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Review

Cyclic nucleotide phosphodiesterases in pancreatic islets

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Abstract

Cyclic nucleotide phosphodiesterases (PDEs) comprise a family of enzymes (PDE1-PDE11) which hydrolyse cyclic AMP and cyclic GMP to their biologically inactive 5′ derivatives. Cyclic AMP is an important physiological amplifier of glucose-induced insulin secretion. As PDEs are the only known mechanism for inactivating cyclic nucleotides, it is important to characterise the PDEs present in the pancreatic islet beta cells. Several studies have shown pancreatic islets or beta cells to contain PDE1C, PDE3B and PDE4, with some evidence for PDE10A. Most evidence suggests that PDE3B is the most important in relation to the regulation of insulin release, although PDE1C could have a role. PDE3-selective inhibitors augment glucose-induced insulin secretion. In contrast, activation of beta-cell PDE3B could mediate the inhibitory effect of IGF-1 and leptin on insulin secretion. In vivo, although PDE3 inhibitors augment glucose-induced insulin secretion, concomitant inhibition of PDE3B in liver and adipose tissue induce insulin resistance and PDE3 inhibitors do not induce hypoglycaemia. The development of PDE3 inhibitors as anti-diabetic agents would require differentiation between PDE3B in the beta cell and that in hepatocytes and adipocytes. Through their effects in regulating beta-cell cyclic nucleotide concentrations, PDEs could modulate beta-cell growth, differentiation and survival; some work has shown that selective inhibition of PDE4 prevents diabetes in NOD mice and that selective PDE3 inhibition blocks cytokine-induced nitric oxide production in islet cells. Further work is required to understand the mechanism of regulation and role of the various PDEs in islet-cell function and to validate them as targets for drugs to treat and prevent diabetes. [Diabetologia (2003) 46:1179–1189]

Keywords Islet beta cell, phosphodiesterase, cyclic AMP, cyclic GMP, insulin secretion.

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Abbreviations: PDE, phosphodiesterase; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon like peptide 1; PDX-1, pancreatic duodenal homeobox-1; IBMX, isobutylmethylxanthine

Introduction

Cyclic nucleotide phosphodiesterases comprise a family of enzymes the function of which is the hydrolysis of cyclic AMP and cyclic GMP to their biologically inactive 5′ derivatives. Currently there are 11 known gene families of CN-PDEs consisting of more than 50 enzymes with differences in their substrate selectivities (cyclic AMP vs cyclic GMP), kinetics, allosteric regulation, tissue distribution and susceptibility to pharmacological inhibition (see reviews [1, 2, 3]). The main properties of some of these enzymes are shown in Table 1 [3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17]. In view of the clear

Table 1.

Enzyme	Tissues	Reference	Substrate	K_m μM	Properties	Inhibitors
PDE1A	thyroid, testis, brain	[4]			Activated by Ca/ calmodulin	zaprinast
PDE1B	brain, lymphocytes	[5]	cGMP	3		vinpocetine
PDE1C	blood vessels, testis	[6]	cAMP	1–30		SCH51866 8-MM-IBMX
PDE2	Brain, heart, platelets, liver, thymocytes	[2] [7]	cGMP	10	Activated by cGMP	EHNA
			cAMP	25		
PDE3A	Heart, blood vessels	[1] [2] [8] [9]	cAMP	0.5	Inhibited by GMP Phosphorylation	milrinone Org 9935
PDE3B	adipocyte hepatocyte lymphocyte				(PKA and insulin-sensitive kinase)	siguazodan cilostamide enoximone OPC3911
PDE4A	lung, inflammatory and immune cells, brain, blood vessels	[10] [11]	cAMP	0.2–4	Phosphorylation by PKA and p42/p44 MAPK (PDE4D3)	rolipram
PDE4B						RP73401
PDE4C						zardaverine
PDE4D						CP80633 CDP840 LAS31025 SB207499
PDE5	smooth muscle	[12]	cGMP	2	Phosphorylation by PKA/PKG	zaprinast sildenafil dipyridamole SCH51866
PDE6	retinal photoreceptors	[13]	cGMP	60–100		zaprinast dipyridamole
PDE7A, PDE7B	brain, lymphocytes, kidney	[14]	cAMP	0.2		
PDE8A, PDE8B	thyroid	[15]	cAMP	0.7	IBMX-insensitive	dipyridamole
PDE9	kidney	[15]	cGMP	0.17	IBMX-insensitive	SCH51866
PDE10A	testis, brain	[3]	cAMP	0.05		SCH51866
			cGMP	3		dipyridamole
PDE11A	??	[3]	cAMP	1		zaprinast
			cGMP	0.5		dipyridamole

importance of cyclic nucleotides, especially cyclic AMP, in the pancreatic islet beta cell and the fact that phosphodiesterase-activity is the only known system for destroying these cyclic nucleotides, it is important to understand the nature and the roles of the phosphodiesterase enzymes expressed in islet beta cells. The aim of this short review is to examine two main questions: (i) what phosphodiesterase (PDE) isoenzymes are expressed in the pancreatic islet beta cell? (ii) which PDE isoenzymes are functionally important in regulating cellular cyclic AMP concentrations in relation to insulin secretion?

Roles of cyclic nucleotides in the pancreatic islet beta cell

cAMP

Insulin secretion

Although glucose-induced insulin secretion does not seem to require increases in islet beta-cell cyclic AMP or the PKA-system [18, 19], intracellular concentrations of cyclic AMP in beta cells are increased by glucose [20, 21]. Moreover, this cyclic nucleotide is generally accepted as an important amplifier of glucose-induced insulin release [22], particularly when its cellular concentrations are increased by various gut hormones implicated as incretins but also by glucose itself [23]. Cyclic AMP augments glucose-induced insulin secretion through a number of mechanisms including increased opening of voltage-sensitive Ca^{2+}

channels [24], calcium-induced Ca^{2+}-release [25], activation of ryanodine receptors in the ER [26, 27], stimulation of beta-cell lipolysis [28] and direct effects on exocytosis [23]. Most actions of cyclic AMP in the beta cell seem to be mediated through protein-kinase A (PKA)-catalysed phosphorylation events but direct effects of the cyclic nucleotide on exocytosis are partly PKA-independent [29]. PKA-independent effects on exocytosis can be mediated by the cyclic AMP-binding protein cAMP-GEFII, interacting with Rim2, a target of the small G-protein Rab3 [30]. Cyclic AMP mediates the insulinotropic action of the incretin factors glucose-dependent insulinotropic peptide (GIP) and glucagon like peptide 1 (GLP-1) [31]. GLP-1 is released during meals and markedly augments the direct effect of glucose on insulin secretion via cyclic AMP-dependent mechanisms.

Other roles in the beta cell

In addition to its role in amplifying glucose-induced insulin secretion, cyclic AMP can mediate or modulate other effects of glucose and insulinotropic hormones in the beta cell. 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 [32]. Whereas the effects of glucose on PDX-1 are not mediated by cyclic AMP, there is strong evidence for the importance of cyclic AMP in GLP-1-dependent stimulation of PDX-1 in the beta cell, as well as its translocation to the nucleus and its activation of the insulin gene promoter [33]. However, the role of cyclic AMP in regulating insulin synthesis is not clear because the adenylyl cyclase activator forskolin, or the cyclic AMP analogue 8-bromo-cyclic AMP suppressed insulin transcription in INS-1 cells in a PKA-independent manner [34]. Cyclic AMP could mediate effects of glucose in stimulating the expression of immediate early response genes such as *c-myc* [35] and *c-fos* [36]. In this context, cyclic AMP can either activate or inhibit the p42/p44 mitogen-activated protein kinase pathway depending on the cell type and conditions [37]. Glucose itself activates the p42/p44 MAPK pathway [38, 39] and this effect is amplified, although not mediated by, cyclic AMP. Indeed, the activation of this pathway by GIP is cyclic AMP and PKA-dependent [40]. Regulation of gene expression by cyclic AMP could have relevance to effects on beta-cell growth, differentiation and apoptosis. For example, increased cyclic AMP concentrations protected rat islets against interleukin-1 beta mediated induction of nitric oxide synthase and nitric oxide production [41]. Excessive nitric oxide production seems to mediate subsequent apoptosis of beta cells. On the other hand, beta-cell lines were made more susceptible to apoptosis after exposure to dibutyryl cyclic AMP [42] or the cyclic AMP-increasing agent forskolin [43].

Cyclic GMP

Although increases in cyclic GMP have been linked with decreases in insulin secretion [44, 45], the role of this nucleotide in the islet beta cell remains unclear [46, 47]. More recent studies suggest that cyclic GMP could mediate the effects of low concentrations of nitric oxide in augmenting glucose-induced Ca^{2+} oscillations and insulin secretion in rat-isolated beta cells [48, 49, 50]. 8-bromoguanosine-cyclic GMP was found to augment glucose-induced Ca^{2+} oscillations in mouse islets and to inhibit K_{ATP} channel activity in mouse-intact beta cells [51]. Cyclic GMP was reported to mediate nitric oxide-induced apoptosis in the insulin-secreting cell line HIT-T15 [52].

PDE isoforms present in the islets

Relatively little is known about the PDE isoenzymes in the pancreatic islet beta cell. Most studies have used pancreatic islets, which contain four endocrine cell types; these comprise the insulin-secreting beta cells, the glucagon-secreting A cells, the somatostatin secreting D cells and the pancreatic polypeptide cells. Blood vessels also permeate the islets. Thus the presence of a particular PDE in islets does not necessarily indicate its presence in the insulin-secreting cells. This technical problem has been circumvented by the use of insulin-secreting cell lines, although these have limitations as models for native beta cells.

PDE1

There are three distinct isoforms of the calcium-calmodulin activated PDE1, termed PDE1A, B and C. These isoforms are encoded by distinct genes and show differential tissue expression. Earlier work showed islets to contain a cytosolic Ca-calmodulin activated PDE, with low and high K_m components for both cAMP and cGMP and a particulate form, which appeared insensitive to Ca-calmodulin [53, 54, 55]. We have recently shown a calcium-calmodulin activated PDE activity in the pellet, but not the supernatant, fraction of homogenates of an insulin-secreting cell line BRIN BD11 [56].

The presence of a Ca-calmodulin activated PDE was also observed in another beta-cell line β-TC3 and furthermore RT-PCR was used to show that this isoform was PDE1C [4]. Thus islets, and possibly beta cells, express PDE1. This is supported to some extent by the observation that the PDE1/PDE5 inhibitor zaprinast (10^{-5}–10^{-4} mol/l) produced a 14 to 30% inhibi-

tion in both membrane and cytosolic fractions of rat islet homogenates [57] and 25% inhibition of cyclic AMP/cyclic GMP PDE activity in BRIN-BD11 cells (IC_{50}~1–5 μmol/l, [56]. A study [4] also showed that the PDE was inhibited by zaprinast (IC_{50} 4.5 μmol/l) or 8-MM-IBMX (IC_{50} 7.5 μmol/l) but not by vinpocetine (IC_{50} >100 μmol/l for PDE1C vs 1.4 μmol/l and 9.8 μmol/l for PDE1A and PDE1B) [5, 6]; these findings were compatible with the presence of PDE1C but not PDE1A or PDE1B. Although the data support the presence of a PDE1, other isoforms are clearly expressed, as evidenced by the presence in islets [57] or beta-cell lines [4, 56] of zaprinast-insensitive PDE activity.

PDE3

Islet PDE activity was inhibited by 60 to 70% using 10 μmol/l cyclic GMP [58]. The pellet fraction of homogenates of BRIN BD11 cells was also found to contain a cyclic AMP PDE activity that was potently (IC_{50}–0.7 μmol/l) inhibited (up to 30–40%) by cyclic GMP [56]. Moreover, the membrane and cytosolic fractions of islet homogenates contained a low K_m (1.4–2.2 μmol/l) cyclic AMP PDE activity [57]. These data are consistent with the presence of PDE3 in insulin-secreting cells. There are two isoforms of PDE3 (PDE3A and PDE3B) and there is convincing evidence that only PDE3B is expressed in the beta cell. For example, the use of a polyclonal antibody against a GST-PD3B, showed a single protein band in western blots of extracts of rat pancreatic islets and the beta-cell line HIT-T15 that corresponded in size to the PDE3B protein found in extracts of rat epididymal adipose tissue [59]. Furthermore, we applied RT-PCR to total RNA of BRIN-BD11 cells and amplified a product using PDE3B-specific primers. The product showed over 97% sequence homology with rat adipose tissue PDE3B [56]. No product was obtained using PDE3A-specific primers. The presence of PDE3B in native beta cells was also supported strongly by immunocytochemistry, showing that in rat islets PDE3B was expressed only in cells that were co-stained with anti-insulin antibodies [59].

Pharmacological characterization of PDE3 has been greatly facilitated by the development of potent, highly selective inhibitors of this isoform, such as SK&F 94836 (siguazodan) [60] and Org 9935 [61]. We found that SK&F 94836 and Org 9935 were very potent inhibitors of rat islet PDE activity, especially in membrane fractions [57] with up to 85% of membrane-bound PDE being inhibited with IC_{50} values of 5.5 and 0.05 μmol/l respectively. Of interest, we have also made similar observations in human islets [62]. This was later confirmed by others [63], who showed that rat and human islets contained a milrinone-sensitive PDE, accounting for up to 70% of total islet PDE activity. In addition, the PDE activity in anti-PDE3B-immunoprecipitates was completely inhibited by milrinone [59]. Again, recent work showed SK&F 94836 and Org 9935 to inhibit cyclic AMP PDE in the BRIN BD11 insulin-secreting cell line [56]. Unlike in islets, we found that these drugs inhibited cyclic AMP hydrolysis only in the membrane fraction of BRIN-BD11 cell homogenates. We proposed that the PDE3 activity found in the soluble fraction of homogenates of rat islets might be PDE3A from blood vessels or other non-beta-cell tissue. PDE3A was shown exclusively in the cytosolic fractions of all tissues studied, whereas PDE3B was expressed in particulate fractions [64].

PDE4

Some experiments showed rolipram to be without effect on cyclic AMP hydrolysis in rat islet homogenates. However, in other experiments homogenate cyclic AMP-PDE activity was inhibited up to 20% by rolipram in concentrations of 10^{-5} to 10^{-4} mol/l [57], although this inhibitory effect is labile. These findings have been confirmed [63], while another study [4] reported a rolipram sensitive cyclic AMP PDE activity in βTC3 cells and showed the expression of PDE4A and PDE4D using RT-PCR. We reported that approximately 25% of the BRIN-BD11 cell homogenate cyclic AMP PDE activity in both cytosolic and membrane fractions were inhibited by rolipram (IC_{50} 0.04–0.1 μmol/l) [56]. Taken together, these results suggest that beta cells express functional PDE4.

Other PDE isoforms

A combination of maximal inhibitory concentrations of Org 9935 (PDE3), rolipram (PDE4) and zaprinast (PDE1/5) inhibited BRIN-BD11 cell cyclic AMP PDE at low substrate concentrations by around 90%. A similar degree of inhibition was obtained by maximal inhibitory concentrations of the non-selective PDE inhibitor IBMX. Thus the total cyclic AMP PDE activity could be largely accounted for by PDE1, PDE3 and PDE4. The ~10% activity not inhibited by IBMX could be attributable to the IBMX-insensitive, cyclic AMP specific enzyme PDE8, although there is no other direct evidence for this. We have also found that Western blotting of islet homogenates showed a protein band corresponding to PDE10A. The significance of this finding has yet to be determined. However, the very low Km for cyclic AMP of PDE10 suggests that these isoforms could function to control basal cyclic AMP under conditions of glucose fasting or in the absence of circulating GLP-1.

Role of islet beta-cell PDE in modulating insulin secretion

Modulation of glucose-induced insulin secretion by PDE inhibitors

The non-selective PDE inhibitor IBMX has been shown by many authors to augment glucose-induced insulin secretion and has been used widely as a tool to investigate the role of cyclic AMP in the beta cell. However, apart from its lack of selectivity for different PDE isoforms, IBMX could have actions that are not related to PDE-inhibitory activity, for example activation of the ryanodine receptor [65] and antagonism at adenosine receptors [66]. We found that several selective PDE3 inhibitors (Org 9935, siguazodan, SK&F 94120, ICI118233) augmented glucose-induced insulin secretion from rat and human islets, whereas selective inhibitors of PDE4 and PDE1/5 did not [57, 62]. Other selective PDE3 inhibitors were also found to augment insulin secretion. Thus, milrinone augmented glucose-induced insulin secretion in rat [4] and human [63] isolated islets and pimobendan augmented glucose-induced insulin secretion in rat islets [67]. Milrinone also augmented glucose-induced insulin secretion by monolayers of neonatal rat pancreatic monolayer cells [59], excluding an indirect effect by modulating the secretion of other islet hormones. Moreover, Org 9935 and siguazodan augmented glucose-induced insulin secretion in the insulin-secreting cell line BRIN-BD11 [56] and OPC3911 produced a similar effect in INS-1(832/13) cells [23]. These data strongly support a role for PDE3, rather than other isoforms, in regulating the cyclic AMP pool that modulates insulin secretion. However, this is complicated by the observations that glucose-induced insulin secretion is augmented in beta-cell lines, as distinct from intact islets, by rolipram, a selective PDE4 inhibitor [4, 56]. There is good evidence in other, non-beta-cell systems for compartmentalization of PDEs [68, 69, 70]. Therefore compartmentalization of PDE4 isoforms might be different between beta-cell lines and native beta cells. In cell lines, PDE4 can interact with relevant cyclic AMP pools regulating insulin secretion due to its relocalisation into compartments containing the insulin secreting machinery. A further and more intriguing complication arises from the observation that 8-MM-IBMX, purported to be a selective inhibitor of the Ca-calmodulin activated PDE1C isoform, augmented glucose-induced insulin secretion in both β-TC3 cells and in islets [4]. Zaprinast, a PDE1/5 inhibitor also augmented glucose-induced insulin secretion in β-TC3 cells. The selectivity of 8-MM-IBMX for PDE1C at the concentrations (150 and 500 μmol/l) used to modulate insulin secretion is not clear. Moreover, the reason for the discrepancy between these observations and those in which zaprinast either had no effect [56, 57] or, in some conditions, inhibited [57] glucose-induced insulin secretion is not known.

Mechanisms underlying the augmentation of glucose-induced insulin secretion by PDE3 inhibitors. Caution must be exercised in interpreting the effects of all "selective" PDE inhibitors because it has not been established that their effects on insulin secretion are mediated solely through inhibition of cyclic AMP hydrolysis. For example, effects of the selective PDE3 inhibitor pimobendan could involve a calcium-sensitising effect unrelated to PDE inhibition [67]. Effects of selective PDE3 inhibitors on glucose-induced insulin secretion can be shown in the absence of a measured increase in cyclic AMP concentrations [57, 67] although the agents clearly increased cyclic AMP when adenylyl cyclase was activated by forskolin [57]. In βTC3 cells rolipram (PDE4) and 8-MM-IBMX (PDE1C) but not milrinone (PDE3) produced modest increases in cyclic AMP [4]. The failure consistently to demonstrate global increases in islet cyclic AMP concentrations in response to the selective PDE3 inhibitors could reflect compartmentalization of the PDEs as has been shown in a number of other systems, including adipocytes and cardiovascular tissues [70, 71, 72]. Moreover, cyclic AMP accumulated as a result of selective inhibition of PDE3 can be destroyed by other PDE isoforms as it diffuses from its site of action. Although detailed studies on the compartmentalization of PDE3B in the islet beta cell have not been carried out, it is clear that the enzyme is membrane bound [23, 56, 57]. Nevertheless, effects of PDE3 inhibitors in the islets are compatible with effects mediated through cyclic AMP. Thus, the selective PDE3 inhibitor Org 9935 increased $[Ca^{2+}]_i$ in rat intact, isolated islets of Langerhans [73]. The selective PDE3 inhibitor OPC3911 augmented Ca^{2+}-induced exocytosis from INS-1(832/13) cells in the presence of normal intracellular concentrations of cyclic AMP but not when cyclic AMP was replaced by the PDE-resistant cyclic AMP analogue Sp-cAMPS [23]. OPC3911 also augmented depolarization-evoked exocytosis when a submaximal concentration of forskolin (500 nmol/l) was present to increase basal cyclic AMP concentrations [23].

Effect of over-expression of PDE3B. Strong evidence supporting a role for PDE3B in the beta cell has been obtained using alternative approaches (thereby validating studies based on pharmacological inhibition) in which adenoviral plasmid constructs were used to over-express PDE3B in insulinoma cells and native rat islets [23]. This resulted in a six- to eightfold over-expression in membrane-associated PDE3B activity. Insulin secretion in response to glucose and GLP-1-mediated augmentation of glucose-induced insulin secretion were markedly reduced, consistent with an opposing action of PDE3B on insulin release. Moreover, cy-

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