Purinergic signalling in the pancreas in health and disease

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Abstract

Pancreatic cells contain specialised stores for ATP. Purinergic receptors (P2 and P1) and ecto-nucleotidases are expressed in both endocrine and exocrine calls, as well as in stromal cells. The pancreas, especially the endocrine cells, were an early target for the actions of ATP. After the historical perspective of purinergic signalling in the pancreas, the focus of this review will be the physiological functions of purinergic signalling in the regulation of both endocrine and exocrine pancreas. Next, we will consider possible interaction between purinergic signalling and other regulatory systems and their relation to nutrient homeostasis and cell survival. The pancreas is an organ exhibiting several serious diseases – cystic fibrosis, pancreatitis, pancreatic cancer and diabetes – and some are associated with changes in life-style and are increasing in incidence. There is upcoming evidence for the role of purinergic signalling in the pathophysiology of the pancreas, and the new challenge is to understand how it is integrated with other pathological processes.

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Introduction

The pancreas performs both exocrine and endocrine functions. The bulk of the pancreas is exocrine, comprising 70–90% acinar cells and 5–25% duct cells, depending on the species. Endocrine cells in the islets of Langerhans contribute only 3–5% of the pancreas. Pancreatic stellate cells (PSCs) comprise <5% of pancreas mass.

Almost 50 years ago, the first reports on the role of purinergic signalling in the endocrine pancreas appeared. Stimulation of secretion of insulin by ATP was first reported in 1963 for rabbit pancreas slices (Rodrigue-Candela et al. 1963) and confirmed later in primates (Levine et al. 1970).

ATP was shown to be released together with insulin from pancreatic secretory granules by exocytosis in 1975, similar to the release of ATP with noradrenaline (NA) from adrenal chromaffin granules (Leitner et al. 1975). ATP was shown to stimulate glucagon and insulin secretion from isolated perfused rat pancreas in 1976, and this was dependent on low and high glucose concentrations respectively (Loubatières-Mariani et al. 1976). The ATP released from secretory granules was shown to be broken down to ADP and AMP (Sussman & Leitner 1977) and ecto-ATPases were identified (Levin et al. 1978). Adenosine, also resulting from ATP breakdown, inhibited insulin secretion stimulated by glucose (Ismail et al. 1977). On the other hand, adenosine, ADP and 5'-AMP released glucagon in isolated perfused rat pancreas (Weir et al. 1975).

Many of the early studies on the role of nucleotides in insulin secretion came from the laboratory of Mme Marie-Madeleine Loubatières-Mariani. For example, it was shown that the relative potency of nucleotides that increased insulin release induced by glucose was ATP ≥ ADP, while AMP and adenosine had only weak activity (about 100-fold less active) and GTP, ITP, CTP and UTP were virtually inactive (Loubatières-Mariani et al. 1979). They showed that 2,2'-pyridylisatogen tosylate, a P2 receptor antagonist, inhibited the insulin-secreting effect of ATP (Chapal & Loubatières-Mariani 1981a). Adenosine stimulated the secretion of glucagon, but not insulin, suggesting that α-cells are more sensitive to adenosine than the β-cells (Loubatières-Mariani et al. 1982).

The work on the role of purinergic regulation of exocrine pancreas and other pancreatic cells started <20 years ago. There have been a number of reviews about purinergic signalling in the pancreas over the years (Tahani 1979, Hillaire-Buys et al. 1994a, Makino et al. 1994, Dubyak 1999, Novak 2003, 2008, Hellman 2009, Petit et al. 2009).

The aim of this review is to update this rapidly developing field and integrate our knowledge about purinergic signalling...
in endocrine and exocrine pancreas, and with other cell types (PSCs, neurons and cells of blood vessels). Any of these cells are potential sources of extracellular nucleotides/sides and ecto-nucleotidases and these can stimulate, amplify or inhibit various processes. The review will first present various roles of purinergic signalling in pancreas physiology. Second, we will focus on the potential role of purinergic signalling in the pathophysiology of the pancreatic diseases that can have more widespread effects, such as in diabetes, cystic fibrosis (CF) and cancer.

Neural regulation of the pancreas

The activities of both endocrine and exocrine cells are regulated by parasympathetic and sympathetic nerves, as well as by hormones, autocrine and paracrine mediators. Intrapancreatic parasympathetic nerves were present at day 14 of gestation in the foetal rat pancreas, but no sympathetic innervation was detected at that stage (de Gasparo et al. 1978). ATP and acetylcholine (ACh) have synergistic effects on insulin release (Bertrand et al. 1986), consistent with their roles as co-transmitters from parasympathetic nerves. Parasympathetic stimulation can evoke secretion from both exocrine acini and ducts (Holst 1993, Love et al. 2007). In addition, ACh and ATP also have synergistic effects on exocrine secretion, though it may involve separate neural and exocrine components (see below).

Effector cells are considered to be innervated when they form close relationships with axonal varicosities (Burnstock 2008) and such relationships have been shown between sympathetic nerve varicosities and both α- and β-cells (Rodriguez-Diaz et al. 2011). Sympathetic nerve stimulation inhibits insulin secretion, perhaps via the α2A-receptor-mediated opening of ATP-dependent K+ channels (Lorrain et al. 1992, Drews et al. 2010). A recent study shows that over-expression of the α2A adrenoceptor contributes to development of type 2 diabetes (Rosengren et al. 2010). Sympathetic nerve stimulation directly regulates exocrine ducts and acinar cells via β-adrenergic receptors (Lingard & Young 1983, 1984, Holst 1993, Novak 1998), though the major effect is on blood vessels where it would cause vasoconstriction (Holst 1993). In addition, sympathetic nerves (probably releasing NA and ATP as co-transmitters) indirectly regulate pancreatic endocrine and exocrine secretions, through actions on the parasympathetic ganglia in the pancreas (Yi et al. 2005).

In the following sections, it will be shown that various pancreatic cell types possess a number of purinergic and adenosine receptors and ecto-nucleotidases, which implicate ATP as a parasympathetic/sympathetic co-transmitter, though direct evidence for neural ATP release in the pancreas is pending.

Pancreatic vasculature

Some of the earliest experiments on ATP-induced insulin release were carried out on isolated perfused pancreas (see above). Apart from reaching islet cells, ATP could also have an effect on vasculature. Evidence for the presence of P2 receptors on vascular smooth muscle in rat pancreas was presented in earlier studies (Chapal & Loubatières-Mariani 1983). P2X receptors mediate vasoconstriction of the rat pancreatic vascular bed (Bertrand et al. 1987), while P2Y receptors mediate vasodilation (Hillaire-Buys et al. 1991), probably via endothelial-derived relaxing factor affecting smooth muscle cells.

Adenosine receptors, probably A2A, mediate vasorelaxation in the pancreatic vascular bed (Yamagishi et al. 1985, Laurent et al. 1999). Adenosine may be protective in pancreatitis (see below) and as indicated by the following experiments. Infusion of homocysteine, a risk factor for atherosclerosis, altered cholinergic endothelium-mediated vasodilation, but did not affect adenosine-mediated endothelial-independent dilation of vascular smooth muscle (Quéré et al. 1997).

Ecto-nucleotidases

There are several types of nucleotide-/side-modifying enzymes expressed in various pancreatic cells. Biochemical studies have shown membrane Mg2+- or Ca2+-activated adenosine triphosphatase activities in rat pancreas (Harper et al. 1978, Lambert & Christophe 1978, Martin & Senior 1980, Hamlyn & Senior 1983). Later, ATP diphosphohydrolase was identified in pig pancreas hydrolysing ATP to ADP and AMP (Laliberté & Beaudoin 1983). Eventually, in 1995, it was possible to purify and identify type-1 ecto-nucleoside triphosphate diphosphohydrolase (denoted NTPDase or CD39 family) in pig pancreas (Sévigny et al. 1995).

Using histochemical lead precipitation methods, earlier studies also searched for localization of enzymes. For example, one study on rat pancreas showed ATPase, ADPase, 5′-nucleotidase and alkaline phosphatase activity in the vasculature, endocrine and exocrine cells (Böck 1989). As Githens reviews, similar studies at that time show that ATP/ADPase activity was strongest in vasculature, ATPase was detected in both endocrine and exocrine cells, while endocrine but not exocrine cells contained alkaline phosphatase (see Githens 1983).

In endocrine pancreas, ATP pyrophosphohydrolase (ecto-NPP) and alkaline phosphatase were shown in isolated mouse pancreatic islets (Capito et al. 1986). A monoclonal antibody has been prepared as a specific inhibitor of human NTPDase-3, which is expressed in all Langerhans islet cells (Munkonda et al. 2009). The following paper from the same group used several methods to demonstrate that NTPDase-3 was expressed in endocrine cells of several species, and it was claimed that ecto-5′-nucleotidase (CD73) was expressed in rats, but not in humans or mice (Lavoie et al. 2010). Furthermore, it was shown that NTPDase-3 can modulate insulin secretion.

Regarding exocrine pancreas, the following information is available. Based on functional and expression studies, it was found that the rat pancreas contains NTPDase-1 in acinar granules and ducts. Upon stimulation, the enzyme is secreted.
in particular form (microvesicles) to pancreatic juice (Sorensen et al. 2003). Indeed, presence of NTPDase-1 in zymogen granules, first detected biochemically (Harper et al. 1978), was confirmed by proteomics and western blot analysis (Chen et al. 2008, Haanes & Novak 2010). Further immunohistochemical studies have shown that NTPDase-1 and -2 (CD39L1) were also localised in mouse pancreas (Kittel et al. 2004). Acinar cells were positive for both NTPDase-1 and -2, but their expression in ductal epithelial cells was weak. In addition, NPTDase-1 was found in blood vessels and NTPDase-2 immunostaining in the basolateral aspect of endothelial cells.

In agreement with the above studies, ecto-ATPase activity was demonstrated by enzyme histochemistry in both pancreatic acini and ducts in rats, but it was not detected in guinea pigs and humans, perhaps indicating species differences in purinergic regulation of pancreatic secretion, or limitations of the detection technique (Kordás et al. 2004). In fact, in a later study on human pancreas, both NTPDase-1 and -2 (CD39 and CD39L1) were detected at the mRNA and protein levels and enzymes were localised in several cell types (Künzli et al. 2007). The presence of NTPDase-1 was confirmed in acini of mouse, rat and human preparations (Lavoie et al. 2010).

Further functional and biochemical studies on rat pancreas have shown that cholecystokinin octapeptide (CCK-8) stimulation of the pancreas causes release of both ATP-consuming enzymes (NTPDase-1 and 5'-nucleotidase, i.e. CD39 and CD73) and ATP-generating enzymes (adenylate kinase and nucleoside diphosphate kinase) into pancreatic juice (Yegutkin et al. 2006). These studies support the idea that intraluminal ATP/adenosine concentrations are regulated within the pancreatic ducal tree and serve to stimulate ductal P2 and adenosine receptors (see below).

Endocrine pancreas

The islets of Langerhans are dispersed throughout the pancreas and comprise four cell types: α-cells containing glucagon, β-cells containing insulin and amylin and δ-cells containing somatostatin and pancreatic polypeptide-containing cells.

β-Cells

The effect of purine compounds, particularly ATP, on insulin secretion is well documented. As early as 1963, it was reported that ATP added to the medium surrounding pieces of rabbit pancreas increased insulin secretion into the medium (Rodrigue-Candela et al. 1963). The stimulatory effect on insulin secretion was later found to also occur when ATP was applied to the isolated perfused rat pancreas (Sussman et al. 1969, Loubatieres et al. 1972, Loubatieres-Mariani et al. 1976, 1979) and hamster pancreas (Feldman & Jackson 1974). The ATP effect was found to be glucose-dependent and exerted via two different types of P2 receptors: P2X receptors on rat pancreatic β-cells transiently stimulated insulin release at low non-stimulated glucose concentration; and P2Y receptors potentiated glucose-induced insulin secretion (Petit et al. 1998). The concentration–response relationship for different P2 receptor agonists and given glucose backgrounds are summarized in a recent review, Table 1 (Petit et al. 2009).

Notably, many later studies indicated that ATP may also have inhibitory effects on insulin release, and this may be exerted via specific P2 receptors with different binding sites, and/or specific intracellular signalling pathways, or indirectly via adenosine receptors after ATP hydrolysis, and there appear to be significant species differences (see below).

ATP binds to P2X and/or P2Y receptors and increases [Ca^{2+}], in many β-cell preparations and models, including human insulin-secreting β-cells (Squires et al. 1994, Jacques-Silva et al. 2010). Various intracellular signalling pathways, K_ATP channel open/closed state, membrane voltage and Ca^{2+} influx eventually lead to release of insulin (Fig. 1). The first phase of the biphasic insulin response to glucose is potentiated by endogenous ATP (Chapal et al. 1993).

Insulin granules also contain ATP (and ADP) (Leitner et al. 1975, Hutton et al. 1983) that is secreted and can be detected as quantal exocytotic release from rat β-cells expressing heterologous P2X2 receptors as ATP biosensors, and concentrations up to 25 μmol/l close to plasma cell membranes have been detected (Hazama et al. 1998, Karanauskaite et al. 2009). Interestingly, small molecules like ATP can be released by a kiss-and-run exocytosis, while insulin is retained in the granule (Obermüller et al. 2005), indicating that the basal release of ATP may have a role as an autocrine regulator. ATP can also be co-released with 5-hydroxytryptamine, γ-aminobutyric acid, glutamate and zinc, which would have further autocrine co-regulatory functions on insulin secretion (Braun et al. 2007, Richards-Williams et al. 2008, Karanauskaite et al. 2009).

Molecular identities of P2 receptors on various preparations of β-cells are summarised in Table 1 and the proposed role in regulation of insulin secretion is depicted in Fig. 1. Regarding P2X receptors, α,β-methylene ATP (α,β-mATP) mimicked ATP effects on insulin secretion (Petit et al. 1987), suggesting that P2X1 or P2X3 receptor subtypes might be involved. RT-PCR and western blots revealed that most of the P2X1–P2X7 receptors are expressed in rat primary islets, β-cells and the INS-1 cell line (Richards-Williams et al. 2008, Santini et al. 2009). Electrophysiological and immunocytochemical studies showed that mouse, human and porcine β-cells express rapidly desensitising P2X1 and P2X3 receptors, and it was suggested that paracrine or neural activation of these receptors contributes to the initial outburst of glucose- and ACh-evoked insulin release (Silva et al. 2008). In turn, ATP liberated together with insulin might participate in positive feedback control of insulin release (Blachier & Malaïsse 1988, da Silva et al. 2007). Recently, P2X3 receptors have been shown to constitute a positive autocrine and amplifying signal for insulin release in the human pancreatic β-cell (Jacques-Silva et al. 2010). In the rat INS-1 cell line the P2X3 receptor had...
<table>
<thead>
<tr>
<th>Compound</th>
<th>Model</th>
<th>Glucose background (mmol/l)</th>
<th>Concentration range (μmol/l)</th>
<th>EC50 (mol/l)</th>
<th>Observation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural ATP</td>
<td>Isolated perfused rat pancreas</td>
<td>8.3</td>
<td>1–300</td>
<td>~2 × 10⁻⁵</td>
<td>ATP:ADP = 3.2</td>
<td>Loubatières-Mariani et al. (1979)</td>
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<tr>
<td></td>
<td>Rat isolated islets INS-1 β-cells</td>
<td>8.3</td>
<td>300–3000</td>
<td>~3.2 × 10⁻⁹</td>
<td></td>
<td>Blachier &amp; Malaisse (1988)</td>
</tr>
<tr>
<td>P2 agonists</td>
<td>Isolated perfused rat pancreas</td>
<td>8.3</td>
<td>1–100</td>
<td>As potent as ATP</td>
<td>Chapal &amp; Loubatières-Mariani (1981b)</td>
<td>Verspohl et al. (2002)</td>
</tr>
<tr>
<td>z,β-Me-ATP</td>
<td>Rat isolated islets INS-1 β-cells</td>
<td>8.3</td>
<td>5–200</td>
<td></td>
<td></td>
<td>Blachier &amp; Malaisse (1988)</td>
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<tr>
<td>P2Y agonists</td>
<td>Isolated perfused rat pancreas</td>
<td>8.3</td>
<td>0.02–2</td>
<td>~0.5 × 10⁻⁶</td>
<td>Analogue: ATP = 45</td>
<td>Bertrand et al. (1987)</td>
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<tr>
<td>2-Methylthio-ATP ADPβS</td>
<td>Isolated perfused rat pancreas</td>
<td>8.3</td>
<td>1–100</td>
<td>15 × 10⁻⁶</td>
<td>Analogue: ATP = 100</td>
<td>Verspohl et al. (2002)</td>
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<td></td>
<td>INS-1 β-cells</td>
<td>8.3</td>
<td>0.005–0.5</td>
<td>~0.2 × 10⁻⁶</td>
<td></td>
<td>Bertrand et al. (1991)</td>
</tr>
<tr>
<td>ADPβS ATPγS</td>
<td>Isolated perfused rat pancreas</td>
<td>8.3</td>
<td>0.01–0.1</td>
<td>~2 × 10⁻⁸</td>
<td>Analogue: ATP ~ 10</td>
<td>Verspohl et al. 2002</td>
</tr>
<tr>
<td>P2Y1 agonists</td>
<td>Isolated perfused rat pancreas</td>
<td>8.3</td>
<td>0.0015–5</td>
<td>2.8 × 10⁻⁸</td>
<td></td>
<td>Hillaire-Buys et al. (2001)</td>
</tr>
<tr>
<td>2-Methylthio-ATP z-B</td>
<td>Isolated mouse islets</td>
<td>8.3</td>
<td>0.001–1</td>
<td>3.2 × 10⁻⁸</td>
<td></td>
<td>Parandeh et al. (2008)</td>
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<tr>
<td>P2Y6 agonists</td>
<td>Isolated mouse islets</td>
<td>8.3</td>
<td>0.001–10</td>
<td>1.6 × 10⁻⁷</td>
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inhibitory effects on insulin secretion at all glucose concentrations tested (Santini et al. 2009).

Evidence for a P2Y receptor mediating the biphasic response in insulin secretion from β-cells was published early (Bertrand et al. 1987, Li et al. 1991). ADP/βS is a potent agonist mediating insulin secretion from perfused rat pancreas and isolated islets (Bertrand et al. 1991, Petit et al. 1998), suggesting that P2Y₁ receptors might also be involved. Furthermore, this ADP analogue enhanced insulin secretion and reduced hyperglycaemia after oral administration to rats and dogs (Hillaire-Buys et al. 1993). Several studies then focussed on P2Y₁ receptors and pharmacological agents were developed (Fischer et al. 1999, Hillaire-Buys et al. 2001, Farret et al. 2006). Data from the P2Y₁ receptor knockout (KO) mice indicated that the receptor is involved in glucose homeostasis; however, insulin secretion was decreased in islets isolated from P2Y₁−/− mice (Léon et al. 2005).

Pancreatic β-cells express a number of other P2Y receptors. The P2U (i.e. P2Y₃) receptor was cloned and characterised from human pancreas (Stam et al. 1996). The P2Y₄ receptor was also demonstrated immunohistochemically in rat β-cells (Coutinho-Silva et al. 2001, 2003). A detailed study with mRNA and protein expression showed that rat insulinoma INS-1 cells express P2Y₁, P2Y₂, P2Y₄, P2Y₆ and P2Y₁₂ receptors (Lugo-Garcia et al. 2007, Santini et al. 2009) and that the P2Y₄ receptor stimulated insulin secretion at all glucose concentrations tested (Santini et al. 2009). However, mouse β-cells do not seem to express P2Y₂ and P2Y₄ receptors (Ohtani et al. 2008, Parandeh et al. 2008).

Although many studies (see above) have demonstrated that ATP/ADP increase insulin release, some early studies demonstrated that ADP also decreased insulin release (Petit et al. 1989, Poulsen et al. 1999). Further studies showed that P2Y receptors, possibly P2Y₁, mediated inhibition of L-type Ca²⁺ channels in rat pancreatic β-cells (Gong et al. 2000). A recent study shows that in mice β-cells ADP inhibits insulin secretion by activation of P2Y₁₃ receptors, but increases insulin secretion via P2Y₁ receptors (Amisten et al. 2010).

**Figure 1** Role of purinergic receptors in regulation of insulin secretion and β-cell survival. The facilitative GLUT2 transporter mediates glucose entry. Glucose metabolism results in production of ATP, which closes the ATP-sensitive channel, K<sub>AATP</sub>. The channel consists of four Kir6.2 and SUR1 subunits. Closure of K<sub>AATP</sub> depolarises the cell membrane potential and thus opens voltage-gated L-type Ca²⁺ channels eventually leading to generation of Ca²⁺ action potentials. Exocytosis of secretory vesicles containing insulin (and ATP) is triggered by increases in the cellular Ca²⁺. ATP can be also released from parasympathetic and sympathetic nerves. Pₐ receptors can boost and amplify signals associated with the glucose effect on insulin secretion and proliferation or apoptosis of β-cells. P₂X receptors facilitate Ca²⁺/Na⁺ influx and membrane depolarisation, and as a result they can elicit insulin secretion even at low glucose concentrations. Some P₂Y receptors increase cellular Ca²⁺ and activate protein kinase C (PKC) pathways. In addition, other P₂Y and adenosine receptors affect the cAMP pathway and possibly Epac signalling. At high adenosine concentrations, adenosine would be transported into the β-cell and exert metabolic effects. Receptors leading to increased insulin secretion are shown in green, those inhibiting insulin secretion are in red. Receptors affecting cell proliferation are in blue and those stimulating apoptosis purple. Receptors depicted here are taken from functional studies and the prefixes refer to rat, mouse or human receptors. A complete list of molecularly identified receptors is given in Table 1 (updated and modified from Novak (2008)).

**Table 1** Molecularly identified receptors. Receptors depicted here are taken from functional studies and the prefixes refer to rat, mouse or human receptors. A complete list of molecularly identified receptors is given (updated and modified from Novak (2008)).
P2Y₁ and P2Y₆ receptors in mouse β-cells inhibited insulin secretion at high glucose concentrations, and were slightly stimulating at 5 mM glucose (Ohtani et al. 2008), while other studies on similar preparations showed clear stimulation of insulin secretion by this receptor at glucose concentrations >8 mM (Parandeh et al. 2008, Balasubramanian et al. 2010). In human pancreatic islets a further two receptors are expressed, P2Y₁₁ and P2Y₁₂ (Lugo-Garcia et al. 2008), and their involvement in stimulation of insulin secretion is postulated. In hamster β-cell line HIT-T15, P2Y₁₁ receptors seem to stimulate insulin secretion while P2X₇ receptors inhibit it and the net effect depends on the glucose concentration (Lee et al. 2008).

Recent studies indicate that P2 receptors are also involved in β-cell survival and sinspanscitic β-cell loss is a key factor in the pathogenesis of diabetes, this issue will be considered in the last section. Pancreatic islet cells express NTPDase-3, and if ecto-5'-nucleotidase is present as shown in some species (Lavoie et al. 2010), one may expect accumulation of adenosine. Indeed, microelectrode recordings from mouse pancreatic β-cells showed that theophylline (a non-selective antagonist) depolarised the β-cell membrane and stimulated insulin release, and in 10 mM glucose, β-cells exhibited slow waves with bursts of spikes in the plateau and increased insulin secretion (Henquin & Meisner 1984). In dog perfused pancreas, the adenosine analogue 5'-N-ethylcarboxamido-adenosine (NECA) inhibited insulin release, though the effect was concentration-dependent (Bacher et al. 1982). Inhibitory A₁ adenosine receptors were then pharmacologically identified on β-cells (Hillaire-Buys et al. 1987, Bertrand et al. 1989a, Verspohl et al. 2002) and in INS-1 cells (Topfer et al. 2008). Notably, the ecto-nucleotidases and A₁ receptors could explain some of the dual effects of ATP.

What is the physiological role of all these P1 and P2 receptors and their apparently different effects on insulin secretion? Secretion of insulin (and glucagon and somatostatin) is pulsatile, as detected in vivo, in vitro pancreas and in isolated islets with coupled β-cells; and pulsatility is also reflected in intracellular Ca²⁺ oscillations and membrane potential changes. One of the possible coordinating mechanisms could be purinergic signalling (Hellman et al. 2004, Novak 2008, Hellman 2009). As discussed above, ATP is intermittently released from neurons and β-cells. Further supporting evidence is that inhibition of the P2Y₁ receptor attenuates glucose-induced insulin oscillations, but increases the total amount of insulin secreted (Salehi et al. 2005). Also A₁ receptor KO increases insulin pulses (and prolongs glucagon and somatostatin pulses and they lose their anti-synchronous relationship) (Salehi et al. 2009). In addition, endothelial cells in the islets can have a tonic inhibitory action on β-cell P2 receptors, resulting in impaired synchronisation of the insulin release pulses (Hellman et al. 2007). Pulsatility of ATP release and differential regulation via various P2 and P1 receptors could contribute to the pattern of insulin release (Fig. 1).

In addition, one could postulate that P2Y receptors mediating stimulation of Gₛ proteins could have similar roles as incretins – glucagon-like peptide (GLP-1) and gastric inhibitory peptide (GIP) – both in augmenting insulin release and in maintaining the β-cell number (Yabe & Seino 2011). One of the important signalling pathways of incretin action involves Epac (exchange proteins activated by cAMP). Whether P2Y or adenosine receptors also stimulate Epac in β-cells is not yet known and should be investigated.

α-Cells

ATP was shown to stimulate secretion of glucagon in isolated perfused rat pancreas in one study, though in another study adenosine and ADP, but not ATP, were successful (Weir et al. 1975, Loubatières-Mariani et al. 1976). Evidence for adenosine A₂ receptors on glucagon-secreting α-cells was presented in several studies (Bacher et al. 1982, Chapal et al. 1984, 1985). The adenosine-stimulating effect on glucagon secretion via A₂ receptors can be potentiated by an α₂-adrenergic agonist (Gross et al. 1987) and NECA, an A₂ receptor agonist, potentiates ACh-induced glucagon secretion (Bertrand et al. 1989b). In mouse α-cells, both A₁ and A₂A receptors were shown by immunohistochemistry and specific stimulation of A₂A receptors with CGS-21680 increased glucagon release, while adenosine decreased it (Tuduri et al. 2008). In A₁ receptor KO mice pulses of glucagon (and somatostatin) were prolonged, indicating that these α-cells (and δ-cells) possess A₁ receptors (Salehi et al. 2009).

Diadenosine tetraphosphate (Ap₄A) stimulated glucagon (as well as insulin) secretion in perfused rat pancreas (Silvestre et al. 1999). Expression and functional studies on mice α-cells show that they express P2 receptors. P2Y₆ receptors, stimulated with UDP½S, increased glucagon release (Parandeh et al. 2008). In contrast, P2Y₁ receptors inhibited Ca²⁺ signalling and glucagon secretion in mice α-cells (Tuduri et al. 2008). In rat islets glucagon secretion was inhibited by P2Y₁ receptor antagonist MRS 2179 (Grapengiesser et al. 2006).

δ-Cells

It was proposed early that δ-cells have local inhibitory effects, via somatostatin, on the release of insulin and glucagon from adjacent α- and β-cells (Hellman & Lernmark 1969). P2 receptor agonists stimulate somatostatin secretion from dog pancreas (Bertrand et al. 1990), especially ADP½S (Hillaire-Buys et al. 1994b). Pulses of somatostatin (and glucagon) are removed by addition of the P2Y₁ receptor antagonist MRS 2179, but the regularity of insulin secretion was maintained (Salehi et al. 2007).

Exocrine pancreas

The exocrine cells form the bulk of the pancreatic tissue and surround the endocrine cells. Although endocrine and
exocrine cells have been regarded as functionally different entities, common points of interests are emerging. Endocrine physiologists are interested in exocrine cells as possible β-cell precursors (Juhl et al. 2010). Recently, it has also been appreciated that exocrine and endocrine diseases are not totally separate entities and may be interdependent (see below). Finally, purinergic signalling has also an important role in the exocrine pancreas and paracrine effects between endocrine, exocrine and other pancreatic cells should be considered (Fig. 2).

Pancreatic acini

Acinar cells secrete fluid containing NaCl and a variety of digestive enzymes, including α-amylase, lipase, colipase, carboxylester lipase, zymogens such as trypsinogen, chymotrypsinogen, procarboxypeptidases, and proelastase as well as trypsin inhibitor pancreatitis-associated protein and lithostathine. This enzyme-rich secretion passes through a series of ducts that secrete a NaHCO3-rich fluid to the duodenum, where together with bile and duodenal secretions it acts on materials entering the duodenum.

Pancreatic acini release ATP in response to various stimuli, including cholinergic and CCK-8 stimulation (Sørensen & Novak 2001). A recent paper showed that ATP accumulates in zymogen granules due to the action of vesicular nucleotide transporter (Haanes & Novak 2010), which belongs to the SLC17A9 solute family and is expressed in the brain and adrenal chromaffin cells (Sawada et al. 2008).

Although pancreatic acini store and release ATP from granules, acini are relatively unaffected by ATP, possessing relatively few functional P2 receptors; the main site of ATP effects are the downstream ducts (Novak et al. 2003). Thus only about 15% of acinar cells in adult rat pancreas respond to UTP and ATP, although transcripts for P2Y2, P2Y4, P2X1 and P2X4 receptors were present (Novak et al. 2002). The authors speculated that the low number of functional P2 receptors in acini might be related to the fact that these cells release ATP and autocrine stimulation should be avoided. A recent study on mouse pancreas pieces confirms very low functionality of P2 receptors in acinar cells compared to surrounding PSC (Won et al. 2011).

ATP released from acini, hydrolysed to adenosine (see above), could stimulate duct or acinar cells. There are several types of adenosine receptors expressed in whole pancreas and real-time PCR shows the following level of expression in rat pancreas: A2A > A2B > A3 > A1 (Novak et al. 2008). It has been known for a long time that adenosine has multiple effects on exocrine pancreas. Here we deal with effects that could be on acinar cells; effects on ducts cells are given below. Adenosine increased amylase secretion in rat pancreatic lobules, but since the effect was inhibited by atropine and could not be reproduced in isolated acini, it was concluded that the adenosine effect was mediated indirectly by release of
neural ACh (Rodriguez-Nodal et al. 1995). Nevertheless, using A₃ receptor agonists and antagonists, functional receptors were identified in mice pancreatic cells and the rat pancreas acini cell line, AR42J (Yamano et al. 2007).

Pancreatic ducts

The principal physiological role of pancreatic ducts is to secrete a bicarbonate-rich isotonic fluid. This is achieved by coordinated action of several $\text{H}^+/\text{HCO}_3^-$ transporters, cAMP- and/or $\text{Ca}^{2+}$-activated Cl$^-$ channels and K$^+$ channels. In contrast to acini, pancreatic duct cells respond very well to ATP and UTP. Early studies show that ATP and UTP applied to the basolateral surface of rat pancreatic duct cells increased $[\text{Ca}^{2+}]_{\text{i}}$, and transiently stimulated K$^+$ and Cl$^-$ conductances (Hug et al. 1994, Christoffersen et al. 1998). ATP and UTP also activated large $\text{Ca}^{2+}$-dependent Cl$^-$ currents, and smaller K$^+$ currents, in CAPAN-1 and CFPAC-1 cells, human pancreatic duct cell lines (Galietta et al. 1994, Chan et al. 1996, Zsembery et al. 2000, Fong et al. 2003). Also in dog pancreatic duct epithelial cells, ATP and UTP stimulated $\text{Ca}^{2+}$-activated Cl$^-$ conductances, again most likely via P2Y$_2$ receptors (Nguyen et al. 1998).

RT-PCR and functional studies showed that pancreatic ducts express P2Y$_2$, P2Y$_4$, P2X$_4$, P2X$_7$ and probably other P2 receptors such as P2Y$_1$ and P2Y$_11$ (Christoffersen et al. 1998, Hede et al. 1999, Luo et al. 1999, Nguyen et al. 2001). As in other epithelia, P2 receptor localisation is difficult to reveal, as the same receptor type can be localised to both luminal and basolateral membranes, though coupled to different ion transporters (Novak 2008, 2011). Thus luminal ATP/UTP, most likely via P2Y$_2$ receptors, stimulates fluid and Cl$^-$/$\text{HCO}_3^-$ secretion (Ishiguro et al. 1999, Steward et al. 2005, Szücs et al. 2006). P2X$_7$ receptors, most likely luminal, are cation channels but also decrease intracellular pH, possibly reflecting $\text{HCO}_3^-$ secretion (Henriksen & Novak 2003). A recent study shows that P2X$_7$ receptors act in conjunction with muscarinic receptors to increase exocrine secretion in pancreas and this secretion was reduced in P2X$_7$ KO mice (Novak et al. 2010). P2 receptors can also down-regulate secretion, e.g. basolateral P2Y$_2$ receptors inhibit K$^+$ channels (KCNMA1, K$_{Ca}$1.1) and thereby ductal secretion (Hede et al. 1999, 2005, Ishiguro et al. 1999, Szücs et al. 2006).

In addition to $\text{HCO}_3^-$ and fluid secretion, larger pancreatic ducts in particular also secrete mucins as demonstrated in dog pancreatic duct epithelial cells. P2Y$_2$ receptors stimulated exocytosis detected by microperometry and cAMP greatly potentiated the $\text{Ca}^{2+}$-mediated effects (Jung et al. 2004, 2010).

RT-PCR and immunohistochemical studies of human pancreatic duct cell lines, PANC-1 and CFPAC-1, demonstrated later the presence of P2Y$_1$, P2Y$_2$, P2Y$_4$, P2Y$_6$, P2Y$_{11-14}$ and P2X$_1$, P2X$_2$, P2X$_4$, P2X$_5$, P2X$_6$ and P2X$_7$ receptors (Hansen et al. 2008) and some were also found in another cell line CAPAN-1 (Szücs et al. 2006). PANC-1 and CFPAC-1 cell lines responded to nucleotides with the following efficacy: UTP $\geq$ ATP = ATP$\gamma$S $> (4\text{-benzoylbenzoyl})\text{ATP}$, ATP, UTP, and single cell $\text{Ca}^{2+}$ measurements indicated functional expression of notably P2Y$_2$, P2X$_4$ and P2X$_7$ receptors. Purinergic receptors mediate Na$^+$/Ca$^{2+}$ exchange in pancreatic duct cells and it is proposed that this plays a role in the regulation of duct lumen $\text{Ca}^{2+}$ content (Hansen et al. 2009).

Pancreatic ducts, both human and rodent, also express functional adenosine receptors, primarily of the A$_{2A}$ and A$_{2B}$ subtypes, stimulation of which results in the opening of Cl$^-$ channels that are required for $\text{HCO}_3^-$ and fluid secretion (Novak et al. 2008). This finding supports nicely earlier studies performed on dog whole pancreas. Although adenosine decreased blood flow, it enhanced secretin-stimulated $\text{HCO}_3^-$ and fluid secretion (Yamagishi et al. 1985, 1986). A pharmacological study indicated that it was the $A_{2A}$ adenosine receptor that was involved in this secretory response in dog pancreas (Iwatsuki 2000).

The above studies show that the purinergic system has a coordinating function in acini-to-duct signalling and a simplified model for this is presented in Fig. 2. Acini secrete ATP and ecto-nucleotidases, and ATP and adenosine (not ADP) are agonists for pancreatic ducts, helping to regulate ion transport and thereby secretion. Neural and mechanically released ATP may also be stimulatory, but possibly at large concentrations it may be inhibitory and down-regulate secretion in order to prevent over-stimulation and distension of ducts. In the case of acinar damage significant amounts of ATP could be released towards the interstitium. At this stage one can only speculate how this might affect endocrine cells, immunoreactive cells, sensory nerves, as well as PSCs.

Pancreatic stellate cells

PSCs are relatively newly discovered cells that play crucial roles in pancreatic inflammation and fibrosis; in addition, it is reputed that they have the potential to become insulin-producing cells. Purinergic signalling has not been extensively investigated yet. First reports show that especially activated PSC respond with increases in $[\text{Ca}^{2+}]_{\text{i}}$, to micromolar concentrations of ATP (Won et al. 2011). Several types of P2Y and P2X receptors are expressed in these cells, mRNA for P2Y$_1$, P2Y$_2$, P2Y$_6$, P2X$_1$, P2X$_4$, P2X$_5$ but not P2X$_7$ was detected (Hennigs et al. 2011), though another study indicates also mRNA for the P2X$_7$ receptor (Künzli et al. 2008). Robust $[\text{Ca}^{2+}]_{\text{i}}$ responses to ATP, UTP and UDP and relative insensitivity to extracellular $\text{Ca}^{2+}$ indicate strong responses from the P2Y$_2$ and P2Y$_6$ receptors (Hennigs et al. 2011). ATP, as well as protease-activated receptors (PAR1 and -2) and platelet-derived growth factor, also results in prominent nuclear $\text{Ca}^{2+}$ signals, which may play a role in PSC proliferation and contributes to the development of pancreatic diseases (Won et al. 2011).
Development and ageing

Changes in expression of P2 receptors in rat and mouse exocrine and endocrine pancreas have been studied during neonatal development and ageing (Coutinho-Silva et al. 2001). P2X1, P2X2, P2Y1, and P2Y2 receptors were expressed by vascular smooth muscle, as detected by immunohistochemistry. P2X3 and P2X4 receptors were absent in the islets of the neonate pancreas, but were progressively up-regulated with age after birth. In contrast, the greatest expression of P2Y1 receptors on cells from the duct system was in neonatal pancreas, and this abated with age. P2X7 receptors were consistently found in α-cells in neonatal and adult pancreas, and only transiently in a few scattered β-cells in stages E12 and E14 (Cheung et al. 2007).

Pathophysiology

The pancreas is affected by a number of serious diseases, including diabetes, CF, acute and chronic pancreatitis and pancreatic cancer. These diseases are considered as separate entities, though there may be a significant overlap in causality and manifestation of the disease. Below, we will review the evidence for implication of purinergic signalling in the pathophysiology of the pancreas and organs affected by pancreatic diseases.

Cystic fibrosis

Mutations of the CF transport conductance regulator (CFTR) gene, which codes for the cAMP-regulated Cl⁻ channel, leads to aberrant ion and fluid transport in many organs including the pancreas. In the pancreas, CFTR is expressed in ducts and faulty Cl⁻, HCO₃⁻ and fluid secretion leads to plugging of ducts by mucus and digestive enzymes, followed by destruction of acini, inflammation, fibrosis and maldigestion (see Novak 2008). More than 80% of CF patients exhibit pancreatic insufficiency at or soon after birth.

CFTR and anion secretion are regulated by A₂A receptors in both rodent and human ducts (Novak et al. 2008). CFTR is regulated also by P2Y₂ and other P2Y receptors, as shown for a number of other epithelia (Novak 2011). In the case of a defect in CFTR, anion (Cl⁻ and HCO₃⁻) secretion may be taken over by Ca²⁺-activated Cl⁻ channels and these are regulated by a number of P2 receptors (see above), and potentially these could be utilised to ‘rescue’ pancreatic function (Wilschanski & Novak 2012).

Purinergic signalling has been studied in the CFPAC-1 cell line, which is a ductal pancreatic adenocarcinoma derived from a patient with the ΔF508 mutation. The following information is available. Purinergic regulation of Cl⁻ and ion transport and Ca²⁺ signalling by CFPAC-1 cells via P2U (P2Y₂ or P2Y₄) receptors has been described (Chan et al. 1996, Cheung et al. 1998, O’Reilly et al. 1998, Zsembery et al. 2000). CFPAC-1 cells express mRNA and proteins for P2Y₁, P2Y₂, P2Y₄, P2Y₆, P2Y₁₁–₁₄ and P2X₁–₇ receptors, similar to CFTR containing cell line PANC-1, though there was a difference in their Ca²⁺ signalling responses (Hansen et al. 2008). Both cell lines express similar levels of mRNA for A₂B > A₂A ≫ A₁ receptors (Novak et al. 2008). Thus the few available studies have not yet revealed differences in P2 and adenosine receptors in these model cell lines. However, defective ATP-dependent mucin secretion was described in CFPAC-1 cells compared with CFTR-expressing cells, where the order of efficacy was ATP > ADP > adenosine > UTP (Montserrat et al. 1996). Also one study showed that in CF ducts NYD-SP27 (phospholipase C (PLC) zeta) was up-regulated, and its inhibition by anti-sense transfection allows ATP to have more sustained effects on Ca²⁺-dependent anion transport (Zhu et al. 2007).

CFTR has been proposed as an ATP release channel and/or regulator of ATP release mechanism (Novak 2011). Whether this could have a consequence for ATP concentrations of normal and CF ducts and thus autoregulation is not known (Fig. 2).

Pancreatitis

Excessive ATP catabolism and depletion occur during acute pancreatitis and both acinar and ductal components are involved (Hegyi et al. 2011). Activation of adenosine A₁ receptors reduces blood flow and induces oedema formation in the rat pancreas, suggesting that adenosine may be involved in the pathogenesis of acute pancreatitis, which may have multi-organ effects and a fatal outcome (Satoh et al. 2000). Nevertheless, adenosine may be cytoprotective. Inhibition of adenosine uptake, and stimulation of A₂A receptors, ameliorates caerulein-induced pancreatitis in mice (Noji et al. 2002). Recurrent acute and chronic pancreatitis have underlying genetic predispositions and a number of genes coding for digestive enzymes and also for CFTR underlie the pathophysiology of pancreatitis (Ooi et al. 2010). Ethanol consumption is a further risk factor, because the pancreas contains high concentrations of non-oxidative synthesize enzymes that combine ethanol with fatty acids, forming fatty acid ethyl esters. Fatty acid ethyl esters cause calcium toxicity via inositol triphosphate receptors and loss of ATP synthesis in acinar cells (Cridle et al. 2006). Intracellular ATP depletion could not abolish toxic Ca²⁺ responses to bile acids (Barrow et al. 2008). The most recent study shows that P2X7 receptors and Toll-like receptor 9, presumably in pancreatic macrophages, are important receptors in mediating inflammatory signals in caerulein pancreatitis (Hoque et al. 2011).

Chronic pancreatitis results in organ fibrosis, pain and exocrine and endocrine insufficiency. PSCs are the key players in organ fibrogenesis. CD39 deletion decreased fibrogenesis in experimental pancreatitis, and it was suggested that extracellular nucleotides are modulators of PSC proliferation and collagen production in pancreatitis (Künzli et al. 2008). Transcripts of the ectonucleotidase, CD39, and P2X₇, P2Y₂ and P2Y₆ receptors are significantly increased in

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chronic pancreatitis (Künzli et al. 2007). It was suggested that these heightened expression patterns infer associations with chronic inflammation and neoplasia of the pancreas. When pancreatic inflammation occurs, PSCs are activated and ATP, acting via both P2X and P2Y receptors (in particular P2Y2 and P2X4 receptors), raise [Ca2+], thereby probably playing a pivotal role in pancreatic fibrogenesis (Hennigs et al. 2011).

Pancreatic cancer

Adenocarcinoma arising from pancreatic ducts is responsible for more than 90% of pancreatic cancers and survival is <5% over a 5-year period. Insulinosmas are relatively rare and have a much better prognosis. What we know about purinergic signalling in these cancer cells is mostly from cultured cancer cell lines, which are often used as model systems.

Insuloinoma cell lines are often compared with isolated islets or β-cells in the same studies and similar conclusions have been reached. For example, ATP at low concentrations promotes insulin secretion from the INS-1 insulinoma cell line and rat islets via P2Y receptors, but inhibits insulin release at high concentrations after being metabolised to adenosine (Verspohl et al. 2002). For a detailed comparison of receptors see Table 2. Also in the CAPAN-1 cell line, derived from human pancreatic adenocarcinoma of ductal origin, ATP and UTP applied to the apical membranes decreased cellular pH indicating HCO3 secretion, but were inhibitory when applied to the basolateral membranes (Sztücs et al. 2006). These findings are in accordance with P2 regulation as revealed in rat and guinea pig ducts, as well as in expression studies (see above).

Cell cycle arrest and induction of apoptosis in pancreatic cancer cells exposed to ATP have been described, and growth inhibition by ATP is adenosine-mediated (Yamada et al. 2002). CD39, and P2X7, P2Y2 and P2Y6 receptors, are significantly increased in biopsies of pancreatic cancer (Künzli et al. 2007). High levels of mRNA for CD39 significantly correlated with better, long-term survival after tumour resection in patients with pancreatic cancer. In contrast, lower levels of P2Y2 receptor expression were advantageous. This study indicates that there may be disturbed purinergic signalling in pancreatic cancer. Studies of other cancer-type models indicate altered purinergic signalling and nucleotide/nucleoside levels at tumors sites (Pellegatti et al. 2008, Ye gutkin et al. 2011).

Diabetes and pancreas

In type 1 diabetes (or insulin-dependent diabetes mellitus) pancreatic β-cells are destroyed/defective and treatment with exogenous insulin is essential. In type 2 diabetes β-cells are unresponsive to glucose, insulin secretion is decreased and/or target tissues are resistant to action of insulin, and one or more metabolic abnormalities develop. Pancreatic diseases (see above) that destroy islets can also lead to diabetes, sometimes referred to as type 3 diabetes. The metabolic syndrome has pronounced effects on small blood vessels, and this leads to many chronic complications in other organ systems.

In diabetes, basic cellular defects in glucose metabolism and changes in intracellular ATP/ADP levels have consequences for cellular energy, cell survival, intracellular signalling, as well as activating membrane-bound ATPases and ion/nutrient transport across cell membranes. It may be expected then that extracellular ATP/nucleotide/nucleoside levels would also be affected and thereby also the components of the purinergic signalling system. Earlier reviews describing the roles of purinergic signalling in insulin secretion and diabetes are available (Loubatières-Mariani et al. 1997, Petit et al. 2001, Farret et al. 2005).

Over the years many animal and cell models have been used to study the basic mechanisms in diabetes. Streptozotocin (STZ)-induced diabetes is an animal model for diabetes and has been widely used (Rakieten et al. 1963), but has been questioned as a valuable model for some aspects of diabetes in man. Other animal models for diabetes include: alloxan-induced diabetes (Jacobs 1937, Rerup 1970); Bio Breeder diabetic rats (BBD), a model of human autoimmune type 1 diabetes (Nakhooda et al. 1977); Zucker diabetic fatty rats (ZDF), a rodent model of non-insulin–dependent diabetes mellitus (Clark et al. 1983); and non-obese diabetic (NOD) mice (Makino et al. 1980).

Early experiments were carried out on the diabetic experimental rat model using alloxan and dithizone (Mikhail & Awadallah 1977, Awadallah et al. 1979). ATP was shown in these studies to have a protective effect, significantly reducing blood sugar levels. ATP injected into the carotid artery increased the sensitivity of alloxan–diabetic rats to glucose and it was suggested that a possible cause of diabetes was a defect in purinergic innervation of the islet cells (Tahani 1979).

In normal pancreatic β-cells, glucose stimulates polyphosphoinositide (PPI) hydrolysis through activation of a phosphoinositide-specific PLC. However, in rats injected with STZ during the neonatal period, glucose-induced PPI hydrolysis is severely diminished and is associated with a reduced insulin-secreting response to glucose (Morin et al. 1996). It has been suggested that the cytotoxic effect of STZ on β-cells is due to a reduction in the intracellular level of ATP (Nukatsuka et al. 1990). KATP channel openers, such as diazoxide, have been explored for beneficial effects on preservation of β-cell function in type 1 diabetes (Grill et al. 2009). Interestingly, a mutation in the KATP channel subunit SUR1 reduces ATPase activity, leading to MgADP activation of the channel, which causes transient neonatal diabetes (de Wet et al. 2008).

STZ-diabetes suppresses the stimulatory effect of adenosine on glucagon secretion from pancreatic α-cells and reduces its vasodilator effect on the vascular bed (Gross et al. 1989, Laurent et al. 1999) via A2 receptors (Gross et al. 1991).

Insulin secretion and increases in [Ca2+]i in pancreatic β-cells are preserved and mediated by P2Y receptors in ZDF rats (Tang et al. 1996). In STZ–diabetic rat pancreatic islets, P2Y1 receptors were shown to be present in intra-islet
capillaries, while P2X4 receptors were found on β- and δ-cells; P2Y1 and P2Y2 receptors were still expressed on pancreatic duct cells and P2X1, P2X3, P2Y1 and P2Y3 receptors in small pancreatic blood vessels (Coutinho-Silva et al. 2003).

P2X7 receptors were expressed on α-cells in healthy pancreas on the periphery of islets; these cells were shown to migrate to the centres of islets to replace the lost β-cells in both STZ-diabetic rats and NOD mice (Coutinho-Silva et al. 2003, 2007).

<table>
<thead>
<tr>
<th>Receptor subtype</th>
<th>Tissue origin</th>
<th>Technique</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>P2X1</td>
<td>Rat and mouse pancreas (progressively up-regulated) Mouse islet cells</td>
<td>Immunohistochemistry</td>
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<td>P2X2</td>
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<td>RT-PCR, western blot analysis and immunohistochemistry</td>
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<td>P2X3</td>
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<td>Rat INS-1e</td>
<td>RT-PCR, siRNA</td>
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<td>Human islets</td>
<td>Immunohistochemistry, RT-PCR, western blot analysis and in situ hybridisation</td>
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<td>P2X4</td>
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<td>Coutinho-Silva et al. (2001)</td>
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<td>RT-PCR, western blot analysis and immunohistochemistry</td>
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<td>Human islets</td>
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<td>Human islets</td>
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<td>P2Y6</td>
<td>INS-1 β-cells Mouse islets and β-cells</td>
<td>RT-PCR, western blot analysis</td>
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<td>Mouse β-TG6 insulinaoma cells</td>
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<td>Mouse MIN6</td>
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<td>Mouse islets and β-cells</td>
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<td>Amiston et al. (2010)</td>
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A study has shown that P2X7 receptors were expressed in β-cells (and also α-cells) and receptors were down-regulated in type 2 diabetes but up-regulated in human obesity (Glas et al. 2009).

There are many therapeutic approaches to treat the primary disorder in diabetes, the insulin secretion purinergic system is one of them. P2 receptors on β-cells may become potential targets for treatment of type 2 diabetes (see Novak 2008, Ahren 2009). It has been suggested that 2-methylthio ATP-αβ, a isomer, a potent and tissue-selective P2Y1 receptor agonist with high efficacy for glucose-dependent insulin secretion, may be a drug candidate for type 2 diabetes (Farret et al. 2006). Dinucleoside polyphosphate analogues, which offer better stability compared to nucleotide, acting through P2Y1 receptors have been developed as insulin secretagogues and it was suggested that they may prove to be an effective and safe treatment for type 2 diabetes (Eliahu et al. 2010).

P1 receptors may be valuable potential targets. Antagonists of A2B receptors may improve insulin secretion (Rusing et al. 2006), and A2B blockers are in development for the treatment of type 2 diabetes and asthma (Fredholm et al. 2011). On the other hand, A2 receptor ligands suppress expression of pro-inflammatory cytokines, ameliorate development of diabetes in model animals and have been claimed as potential candidates for the treatment of type 1 diabetes (Németh et al. 2007).

In addition to purinergic signalling, the energy/nucleotide status of pancreatic cells is of importance. The intracellular ATP/ADP ratio serves as a coupling factor between glucose metabolism and insulin release (Detimary et al. 1995). Advanced glycation end products, which are implicated in diabetic complications, inhibit cytochrome c oxidase and ATP production, leading to impairment of glucose-stimulated insulin secretion (Zhao et al. 2009). Biotin, a member of the vitamin B group, enhances ATP synthesis in rat pancreatic islets, resulting in reinforcement of glucose-induced insulin secretion (Sone et al. 2004). Another approach is direct delivery of intracellular ATP (via lipid vesicles), and one study reports improved healing of skin wounds in diabetic rabbits (Wang et al. 2010).

One of the key factors in the pathogenesis of diabetes is the pancreatic β-cell mass. Incretins GLP-1 and GIP, in addition to augmenting of insulin secretion, have also proliferative and anti-apoptotic effects on β-cells mass and some of the intracellular signalling pathways are well characterised (Yabe & Seino 2011). Pro- and anti-inflammatory signalling molecules such as cytokines influence proliferation and apoptosis of β-cells (Maedler et al. 2009). A number of

![Figure 3](https://example.com/figure3.png)

**Figure 3** Distribution of purinoceptor subtypes on pancreatic endocrine, exocrine and stellate cells, as well as pancreatic blood vessels and immunoreactive cells. Receptor identified by molecular and functional studies for rodent and human preparations is included and asterisks indicate functionally dominant receptor subtypes on β-cells and ducts. The figure also depicts the integrated role of pancreas and gut in nutrient homeostasis – nutrient sensing on gut and blood side, gut hormone and incretin release, digestive processes and pancreatic hormone release. In addition, cell numbers would be regulated via purinergic signalling, cytokines and growth factors.
purinergic receptors have similar abilities to mediate cell proliferation and apoptosis and studies indicate that the P2X7 receptor, possibly different variants, may be able to support both functions (Lenertz et al. 2011). It is only recently that studies addressing the question of purinergic signalling and β-cell survival became available. It was shown that P2Y6 receptor agonists not only increase insulin secretion, but prevent β-cell death induced by tumour necrosis factor-α (Balasubramanian et al. 2010). On the other hand, it seems that activation of the P2Y13 receptor of the mouse pancreatic insuloma cell line, MIN6C4, has a pronounced pro-apoptotic effect; 2-methylthio ADP reduced cell proliferation and increased caspase-3 activity, effects that were reversed by the P2Y13 receptor antagonist, MRS2211 (Tan et al. 2010). Extracellular ATP (1 μM) increased insulin secretion in mouse β-cell lines, probably via P2Y1 and P2X4 receptors, though at higher ATP concentrations in the medium, cell viability decreased (Ohtani et al. 2011). In human islets the P2X7 receptor seems to be involved in secretion of insulin and interleukin-1 (Glas et al. 2009). This study showed further that P2X7 KO mice had lower β-cells mass, impaired glucose tolerance and a defect in insulin and interleukin secretion. Extracellular ATP-induced nuclear Ca2+ transients are mediated by 1,4,5-trisphosphate receptors in mouse β-cells (Chen et al. 2009) and possible influence on regulation of gene expression needs to be addressed. It is clear that in order to understand β-cell function and survival on the integrative level, it will be necessary to explore mechanisms of purinergic signalling together with incretins and inflammatory signals.

Perspectives and conclusions

In recent years, it has been estimated that up to 50% of patients with diabetes, i.e. endocrine insufficiency, also have exocrine insufficiency, most commonly due to chronic pancreatitis and CF (Andren-Sandberg & Hardt 2008, Hardt et al. 2008). This may not be surprising as there are close morphological and functional interactions between endocrine and exocrine cells. For example, insulin has a significant regulator effect on exocrine secretion (Lee et al. 2009), and exocrine cells can differentiate into β-cells (Juhl et al. 2010). Some of the recently described genetic mutations that cause both endocrine and exocrine pathologies underscore the interdependence of the two systems (Raeder et al. 2006, Andren-Sandberg & Hardt 2008). Purinergic signalling is well described for the two systems separately. It is timely to see the interaction between the two systems, as there are obviously rich sites for ATP release β-cells, acini and ducts (Figs 1, 2 and 3).

The pancreas is a central organ in nutrient and energy homeostasis with both exocrine and endocrine cells, which participate in complex processes that have consequences for whole body physiology (Fig. 3). Thus, it will be important to understand how purinergic signalling, together with gut hormones, incretins and cytokines, regulates exocrine and endocrine functions and how this participates in the nutrient breakdown, assimilation and nutrient sensing, cell to cell interaction and survival. Drugs designed to target specific components of the purinergic system may become of relevance to pancreatic diseases including diabetes. This review points to a significant modulatory role of purinergic signalling in pancreatic physiology and pathophysiology.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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