

<Review Article>

## Phosphodiesterases in the pathophysiology of diabetes mellitus

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**Summary** Diabetes Mellitus is a serious progressive disorder caused by an absence of or insufficient amount of insulin in the bloodstream. It is associated with risk of cardiovascular disease as well as specific microvascular complications [such as retinopathy, nephropathy and neuropathy]. Primarily there are two main types of diabetes classified as type 1 and type 2. Type 1 diabetes mellitus, is characterized by the inability of the pancreas to secrete insulin because of autoimmune destruction of the  $\beta$ -cells. Type 2 is insulin resistance and the most common form of diabetes, and most individuals with the disease are adults. Cyclic 3'5'-AMP (cAMP) is an important physiological amplifier of glucose-induced insulin secretion by the pancreatic islet  $\beta$ -cell. It is formed by the activity of adenylyl cyclase. Nine families of adenylyl cyclases catalyze the synthesis of the second messenger cAMP and protein kinases A. Cyclic nucleotide levels are regulated through catabolic processes directed by phosphodiesterases (PDEs) that breaks a phosphodiester bond in the second messenger molecules cAMP and cGMP. PDEs, which are ubiquitously distributed in mammalian tissues, contains at least 11 gene families. PDE inhibitors may be of value in preventing  $\beta$ -cell loss in both type 1 and type 2 diabetes. Comparative studies on PDEs would be very important to evaluate the involvement of each PDE in specific cellular function and to understand regulation of cyclic nucleotide signaling. In this overview, we highlight recent studies to define PDE expression and to provide evidence that PDE inhibitors may be effective agents in delay or in treatment of diabetes mellitus.

**Key words:** Phosphodiesterases, Diabetes mellitus, Cyclic AMP

Diabetes Mellitus is a disorder characterized by hyperglycaemia that results from defects in insulin secretion, or action, or both. It is a chronic disease that requires long-term medication, both to limit the development of its devastating complications and to manage them when they do occur. WHO has designated diabetes mellitus the only non infectious disease as an epidemic. The number of diabetics is increasing by 4-

5% per year<sup>1)</sup>, while the prevalence of all types of diabetes is estimated to be 2.3% of the world's population. Though there is an increase in the prevalence of type 1 diabetes, the more common form of diabetes, is type 2 diabetes, which accounts for more than 90 per cent of all diabetic cases. In the recent years the onset of diabetes is occurring at a younger age, causing devastating complications<sup>2)</sup>. The primary symptoms of

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diabetes are hyperglycaemia, accompanied by glucosuria and polyuria. A secondary set of symptoms arise in chronic or long standing diabetes; these include degeneration of the walls of blood vessels, particularly of fine capillaries and their basement membranes. It is a disorder that primarily affects the micro vascular circulation<sup>3</sup>.

Primarily there are two main types of diabetes classified as type 1 and type 2. Type 1 diabetes mellitus irrespective and unrelated to age, is characterized by the inability of the pancreas to secrete insulin because of autoimmune destruction of the  $\beta$ -cells. It commonly occurs in children, but, newer antibody tests have allowed for the identification of more people with the new-onset adult form of type 1 diabetes mellitus called latent autoimmune diabetes of the adult (LADA). In case of type 1 diabetes patients, if insulin is withdrawn, ketosis and eventually ketoacidosis develop. Therefore, these patients are dependent on exogenous insulin<sup>4</sup> and it is termed as IDDM. Type 2 diabetes mellitus is due to the interaction of multiple genes and by various environmental factors<sup>5</sup> and was once called as adult-onset diabetes. Recent studies suggest that because of the epidemic of obesity and inactivity in children, it is occurring at younger and younger ages and has been diagnosed in children as young as 2 years of age who have a family history of diabetes. Obesity, endogenous glucose output [EGO], impaired insulin action and insulin secretory dysfunctions are the prime abnormalities which characterize type 2 diabetes mellitus<sup>6</sup>.

Production of cyclic 3'5'-AMP (cAMP) formed by the enzyme adenylyl cyclase in the pancreatic islet  $\beta$ -cell in response to the glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic peptide, causes insulin secretion<sup>7</sup>. Glucose-induced insulin secretion via activation of  $\beta$ -cell G-protein-coupled receptors (GPCRs)<sup>7-10</sup> leading to an increase in islet  $\beta$ -cell cAMP is augmented by the hormones secreted in response to nutrients<sup>8</sup>. Release of insulin in response to glucose stimulation, and  $\beta$ -cell growth are important features of pancreatic islet  $\beta$ -cell biology<sup>11</sup>. The synthesis of the second messenger cAMP and protein kinases A, especially incretin hormones is catalysed by the nine families of adenylyl cyclase.

Recent progress in the field of cyclic nucleotides has shown that a large array of closely related proteins is involved in each step of the signal transduction cascade. These cyclic nucleotides are recognized as critical mediators and signalling is highly compartmentalized through plasma membrane localization of adenylyl cyclase and expression of scaffolding proteins that anchor protein kinase A to specific intracellular locations. Cyclic AMP influences many steps involved in glucose induced insulin secretion and may be important in regulating pancreatic islet  $\beta$ -cell differentiation, growth and survival. This itself is rapidly degraded in the pancreatic islet  $\beta$ -cell by cyclic nucleotide phosphodiesterase enzymes (PDEs).

In this overview, we highlight recent studies to define PDE expression and to provide evidence that PDE inhibitors may be effective agents in delay or in treatment of diabetes mellitus. Cyclic nucleotide levels are only regulated through catabolic processes directed by phosphodiesterases that breaks a phosphodiester bond in the second messenger molecules cAMP and cGMP. Therefore characterization of PDEs in pancreatic islet  $\beta$ -cell is important<sup>12</sup>. PDEs control the cellular concentrations of the second messenger's cAMP and cGMP and as such have recently been recognized as new target classes in drug discovery. They regulate the amplitude and duration of responses triggered by the second messengers, cAMP and cGMP. In doing so, they regulate a wide range of biological responses triggered by light, hormones, neurotransmitters and odorants.

Individual PDE genes lack the selective recognition for the cyclic nucleotide substrates. Therefore, newer mechanisms are required to elucidate these specific recognition for the cyclic nucleotides substrates by the PDE families<sup>13</sup>. Cyclic nucleotide phosphodiesterases, which are ubiquitously distributed in mammalian tissues, contains at least 11 gene families {PDE1 to PDE11}, which are functionally distinct, highly regulated, and structurally related<sup>13</sup>. Each PDE gene contains several distinct transcriptional units that give rise to proteins with subtle structural differences. PDE families differ in their primary sequences, substrate affinities and catalytic properties, sensitivity to effectors and inhibitors, responses to

regulatory molecules, and cellular functions. Hence PDEs are critical determinants that characterize cyclic nucleotide signalling pathways. Most cells contain representatives of more than one PDE gene family (and different variants of the same family) but in different amounts, proportions, and sub cellular locations. By virtue of their distinct intrinsic characteristics, their intracellular targeting to different sub cellular locations, and their interactions with molecular scaffolds, cellular structural elements, and regulatory partners, different PDEs integrate multiple cellular inputs and modulate the intracellular diffusion and functional compartmentalization, as well as the amplitude, duration, termination, and specificity of cyclic nucleotide signals and actions. It has been established that PDE1 to PDE6 were the first to be characterized because of their distribution in various tissues but however, their tissue function and their regulation in pathophysiology remains a puzzle<sup>14</sup>. The unique cell- and tissue-specific distribution of PDEs has prompted the development of highly specific PDE inhibitors to treat a variety of inflammatory conditions. In many pathologies, such as inflammation, neurodegeneration, and cancer, alterations in intracellular signalling related to PDE deregulation may explain the difficulties observed in the prevention and treatment of these pathologies.

By inhibiting specifically the up-regulated PDE isozyme (s) with newly synthesized potent and isozyme-selective PDE inhibitors, it may be potentially possible to restore normal intracellular signalling selectively, providing therapy with reduced adverse effects. The past decades have witnessed a rapid rise in the prevalence of diabetes. The fact that there is a shift in age of onset to younger age groups is alarming, hence the early identification of at risk individuals and appropriate intervention could greatly help to prevent, or at least delay, the onset of diabetes. The selective targeting of  $\beta$ -cell cAMP is most likely to be achieved through the activation of GLP-1 or GIP receptors on the  $\beta$ -cell, as the main actions of these hormones are mediated through cAMP.

PDE inhibitors may be of value in preventing  $\beta$ -cell loss in both type 1 and type 2 diabetes. Earlier studies have shown pancreatic islets or  $\beta$ -cells to

contain PDE1C, PDE3B and PDE4, along with PDE10A. Several reports suggest 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<sup>12</sup>. There are several PDE3 inhibitors available for clinical use, such as cilostamide, cilostazol, milrinone, and amrinone. Future studies should therefore focus on development of whether PDE3B inhibitors induce long term up regulation of PDE3B expression to ultimately blunt the effects of these inhibitors on insulin secretion and also to establish whether alteration in PDE3B gene expression can modulate insulin secretion or glucose tolerance in models of Type 2 diabetes. Cytokines (Interleukin 1 (IL-1), IFN, and TNF), appear to be important mediators of islet  $\beta$ -cell dysfunction and destruction<sup>15, 16</sup>. Though the molecular mechanisms of toxicity are not well understood it has been suggested that cytokines augment inducible nitric oxide synthase (iNOS) expression leading to abnormal nitric oxide (NO) production which might have contributed to islet cell damage<sup>17</sup>. NO inactivates important enzymes in cells by nitrosylation of target iron sulfur proteins, e.g., mitochondrial aconitase, required for glucose oxidation and insulin release which impairs cell metabolism, diminishes the capacity of insulin secretion, and may eventually cause cell death<sup>18, 19</sup>.

Beshay et al. demonstrated that the general phosphodiesterase (PDE) inhibitor pentoxifylline (PTX), PDE III inhibitor cilostamide (CIL) and PDE type IV inhibitor rolipram (ROL), increase intracellular cAMP levels and suppress inflammatory cytokine release partly by blocking nitric oxide (NO) production<sup>20</sup>. Further it has been suggested that the PDE inhibitors may present a novel therapeutic approach for the treatment of diabetes and other autoimmune and/or inflammatory diseases<sup>21-24</sup>. Moreover, comparative studies on PDEs would be very important to evaluate the involvement of each PDE in specific cellular function and to understand regulation of cyclic nucleotide signalling. Establishment of a standardized platform for PDE research is necessary to interpret further intriguing observations and to validate them as targets for drugs to treat and prevent diabetes.

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