

# Purinergic signalling and diabetes

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**Abstract** The pancreas is an organ with a central role in nutrient breakdown, nutrient sensing and release of hormones regulating whole body nutrient homeostasis. In diabetes mellitus, the balance is broken—cells can be starving in the midst of plenty. There are indications that the incidence of diabetes type 1 and 2, and possibly pancreatogenic diabetes, is rising globally. Events leading to insulin secretion and action are complex, but there is emerging evidence that intracellular nucleotides and nucleotides are not only important as intracellular energy molecules but also as extracellular signalling molecules in purinergic signalling cascades. This signalling takes place at the level of the pancreas, where the close apposition of various cells—endocrine, exocrine, stromal and immune cells—contributes to the integrated function. Following an introduction to diabetes, the pancreas and purinergic signalling, we will focus on the role of purinergic signalling and its changes associated with diabetes in the pancreas and selected tissues/organ systems affected by hyperglycaemia and other stress molecules of diabetes. Since this is the first review of this kind, a comprehensive historical angle is taken, and common and divergent roles of receptors

for nucleotides and nucleosides in different organ systems will be given. This integrated picture will aid our understanding of the challenges of the potential and currently used drugs targeted to specific organ/cells or disorders associated with diabetes.

**Keywords** Adenosine 5'-triphosphate ·  $\beta$  cells · Insulin · Obesity · Nephropathy · Pain · Exocrine pancreas · Cardiovascular disease

## Introduction

### Diabetes

The incidence of diabetes mellitus (types 1 and 2) is globally rising, and its appearance is shifting towards a younger age group. Type 1 diabetes (T1D), or insulin-dependent diabetes mellitus, is an autoimmune disease that precipitates in genetically predisposed individuals by environmental factors, predominantly viral infections. As a result, pancreatic  $\beta$ -cell mass and function deteriorate and patients become dependent on exogenous insulin [1]. In type 2 diabetes (T2D), insulin secretion may be close to normal, at least in the beginning, but target tissues may be resistant to insulin. With progression of the disease, metabolic stress factors and cytokines, such as interleukin IL-1 $\beta$ , contribute to decrease in  $\beta$ -cell mass and function [2, 3]. T2D is usually a later onset disease; it is often associated with obesity and a low-grade inflammation of adipose tissue and auto-inflammation in islets, and subsequently altered adipokines profiles may in part contribute to an induction of hepatic and skeletal muscle insulin resistance [4, 5].

Another, less well recognised, form of diabetes mellitus is due to exocrine pancreatic dysfunction; the disease accounts for about 10% of diabetic patients, but prevalence may depend on region/population. This pancreatogenic diabetes, referred to as type 3c diabetes, occurs due to inherited

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or acquired pancreatic disease and has a unique pattern of hormonal and metabolic characteristics and diagnosis may be difficult [6–9]. Links between concurrent exocrine and endocrine pancreatic disease and contributing factors are poorly characterised. Usually, the exocrine pathology is explained as a result of local insulin deficiency or neuropathological changes in pancreas as a result of diabetes or that autoimmune disease could involve both endocrine and exocrine parts of the gland. The close morphological association between exocrine cells, especially ducts, and islets and release of various cytokines from exocrine cells suggests that the exocrine–endocrine axis is important [10–12]. Recent studies also show that genetic mutation coding for the acinar digestive enzyme, carboxyl ester lipase, leads to diabetes [6]. It is proposed that pancreatogenic diabetes may be more common and frequently associated with inflammation and sub-clinical chronic pancreatitis [13, 14].

Generally, diabetic mellitus diseases have in common inadequate insulin-regulated glucose transport and metabolism in major target tissues—liver, skeletal muscle and adipose tissue. This results in high circulating levels of glucose, free fatty acids and pro-inflammatory cytokines that cause serious problems in many organs, such as cardiovascular diseases, neuropathy and pain, renal disease, disturbances in the urogenital and gastrointestinal system, skin healing problems and skeletal muscle weakness. Events leading to insulin production and action are complex, but there is emerging evidence that various components of purinergic signalling may be important regulatory factors, both at the level of the pancreas and at the level of the organs affected. Following the introduction to the pancreas and purinergic signalling, we will focus on purinergic signalling associated with diabetes in selected organ systems.

In order to study the basic mechanism of diabetes, many cell and animal models have been developed (see for reviews [15, 16]). Here, we mention a few common animal models, which have been of particular use in studying the complexity of organ defects in purinergic signalling. Streptozotocin (STZ)-induced diabetes in rats has been widely used [17], but has been questioned as a valuable model for some aspects of diabetes in man. Other animal models include alloxan-induced diabetes [18, 19], Bio Breeder diabetic rats (BBD) [20], non-obese diabetic (NOD) mice [21] and the murine model of T1D, the RIP-I/hIFN $\beta$  transgenic mouse treated with very low doses of STZ [22]. For T2D diabetes, leptin-deficient or leptin-resistant mice (ob/ob and db/db) and Zucker diabetic fatty rats (ZDF) [23] are the most common models.

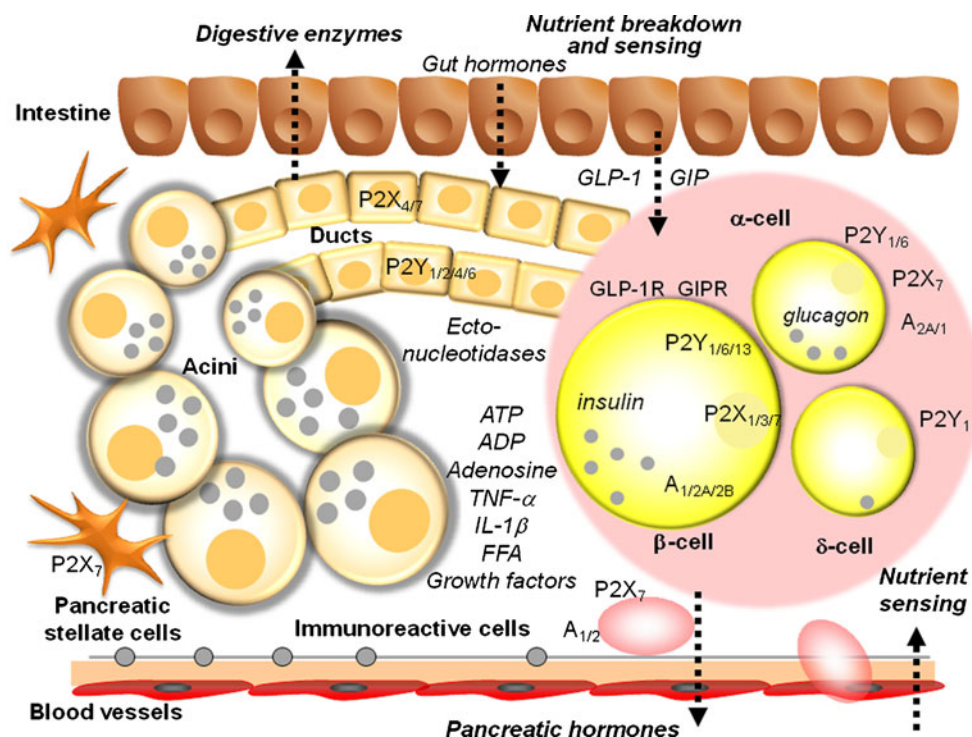
In diabetes mellitus, the basic cellular defects in metabolism lead to altered intracellular nucleotide levels. The next section will show that nucleotides have also important regulatory roles on the outside of the cell.

## Purinergic signalling

The purinergic system is a signalling system, where the purine nucleotides, ATP and ADP, and the nucleoside, adenosine, act as extracellular messengers. This concept, which was first proposed 40 years ago [24], met with considerable resistance for many years, largely because ATP had been established as an intracellular energy source involved in various metabolic cycles, and it was thought that such a ubiquitous molecule was unlikely to be involved in selective extracellular signalling. However, ATP was one of the first molecules to appear in biological evolution so that it is not really surprising that it should have been utilised early for extracellular, as well as intracellular, purposes. The existence of potent extracellular enzymes that regulate the amount of ATP and adenosine available for signalling also provides support that ATP has extracellular actions [25]. Implicit in purinergic signalling is the presence of receptors for ATP. A basis for distinguishing adenosine receptors (P1), from ATP/ADP receptors (P2), was proposed in 1978 [26]. The turning point in acceptance of the concept of purinergic signalling was in the early 1990 s, when receptor subtypes were cloned and characterised. Four subtypes of P1 receptors have been cloned, namely, A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub>. A<sub>1</sub> and A<sub>3</sub> receptors preferentially couple to G<sub>i</sub> proteins and inhibit adenylate cyclase, while A<sub>2A</sub> and A<sub>2B</sub> couple to G<sub>s</sub> and G<sub>o</sub> and stimulate production of cyclic AMP (cAMP). P2 receptors belong to two families based on molecular structure and second messenger systems, namely P2X ionotropic ligand-gated ion channel receptors and P2Y metabotropic G protein-coupled receptors [27, 28]. There are currently seven subtypes of P2X receptors and eight subtypes of P2Y receptors identified and characterised in mammals [29]. Most P2Y receptors couple to G<sub>q</sub>/G<sub>11</sub> proteins and thus activate PLC- $\beta$ , except for P2Y<sub>12</sub>, P2Y<sub>13</sub> and P2Y<sub>14</sub> that couple to G<sub>i</sub> proteins and inhibit adenylate cyclase, and P2Y<sub>11</sub> couples to G<sub>s</sub> and G<sub>q</sub> [30]. Functional purinoceptors are expressed by neurons and most non-neuronal cells (see [31]), including various cells in the pancreas. Two of the agonist-bound receptors, A<sub>2A</sub> and P2X<sub>4</sub>, have been crystallised [32, 33]. Extracellular nucleotide and nucleoside concentrations are regulated by a large number of ecto-nucleotidases, and some of those have also been crystallised [34].

## Purinergic signalling in healthy pancreas

The pancreas is an organ with a central role in nutrient breakdown, nutrient sensing and release of hormones regulating whole body nutrient homeostasis. The close apposition of various cells types, and indications that there is an interrelation between endocrine and exocrine diseases, warrants analysis of the integrated picture and the possibility that purinergic signalling may play a coordinating role (Fig. 1). There have been



**Fig. 1** Integrated function of pancreas in nutrient breakdown, nutrient sensing and release of pancreatic hormones. Purinergic signalling plays significant roles in physiological responses as well as in diabetes. Distribution of key receptors in pancreatic cells is shown and also locally produced and blood-borne factors that could affect insulin release and/or  $\beta$ -cell viability. Pancreatic acini secrete digestive enzymes and ATP. Pancreatic ducts express receptors that are involved in

regulation of bicarbonate-rich fluid secretion. Both exocrine cells can contribute to the interstitial milieu in the form of nucleotides/nucleosides or secreted cytokines.  $\beta$  Cells secrete insulin and ATP and purinergic receptors stimulate or inhibit insulin secretion, while others regulate cell viability. In addition,  $\alpha$  cells express receptors that regulate glucagon secretion. Figure also shows GLP-1 and GIP that regulate both insulin secretion and  $\beta$ -cell mass. For details, see the text

a number of reviews on the role of purinergic signalling in both endocrine and exocrine pancreas [35–39]. Here, we include only a brief overview, with the latest updates.

There are numerous sources of nucleotides/side within the pancreas. ATP/ADP is most likely a cotransmitter in both sympathetic and parasympathetic nerves supplying the pancreas and nucleotides are stored and released from hormone-containing vesicles from endocrine cells [40]. In addition, ATP is released from enzyme-containing zymogen granules from acini, where it is accumulated by the vesicular nucleotide transporter VNUT [41]. Apart from vesicle/granule exocytosis, there may be other mechanisms for ATP release, and this is highly debated and currently an active research field [42]. A number of early biochemical studies have shown that the pancreas expresses several types of ecto-nucleotidases enabling conversion of ATP/ADP to adenosine. In pancreatic islets, ATP pyrophosphatase, alkaline phosphatase, ecto-5'-nucleotidase as well as NTPDase-3 were found [43]. In exocrine pancreas, NTPDase-1 and ecto-5'-nucleotidase were found in acini, in particular in zymogen granules, and these were secreted into pancreatic juice in a particular form (microvesicles). In addition, there are also ATP-generating enzymes, adenylate kinase and nucleoside diphosphate

kinase, found in pancreatic juice [44]. NTPDase-1 and NTPDase-2 are also expressed in duct cells, as well as on blood vessels where the ATP/ADPase activity was strongest. For the latest review on the cellular and molecular action of ecto-nucleotidases, see Zimmermann et al. [34].

It has been shown that human  $\beta$  cells express P2X<sub>3</sub>, P2X<sub>5</sub>, P2X<sub>7</sub> and P2Y<sub>11</sub> and P2Y<sub>12</sub> receptors. A large number of studies on rodent pancreas, islet and cell preparations and  $\beta$ -cell lines revealed that these express the following P2 receptor subtypes: P2X<sub>1</sub>–P2X<sub>7</sub>, PY<sub>1</sub>, P2Y<sub>2</sub>, P2Y<sub>4</sub> and P2Y<sub>6</sub>, P2Y<sub>11</sub>–P2Y<sub>13</sub>; although the functions of some are not known and there are likely to be differences between species. In general, some P2 receptors mediate stimulation of insulin release already in non-stimulating glucose concentrations, while others may mediate potentiation of glucose-induced insulin secretion (see [38, 39]). Furthermore, some P2 receptor subtypes (e.g. mP2Y<sub>13</sub>, rP2X<sub>3</sub>) have inhibitory effects on insulin secretion, probably by stimulating different signalling pathways. Regarding adenosine receptors, earlier studies established that  $\beta$  cells also express A<sub>1</sub> receptors that mediate inhibition of insulin secretion (see [39]). Recent studies indicate that A<sub>2B</sub> receptors might also be expressed on  $\beta$  cells where they mediate inhibition of insulin secretion directly [45] or via the

immune system [46]. Another study also implicates  $A_{2A}$  receptors, which mediate increase in  $\beta$ -cell proliferation [47]. Together, activation of P1 and P2 receptors could exert pulsatile and synchronising effects on secretion of insulin and glucagon and thus contribute to balanced blood glucose regulation [35, 37, 48]. Recent studies also show that P2 receptors may mediate regulation of  $\beta$ -cell mass, which would be highly relevant to diabetes and is discussed below.

In contrast to  $\beta$  cells, there is less information about purinergic regulation of other islet cells.  $\alpha$  Cells express  $A_{2A}$  receptors, which mediate stimulation of glucagon release, while  $A_1$  receptors mediate inhibition. The  $P2Y_6$  receptor also mediates stimulation of glucagon release, while the  $P2Y_1$  receptor mediates inhibition. ADP analogues stimulate somatostatin secretion from  $\delta$  cells most likely via the  $P2Y_1$  receptor.  $P2X_7$  receptors have been immunolocalised on  $\alpha$  cells [49].  $\delta$  Cells express  $P2Y_1$  receptors [50].

In exocrine pancreas, acini are a rich site of ATP release, and they also express and release ectonucleotide/side breakdown enzymes (see above). Rodent acini contain transcripts for  $P2Y_2$ ,  $P2Y_4$ ,  $P2X_1$  and  $P2X_4$  receptors, but their functionality is low, perhaps indicating a protective mechanism curbing release of digestive enzymes. Secreted luminal ATP, and adenosine, and also ATP released from nerve endings and duct cells can regulate pancreatic duct function, which is secretion of  $HCO_3^-$  rich fluid that together with acinar enzymes contributes to digestive processes within the small intestine. Pancreatic ducts from rodents and human cell lines express  $P2Y_1$ ,  $P2Y_2$ ,  $P2Y_4$ ,  $P2Y_6$  and  $P2Y_{11-14}$ , and  $P2X_1$ ,  $P2X_2$  and  $P2X_4$ – $P2X_7$  receptors. Functional studies show that luminal  $P2Y$  receptors mediate stimulation of mucin secretion and  $HCO_3^-/H^+$  transport, and in particular, they stimulate fluid secretion by activating  $Cl^-$  channels (TMEM16A and CFTR), as well as  $K^+$  channels (IK,  $K_{Ca3.1}$ ), which potentiate the secretory effect [51].  $P2X_7$  receptors also mediate regulation of pancreatic secretion. On the basolateral membrane some receptors, e.g.  $P2Y_2$ , mediate down-regulation of secretion, probably a safety mechanism in over-distended duct. A recent study on the human adenocarcinoma cell line, PANC-1, shows that  $P2Y_1$  and  $P2Y_6$  receptors mediate increase in proliferation [52]. Pancreatic ducts also express  $A_{2A}$  and  $A_{2B}$  receptors that mediate stimulation of CFTR  $Cl^-$  channels and therefore secretion.

Pancreatic stellate cells (PSCs) are thought to be important in inflammation and fibrosis, and their role in pancreas cancer and chronic pancreatitis has been well studied, but their role in diabetes is less clear. At the messenger RNA (mRNA) level, PSCs express  $P2Y_1$ ,  $P2Y_2$ ,  $P2Y_6$ ,  $P2X_1$ ,  $P2X_4$  and  $P2X_6$  receptors. Micromolar concentrations of ATP stimulate nuclear  $Ca^{2+}$  signals, which may stimulate proliferation of this cell type [53]. A recent study shows that the  $P2X_7$  receptor on PSC also can stimulate proliferation of these cells but also cell death, depending on the ATP concentrations [54].

## Purinergic signalling in diabetes

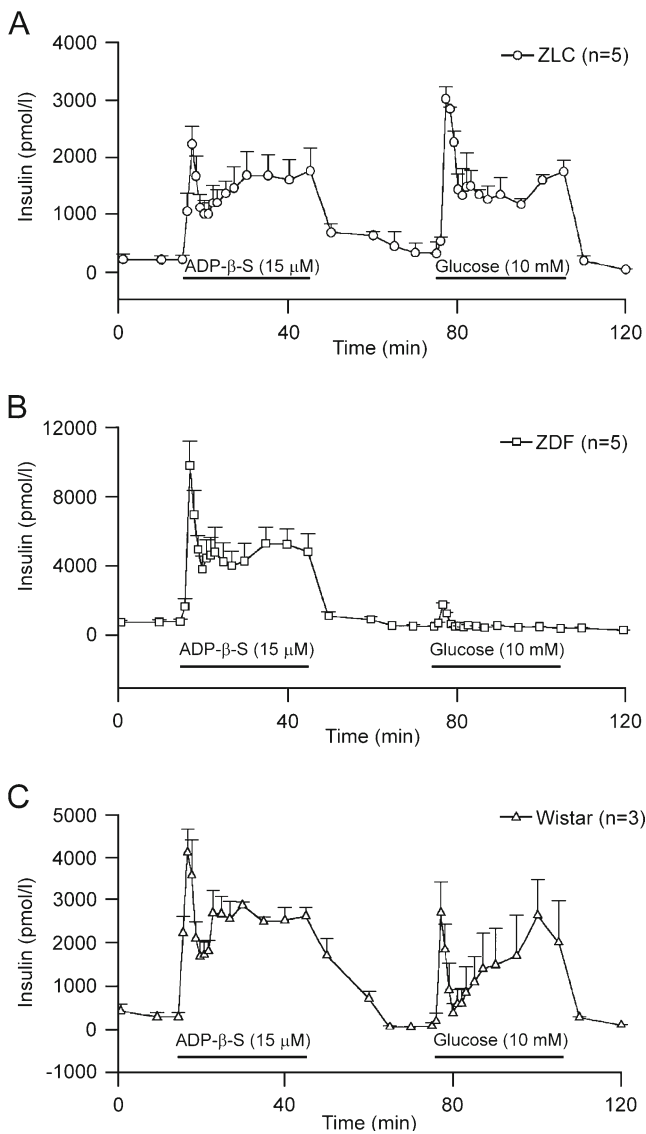
### Pancreas

Let us start with intracellular events in  $\beta$  cells, where the primary defect in intracellular signalling, membrane transporters, metabolic processes or regulation thereof, may lead to a decrease in insulin secretion and, in the worst case, cell death. In normal  $\beta$  cells, glucose entry via GLUT (GLUT2 in rodents, GLUT1/3 in humans) and its metabolism leads to the production of ATP, which closes ATP sensitive  $K^+$  channels ( $K_{ATP}$ ), and this in turn leads to depolarisation of the plasma membrane, influx of  $Ca^{2+}$  and a chain of signalling events that culminate in exocytosis of insulin (and ATP). Two incretins, GIP and GLP-1, potentiate insulin secretion and synthesis, as well as preserving  $\beta$ -cell mass [55]. In normal pancreatic  $\beta$  cells, glucose also stimulates polyphosphoinositide (PPI) hydrolysis via activation of a phosphoinositide-specific phospholipase C. In STZ-injected neonatal rats, glucose-induced PPI hydrolysis was severely diminished and was associated with reduced insulin-secreting responses to glucose [56]. A further cytotoxic effect of STZ on  $\beta$  cells may be due to a reduction in the intracellular level of ATP and thus activation of  $K_{ATP}$ , hyperpolarisation of cell membrane and reduction of insulin release [57]. Nevertheless,  $K_{ATP}$  channel openers, such as diazoxide, can counteract chronic over-stimulation of  $\beta$  cells and have been proposed to have beneficial effects in a subgroup of T2D [58]. Gain-of-function mutations in  $K_{ATP}$  channel subunits SUR1 or Kir6.2 can cause transient or permanent neonatal diabetes, for example, as demonstrated in a study of R826W mutation in SUR1 [59]. The intracellular ATP/ADP ratio is a coupling factor between glucose metabolism and insulin release [60]. Glycation end products, which are implicated in diabetic complications, inhibit cytochrome c oxidase and ATP production, resulting in impairment of glucose-stimulated insulin secretion [61].

In addition to intracellular functions, extracellular nucleotides/sides regulate pancreatic islet cells (Fig. 1), as well as a range of organs affected in diabetes. Evidence for the role of purinergic signalling in relation to diabetes was obtained in early experiments in the 1970–1980 s. Studies on a diabetic rat model using alloxan and dithizone [62, 63] showed that ATP was protective by reducing blood sugar levels. Infusion of ATP into the carotid artery increased the sensitivity of alloxan-diabetic rats to glucose, suggesting that a possible cause of diabetes was a defect in purinergic innervation of the islet cells [64].

Two early studies on different animal models of T1D and T2D showed that, although the insulin response to glucose was lost, insulin secretion to P2 receptor agonists was preserved (Fig. 2). In the isolated perfused pancreas of STZ-rats, ADP $\beta$ S, a potent  $P2Y_1$  receptor agonist induced insulin





**Fig. 2** Perfusion of isolated rat pancreas with ADP $\beta$ S and glucose. **a** Pancreata isolated from Zucker lean controls (ZLC) were perfused for 30 min with perfusate containing 5 mM glucose (first 15 min not shown). ADP $\beta$ S (15  $\mu$ M) was applied into perfusate as shown by horizontal bar. ADP $\beta$ S produced a biphasic insulin release. Raising glucose to 10 mM produced a biphasic insulin secretion. **b** Pancreata isolated from Zucker diabetic fatty (ZDF) rats were perfused using the same protocol as in **a**. ADP $\beta$ S (15  $\mu$ M) caused a much larger biphasic insulin secretion while glucose induced a small and transient insulin release. **c** Perfusion of isolated rat pancreas from Wistar rats with ADP $\beta$ S and glucose. (Reproduced from [66], with permission)

release that was similar to that in control rats, and this was independent of glucose concentration 5–28 mM [65, 66]. In ZDF rats, increases in  $[Ca^{2+}]_i$  and insulin secretion by pancreatic  $\beta$  cells were preserved and mediated by P2Y receptors, again with ADP $\beta$ S being most potent [66]. Consequently, a number of studies focussed on developing stable P2Y<sub>1</sub> receptor agonists as potential insulin secretagogues (e.g. 2-methylthio ATP- $\alpha$ - $\beta$ , A isomer and stable dinucleoside

polyphosphate analogues); however, a vasodilatory effect was a risk factor [67–70]. Also using a mouse model, another pattern regarding the P2Y<sub>1</sub> receptor appears. In islets isolated from P2Y<sub>1</sub><sup>-/-</sup> mice, insulin secretion was significantly increased at high stimulating glucose concentrations compared to wild type [71].

In a histochemical study on STZ-diabetic rats, the following picture was revealed. In pancreatic islets, P2Y<sub>1</sub> receptors were present in intra-islet capillaries, while P2X<sub>4</sub> receptors were present on  $\beta$  and  $\delta$  cells. Pancreatic duct cells still expressed P2Y<sub>1</sub> and P2Y<sub>2</sub> receptors, while P2X<sub>1</sub>, P2X<sub>3</sub>, P2Y<sub>1</sub> and P2Y<sub>2</sub> receptors were expressed in small pancreatic blood vessels [72].  $\alpha$  Cells expressed P2X<sub>7</sub> receptors in healthy pancreas on the periphery of islets. These P2X<sub>7</sub>-immunoreactive cells migrated to the centres of islets to replace the lost  $\beta$  cells in both STZ-diabetic rats and NOD mice [72, 73]. In another study, P2X<sub>7</sub> receptors were also claimed to be expressed  $\beta$  cells; P2X<sub>7</sub> receptors were down-regulated in T2D, but up-regulated in human obesity [74]. In human islets, the receptor seems to be involved in secretion of insulin and the IL-1 receptor antagonist IL-1Ra.

Regarding adenosine receptors, older studies showed that STZ diabetes suppressed the stimulatory action of adenosine on glucagon secretion from pancreatic  $\alpha$  cells and reduced vasodilation of the vascular bed [75, 76], via A<sub>2</sub> receptors [77]. Several studies showed that a nonspecific adenosine receptor agonist, adenosine-5'-N-ethylcarboxamide (NECA), decreased insulin secretion and increased blood glucose and decreased glucose uptake. Studies on A<sub>1</sub><sup>-/-</sup> mice showed increased insulin and glucagon secretion [78]. A recent study on A<sub>1</sub> receptor knockout mice showed that fasting glucose and insulin secretion were significantly higher, but insulin sensitivity was impaired as reflected by reduced glucose uptake in muscles and adipose tissue [79]. However, the effects could not simply be explained by an A<sub>1</sub> receptor effect on insulin secretion of  $\beta$  cells. Several studies with non-selective adenosine receptor agonists showed the following. NECA decreased blood glucose in STZ-diabetic and cyclophosphamide-treated NOD mice; it increased pancreas insulin content and suppressed expression of pancreatic proinflammatory cytokines (TNF- $\alpha$ , MIP-1 $\alpha$ , IL-12 and INF- $\gamma$ ) and in immunoreactive cells [80]. This effect was most likely mediated via the A<sub>2B</sub> receptor, although the authors proposed that this was not at the level of the pancreas. In other studies, infusion of specific A<sub>2B</sub> receptor blockers increased insulin secretion in INS-1 cells and also in T2D model of Goto-Kakizaki rats, indicating that the effect could be at the pancreas level [45]. Furthermore, a study on a mouse model for T2D suggested that the increased expression of A<sub>2B</sub> receptors on endothelial cells and macrophages enhances production of IL-6 and that this results in stimulation of an inflammatory response and insulin resistance in skeletal muscle, adipose tissue and liver; effects on pancreas were also considered [46].

A key factor in the pathogenesis of diabetes is the pancreatic  $\beta$ -cell mass (especially T1D). In order to understand  $\beta$ -cell function and survival at the integrative level, exploration of the mechanisms of purinergic signalling together with incretins and inflammatory signals will be necessary. Here, are some trends in these directions. Pro- and anti-inflammatory cytokines, originating from various local or invading cells or from other organs, can influence proliferation and apoptosis of  $\beta$  cells [81]. A number of purinergic receptors have similar abilities to mediate cell proliferation and apoptosis and activities via the P2X<sub>7</sub> receptor may be able to support both functions [82, 83]. The P2X<sub>7</sub> receptor and cytokine systems can be related. For example, in macrophages, the P2X<sub>7</sub> receptor is involved IL-1 $\beta$  secretion, and in T cells, it caused MHC-I shedding and extravasation [82]. The two incretins, GLP-1 and GIP, augment insulin secretion, but also have proliferative and anti-apoptotic effects on  $\beta$ -cell mass [55].

Recently, there have been studies addressing the question of purinergic signalling and  $\beta$ -cell survival, and the summary of data is depicted in Fig. 3. P2Y<sub>6</sub> receptor agonists not only increase insulin secretion in MIN6 mouse  $\beta$  cells, but they also prevent  $\beta$ -cell death induced by tumour necrosis factor- $\alpha$  [84]. In contrast, activation of the P2Y<sub>13</sub> receptor of the mouse pancreatic insulinoma cell line, MIN6C4, has pro-apoptotic effects [85]. Furthermore, high glucose and free fatty acids induce  $\beta$ -cell apoptosis via autocrine ADP action on the P2Y<sub>13</sub> receptor [86]. Extracellular ATP (1  $\mu$ M) increased insulin secretion in mouse  $\beta$ -cell lines, but at higher ATP concentrations, cell viability decreased and P2Y<sub>1</sub> and P2X<sub>4</sub> receptors were implicated [87]. The P2X<sub>7</sub> receptor knockout mice had lower  $\beta$ -cell mass, impaired glucose tolerance and defective insulin and

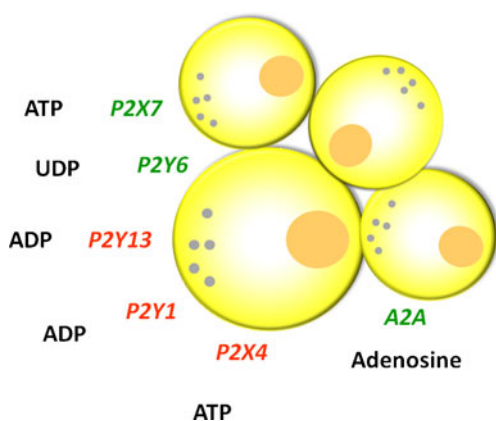
interleukin secretion [74]. Recent studies address the question of adenosine in  $\beta$ -cell mass using screening assays. Using a zebra fish model of diabetes, it was found that the non-specific adenosine agonist, NECA, increased proliferation of  $\beta$  cells but not other endocrine cells, and the data suggest that this was via A<sub>2Aa</sub> receptors. In STZ-diabetic mice, NECA also increased the number of  $\beta$  cells and improved glucose control [47]. In another screening study, it was found that adenosine kinase inhibitors increased rodent and porcine  $\beta$ -cell replication. It was proposed that the nuclear enzyme regulates adenosine levels and the mTOR cell proliferation pathway [88].

Earlier reviews describing the roles of purinergic signalling in insulin secretion and diabetes in relation to the pancreas are available [89–91]. Below, we will review evidence for the role of purinergic signalling in various organs affected by hyperglycaemia in diabetes and indicate whether any of those can be potential targets for organ-specific treatments in diabetes.

### Cardiovascular system

Problems associated with diabetes and the cardiovascular system are many and include hypertension, atherosclerosis, cardiac disease, microvascular pathology in several organs and disturbances in blood cells. In particular adenosine receptors, but also P2 receptors, nucleotide/side converting enzymes and transporters, are affected in the diabetic vascular system; effects vary depending on the organ and local regulatory system. In general, in a healthy vessel, there is P2X receptor-mediated vasoconstriction and P2Y receptor-mediated vasodilation via stimulation of nitric oxide (NO) synthase and NO release from endothelial cells. On endothelial cells, A<sub>1</sub> receptors also mediate stimulation of NO release in some vessels. In the heart, adenosine is cytoprotective, and it slows sinoatrial and atrioventricular conduction, resulting in decreased heart rate, coronary vasodilatation, and it attenuates the functional and metabolic effects of  $\beta$ -adrenergic receptor stimulation, and in particular it has significant effects on glucose and fatty acid metabolism [92, 93]. Thus, adenosine helps to restore the balance in myocardial O<sub>2</sub> supply–demand, and there is evidence that all four adenosine receptor subtypes expressed in various cells in the heart exert cardioprotective effects [92]. In the following paragraphs, we will review the original studies that support the notion that purinergic signalling is involved in the diabetic cardiovascular system.

Both microvascular pathology and sympathetic denervation are present in alloxan-induced diabetes in rats [94]. Twelve weeks after induction of STZ diabetes, there was prejunctional impairment of sympathetic transmission via P1 receptors and impaired endothelium-mediated vasodilation by ATP of the rat mesenteric arterial bed [95]. In contrast, at



**Fig. 3** Purinergic receptors have effects on  $\beta$ -cell mass. Receptors marked in *green* increase  $\beta$ -cell mass (proliferation/replication), while those marked in *red* mediate  $\beta$ -cell death (apoptosis). Some purinergic receptors exert cytoprotective actions when cells are exposed to other factors, e.g. cytokines. The effects of P1 and P2 receptor stimulation on cell viability and/or insulin release may be dependent on concentrations of nucleotides/sides. For details, see the text

8 weeks STZ diabetes, the functions were unimpaired, although sensory-motor nerve-mediated vasodilation was attenuated [96]. Enhanced ATP-induced contraction of mesenteric arteries from diabetic Goto-Kakizaki rats at the chronic stage of diabetes was shown to be due to increased cPLA<sub>2</sub>/COX pathway activity in smooth muscle [97]. It was shown further that the angiotensin II type 1 antagonist, losartan, normalises the P2Y receptor-mediated contraction. P2Y receptor-mediated insulin stimulating responses of  $\beta$  cells and of the pancreas vascular bed were preserved in STZ-diabetic rat pancreas [65]. In the tail artery of STZ-diabetic rats, there is an increased neurotransmitter role for ATP compared to its cotransmitter noradrenaline (NA) in sympathetic nerves and an increased potency of ATP via P2X receptors [98].

The sensitivity of platelet aggregation by ADP is increased in diabetic patients, and this may contribute to microangiopathy [99]. Platelets of T2D patients were characterised by high ATP content [100]. The activity of both NTPDase and 5'-nucleotidase of platelets (and synaptosomes) showed increased activity in alloxan-induced diabetes [101, 102]. Adenosine deaminase and 5'-nucleotidase activities were higher in platelets in diabetic patients than control subjects [103]. In erythrocytes, ATP concentration is influenced by insulin levels in plasma [104], but there is impairment of ATP release from human erythrocytes in T2D, which may contribute to the vascular disease [105]. Interestingly, in blood serum of STZ-treated rats, nucleotide hydrolysis rates were increased, but these could return to control in rats subjected to physical training [106].

In human subjects with T2D, the vasodilator actions of ATP, UTP and adenosine in skeletal muscle were diminished by 50% compared to controls, and this effect was most likely due to altered receptor sensitivity [107]. A similar conclusion was reached in a study of another vascular bed; soon after the onset of alloxan-induced diabetes in rabbits, retinal blood flow velocity decreased following ATP or 2'(3')-O-(4-benzoylbenzoyl) adenosine 5'-triphosphate infusion and P2X<sub>7</sub> receptors were implicated [108]. However, vasodilator effects of adenosine on retinal arterioles were preserved in STZ-diabetic rats [109].

Adenosine receptors are major regulators of vascular beds in many organs. Adenosine and AMP enhanced the NO synthase response to inflammatory cytokines in diabetic vascular smooth muscle cells from rat aorta [110]. Relaxation of the rat aorta by adenosine via A<sub>2</sub> receptors and endothelial release of endothelium-derived relaxing factor (EDRF) was attenuated in the STZ-diabetic rat [111], although an earlier study found similar release of EDRF in response to cholinergic stimulation, but enhanced release of oxygen free radicals in diabetic preparations [112]. It has been proposed that foetal endothelial dysfunction in gestational diabetes mellitus involves a functional link between adenosine and insulin signalling [113]. Adenosine inhibited

the enhanced growth of aortic smooth muscle cells in STZ-diabetic rats [114].

In the heart, adenosine protects the myocardium against ischaemic and reperfusion injury; it has negative inotropic and chronotropic effects and attenuates proliferation of fibroblasts. Diabetic patients have ventricular hypertrophy and reduced tolerance to stress. Atria from 6 week STZ-diabetic rats exhibited supersensitivity to the negative inotropic and chronotropic effect of adenosine, postulated to be due to impairment of the adenosine uptake mechanism on plasma membranes [115]. Also in diabetic rat cardiac fibroblasts, altered expression of nucleoside transporters was detected, and this was proposed to lead to increased uptake but decreased release of adenosine [116].

Myocardial over-expression of adenine nucleotide translocase 1 on inner mitochondrial membranes accelerates mitochondrial ATP/ADP exchange and ameliorates diabetic cardiomyopathy in mice, a promising target for diabetic cardiomyopathy [117]. Diabetic cardiomyocytes from rats treated with losartan maintained the capacity to respond to ATP depletion leading to contractile failure [118]. STZ diabetes in rats resulted in an increase in A<sub>1</sub> and A<sub>3</sub> receptor protein levels in cardiac myocytes, while A<sub>2A</sub> receptor protein expression remained unchanged [119]. Heart rate response to adenosine infusion (increase due to A<sub>2</sub> receptors) is diminished in patients with diabetes mellitus, probably due to cardiovascular autonomic neuropathy [120, 121]. In accordance, a preliminary study showed that ATP and ADP hydrolysis was decreased in cardiac synaptosomes of STZ-rats, and this effect was reversed with insulin treatment [122].

One of the receptors linked to many diseases is the P2X<sub>7</sub> receptor. It was hypothesised that P2X<sub>7</sub> receptors participate in the pathogenesis of vascular complications in diabetes, based on experiments showing that high glucose concentrations triggered the assembly of P2X<sub>7</sub> receptors and apoptosis in skin fibroblasts that share some of the features with smooth muscle cells [123]. Accordingly, human fibroblasts from diabetic patients had enhanced P2X<sub>7</sub> receptor activity [124]. Nevertheless, another paper from this group also showed that P2Y receptor-dependent GLUT1 activation was defective in fibroblasts from the T2D patients [125].

Gestational diabetes is first recognised in pregnancy and is associated with abnormal foetal development and perinatal complications. There is a low capacity of adenosine transport by the foetal endothelium of umbilical vein in gestational diabetes leading to accumulation of extracellular adenosine and its action on endothelial A<sub>2A</sub> receptors [126–128]) and insulin reversed these effects [129].

#### Nervous system

Diabetic neuropathy, characterised by nerve fibre atrophy and loss, was recognised early (for reviews, see [130–133]).

In T2D animal models, there was early evidence for increased sympathetic activity in STZ-diabetic rats. Mice with spontaneous diabetes show changes of sympathetic function similar to those found in diabetic patients with autonomic nerve pathology [134]. In addition, sympathetic nerves in pancreatic islets are impaired in BBD rats [135]. STZ-diabetic mice at 7 weeks showed reduced cutaneous sensory innervation and reduced expression of P2X<sub>3</sub> receptors in footpad skin [136].

Painful diabetic neuropathy is a complication of diabetes; it causes hyperalgesia and allodynia [137]. Modulation of cutaneous polymodal receptors in diabetic rats by sympathetic nerves (which release ATP and NA as cotransmitters) has been reported [138]. Adenosine seems to be protective. An adenosine kinase inhibitor, which increases extracellular levels of adenosine, attenuates tactile allodynia in a rat model of diabetic neuropathic pain [139]. Allosteric enhancers for A<sub>1</sub> receptors are targets for neuropathic drug development [140]. Peripheral neuropathy, vascular disease and oedema are some of the factors responsible for impaired healing after trauma and infection. Adenosine receptor agonists have been proposed for promotion of dermal wound healing, particularly for diabetic foot ulcers [141]. In some neuropathic pain models, there is activation of dorsal horn microglia and P2 receptors (P2X<sub>4</sub> and P2Y<sub>12</sub>) [142]. In STZ-diabetic mice, the levels of P2X<sub>2</sub> and P2X<sub>3</sub> receptor mRNA were significantly increased in dorsal root ganglion, suggesting that the up-regulation of these receptors is associated with mechanical allodynia [143]. A substantial enhancement of P2X<sub>3</sub> receptor activity and an increase in expression of P2X<sub>3</sub> receptors was reported recently and claimed to contribute to the development of chronic pain in STZ-induced diabetic rats [144]. As another approach, the protective actions of adenosine were investigated in STZ-diabetic rat models of neuropathic pain. Using antagonists, it was concluded that analgesic actions of adenosine were exerted via A<sub>1</sub> receptors [145].

Regarding the effect on the brain, in alloxan-diabetic rats, there are abnormalities in activity but not expression of G proteins in the striatum [146]. Diabetic encephalopathy results in cognitive impairment and modification of hippocampal function. In STZ-diabetic rats, there was a decrease in ATP concentrations in cerebrospinal fluid, decrease in density of P2X<sub>3,5,7</sub> and P2Y<sub>2,6,11</sub> receptors in hippocampal nerve terminals, but an increase in P2X<sub>1,2,5,6,7</sub> and P2Y<sub>6</sub> (but not P2Y<sub>2</sub>) receptors in membranes of astrocytes/neurons, indicating changes neuro- and gliotransmission [147]. There is decreased adenosine uptake in hippocampus of STZ-diabetic rats. This can increase adenosine sensitivity of synaptic potentials [148] and accelerate ischaemic block of population spikes in hippocampal slices [149]. The balance between inhibitory A<sub>1</sub> and facilitatory A<sub>2A</sub> receptor activation was modified in the hippocampus of STZ-diabetic rats; A<sub>1</sub> receptors were down-

regulated, while A<sub>2A</sub> receptors were up-regulated [150]. In patients with diabetic neuropathy, there are abnormalities of Ca<sup>2+</sup>/Mg<sup>2+</sup> ATPase activity in erythrocytes, and the results were interpreted in favour of altered Ca<sup>2+</sup> homeostasis and microangiopathy playing a role in the pathogenesis of diabetic neuropathy [151].

#### Retina

Diabetic retinopathy, involving capillary abnormalities, is often seen in the early stages of diabetes (see [152]). The involvement of damaged sympathetic nerves (that release the cotransmitters NA and ATP) in the deterioration of capillaries and loss of ganglion cells was proposed [153]. High glucose alters the purinergic signalling system in the retina. Firstly, it increases the exocytotic release of ATP from cultured retinal cells and also decreases its extracellular degradation, both of which result in high levels of ATP [154]. Second, retinal neurons and microglia cultured in high glucose media augmented Ca<sup>2+</sup> responses to P2 receptor stimulation, which may increase release of neurotransmitters and inflammatory mediators and thus lead to the inflammation involved in the pathogenesis of diabetic retinopathy [155].

ATP and ADP but not adenosine stimulate phosphoinositide metabolism in endothelial cells from bovine retinal microvessels, and it was proposed that this may be involved in the pathophysiology of diabetic retinopathy [156]. In addition, the formation of P2X<sub>7</sub> receptor pores is enhanced in retinal microvessels early in the course of experimental diabetes [157]. Extracellular ATP induces cell death of retinal microvessels via P2X<sub>7</sub> receptors and voltage-activated Ca<sup>2+</sup> channels, and it was proposed that activation of P2Y<sub>4</sub> receptors triggers a series of events that prevents P2X<sub>7</sub> receptor-mediated pores and toxicity in retinal microvessels [108, 158]. Diabetic retinopathy is associated with macula oedema, which may be due to breakdown of blood–brain barrier and other effects, such as dysfunction of glial cell volume regulation. Activation of A<sub>1</sub> receptors restores cell volume regulation of glial cells in the diabetic rat retina [159].

#### Kidney

Glomerular hyperfiltration, hypertrophy and microvascular dysfunction are the leading hallmarks of early diabetes leading to progressive nephropathy and hypertension. Diabetic nephropathy leads to end-stage renal disease, morbidity and mortality. The kidney is an interface between vascular and epithelial systems, and both are regulated by P1 and P2 receptors. Regarding the vasculature, adenosine signalling has a prominent but complex role. In STZ-diabetic rats, adenosine enhanced vasoconstriction of the kidney vascular bed via A<sub>1</sub> receptors [160]. It was suggested that the increased vasoconstrictor effect of adenosine on the diabetic renal vasculature



was caused by defective NO-dependent renal vasodilation of the afferent arterioles [161]. A<sub>1</sub> receptor knockout mice developed diabetic-induced glomerular hyperfiltration, which suggested that the tubuloglomerular feedback (TGF) mechanism is not involved in the development of hyperfiltration [162]. However, in a later paper, it was shown that A<sub>1</sub> receptor knockout blunts glomerular hyperfiltration and the salt paradox in early STZ diabetes [163]. Furthermore, a study on an A<sub>1</sub> receptor over-expression mouse model shows that it is a crucial receptor for regulation of afferent arteriole tone [164]. The general scenario is that in response to salt load, macula densa cells release ATP, which can be hydrolyzed to adenosine, and A<sub>1</sub> (and P2X<sub>1</sub>) receptors on the afferent arteriole mediate TGF responses (see [165, 166]). The tubulo-centric hypothesis, which requires further support, states that increased glucose load and absorption in proximal tubule (as in diabetes mellitus) decreases electrolyte to macula densa and thus down-regulated TGF causes increased GFR and hyperfiltration. Vasodilatory effects are mediated via A<sub>2</sub> receptors, A<sub>2A</sub> on endothelial cells increase NO production; A<sub>2B</sub> receptors are expressed in podocytes. In addition, A<sub>2A</sub> receptors are also general anti-inflammatory receptors (see [167]).

There is evidence for altered adenosine level and adenosine receptor expression in diabetes. Reasoning that glomerular hyperfiltration in diabetes may be due to decreased vasoconstriction by adenosine, it was shown that inhibiting adenosine uptake by dipyridamole prevented early alterations in kidney function associated with diabetes [168]. In addition, administration of an adenosine analogue decreased diuresis and glycosuria in STZ-diabetic rats, although the non-specific adenosine receptor antagonist seemed to have different effects [169]. Agonists to adenosine receptors attenuated glucose and protein excretion in diabetic Wistar rat kidneys [170]. However, if adenosine levels are too high and low sensitivity A<sub>2</sub> receptors are activated, the effects may become different. There is a significant increase in levels of adenosine (and purine metabolites) in plasma of patients with diabetic nephropathy compared to T2D patients without nephropathy [171]. Glomeruli of diabetic rats accumulate six times more adenosine than control tissues, and this is due to decreased nucleoside uptake activity and increased AMP hydrolysis; a possible consequence is activation of A<sub>2B</sub> receptors that then cause release of transforming growth factor β1 (TGF-1β) that may contribute to glomerulopathy [172]. In contrast, A<sub>2A</sub> receptor activation attenuates inflammation, injury and diabetic nephropathy [173].

In STZ-diabetic rats, A<sub>1</sub> and A<sub>3</sub> receptor mRNA and protein increased in both kidney cortex and medulla; A<sub>2A</sub> receptor expression increased in the cortex, but not medulla; A<sub>2B</sub> receptor expression was unchanged; and immunohistochemistry showed receptor localisation mainly on renal tubules [174]. cAMP-mediated inhibition of distal phosphate transport may explain the observation that adenosine

enhances the antiphosphaturic effect in STZ diabetes [175]. In general, purinergic receptors on renal tubules exert negative regulator effects on electrolyte transport [176].

Adenosine receptors are being suggested as a therapeutic option for diabetic nephropathy [167, 172]. The vascular ectonucleotidase ENTPD-1 is a novel factor considered, as it prevents chronic microvascular injury, inflammation and thromboregulation, in STZ-mice [177]. For both approaches, differential effects of adenosine via A<sub>1</sub> vs A<sub>2B</sub> receptors should be considered.

#### Urinogenital system

A high incidence of bladder dysfunction has been reported in patients with diabetes mellitus; the symptoms may progress with time and range from an overactive bladder and hypercontractive detrusor to voiding problems with urinary retention and acontractile detrusor. It is not certain whether bladder dysfunction is secondary to neuropathology or bladder overdistension with smooth muscle and urothelial dysfunction. Again, animal studies have been useful. In STZ-induced diabetic rats, there is bladder hypertrophy and distension [178]. After 3 months, STZ-treated rats showed reduced contractile responses to nerve stimulation, but no change in sensitivity to acetylcholine and ATP [179]. Later, it was claimed that there was a reduction in the non-cholinergic contractile component of parasympathetic nerve stimulation in 12 weeks STZ-rats, probably caused by a reduction in release of the non-cholinergic transmitter [180]. It was proposed that cholinergic and purinergic parasympathetic nerve components of contraction were minimally affected by STZ treatment, but in M<sub>2</sub>-muscarinic knockout mouse bladder, STZ treatment reduced both the cholinergic and purinergic components [181]. There are conflicting reports about the changes in ATP-mediated neural responses in STZ-diabetic bladder. This appears to depend largely on the time course; it seems likely that there is an increase in the purinergic component in the early stages (1–8 weeks), but decreased responses after 8 weeks. Up-regulation of P2X<sub>1</sub> receptors was claimed in the early stages of STZ diabetes and down-regulation of P2X<sub>2</sub> receptors in the later stages [182, 183]. Similarly, there was increased expression of P2Y<sub>2</sub>, P2Y<sub>4</sub> and P2X<sub>4</sub> receptors in STZ-rats in 2–4 months, but not at 8 months [184]. Furthermore, 6 months after alloxan-induced diabetes in rabbits, there was enhancement of purinergic, but reduction of cholinergic neurotransmission to the detrusor muscle of the bladder [185]. The P2X<sub>3</sub> receptor is important for afferent pathways controlling urinary bladder volume reflexes [173], and this may be a candidate regulator. Impairment of the initiation of voiding reflexes via sensory nerve pathways activated by ATP released from urothelial cells in the bladder in diabetes has also been implicated [186–188]. In addition, there is

impaired ATP-induced release of prostaglandins from urothelial cells [189], and urothelium itself releases ATP [190]. Both ATP and NO are released from the urothelium in the bladder. In early diabetic bladders from STZ-treated rats showing overactivity and a ‘diuretic’ underactivity model, the release ratio of ATP and NO was correlated with bladder contraction frequency, being enhanced in overactive and diminished in underactive bladders [191]. It was suggested that the ATP/NO ratio could be used to monitor changes in bladder activity during drug therapy.

Adenosine, a direct vasodilator of corpus cavernosum, was recommended for the treatment of diabetic erectile impotence [192]. Adenosine-induced inhibition of sympathetic nerve-mediated contractile responses of mouse corpus cavernosum is impaired in T2D db/db mice [193]. It was suggested that the relaxant response of the corpus cavernosum to adenosine and ATP in both men and rats was largely endothelium-dependent via release of NO [194]. A functional study of purinergic signalling in the alloxan-diabetic rabbit corpus cavernosum led to the conclusion that relaxations mediated by both P2Y<sub>1</sub> receptors (via ADP and ATP) on endothelial cells and P2Y<sub>4</sub> receptors (via UTP and ATP) on cavernosal smooth muscle, were impaired [195]. Another paper showed that ATP can contract corpus cavernosum smooth muscle via P2X<sub>1</sub> receptors and relax via P2Y<sub>1</sub> and P2Y<sub>2</sub> receptors and that the P2Y receptor relaxant effects were significantly decreased in STZ-diabetic rats [196].

### Gastrointestinal system

Many gastrointestinal complications of diabetes seem to be related to dysfunction of the neurons supplying the enteric nervous system. Thus, delayed gastric emptying, abnormal motility, secretion or absorption could lead to some of the symptoms of pain, constipation, diarrhoea, irritable bowel, ulcers, etc. Non-adrenergic, non-cholinergic (NANC) relaxant responses of the gastric fundus were impaired in 8 and 12 week STZ-induced diabetic rats [197, 198]. However, in 8 week STZ-diabetic rats, there appeared to be an increase in the purinergic component of the vagal NANC responses of the gastric fundus to vagal nerve stimulation [199]. Another study described impairment of the nitric component of NANC nerve-mediated relaxations of the rat gastric fundus [200]. Moreover, impairment of the nitric-mediated relaxation of rat duodenum was described in 3–4 week STZ-rats; responses to the NO donor sodium nitroprusside were unchanged, but the response to ATP was enhanced [201]. It was suggested that reduction in NO synthase activity was associated with the impairment of NANC relaxation. Impairment of both purinergic and nitric components of NANC inhibitory neurotransmission was claimed in the gastrointestinal tract of T1D diabetic RIP-I/hIFN $\beta$  transgenic mice [22]. Electrophysiological responses to NA, but not to acetylcholine or ATP,

were potentiated in the caecum of 8 week STZ-diabetic rats, perhaps resulting from supersensitivity of  $\alpha$ -adrenoceptors after sympathetic nerve damage [202].

Fatty infiltrations of the liver are common in T2D patients and liver tests are abnormal, but it is not clear whether this is due to obesity. ATP administered to alloxan-diabetic rats lowered blood glucose, decreased liver fat, increased serum albumin and decreased  $\beta$ -globulin [203]. Development of STZ diabetes in rats resulted in a significant increase in expression of A<sub>2A</sub> and A<sub>3</sub> (but not A<sub>1</sub>) receptor mRNA levels and protein content in the rat liver, while expression of A<sub>2B</sub> was markedly decreased [204]. Adenosine induced hepatic glucose production, and this was inhibited by an A<sub>2B</sub> receptor antagonist that also reduced blood glucose in KK-A<sup>y</sup> T2D diabetic mice [205]. In another study on the liver of STZ-induced diabetic rats, an increase in adenosine A<sub>1</sub> receptor expression was detected in hepatocytes, as well as increased glycogen synthesis with the adenosine analogue, cyclopentyladenosine [206].

ATP reduced binding to insulin receptor degradation in rat adipocytes [207]. A similar process may happen in hepatocytes that are the main site of insulin degradation. However, in later studies, it was shown that it was cellular ATP that had a direct allosteric effect on insulin-degrading enzyme [208, 209].

### Adipocytes

One of the manifestations of diabetes is an enhancement of the lipolytic process, release of free fatty acids, adipokines and cytokines. Adenosine is known to have anti-lipolytic effects; it stimulates lipogenesis, modulates insulin sensitivity, and mediates leptin secretion in isolated adipocytes. Activation of A<sub>1</sub> receptors on adipocytes for the treatment of non-insulin-dependent diabetes mellitus was proposed early [210]. Accordingly, over-expression of A<sub>1</sub> receptors in adipose tissue protects mice from obesity-related insulin resistance [211]. Knockdown of A<sub>1</sub> receptors impaired insulin sensitivity of glucose uptake by adipocytes [79]. Although A<sub>1</sub> agonists, by inhibiting adenylate cyclase, inhibited lipolysis in vitro and in vivo in STZ-diabetic animals, oral administration also produced significant bradycardia [212]. Recent reviews summarise adenosine receptor physiology and challenges in therapeutic approach for treatment of diabetes and obesity [213, 214]. In addition, A<sub>1</sub> receptors stimulate adipocyte differentiation, while A<sub>2B</sub> receptors inhibit adipogenesis, and targeting these may be useful in management of obesity and diabetes [215].

ATP originates from sympathetic nerves and seems to have dual effect on white adipocyte: stimulating lipolysis and inhibiting insulin-induced leptin secretion [216]. Although adipocytes express several P2 receptors (P2Y<sub>1,2,4,6,11</sub>), the leptin effect may be due to P2Y<sub>1</sub> receptors, since specific

antagonist reduced leptin release in isolated adipocytes and circulating levels of leptin was lower in P2Y<sub>1</sub> knockout mice [217]. High ATP dosages stimulated inflammatory responses and insulin resistance in rat adipocytes [218]. Human adipocytes express P2X<sub>7</sub> receptors that modulate the release of inflammatory cytokines, and interestingly, patients with metabolic syndrome showed enhanced expression of P2X<sub>7</sub> receptors [219]. Brown adipocytes that have a capacity to convert metabolic energy to heat also express a number of P2 receptors (P2Y<sub>2</sub>, P2Y<sub>6</sub>, P2Y<sub>12</sub>, P2X<sub>1–7</sub>), that can increase Ca<sup>2+</sup> signalling and membrane trafficking [220], and their role in metabolic syndromes needs to be evaluated. In patients with T2D, insulin resistance is related to lowered ATP synthesis in liver, which is at least partly accounted for by fat deposits assessed from waist circumference [221].

#### Skeletal muscle

Skeletal muscles are the major site of insulin-sensitive glucose uptake, and therefore, insulin resistance has a profound effect on hyperglycaemia and glucose intolerance in T2D. Diabetes also leads to muscle weakness that is linked most strongly to impairment of glucose uptake via the insulin-sensitive GLUT4 transporter and subsequent metabolic disturbances. For example, ATP synthesis is impaired in isolated mitochondria from myotubes isolated from T2D patients [222]. There are a number of studies indicating that purinergic receptors also regulate glucose transport into muscles and vascular perfusion, which can alter muscle performance. There are studies that show that A<sub>1</sub> receptors are involved in insulin sensitivity of glucose uptake and utilisation in isolated muscle fibres [223, 224]. In addition, extracellular ATP stimulates translocation of GLUT4 to plasma membranes of muscle fibres [225]. Exercise increases interstitial concentrations of ATP [226], and a recent study showed immunohistochemical localisation of several P2 receptors on skeletal muscle plasma membrane, and on blood vessels, although the distribution was similar in samples from normal and T2D individuals [227].

On the issue related to the vascular supply of skeletal muscles, there are also a number of studies implicating purinergic signalling. Pre-diabetic ZDF rats have high insulin levels, which impair the ability of red blood cells to release ATP in response to low pO<sub>2</sub>. It was suggested that this O<sub>2</sub>-dependent release of ATP may contribute to the failure in the regulation of O<sub>2</sub> supply to meet the demand in skeletal muscle in pre-diabetes [228]. ATP release was also shown to be impaired in erythrocytes of humans with T2D, and it was suggested that this could contribute to peripheral vascular disease in skeletal muscle in T2D [229]. In human experiments, it was shown that the vasodilator action of ATP, UTP and adenosine in skeletal muscle was diminished by 50% in T2D patients. This does not seem to be due to altered receptor

expression, but rather to their altered sensitivity, and it may underlie reduced vascular function to limit exercise capacity [107]. A recent study showed that 7-day bed rest induced insulin resistance and lowered content/activity of proteins responsible for glucose transport, phosphorylation and storage in muscles [230].

#### Skin

Impaired wound healing is one of the major problems in patients with diabetes, and it can lead to ulcers, pain and eventually amputation. The exact mechanisms are not understood, but eventually, alterations in fibroblast proliferation lead to formation of granulation tissue that delays wound healing. It has been suggested that adenosine A<sub>2A</sub> receptor activation by polydeoxyribonucleotide might represent a therapeutic strategy to overcome the diabetes-impaired cell-cycle machinery during impaired skin wound healing in genetically diabetic mice [231]. Improved healing of skin wounds in diabetic rabbits has been reported during direct delivery of intracellular ATP via lipid vesicles [232].

#### Therapeutic approaches

Purinergic signalling offers potential for the development of novel therapeutic approaches to treat the primary disorder in diabetes, i.e. the pancreas, as well as diabetes-related problems in other organs/tissues. Strategies for the pancreas could include drugs that would increase insulin secretion, protect and support  $\beta$ -cell regeneration, curb inflammation within the pancreas, and for T1D, pretreatment of islets before transplantation and stem cell therapy (the latter two are beyond this review). There are a number of promising studies on model animals and systems that could be translated to human applications.

In order to increase insulin secretion for treatment of T2D, stable and tissue specific analogues for the P2Y<sub>1</sub> receptor have been developed, although no drug is in clinical trials (see above). Other P2Y receptors that could be considered are P2Y<sub>6</sub> receptors that mediate increase in insulin secretion and prevent  $\beta$ -cell death [84]. Inhibition of the pro-apoptotic P2Y<sub>13</sub> receptor may also be considered [85]. The P2X<sub>7</sub> receptor may be an interesting target for diabetes and obesity, but due to its wide expression in several pancreatic cells, inflammatory cells and CNS, more basic knowledge is needed.

Adenosine is a potent endogenous autocrine anti-inflammatory and immunosuppressive molecule, it is released or formed after breakdown of at the site of injury and therefore adenosine receptors are also potential targets. The methylxanthine, caffeine, is a nonspecific adenosine

receptor blocker, and it is no surprise that a role of coffee consumption in lowering the risk of T2D has been widely debated. Well-controlled studies in diabetic mice models provide support for human studies [233]. Nevertheless, more specific adenosine receptor drugs would provide a better control. A<sub>2A</sub> receptor agonists may be exploited for  $\beta$ -cell regeneration [47]. Antagonists of A<sub>2B</sub> receptors would improve insulin secretion as well as decrease the inflammatory response and improve insulin resistance [45, 46]. In fact, A<sub>2B</sub> blockers are being developed for reversal of insulin resistance in T2D: ATL 844 as a joint venture by Clinical Data Inc. and Novartis; and GS 6201 (CVT-6883) by Gilead Sciences [234, 235].

Regarding other diabetic associated maladies, Sandoz/Novartis is developing an A<sub>1</sub> receptor agonist SDZ WAG94; Gilead has GS 9667 in clinical I trials for the treatment of hypertriglyceridemia associated with diabetes. The specific A<sub>2A</sub> agonist BVT.115959 by Biovitrum is in clinical II trials for diabetic neuropathic pain, and Sonedenoson by King is in clinical trials for diabetic foot ulcers and wound healing [235].

In addition to purinergic signalling, the energy/nucleotide status of pancreatic and other cells could be considered for therapeutic approaches. Enhancement of ATP synthesis in pancreatic islets, e.g. by biotin, reinforces glucose-induced insulin secretion [236]. Direct delivery of intracellular ATP via lipid vesicles appears to be possible, at least in the skin [232].

## Conclusions and perspectives

The pancreas is a central organ in nutrient and energy homeostasis with endocrine, exocrine, stromal and immunoreactive cells, which participate in complex processes that have consequences for whole body physiology (Fig. 1). This review has focussed on the role of purinergic signalling in the regulation of insulin secretion and  $\beta$ -cell viability, and in the regulation of various tissues/organs that are affected by diabetes. The enormous flexibility and diversity of the purinergic system can be exploited in drug design for the treatment of primary and secondary sites of diabetes, although integrated understanding is needed. Some purinergic drugs are already in clinical trials, and it is hoped that finer regulation of diabetes 2 and 1 and their complications will be possible in the near future.

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