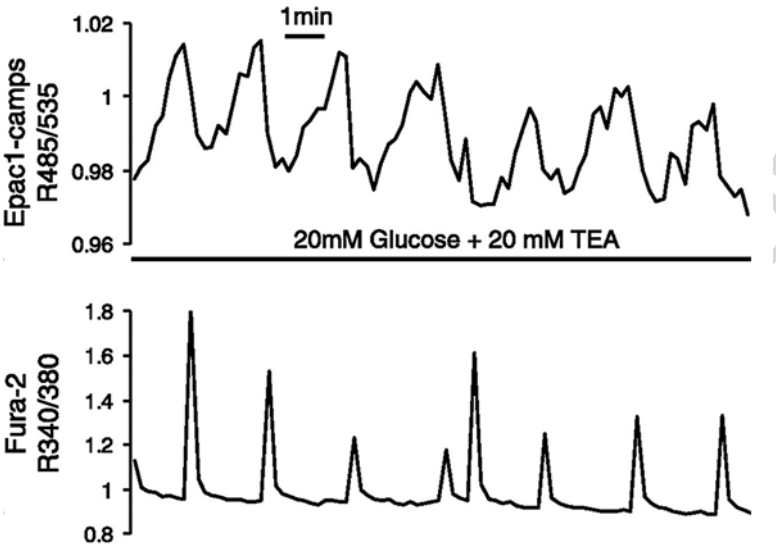

Fig. 13.2 Ca^{2+} and cAMP oscillations in glucose-stimulated MIN6 cells. Simultaneous imaging of cytosolic cAMP concentration ($[cAMP]_i$; *top trace*, $R_{485/535}$) and cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$; *bottom trace*, $R_{340/380}$) in a single MIN6 cell stimulated with 20 mM glucose and 20 mM tetraethylammonium chloride (TEA). Note that second messenger oscillations were out of phase, with each $[Ca^{2+}]_i$ spike coupled to a rapid and transient reduction in $[cAMP]_i$. (Reproduced from Fridlyand LE, Harbeck MC, Roe MW, Philipson LH. Regulation of cAMP dynamics by 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 β-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 $[Ca^{2+}]_i$ 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. Localgeneration,

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 β-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 β-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 β-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 β-Cell

cAMP modulates a number of β-cell functions including insulin secretion, insulin synthesis, β-cell replication, and β-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 β-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 β-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 β-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 β-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 β-cell augment glucose-induced insulin secretion at several sites in the secretory pathway.

13.3.1.1 Effects on the β-Cell ATP-Sensitive Potassium Channel

The β-cell ATP-sensitive potassium channel (K_{ATP} channel) plays a fundamental role in glucose-induced insulin secretion. Elevation of cAMP in the β-cell using GLP-1, forskolin, or the non-selective PDE inhibitor IBMX inhibits the β-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 β-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 β-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 β-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 β-cell line βTC3 [110].

Mobilization of Ca^{2+} from Intracellular Stores

Calcium-Induced Calcium Release

In addition to facilitating calcium entry, agents that elevate β-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 β-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 PKCα and PKCϵ in INS-1 cells and its effects are

mimicked by forskolin. This activation was Ca^{2+} dependent, and it was hypothesized that it was effected through mobilization of Ca^{2+} as a result, for example, of PKA sensitization of the IP_3 channel and consequent Ca^{2+}-mediated activation of phospholipase C [129].

13.4 Role of cAMP in Insulin Synthesis and in β-Cell Differentiation, Proliferation, and Survival

The incretin GLP-1, acting to an important extent through cAMP effector mechanisms, increases insulin synthesis, promotes β-cell proliferation and inhibits β-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 β-cell-specific deficiency in the α subunit of G_s showed reduced β-cell mass, reduced islet content of insulin, reduced β-cell proliferation, and increased β-cell apoptosis, and marked hyperglycaemia suggests the fundamental importance of responsiveness to incretin hormones [131] in β-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 β-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 a 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 β-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 β-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 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 β-Cell Apoptosis and Stimulation of β-Cell Proliferation

There is abundant evidence for suppression of β-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 β-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 β-cells [154] may also be implicated.

In addition to preventing apoptosis of β-cells, the incretin hormones and other agents elevating cAMP promote β-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 β-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 β-cell proliferation was inhibited by blocking β-catenin or the transcription factor TCF7L2, critical mediators of Wnt signalling [159].

An additional mechanism whereby cAMP modulates β-cell proliferation may be through regulation of the CREB antagonists cAMP response element modulator CREM-α and ICERI 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 β-cell line and in rat primary β-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-α or DUSP14, or expression of a dominant-negative DUSP14, augmented GLP-1-induced β-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 α-cells; this effect was accompanied by elevations in intracellular cAMP, increases in Ca^{2+} currents and was mediated by PKA [161]. Exposure of an α-cell line (INRl-G9) expressing recombinant GLP-1 receptors to GLP-1 increased the formation of cAMP and elevated free cytosolic [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 β-adrenoceptors, augmented depolarization-evoked glucagon secretion from rat primary α-cells [170]. This effect was mimicked by forskolin and was PKA dependent. As in the β-cell the PKA-dependent effects appear to involve more than one mechanism, including increased Ca^{2+} entry and augmentation of the effects of Ca^{2+}. Photo release of caged cAMP increased exocytosis even when intracellular [Ca^{2+}] was clamped [170]. These data were supported by observations using mouse primary α-cells, in which adrenaline-induced increases in α-cell $[Ca^{2+}]_i$ 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 β-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].

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