REVIEW

Purinergic signalling in the pancreas in health and disease

G Burnstock and I Novak¹

University College Medical School, Autonomic Neuroscience Centre, Rowland Hill Street, London NW3 2PF, UK

¹Molecular and Integrative Physiology, Department of Biology, University of Copenhagen, August Krogh Building, Universitetsparken 13, DK 2100 Copenhagen Ø, Denmark

(Correspondence should be addressed to G Burnstock; Email: g.burnstock@ucl.ac.uk)

Abstract

Pancreatic cells contain specialised stores for ATP. Purinergic receptors (P2 and P1) and ecto-nucleotidases are expressed in both endocrine and exocrine cells, 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. Intrapaneatic 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 δ -cells, but less so with β -cells (Rodríguez-Díaz *et al.* 2011). Sympathetic nerve stimulation inhibits insulin secretion, perhaps via the α_{2A} -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 α_{2A} 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 A_{2A} , 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 (Quérel *et al.* 1997).

Ecto-nucleotidases

There are several types of nucleotide-/side-modifying enzymes expressed in various pancreatic cells. Biochemical studies have shown membrane Mg^{2+} - or Ca^{2+} -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 (Laliberte & 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 (Sévin *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 (Sørensen *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, NTPDase-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 ductal 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, Loubatières *et al.* 1972, Loubatières-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 $[Ca^{2+}]_i$ 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 μ mol/l close to plasma cell membranes have been detected (Hazama *et al.* 1998, Karanaukaite *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, Karanaukaite *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 -meATP) 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- or 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 & Malaisse 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 1 Concentration–response relationships for different P2 receptor agonists increasing insulin secretion. From Petit *et al.* (2009)

Compound	Model	Glucose background (mmol/l)	Concentration range (μmol/l)	EC ₅₀ (mol/l)	Observation	Reference
Natural ATP	Isolated perfused rat pancreas	8.3	1–300	$\sim 2 \times 10^{-5}$	ATP:ADP = 3:2	Loubatières-Mariani <i>et al.</i> (1979)
P2 agonists α,β-Me-ATP	Rat isolated islets	8.3	300–3000	$\sim 3.2 \times 10^{-9}$		Blachier & Malaisse (1988)
	INS-1 β-cells	5.6 and 8.3	0.001–0.01			Verspohl <i>et al.</i> (2002)
P2Y agonists 2-Methylthio-ATP	Isolated perfused rat pancreas	8.3	1–100		As potent as ATP	Chapal & Loubatières-Mariani (1981b)
	Rat isolated islets	8.3	5–200			Blachier & Malaisse (1988)
P2Y agonists 2-Methylthio-ATP	Isolated perfused rat pancreas	8.3	0.02–2	$\sim 0.5 \times 10^{-6}$	Analogue: ATP = 45	Bertrand <i>et al.</i> (1987)
	INS-1 β-cells	8.3	1–100	15×10^{-6}	Analogue: ATP = 100	Verspohl <i>et al.</i> (2002)
ADPβS	Isolated perfused rat pancreas	8.3	0.005–0.5	$\sim 0.2 \times 10^{-6}$		Bertrand <i>et al.</i> (1991)
ADPβS ATPαS	INS-1 β-cells	8.3	0.01–0.1	$\sim 2 \times 10^{-8}$	Analogue: ATP ~ 10	Verspohl <i>et al.</i> 2002
	Isolated perfused rat pancreas	8.3	0.15–15	$\sim 1.5 \times 10^{-6}$		Hillaire-Buys <i>et al.</i> (2001)
P2Y ₁ agonists 2-Methylthio-ATP-α-B	Isolated perfused rat pancreas	8.3	0.0015–5	2.8×10^{-8}		Farret <i>et al.</i> (2006)
P2Y ₆ agonists UDPβS	Isolated mouse islets	8.3	0.001–1	3.2×10^{-8}		Parandeh <i>et al.</i> (2008)
		20	0.01–10	1.6×10^{-7}

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_{ATP} . The channel consists of four Kir6.2 and SUR1 subunits. Closure of K_{ATP} depolarises the cell membrane potential and thus opens voltage-gated L-type Ca^{2+} channels eventually leading to generation of Ca^{2+} action potentials. Exocytosis of secretory vesicles containing insulin (and ATP) is triggered by increases in the cellular Ca^{2+} . ATP can be also released from parasympathetic and sympathetic nerves. P_2 receptors can boost and amplify signals associated with the glucose effect on insulin secretion and proliferation or apoptosis of β -cells. P_2X receptors facilitate Ca^{2+}/Na^{+} influx and membrane depolarisation, and as a result they can elicit insulin secretion even at low glucose concentrations. Some P_2Y receptors increase cellular Ca^{2+} and activate protein kinase C (PKC) pathways. In addition, other P_2Y 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)).

inhibitory effects on insulin secretion at all glucose concentrations tested (Santini *et al.* 2009).

Evidence for a P_2Y receptor mediating the biphasic response in insulin secretion from β -cells was published early (Bertrand *et al.* 1987, Li *et al.* 1991). ADP β S is a potent agonist mediating insulin secretion from perfused rat pancreas and isolated islets (Bertrand *et al.* 1991, Petit *et al.* 1998), suggesting that P_2Y_1 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 P_2Y_1 receptors and pharmacological agents were developed (Fischer *et al.* 1999, Hillaire-Buys *et al.* 2001, Farret *et al.* 2006). Data from the P_2Y_1 receptor knockout (KO) mice indicated that the receptor is involved in glucose homeostasis; however, insulin secretion was decreased in islets isolated from $P_2Y_1^{-/-}$ mice (Léon *et al.* 2005).

Pancreatic β -cells express a number of other P_2Y receptors. The P_2U (i.e. P_2Y_2) receptor was cloned and characterised

from human pancreas (Stam *et al.* 1996). The P_2Y_4 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 P_2Y_1 , P_2Y_2 , P_2Y_4 , P_2Y_6 and P_2Y_{12} receptors (Lugo-Garcia *et al.* 2007, Santini *et al.* 2009) and that the P_2Y_4 receptor stimulated insulin secretion at all glucose concentrations tested (Santini *et al.* 2009). However, mouse β -cells do not seem to express P_2Y_2 and P_2Y_4 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 P_2Y receptors, possibly P_2Y_1 , mediated inhibition of L-type Ca^{2+} 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 P_2Y_{13} receptors, but increases insulin secretion via P_2Y_1 receptors (Amisten *et al.* 2010).

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 islet 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 & Meissner 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 (Töpfer *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 α_2 -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 A_{2A} receptors were shown by immunohistochemistry and specific stimulation of A_{2A} 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 UDP β S, 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 ADP β S (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 NaHCO_3 -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 P2Y_2 , P2Y_4 , P2X_1 and P2X_4 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: $\text{A}_{2\text{A}} > \text{A}_{2\text{B}} \geq \text{A}_3 \gg \text{A}_1$ (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

Figure 2 Integrated model for purinergic signalling in the pancreas. Pancreatic acini secrete ATP and also nucleotidases (CD39 and CD73), which hydrolyse ATP to adenosine (Ado). Intraluminal ATP binds to luminal P2X and P2Y receptors and regulates ductal secretion. ATP released from nerves and islet cells, e.g. β -cells, could act on P2Y receptors on the basolateral side of ducts. In addition, ATP released from distended ducts and damaged acini could affect surrounding endocrine, exocrine and stromal cells. Receptors with functional identification are shown here (modified from Novak (2008)).

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 H⁺/HCO₃⁻ transporters, cAMP- and/or Ca²⁺-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 [Ca²⁺]_i and transiently stimulated K⁺ and Cl⁻ conductances (Hug *et al.* 1994, Christoffersen *et al.* 1998). ATP and UTP also activated large Ca²⁺-dependent Cl⁻ currents, and smaller K⁺ currents, in CAPAN-1 and CFPAC-1 cells, human pancreatic duct cell lines (Galiotta *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 Ca²⁺-activated Cl⁻ and K⁺ conductances, again most likely via P2Y₂ receptors (Nguyen *et al.* 1998).

RT-PCR and functional studies showed that pancreatic ducts express P2Y₂, P2Y₄, P2X₁, P2X₄, P2X₇ and probably other P2 receptors such as P2Y₁ and P2Y₁₁ (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 P2Y₂ receptors, stimulates fluid and Cl⁻/HCO₃⁻ secretion (Ishiguro *et al.* 1999, Steward *et al.* 2005, Szücs *et al.* 2006). P2X₇ receptors, most likely luminal, are cation channels but also decrease intracellular pH, possibly reflecting HCO₃⁻ secretion (Henriksen & Novak 2003). A recent study shows that P2X₇ receptors act in conjunction with muscarinic receptors to increase exocrine secretion in pancreas and this secretion was reduced in P2X₇ KO mice (Novak *et al.* 2010). P2 receptors can also down-regulate secretion, e.g. basolateral P2Y₂ 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 HCO₃⁻ and fluid secretion, larger pancreatic ducts in particular also secrete mucins as demonstrated in dog pancreatic duct epithelial cells. P2Y₂ receptors stimulated exocytosis detected by microamperometry and cAMP greatly potentiated the Ca²⁺-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 P2Y₁, P2Y₂, P2Y₄, P2Y₆, P2Y₁₁₋₁₄ and P2X₁, P2X₂, P2X₄, P2X₅, P2X₆ and P2X₇ 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 ≥ ATP = ATPγS > 2'/(3')-O-(4-benzoylbenzoyl)ATP (BzATP), ATP, UTP, and single cell Ca²⁺ measurements indicated functional expression of notably P2Y₂, P2X₄ and P2X₇ receptors. Purinergic receptors mediate Na⁺/Ca²⁺ exchange in pancreatic duct cells and it is proposed that this plays a role in the regulation of duct lumen Ca²⁺ 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 HCO₃⁻ 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 HCO₃⁻ 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 [Ca²⁺]_i to micromolar concentrations of ATP (Won *et al.* 2011). Several types of P2Y and P2X receptors are expressed in these cells, mRNA for P2Y₁, P2Y₂, P2Y₆, P2X₁, P2X₄, P2X₅ but not P2X₇ was detected (Hennings *et al.* 2011), though another study indicates also mRNA for the P2X₇ receptor (Künzli *et al.* 2008). Robust [Ca²⁺]_i responses to ATP, UTP and UDP and relative insensitivity to extracellular Ca²⁺ indicate strong responses from the P2Y₂ and P2Y₆ receptors (Hennings *et al.* 2011). ATP, as well as protease-activated receptors (PAR1 and -2) and platelet-derived growth factor, also results in prominent nuclear Ca²⁺ 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. P2X₁ 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 A_{2A} 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 Δ F508 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, Zsemberly *et al.* 2000). CFPAC-1 cells express mRNA and proteins for

P2Y₁, P2Y₂, P2Y₄, P2Y₆, P2Y_{11–14} and P2X_{1–7} 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 A_{2B} > A_{2A} >> A₃ > 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 A_{2A} receptors, ameliorates caerulein-induced pancreatitis in mice (Noji *et al.* 2002). Recurrent acute and chronic pancreatitis have underlying genetic predilections 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 synthase enzymes that combine ethanol with fatty acids, forming fatty acid ethyl esters. Fatty acid ethyl esters cause calcium toxicity via inositol trisphosphate receptors and loss of ATP synthesis in acinar cells (Criddle *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

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 $[Ca^{2+}]_i$, 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. Insulinomas 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.

Insulinoma 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 HCO_3^- secretion, but were inhibitory when applied to the basolateral membranes (Szűcs *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, Yegutkin *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 (Nakhoda *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). K_{ATP} 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 K_{ATP} 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 $[Ca^{2+}]_i$ 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

Table 2 Molecular identity of P2 receptor subtypes expressed in pancreatic β -cells. Other functional and pharmacological evidence for P2 receptors is given in the text. Modified and updated from Petit *et al.* (2009)

Receptor subtype	Tissue origin	Technique	Reference
P2X ₁	Rat and mouse pancreas (progressively up-regulated)	Immunohistochemistry	Coutinho-Silva <i>et al.</i> (2001)
	Mouse islet cells	Immunocytochemistry	Silva <i>et al.</i> (2008)
	Rat INS-1e	RT-PCR	Santini <i>et al.</i> (2009)
P2X ₂	Rat islets, rat (INS-1) and mouse (β TC3) β -cell models	RT-PCR, western blot analysis and immunohistochemistry	Richards-Williams <i>et al.</i> (2008)
	Rat INS-1e	RT-PCR	Santini <i>et al.</i> (2009)
P2X ₃	Mouse islet cells	Immunocytochemistry	Silva <i>et al.</i> (2008)
	Rat islets, rat (INS-1) and mouse (β TC3) β -cell models	RT-PCR, western blot analysis and immunohistochemistry	Richards-Williams <i>et al.</i> (2008)
	Rat INS-1e	RT-PCR, siRNA	Santini <i>et al.</i> (2009)
	Human islets	Immunohistochemistry, RT-PCR, western blot analysis and <i>in situ</i> hybridisation	Jacques-Silva <i>et al.</i> (2010)
P2X ₄	Rat islets, RINm5F and HIT-T15 cells	mRNA blot analysis	Wang <i>et al.</i> (1996)
	Rat and mouse pancreas (progressively up-regulated)	Immunohistochemistry	Coutinho-Silva <i>et al.</i> (2001)
	Rat islets, rat (INS-1) and mouse (β TC3) β -cell models	RT-PCR, western blot analysis and immunohistochemistry	Richards-Williams <i>et al.</i> (2008)
	Rat INS-1e	RT-PCR	Santini <i>et al.</i> (2009)
P2X ₅	Human islets	<i>In situ</i> hybridisation	Jacques-Silva <i>et al.</i> (2010)
P2X ₆	Rat islets, rat (INS-1) and mouse (β TC3) β -cell models	RT-PCR, western blot analysis and immunohistochemistry	Richards-Williams <i>et al.</i> (2008)
	Rat INS-1e	RT-PCR	Santini <i>et al.</i> (2009)
P2X ₇	HIT-T15 cells	Western blot analysis	Lee <i>et al.</i> (2008)
	Rat INS-1e	RT-PCR	Santini <i>et al.</i> (2009)
	Human islets	<i>In situ</i> hybridisation	Jacques-Silva <i>et al.</i> (2010)
	Mouse wild-type (WT) and KO islets and pancreas	RT-PCR, western blot analysis, immunohistochemistry and functional studies	Glas <i>et al.</i> (2009)
P2Y ₁	Human islets	RT-PCR and western blot analysis	Lugo-Garcia <i>et al.</i> (2007)
	INS-1 β -cells	RT-PCR	Parandeh <i>et al.</i> (2008)
	Mouse islets and β -cells	RT-PCR	Ohtani <i>et al.</i> (2008)
	Mouse β -TC6 insulinoma cells	RT-PCR	Santini <i>et al.</i> (2009)
	Rat INS-1e	RT-PCR	Balasubramanian <i>et al.</i> (2010)
	Mouse MIN6	RT-PCR	Léon <i>et al.</i> (2005)
	Mouse WT and KO whole body	Functional studies	Léon <i>et al.</i> (2005)
P2Y ₂	INS-1 β -cells	RT-PCR and western blot analysis	Lugo-Garcia <i>et al.</i> (2007)
	Rat INS-1e	RT-PCR	Santini <i>et al.</i> (2009)
P2Y ₄	Pancreatic β -cells (normal and diabetic rats)	Immunohistochemistry	Coutinho-Silva <i>et al.</i> (2001)
	Rat islets, INS-1 and RIN cells	RT-PCR and western blot analysis	Bokvist <i>et al.</i> (2003)
	INS-1 β -cells	RT-PCR and western blot analysis	Lugo-Garcia <i>et al.</i> (2007)
	Rat INS-1e	RT-PCR, siRNA	Santini <i>et al.</i> (2009)
P2Y ₆	INS-1 β -cells	RT-PCR and western blot analysis	Lugo-Garcia <i>et al.</i> (2007)
	Mouse islets and β -cells	RT-PCR	Parandeh <i>et al.</i> (2008)
	Mouse β -TC6 insulinoma cells	RT-PCR	Ohtani <i>et al.</i> (2008)
	Rat INS-1e	RT-PCR	Santini <i>et al.</i> (2009)
	Mouse MIN6	RT-PCR	Balasubramanian <i>et al.</i> (2010)
P2Y ₁₁	Human β -cells	RT-PCR, western blot analysis, immunofluorescence	Lugo-Garcia <i>et al.</i> (2008)
	HIT-T15 cells	Western blot analysis	Lee <i>et al.</i> (2008)
P2Y ₁₂	INS-1 β -cells	RT-PCR and western blot analysis	Lugo-Garcia <i>et al.</i> (2007)
	Human β -cells	RT-PCR, western blot analysis, immunofluorescence	Lugo-Garcia <i>et al.</i> (2008)
	Rat INS-1e	RT-PCR	Santini <i>et al.</i> (2009)
P2Y ₁₃	Mouse islets and β -cells	RT-PCR	Amisten <i>et al.</i> (2010)

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 P2Y₂ 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).

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- α - β , 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 A_{2B} receptors may improve insulin secretion (Rusing *et al.* 2006), and A_{2B} 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 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 insulinoma 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 μ M) 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.* 1990), 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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References

- Ahren B 2009 Islet G protein-coupled receptors as potential targets for treatment of type 2 diabetes. *Nature Reviews. Drug Discovery* **8** 369–385. (doi:10.1038/nrd2782)
- Amisten S, Meidute-Abaraviciene S, Tan C, Olde B, Lundquist I, Salehi A & Erlinge D 2010 ADP mediates inhibition of insulin secretion by activation of P2Y₁₃ receptors in mice. *Diabetologia* **53** 1927–1934. (doi:10.1007/s00125-010-1807-8)
- Andren-Sandberg A & Hardt PD 2008 Second Giessen International Workshop on Interactions of Exocrine and Endocrine Pancreatic Diseases, Castle of Rauischholzhausen of the Justus-Liebig-University, Giessen (Rauischholzhausen), Germany. March 7–8, 2008. *Journal of the Pancreas* **9** 541–575.
- Awadallah R, Tahani HM & El-Dessoukey EA 1979 Serum mineral changes due to exogenous ATP and certain trace elements in experimental diabetes. *Zeitschrift für Ernährungswissenschaft* **18** 1–7. (doi:10.1007/BF02026530)
- Bacher S, Kraupp O, Conca W & Raberger G 1982 The effects of NECA (adenosine-5'-N-ethylcarboxamide) and of adenosine on glucagon and insulin release from the *in situ* isolated blood-perfused pancreas in anesthetized dogs. *Naunyn-Schmiedeberg's Archives of Pharmacology* **320** 67–71. (doi:10.1007/BF00499075)
- Balasubramanian R, de Azua IR, Wess J & Jacobson KA 2010 Activation of distinct P2Y receptor subtypes stimulates insulin secretion in MIN6 mouse pancreatic β cells. *Biochemical Pharmacology* **79** 1317–1326. (doi:10.1016/j.bcp.2009.12.026)
- Barrow SL, Voronina SG, daSilva Xavier G, Chvanov MA, Longbottom RE, Gerasimenko OV, Petersen OH, Tepikin GA & Tepikin AV 2008 ATP depletion inhibits Ca²⁺ release, influx and extrusion in pancreatic acinar cells but not pathological Ca²⁺ responses induced by bile. *Pflügers Archiv* **455** 1025–1039. (doi:10.1007/s00424-007-0360-x)
- Bertrand G, Chapal J & Loubatières-Mariani MM 1986 Potentiating synergism between adenosine diphosphate or triphosphate and acetylcholine on insulin secretion. *American Journal of Physiology* **251** E416–E421.
- Bertrand G, Chapal J, Loubatières-Mariani MM & Roye M 1987 Evidence for two different P₂-purinoceptors on β cell and pancreatic vascular bed. *British Journal of Pharmacology* **91** 783–787.
- Bertrand G, Petit P, Bozem M & Henquin JC 1989a Membrane and intracellular effects of adenosine in mouse pancreatic β -cells. *American Journal of Physiology* **257** E473–E478.
- Bertrand G, Gross R, Petit P & Loubatières-Mariani MM 1989b An A2-purinoceptor agonist, NECA, potentiates acetylcholine-induced glucagon secretion. *British Journal of Pharmacology* **96** 500–502.

- Bertrand G, Gross R, Ribes G & Loubatières-Mariani MM 1990 P₂ purinoceptor agonists stimulate somatostatin secretion from dog pancreas. *European Journal of Pharmacology* **182** 369–373. (doi:10.1016/0014-2999(90)90296-1)
- Bertrand G, Chapal J, Puech R & Loubatières-Mariani MM 1991 Adenosine-5'-O-(2-thiodiphosphate) is a potent agonist at P₂ purinoceptors mediating insulin secretion from perfused rat pancreas. *British Journal of Pharmacology* **102** 627–630.
- Blachier F & Malaisse WJ 1988 Effect of exogenous ATP upon inositol phosphate production, cationic fluxes and insulin release in pancreatic islet cells. *Biochimica et Biophysica Acta* **970** 222–229. (doi:10.1016/0167-4889(88)90182-6)
- Böck P 1989 Fate of ATP in secretory granules: phosphohydrolase studies in pancreatic vascular bed. *Archives of Histology and Cytology* **52** (Suppl) 85–90. (doi:10.1679/aohc.52.Suppl_85)
- Bokvist K, Efanov AM, Sandusky G, Sewing S, Treinies I & Gromada J 2003 The P2Y₄ pyrimidinergic receptor is important for nucleotide stimulation of insulin secretion in rat pancreatic β -cells. *Diabetes & Metabolism* **29** 4S77–4S78.
- Braun M, Wendt A, Karanauskaitė J, Galvanovskis J, Clark A, MacDonald PE & Rorsman P 2007 Corelease and differential exit via the fusion pore of GABA, serotonin, and ATP from LDCV in rat pancreatic β cells. *Journal of General Physiology* **129** 221–231. (doi:10.1085/jgp.200609658)
- Burnstock G 2008 Non-synaptic transmission at autonomic neuroeffector junctions. *Neurochemistry International* **52** 14–25. (doi:10.1016/j.neuint.2007.03.007)
- Capito K, Hansen SE, Hedeskov CJ & Thams P 1986 Presence of ATP-pyrophosphohydrolase in mouse pancreatic islets. *Diabetes* **35** 1096–1100. (doi:10.2337/diabetes.35.10.1096)
- Chan HC, Cheung WT, Leung PY, Wu LJ, Chew SB, Ko WH & Wong PY 1996 Purinergic regulation of anion secretion by cystic fibrosis pancreatic duct cells. *American Journal of Physiology* **271** C469–C477.
- Chapal J & Loubatières-Mariani MM 1981a Attempt to antagonize the stimulatory effect of ATP on insulin secretion. *European Journal of Pharmacology* **74** 127–134. (doi:10.1016/0014-2999(81)90522-7)
- Chapal J & Loubatières-Mariani MM 1981b Effects of phosphate-modified adenine nucleotide analogues on insulin secretion from perfused rat pancreas. *British Journal of Pharmacology* **73** 105–110.
- Chapal J & Loubatières-Mariani MM 1983 Evidence for purinergic receptors on vascular smooth muscle in rat pancreas. *European Journal of Pharmacology* **87** 423–430. (doi:10.1016/0014-2999(83)90081-X)
- Chapal J, Loubatières-Mariani MM, Roye M & Zerbib A 1984 Effects of adenosine, adenosine triphosphate and structural analogues on glucagon secretion from the perfused pancreas of rat *in vitro*. *British Journal of Pharmacology* **83** 927–933.
- Chapal J, Loubatières-Mariani MM, Petit P & Roye M 1985 Evidence for an A₂-subtype adenosine receptor on pancreatic glucagon secreting cells. *British Journal of Pharmacology* **86** 565–569.
- Chapal J, Bertrand G, Hillaire-Buys D, Gross R & Loubatières-Mariani MM 1993 Prior glucose deprivation increases the first phase of glucose-induced insulin response: possible involvement of endogenous ATP and (or) ADP. *Canadian Journal of Physiology and Pharmacology* **71** 611–614. (doi:10.1139/y93-086)
- Chen X, Ulintz PJ, Simon ES, Williams JA & Andrews PC 2008 Global topology analysis of pancreatic zymogen granule membrane proteins. *Molecular and Cellular Proteomics* **7** 2323–2336. (doi:10.1074/mcp.M700575-MCP200)
- Chen Z, Li Z, Peng G, Chen X, Yin W, Kotlikoff MI, Yuan ZQ & Ji G 2009 Extracellular ATP-induced nuclear Ca²⁺ transient is mediated by inositol 1,4,5-trisphosphate receptors in mouse pancreatic β -cells. *Biochemical and Biophysical Research Communications* **382** 381–384. (doi:10.1016/j.bbrc.2009.03.030)
- Cheung CY, Wang XF & Chan HC 1998 Stimulation of HCO₃⁻ secretion across cystic fibrosis pancreatic duct cells by extracellular ATP. *Biological Signals and Receptors* **7** 321–327. (doi:10.1159/000014555)
- Cheung KK, Coutinho-Silva R, Chan WY & Burnstock G 2007 Early expression of adenosine 5'-triphosphate-gated P2X₇ receptors in the developing rat pancreas. *Pancreas* **35** 164–168. (doi:10.1097/MPA.0b013e318053e00d)
- Christoffersen BC, Hug MJ & Novak I 1998 Different purinergic receptors lead to intracellular calcium increases in pancreatic ducts. *Pflügers Archiv* **436** 33–39. (doi:10.1007/s004240050601)
- Clark JB, Palmer CJ & Shaw WN 1983 The diabetic Zucker fatty rat. *Proceedings of the Society for Experimental Biology and Medicine* **173** 68–75.
- Coutinho-Silva R, Parsons M, Robson T & Burnstock G 2001 Changes in expression of P₂ receptors in rat and mouse pancreas during development and aging. *Cell and Tissue Research* **306** 373–383. (doi:10.1007/s004410100458)
- Coutinho-Silva R, Parsons M, Robson T, Lincoln J & Burnstock G 2003 P2X and P2Y purinoceptor expression in pancreas from streptozotocin-diabetic rats. *Molecular and Cellular Endocrinology* **204** 141–154. (doi:10.1016/S0303-7207(03)00003-0)
- Coutinho-Silva R, Robson T, Beales PE & Burnstock G 2007 Changes in expression of P2X₇ receptors in NOD mouse pancreas during the development of diabetes. *Autoimmunity* **40** 108–116. (doi:10.1080/08916930601118841)
- Criddle DN, Murphy J, Fistetto G, Barrow S, Tepikin AV, Neoptolemos JP, Sutton R & Petersen OH 2006 Fatty acid ethyl esters cause pancreatic calcium toxicity via inositol trisphosphate receptors and loss of ATP synthesis. *Gastroenterology* **130** 781–793. (doi:10.1053/j.gastro.2005.12.031)
- Detimary P, Jonas J-C & Henquin J-C 1995 Possible links between glucose-induced changes in the energy state of pancreatic B cells and insulin release. *Journal of Clinical Investigation* **96** 1738–1745. (doi:10.1172/JCI118219)
- Drews G, Krippeit-Drews P & Dufer M 2010 Electrophysiology of islet cells. *Advances in Experimental Medicine and Biology* **654** 115–163. (doi:full_text)
- Dubyak GR 1999 Focus on "multiple functional P2X and P2Y receptors in the luminal and basolateral membranes of pancreatic duct cells". *American Journal of Physiology* **277** C202–C204.
- Eliahu S, Barr HM, Camden J, Weisman GA & Fischer B 2010 A novel insulin secretagogue based on a dinucleoside polyphosphate scaffold. *Journal of Medicinal Chemistry* **53** 2472–2481. (doi:10.1021/jm901621h)
- Farret A, Lugo-Garcia L, Galtier F, Gross R & Petit P 2005 Pharmacological interventions that directly stimulate or modulate insulin secretion from pancreatic beta-cell: implications for the treatment of type 2 diabetes. *Fundamental & Clinical Pharmacology* **19** 647–656. (doi:10.1111/j.1472-8206.2005.00375.x)
- Farret A, Filhol R, Linck N, Manteghetti M, Vignon J, Gross R & Petit P 2006 P2Y receptor mediated modulation of insulin release by a novel generation of 2-substituted-5'-O-(1-boranotriphosphate)-adenosine analogues. *Pharmaceutical Research* **23** 2665–2671. (doi:10.1007/s11095-006-9112-4)
- Feldman JM & Jackson TB 1974 Specificity of nucleotide-induced insulin secretion. *Endocrinology* **94** 388–394. (doi:10.1210/endo-94-2-388)
- Fischer B, Chulkin A, Boyer JL, Harden KT, Gendron FP, Beaudoin AR, Chapal J, Hillaire-Buys D & Petit P 1999 2-Thioether 5'-O-(1--thiotriphosphate)adenosine derivatives as new insulin secretagogues acting through P2Y₂-Receptors. *Journal of Medicinal Chemistry* **42** 3636–3646. (doi:10.1021/jm990158y)
- Fong P, Argent BE, Guggino WB & Gray MA 2003 Characterization of vectorial chloride transport pathways in the human pancreatic duct adenocarcinoma cell line, HPAF. *American Journal of Physiology. Cell Physiology* **285** C433–C445. (doi:10.1152/ajpcell.00509.2002)
- Fredholm BB, Ijzerman AP, Jacobson KA, Linden J & Muller CE 2011 International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors – an update. *Pharmacological Reviews* **63** 1–34. (doi:10.1124/pr.110.003285)
- Galletta LJ, Zegarra-Moran O, Mastrocola T, Wöhrle C, Rugolo M & Romeo G 1994 Activation of Ca²⁺-dependent K⁺ and Cl⁻ currents by UTP and ATP in CFPAC-1 cells. *Pflügers Archiv* **426** 534–541. (doi:10.1007/BF00378531)
- de Gasparo M, Krinke G, Milner GR & Milner RD 1978 Influence of autonomic innervation on the foetal rat pancreas *in vitro*. *Journal of Endocrinology* **79** 49–58. (doi:10.1677/joe.0.0790049)

- Githens S 1983 Localization of alkaline phosphatase and adenosine triphosphatase in the mammalian pancreas. *Journal of Histochemistry and Cytochemistry* **31** 697–705. (doi:10.1177/31.5.6221048)
- Glas R, Sauter NS, Schulthess FT, Shu L, Oberholzer J & Maedler K 2009 Purinergic P2X₇ receptors regulate secretion of interleukin-1 receptor antagonist and beta cell function and survival. *Diabetologia* **52** 1579–1588. (doi:10.1007/s00125-009-1349-0)
- Gong Q, Kakei M, Koriyama N, Nakazaki M, Morimitsu S, Yaekura K & Tei C 2000 P2Y-purinoreceptor mediated inhibition of L-type Ca²⁺ channels in rat pancreatic β -cells. *Cell Structure and Function* **25** 279–289. (doi:10.1247/csf.25.279)
- Grapengiesser E, Salehi A, Qader SS & Hellman B 2006 Glucose induces glucagon release pulses antisynchronous with insulin and sensitive to purinoreceptor inhibition. *Endocrinology* **147** 3472–3477. (doi:10.1210/en.2005-1431)
- Grill V, Radtke M, Qvigestad E, Kollind M & Björklund A 2009 Beneficial effects of K-ATP channel openers in diabetes: an update on mechanisms and clinical experiences. *Diabetes, Obesity and Metabolism* **11** (Suppl 4) 143–148. (doi:10.1111/j.1463-1326.2009.01119.x)
- Gross R, Bertrand G, Ribes G & Loubatières-Mariani MM 1987 α_2 -Adrenergic potentiation of adenosine-stimulating effect on glucagon secretion. *Endocrinology* **121** 765–769. (doi:10.1210/endo-121-2-765)
- Gross R, Hillaire-Buys D, Bertrand G, Ribes G & Loubatières-Mariani MM 1989 Diabetes and impaired response of glucagon cells and vascular bed to adenosine in rat pancreas. *Diabetes* **38** 1291–1295. (doi:10.2337/diabetes.38.10.1291)
- Gross R, Hillaire-Buys D, Ribes G & Loubatières-Mariani MM 1991 Diabetes alters the responses of glucagon secreting cells and vascular bed to isoprenaline and forskolin *in vitro* in rat pancreas. *Life Sciences* **48** 2349–2358. (doi:10.1016/0024-3205(91)90272-D)
- Haanes KA & Novak I 2010 ATP storage and uptake by isolated pancreatic zymogen granules. *Biochemical Journal* **429** 303–311. (doi:10.1042/BJ20091337)
- Hamlyn JM & Senior AE 1983 Evidence that Mg²⁺- or Ca²⁺-activated adenosine triphosphatase in rat pancreas is a plasma-membrane ectoenzyme. *Biochemical Journal* **214** 59–68.
- Hansen MR, Krabbe S & Novak I 2008 Purinergic receptors and calcium signalling in human pancreatic duct cell lines. *Cellular Physiology and Biochemistry* **22** 157–168. (doi:10.1159/000149793)
- Hansen MR, Krabbe S, Ankori-Stark I & Novak I 2009 Purinergic receptors stimulate Na⁺/Ca²⁺ exchange in pancreatic duct cells: possible role of proteins handling and transporting Ca²⁺. *Cellular Physiology and Biochemistry* **23** 387–396. (doi:10.1159/000218185)
- Hardt PD, Brendel MD, Kloer HU & Bretzel RG 2008 Is pancreatic diabetes (type 3c diabetes) underdiagnosed and misdiagnosed? *Diabetes Care* **31** (Suppl 2) S165–S169. (doi:10.2337/dc08-s244)
- Harper F, Lamy F & Calvert R 1978 Some properties of a Ca²⁺- and (or) Mg²⁺-requiring nucleoside di- and tri-phosphatase(s) associated with the membranes of rat pancreatic zymogen granules. *Canadian Journal of Biochemistry* **56** 565–576. (doi:10.1139/c78-086)
- Hazama A, Hayashi S & Okada Y 1998 Cell surface measurements of ATP release from single pancreatic beta cells using a novel biosensor technique. *Pflügers Archiv* **437** 31–35. (doi:10.1007/s004240050742)
- Hede SE, Amstrup J, Christoffersen BC & Novak I 1999 Purinoreceptors evoke different electrophysiological responses in pancreatic ducts. P2Y inhibits K⁺ conductance, and P2X stimulates cation conductance. *Journal of Biological Chemistry* **274** 31784–31791. (doi:10.1074/jbc.274.45.31784)
- Hede SE, Amstrup J, Klaerke DA & Novak I 2005 P2Y₂ and P2Y₄ receptors regulate pancreatic Ca²⁺ activated K⁺ channels differently. *Pflügers Archiv: European Journal of Physiology* **450** 429–436. (doi:10.1007/s00424-005-1433-3)
- Hegyi P, Pandol S, Venglovecz V & Rakonczay Z Jr 2011 The acinar-ductal tango in the pathogenesis of acute pancreatitis. *Gut* **60** 544–552. (doi:10.1136/gut.2010.218461)
- Hellman B 2009 Pulsatility of insulin release – a clinically important phenomenon. *Upsala Journal of Medical Sciences* **114** 193–205. (doi:10.3109/03009730903366075)
- Hellman B & Lernmark A 1969 Inhibition of the *in vitro* secretion of insulin by an extract of pancreatic α_1 -cells. *Endocrinology* **84** 1484–1488. (doi:10.1210/endo-84-6-1484)
- Hellman B, Dansk H & Grapengiesser E 2004 Pancreatic β -cells communicate via intermittent release of ATP. *American Journal of Physiology. Endocrinology and Metabolism* **286** E759–E765. (doi:10.1152/ajpendo.00452.2003)
- Hellman B, Jansson L, Dansk H & Grapengiesser E 2007 Effects of external ATP on Ca²⁺ signalling in endothelial cells isolated from mouse islets. *Endocrine* **32** 33–40. (doi:10.1007/s12020-007-9004-3)
- Hennigs JK, Seiz O, Spiro J, Berna MJ, Baumann HJ, Klose H & Pace A 2011 Molecular basis of P₂-receptor-mediated calcium signaling in activated pancreatic stellate cells. *Pancreas* **40** 740–746. (doi:10.1097/MPA.0b013e31821b5b68)
- Henquin JC & Meissner HP 1984 Effects of theophylline and dibutyl cyclic adenosine monophosphate on the membrane potential of mouse pancreatic β -cells. *Journal of Physiology* **351** 595–612.
- Henriksen KL & Novak I 2003 Effect of ATP on intracellular pH in pancreatic ducts involves P2X₇ receptors. *Cellular Physiology and Biochemistry* **13** 93–102. (doi:10.1159/000070253)
- Hillaire-Buys D, Bertrand G, Gross R & Loubatières-Mariani MM 1987 Evidence for an inhibitory A₁ subtype adenosine receptor on pancreatic insulin-secreting cells. *European Journal of Pharmacology* **136** 109–112. (doi:10.1016/0014-2999(87)90786-2)
- Hillaire-Buys D, Chapal J, Petit P & Loubatières-Mariani MM 1991 Dual regulation of pancreatic vascular tone by P2X and P2Y purinoreceptor subtypes. *European Journal of Pharmacology* **199** 309–314. (doi:10.1016/0014-2999(91)90494-B)
- Hillaire-Buys D, Bertrand G, Chapal J, Puech R, Ribes G & Loubatières-Mariani MM 1993 Stimulation of insulin secretion and improvement of glucose tolerance in rat and dog by the P₂_Y-purinoreceptor agonist, adenosine-5'-O-(2-thiodiphosphate). *British Journal of Pharmacology* **109** 183–187.
- Hillaire-Buys D, Bertrand G, Petit P & Loubatières-Mariani MM 1994a Purinergic receptors on insulin-secreting cells. *Fundamental & Clinical Pharmacology* **8** 117–127. (doi:10.1111/j.1472-8206.1994.tb00788.x)
- Hillaire-Buys D, Gross R, Parés-Herbuté N, Ribes G & Loubatières-Mariani MM 1994b *In vivo* and *in vitro* effects of adenosine-5'-O-(2-thiodiphosphate) on pancreatic hormones in dogs. *Pancreas* **9** 646–651. (doi:10.1097/00006676-199409000-00016)
- Hillaire-Buys D, Shahar L, Fischer B, Chulkin A, Linck N, Chapal J, Loubatières-Mariani MM & Petit P 2001 Pharmacological evaluation and chemical stability of 2-benzylthioether-5'-O-(1-thiotriphosphate)-adenosine, a new insulin secretagogue acting through P2Y receptors. *Drug Development Research* **53** 33–43. (doi:10.1002/ddr.1167)
- Holst JJ 1993 Neural regulation of pancreatic exocrine function. In *The Pancreas. Biology, Pathobiology, and Disease*, 2nd edn, pp 381–402. Eds V LW Go, EP DiMaggio, JD Gardner, E Lebenthal, HA Reber & GA Scheele. New York, NY: Raven Press.
- Hoque R, Sohail M, Malik A, Sarwar S, Luo Y, Shah A, Barrat F, Flavell R, Gorelick F, Husain S *et al.* 2011 TLR9 and the NLRP3 inflammasome link acinar cell death with inflammation in acute pancreatitis. *Gastroenterology* **141** 358–369. (doi:10.1053/j.gastro.2011.03.041)
- Hug M, Pahl C & Novak I 1994 Effect of ATP, carbachol and other agonists on intracellular calcium activity and membrane voltage of pancreatic ducts. *Pflügers Archiv* **426** 412–418. (doi:10.1007/BF00388304)
- Hutton JC, Penn EJ & Peshavaria M 1983 Low-molecular-weight constituents of isolated insulin-secreting granules. Bivalent cations, adenine nucleotides and inorganic phosphate. *Biochemical Journal* **210** 297–305.
- Ishiguro H, Naruse S, Kitagawa M, Hayakawa T, Case RM & Steward MC 1999 Luminal ATP stimulates fluid and HCO₃⁻ secretion in guinea-pig pancreatic duct. *Journal of Physiology* **519** 551–558. (doi:10.1111/j.1469-7793.1999.0551m.x)
- Ismail NA, El Denshary EE & Montague W 1977 Adenosine and the regulation of insulin secretion by isolated rat islets of Langerhans. *Biochemical Journal* **164** 409–413.
- Iwatsuki K 2000 Subtypes of adenosine receptors on pancreatic exocrine secretion in anaesthetized dogs. *Fundamental & Clinical Pharmacology* **14** 203–208. (doi:10.1111/j.1472-8206.2000.tb00017.x)

- Jacobs HR 1937 Hyperglycemic actions of alloxan. *Proceedings of the Society for Experimental Biology and Medicine* **37** 404–409.
- Jacques-Silva MC, Correa-Medina M, Cabrera O, Rodriguez-Diaz R, Makeeva N, Fachado A, Diez J, Berman DM, Kenyon NS, Ricordi C *et al.* 2010 ATP-gated P2X₃ receptors constitute a positive autocrine signal for insulin release in the human pancreatic β cell. *PNAS* **107** 6465–6470. (doi:10.1073/pnas.0908935107)
- Juhl SR, Bonner-Weir S & Sharma A 2010 Regenerating pancreatic beta-cells: plasticity of adult pancreatic cells and the feasibility of *in-vivo* neogenesis. *Current Opinion in Organ Transplantation* **15** 79–85. (doi:10.1097/MOT.0b013e3283344932)
- Jung SR, Kim MH, Hille B, Nguyen TD & Koh DS 2004 Regulation of exocytosis by purinergic receptors in pancreatic duct epithelial cells. *American Journal of Physiology. Cell Physiology* **286** C573–C579. (doi:10.1152/ajpcell.00350.2003)
- Jung SR, Hille B, Nguyen TD & Koh DS 2010 Cyclic AMP potentiates Ca²⁺-dependent exocytosis in pancreatic duct epithelial cells. *Journal of General Physiology* **135** 527–543. (doi:10.1085/jgp.200910355)
- Karanauskaitė J, Hoppa MB, Braun M, Galvanovskis J & Rorsman P 2009 Quantal ATP release in rat β -cells by exocytosis of insulin-containing LDCVs. *Pflügers Archiv* **458** 389–401. (doi:10.1007/s00424-008-0610-6)
- Kittel A, Pelletier J, Bigonnesse F, Guckelberger O, Kordás K, Braun N, Robson SC & Sévigny J 2004 Localization of nucleoside triphosphate diphosphohydrolase-1 (NTPDase1) and NTPDase2 in pancreas and salivary gland. *Journal of Histochemistry and Cytochemistry* **52** 861–871. (doi:10.1369/jhc.3A6167.2004)
- Kordás KS, Sperlách B, Tihanyi T, Topa L, Steward MC, Varga G & Kittel A 2004 ATP and ATPase secretion by exocrine pancreas in rat, guinea pig, and human. *Pancreas* **29** 53–60. (doi:10.1097/00006676-200407000-00056)
- Künzli BM, Berberat PO, Giese T, Csizmadia E, Kaczmarek E, Baker C, Halaceli I, Buchler MW, Friess H & Robson SC 2007 Upregulation of CD39/NTPDases and P2 receptors in human pancreatic disease. *American Journal of Physiology. Gastrointestinal and Liver Physiology* **292** G223–G230. (doi:10.1152/ajpgi.00259.2006)
- Künzli BM, Nuhn P, Enyoji K, Banz Y, Smith RN, Csizmadia E, Schuppan D, Berberat PO, Friess H & Robson SC 2008 Disordered pancreatic inflammatory responses and inhibition of fibrosis in CD39-null mice. *Gastroenterology* **134** 292–305. (doi:10.1053/j.gastro.2007.10.030)
- Laliberte JF & Beaudoin AR 1983 Sequential hydrolysis of the γ - and β -phosphate groups of ATP by the ATP diphosphohydrolase from pig pancreas. *Biochimica et Biophysica Acta* **742** 9–15. (doi:10.1016/0167-4838(83)90352-7)
- Lambert M & Christophe J 1978 Characterization of (Mg,Ca)-ATPase activity in rat pancreatic plasma membranes. *European Journal of Biochemistry* **91** 485–492. (doi:10.1111/j.1432-1033.1978.tb12701.x)
- Laurent F, Hillaire-Buys D, Chapal J, Dietz S, Portet K, Cros G, Petit P & Michel A 1999 Contrasting effects of streptozotocin-induced diabetes on the *in vitro* relaxant properties of adenosine in rat pancreatic vascular bed and thoracic aorta. *Naunyn-Schmiedeberg's Archives of Pharmacology* **360** 309–316. (doi:10.1007/s002109900061)
- Lavoie EG, Fausther M, Kauffenstein G, Kukulska F, Kunzli BM, Friess H & Sevigny J 2010 Identification of the ectonucleotidases expressed in mouse, rat, and human Langerhans islets: potential role of NTPDase3 in insulin secretion. *American Journal of Physiology. Endocrinology and Metabolism* **299** E647–E656. (doi:10.1152/ajpendo.00126.2010)
- Lee KY, Zhou L, Ren XS, Chang TM & Chey WY 1990 An important role of endogenous insulin on exocrine pancreatic secretion in rats. *American Journal of Physiology* **258** G268–G274.
- Lee DH, Park KS, Kim DR, Lee JW & Kong ID 2008 Dual effect of ATP on glucose-induced insulin secretion in HIT-T15 cells. *Pancreas* **37** 302–308. (doi:10.1097/MPA.0b013e318168daaa)
- Leitner JW, Sussman KE, Vatter AE & Schneider FH 1975 Adenine nucleotides in the secretory granule fraction of rat islets. *Endocrinology* **96** 662–677. (doi:10.1210/endo-96-3-662)
- Lenertz LY, Gavala ML, Zhu Y & Bertics PJ 2011 Transcriptional control mechanisms associated with the nucleotide receptor P2X₇, a critical regulator of immunologic, osteogenic, and neurologic functions. *Immunologic Research* **50** 22–38. (doi:10.1007/s12026-011-8203-4)
- Léon C, Freund M, Latchoumanan O, Farret A, Petit P, Cazenave JP & Gachet C 2005 The P2Y₁ receptor is involved in the maintenance of glucose homeostasis and in insulin secretion in mice. *Purinergic Signalling* **1** 145–151. (doi:10.1007/s11302-005-6209-x)
- Levin SR, Kasson BG & Driessen JF 1978 Adenosine triphosphatases of rat pancreatic islets: comparison with those of rat kidney. *Journal of Clinical Investigation* **62** 692–701. (doi:10.1172/JCI109177)
- Levine RA, Oyama S, Kagan A & Glick SM 1970 Stimulation of insulin and growth hormone secretion by adenine nucleotides in primates. *Journal of Laboratory and Clinical Medicine* **75** 30–36.
- Li GD, Milani D, Dunne MJ, Pralong WF, Theler JM, Petersen OH & Wollheim CB 1991 Extracellular ATP causes Ca²⁺-dependent and -independent insulin secretion in RINm5F cells. Phospholipase C mediates Ca²⁺ mobilization but not Ca²⁺ influx and membrane depolarization. *Journal of Biological Chemistry* **266** 3449–3457.
- Lingard JM & Young JA 1983 β -Adrenergic control of exocrine secretion by perfused rat pancreas *in vitro*. *American Journal of Physiology* **245** G690–G696.
- Lingard JM & Young JA 1984 Adrenergic secretomotor control of the rat pancreas. In *Secretion: Mechanism and Control*, pp 271–276. Eds RM Case, JM Lingard & JA Young. Manchester: Manchester University Press.
- Lorrain J, Angel I, Duval N, Eon MT, Oblin A & Langer SZ 1992 Adrenergic and nonadrenergic cotransmitters inhibit insulin secretion during sympathetic stimulation in dogs. *American Journal of Physiology* **263** E72–E78.
- Loubatières AL, Loubatières-Mariani MM & Chapal J 1972 Adenosine triphosphate (ATP), cyclic adenosine 3'5' monophosphate (cyclic 3'5' AMP) and insulin secretion. *Comptes Rendus des Séances de la Société de Biologie et de ses filiales* **166** 1742–1746.
- Loubatières-Mariani MM, Loubatières AL, Chapal J & Valette G 1976 Adenosine triphosphate (ATP) and glucose. Action on insulin and glucagon secretion. *Comptes Rendus des Séances de la Société de Biologie et de ses filiales* **170** 833–836.
- Loubatières-Mariani MM, Chapal J, Lignon F & Valette G 1979 Structural specificity of nucleotides for insulin secretory action from the isolated perfused rat pancreas. *European Journal of Pharmacology* **59** 277–286. (doi:10.1016/0014-2999(79)90291-7)
- Loubatières-Mariani MM, Chapal J & Roye M 1982 Effects of adenosine on the secretions of glucagon and insulin of isolated ad perfused pancreas of the rat. *Comptes Rendus des Séances de la Société de Biologie et de ses filiales* **176** 663–669.
- Loubatières-Mariani MM, Hillaire-Buys D, Chapal J, Bertrand G & Petit P 1997 P₂ purinoceptor agonists: new insulin secretagogues potentially useful in the treatment of non-insulin-dependent diabetes mellitus. In *Purinergic Approaches in Experimental Therapeutics*, pp 253–260. Eds KA Jacobson & MF Jarvis. New York, NY: Wiley-Liss.
- Love JA, Yi E & Smith TG 2007 Autonomic pathways regulating pancreatic exocrine secretion. *Autonomic Neuroscience* **133** 19–34. (doi:10.1016/j.autneu.2006.10.001)
- Lugo-García L, Filhol R, Lajoix AD, Gross R, Petit P & Vignon J 2007 Expression of purinergic P2Y receptor subtypes by INS-1 insulinoma β -cells: a molecular and binding characterization *European Journal of Pharmacology* **568** 54–60. (doi:10.1016/j.ejphar.2007.04.012)
- Lugo-García L, Nadal B, Gomis R, Petit P, Gross R & Lajoix AD 2008 Human pancreatic islets express the purinergic P2Y₁₁ and P2Y₁₂ receptors. *Hormone and Metabolic Research* **40** 827–830. (doi:10.1055/s-0028-1082050)
- Luo X, Zheng W, Yan M, Lee MG & Muallem S 1999 Multiple functional P2X and P2Y receptors in the luminal and basolateral membranes of pancreatic duct cells. *American Journal of Physiology* **277** C205–C215.
- Maedler K, Dharmadhikari G, Schumann DM & Storling J 2009 Interleukin-1 beta targeted therapy for type 2 diabetes. *Expert Opinion on Biological Therapy* **9** 1177–1188. (doi:10.1517/14712590903136888)
- Makino S, Kunitomo K, Muraoka Y, Mizushima Y, Katagiri K & Tochino Y 1980 Breeding of a non-obese, diabetic strain of mice. *Jikken Dobutsu* **29** 1–13.
- Makino H, Manganiello VC & Kono T 1994 Roles of ATP in insulin actions. *Annual Review of Physiology* **56** 273–295. (doi:10.1146/annurev.ph.56.030194.001421)

- Martin SS & Senior AE 1980 Membrane adenosine triphosphatase activities in rat pancreas. *Biochimica et Biophysica Acta* **602** 401–418. (doi:10.1016/0005-2736(80)90320-X)
- Mikhail TH & Awadallah R 1977 The effect of ATP and certain trace elements on the induction of experimental diabetes. *Zeitschrift für Ernährungswissenschaft* **16** 176–183. (doi:10.1007/BF02024790)
- Montserrat C, Merten M & Figarella C 1996 Defective ATP-dependent mucin secretion by cystic fibrosis pancreatic epithelial cells. *FEBS Letters* **393** 264–268. (doi:10.1016/0014-5793(96)00900-3)
- Morin L, Giroix MH & Portha B 1996 Decreased ATP-induced synthesis and Ca^{2+} -stimulated degradation of polyphosphoinositides in pancreatic islets from neonatally streptozotocin-diabetic rats. *Biochemical and Biophysical Research Communications* **228** 573–578. (doi:10.1006/bbrc.1996.1700)
- Munkonda MN, Pelletier J, Ivanenkov VV, Fausther M, Tremblay A, Künzli B, Kirley TL & Sévigny J 2009 Characterization of a monoclonal antibody as the first specific inhibitor of human NTP diphosphohydrolase-3: partial characterization of the inhibitory epitope and potential applications. *FEBS Journal* **276** 479–496. (doi:10.1111/j.1742-4658.2008.06797.x)
- Nakhoda AF, Like AA, Chappel CI, Murray FT & Marliss EB 1977 The spontaneously diabetic Wistar rat. Metabolic and morphologic studies. *Diabetes* **26** 100–112. (doi:10.2337/diabetes.26.2.100)
- Németh ZH, Bleich D, Csóka B, Pacher P, Mabley JG, Himer L, Vizi ES, Deitch EA, Szabó C, Cronstein BN *et al.* 2007 Adenosine receptor activation ameliorates type 1 diabetes. *FASEB Journal* **21** 2379–2388. (doi:10.1096/fj.07-8213com)
- Nguyen TD, Moody MW, Savard CE & Lee SP 1998 Secretory effects of ATP on nontransformed dog pancreatic duct epithelial cells. *American Journal of Physiology* **275** G104–G113.
- Nguyen TD, Meichle S, Kim US, Wong T & Moody MW 2001 P2Y₁₁, a purinergic receptor acting via cAMP, mediates secretion by pancreatic duct epithelial cells. *American Journal of Physiology. Gastrointestinal and Liver Physiology* **280** G795–G804.
- Noji T, Nan-ya K, Mizutani M, Katagiri C, Sano J, Takada C, Nishikawa S, Karasawa A & Kusaka H 2002 KF24345, an adenosine uptake inhibitor, ameliorates the severity and mortality of lethal acute pancreatitis via endogenous adenosine in mice. *European Journal of Pharmacology* **454** 85–93. (doi:10.1016/S0014-2999(02)02476-7)
- Novak I 1998 β -Adrenergic regulation of ion transport in pancreatic ducts: patch-clamp study of isolated rat pancreatic ducts. *Gastroenterology* **115** 1–9. (doi:10.1016/S0016-5085(98)70151-9)
- Novak I 2003 ATP as a signaling molecule – the exocrine focus. *News in Physiological Sciences* **18** 12–17. (doi:10.1152/nips.01409.2002)
- Novak I 2008 Purinergic receptors in the endocrine and exocrine pancreas. *Purinergic Signalling* **4** 237–253. (doi:10.1007/s11302-007-9087-6)
- Novak I 2011 Purinergic signalling in epithelial ion transport – regulation of secretion and absorption. *Acta Physiologica* **202** 501–522. (doi:10.1111/j.1748-1716.2010.02225.x)
- Novak I, Nitschke R & Amstrup J 2002 Purinergic receptors have different effects in rat exocrine pancreas. Calcium signals monitored by fura-2 using confocal microscopy. *Cellular Physiology and Biochemistry* **12** 83–92. (doi:10.1159/000063784)
- Novak I, Amstrup J, Henriksen KL, Hede SE & Sørensen CE 2003 ATP release and effects in pancreas. *Drug Development Research* **59** 128–1357. (doi:10.1002/ddr.10192)
- Novak I, Hede SE & Hansen MR 2008 Adenosine receptors in rat and human pancreatic ducts stimulate chloride transport. *Pflügers Archiv* **456** 437–447. (doi:10.1007/s00424-007-0403-3)
- Novak I, Jans IM & Wohlfahrt L 2010 Effect of P2X₇ receptor knockout on exocrine secretion of pancreas, salivary glands and lacrimal glands. *Journal of Physiology* **588** 3615–3627. (doi:10.1113/jphysiol.2010.190017)
- Nukatsuka M, Yoshimura Y, Nishida M & Kawada J 1990 Importance of the concentration of ATP in rat pancreatic β cells in the mechanism of streptozotocin-induced cytotoxicity. *Journal of Endocrinology* **127** 161–165. (doi:10.1677/joe.0.1270161)
- Obermüller S, Lindqvist A, Karanauskaitė J, Galvanovskis J, Rorsman P & Barg S 2005 Selective nucleotide-release from dense-core granules in insulin-secreting cells. *Journal of Cell Science* **118** 4271–4282. (doi:10.1242/jcs.02549)
- Ohtani M, Suzuki J, Jacobson KA & Oka T 2008 Evidence for the possible involvement of the P2Y₆ receptor in Ca^{2+} mobilization and insulin secretion in mouse pancreatic islets. *Purinergic Signalling* **4** 365–375. (doi:10.1007/s11302-008-9122-2)
- Ohtani M, Ohura K & Oka T 2011 Involvement of P2X receptors in the regulation of insulin secretion, proliferation and survival in mouse pancreatic β -cells. *Cellular Physiology and Biochemistry* **28** 355–366. (doi:10.1159/000331752)
- Ooi CY, Gonska T, Durie PR & Freedman SD 2010 Genetic testing in pancreatitis. *Gastroenterology* **138** 2202–6, 2206. (doi:10.1053/j.gastro.2010.04.022)
- O'Reilly CM, O'Farrell AM & Ryan MP 1998 Purinoceptor activation of chloride transport in cystic fibrosis and CFTR-transfected pancreatic cell lines. *British Journal of Pharmacology* **124** 1597–1606. (doi:10.1038/sj.bjp.0701990)
- Parandeh F, Abaraviciene SM, Amisten S, Erlinge D & Salehi A 2008 Uridine diphosphate (UDP) stimulates insulin secretion by activation of P2Y₆ receptors. *Biochemical and Biophysical Research Communications* **370** 499–503. (doi:10.1016/j.bbrc.2008.03.119)
- Pellegatti P, Raffaghello L, Bianchi G, Piccardi F, Pistoia V & Di Virgilio F 2008 Increased level of extracellular ATP at tumor sites: *in vivo* imaging with plasma membrane luciferase. *PLoS ONE* **3** e2599. (doi:10.1371/journal.pone.0002599)
- Petit P, Manteghetti M, Puech R & Loubatières-Mariani MM 1987 ATP and phosphate-modified adenine nucleotide analogues. Effects on insulin secretion and calcium uptake. *Biochemical Pharmacology* **36** 377–380. (doi:10.1016/0006-2952(87)90297-8)
- Petit P, Bertrand G, Schmeer W & Henquin JC 1989 Effects of extracellular adenine nucleotides on the electrical, ionic and secretory events in mouse pancreatic beta-cells. *British Journal of Pharmacology* **98** 875–882.
- Petit P, Hillaire-Buys D, Manteghetti M, Debrus S, Chapal J & Loubatières-Mariani MM 1998 Evidence for two different types of P2 receptors stimulating insulin secretion from pancreatic B cell. *British Journal of Pharmacology* **125** 1368–1374. (doi:10.1038/sj.bjp.0702214)
- Petit P, Hillaire-Buys D, Loubatières-Mariani MM & Chapal J 2001 Purinergic receptors and the pharmacology of type 2 diabetes. In *Handbook of Experimental Pharmacology. Purinergic and Pyrimidinergic Signalling II – Cardiovascular, Respiratory, Immune, Metabolic and Gastrointestinal Tract Function*, 151/11nd edn, pp 337–391. Eds MP Abbracchio & M Williams. Berlin: Springer-Verlag.
- Petit P, Lajoie AD & Gross R 2009 P2 purinergic signalling in the pancreatic beta-cell: control of insulin secretion and pharmacology. *European Journal of Pharmaceutical Sciences* **37** 67–75. (doi:10.1016/j.ejps.2009.01.007)
- Poulsen CR, Bokvist K, Olsen HL, Hoy M, Capito K, Gilon P & Gromada J 1999 Multiple sites of purinergic control of insulin secretion in mouse pancreatic beta-cells. *Diabetes* **48** 2171–2181. (doi:10.2337/diabetes.48.11.2171)
- Quéré I, Hillaire-Buys D, Brunschwig C, Chapal J, Janbon C, Blayac JP, Petit P & Loubatières-Mariani MM 1997 Effects of homocysteine on acetylcholine- and adenosine-induced vasodilatation of pancreatic vascular bed in rats. *British Journal of Pharmacology* **122** 351–357. (doi:10.1038/sj.bjp.0701358)
- Raeder H, Johansson S, Holm PI, Haldorsen IS, Mas E, Sbarra V, Nermoen I, Eide SA, Grevle L, Bjorkhaug L *et al.* 2006 Mutations in the CEL VNTR cause a syndrome of diabetes and pancreatic exocrine dysfunction. *Nature Genetics* **38** 54–62. (doi:10.1038/ng1708)
- Rakieten N, Rakieten ML & Nadkarni MV 1963 Studies on the diabetogenic action of streptozotocin (Nsc-37917). *Cancer Chemotherapy Reports* 91–98.
- Rerup CC 1970 Drugs producing diabetes through damage of the insulin secreting cells. *Pharmacological Reviews* **22** 485–518.
- Richards-Williams C, Contreras JL, Berecek KH & Schwiebert EM 2008 Extracellular ATP and zinc are co-secreted with insulin and activate multiple P2X purinergic receptor channels expressed by islet beta-cells to potentiate insulin secretion. *Purinergic Signalling* **4** 393–405. (doi:10.1007/s11302-008-9126-y)
- Rodrigue-Candela JL, Martin-Hernandez D & Castilla-Cortazar T 1963 Stimulation of insulin secretion *in vitro* by adenosine triphosphate. *Nature* **197** 1304. (doi:10.1038/1971304a0)

- Rodriguez-Diaz R, Abdulreda MH, Formoso AL, Gans I, Ricordi C, Berggren PO & Caicedo A 2011 Innervation patterns of autonomic axons in the human endocrine pancreas. *Cell Metabolism* **14** 45–54. (doi:10.1016/j.cmet.2011.05.008)
- Rodriguez-Nodal F, San Roman JI, Lopez-Novoa JM & Calvo JJ 1995 Effect of adenosine and adenosine agonists on amylase release from rat pancreatic lobules. *Life Sciences* **57** L253–L258. (doi:10.1016/0024-3205(95)02140-E)
- Rosengren AH, Jokubka R, Tojjar D, Granhall C, Hansson O, Li DQ, Nagaraj V, Reinbothe TM, Tuncel J, Eliasson L *et al.* 2010 Overexpression of alpha2A-adrenergic receptors contributes to type 2 diabetes. *Science* **327** 217–220. (doi:10.1126/science.1176827)
- Rusing D, Muller CE & Verspohl EJ 2006 The impact of adenosine and A_{2B} receptors on glucose homeostasis. *Journal of Pharmacy and Pharmacology* **58** 1639–1645. (doi:10.1211/jpp.58.12.0011)
- Salehi A, Qader SS, Grapengiesser E & Hellman B 2005 Inhibition of purinoceptors amplifies glucose-stimulated insulin release with removal of its pulsatility. *Diabetes* **54** 2126–2131. (doi:10.2337/diabetes.54.7.2126)
- Salehi A, Qader SS, Grapengiesser E & Hellman B 2007 Pulses of somatostatin release are slightly delayed compared with insulin and antisynchronous to glucagon. *Regulatory Peptides* **144** 43–49. (doi:10.1016/j.regpep.2007.06.003)
- Salehi A, Parandeh F, Fredholm BB, Grapengiesser E & Hellman B 2009 Absence of adenosine A1 receptors unmasks pulses of insulin release and prolongs those of glucagon and somatostatin. *Life Sciences* **85** 470–476. (doi:10.1016/j.lfs.2009.08.001)
- Santini E, Cuccato S, Madec S, Chimenti D, Ferrannini E & Solini A 2009 Extracellular adenosine 5'-triphosphate modulates insulin secretion via functionally active purinergic receptors of X and Y subtype. *Endocrinology* **150** 2596–2602. (doi:10.1210/en.2008-1486)
- Satoh A, Shimosegawa T, Satoh K, Ito H, Kohno Y, Masamune A, Fujita M & Toyota T 2000 Activation of adenosine A1-receptor pathway induces edema formation in the pancreas of rats. *Gastroenterology* **119** 829–836. (doi:10.1053/gast.2000.16502)
- Sawada K, Echigo N, Nuge N, Miyaji T, Otsuka M, Omote H, Yamamoto A & Moriyama Y 2008 Identification of a vesicular nucleotide transporter. *PNAS* **105** 5683–5686. (doi:10.1073/pnas.0800141105)
- Sévigny J, Côté YP & Beaudoin AR 1995 Purification of pancreas type-I ATP diphosphohydrolase and identification by affinity labelling with the 5'-p-fluorosulphonylbenzoyl adenosine ATP analogue. *Biochemical Journal* **312** 351–356.
- da Silva MCJ, Cabrera O, Ricordi C, Berggren PO & Caicedo A 2007 Extracellular ATP is a positive autocrine signal for insulin release in the human pancreatic beta-cell. *FASEB Journal* **21** A829–A830.
- Silva AM, Rodrigues RJ, Tome AR, Cunha RA, Misler S, Rosario LM & Santos RM 2008 Electrophysiological and immunocytochemical evidence for P2X purinergic receptors in pancreatic β cells. *Pancreas* **36** 279–283. (doi:10.1097/MPA.0b013e31815a8473)
- Silvestre RA, Rodríguez-Gallardo J, Egidio EM & Marco J 1999 Stimulatory effect of exogenous diadenosine tetraphosphate on insulin and glucagon secretion in the perfused rat pancreas. *British Journal of Pharmacology* **128** 795–801. (doi:10.1038/sj.bjp.0702837)
- Sone H, Sasaki Y, Komai M, Toyomizu M, Kagawa Y & Furukawa Y 2004 Biotin enhances ATP synthesis in pancreatic islets of the rat, resulting in reinforcement of glucose-induced insulin secretion. *Biochemical and Biophysical Research Communications* **314** 824–829. (doi:10.1016/j.bbrc.2003.12.164)
- Sørensen CE & Novak I 2001 Visualization of ATP release in pancreatic acini in response to cholinergic stimulus. Use of fluorescent probes and confocal microscopy. *Journal of Biological Chemistry* **276** 32925–32932. (doi:10.1074/jbc.M103313200)
- Sørensen CE, Amstrup J, Rasmussen HN, Ankori-Stark I & Novak I 2003 Rat pancreas secretes particulate ecto-nucleotidase CD39. *Journal of Physiology* **551** 881–892. (doi:10.1113/jphysiol.2003.049411)
- Squires PE, James RF, London NJ & Dunne MJ 1994 ATP-induced intracellular Ca²⁺ signals in isolated human insulin-secreting cells. *Pflügers Archiv* **427** 181–183. (doi:10.1007/BF00585959)
- Stam NJ, Klomp J, Van de Heuvel N & Olijve W 1996 Molecular cloning and characterization of a novel orphan receptor (P_{2U}) expressed in human pancreas that shows high structural homology to the P_{2U} purinoceptor. *FEBS Letters* **384** 260–264. (doi:10.1016/0014-5793(96)00321-3)
- Steward MC, Ishiguro H & Case RM 2005 Mechanisms of bicarbonate secretion in the pancreatic duct. *Annual Review of Physiology* **67** 377–409. (doi:10.1146/annurev.physiol.67.031103.153247)
- Sussman KE & Leitner JW 1977 Conversion of ATP into other adenine nucleotides within isolated islet secretory vesicles. Effect of cyclic AMP on phosphorus translocation. *Endocrinology* **101** 694–701. (doi:10.1210/endo-101-3-694)
- Sussman KE, Vaughan GD & Stjernholm MR 1969 Factors controlling insulin secretion in the perfused isolated rat pancreas. In *Diabetes: Proceedings of the 6th Congress of the International Diabetes Federation*, pp 123. Ed J Ostman. Amsterdam: Excerpta Medica Foundation.
- Szűcs A, Demeter I, Burghardt B, Óvári G, Case RM, Steward MC & Varga G 2006 Vectorial bicarbonate transport by Capan-1 cells: a model for human pancreatic ductal secretion. *Cellular Physiology and Biochemistry* **18** 253–264. (doi:10.1159/000097672)
- Tahani HM 1979 The purinergic nerve hypothesis and insulin secretion. *Zeitschrift für Ernährungswissenschaft* **18** 128–138. (doi:10.1007/BF02023727)
- Tan C, Salehi A, Svensson S, Olde B & Erlinge D 2010 ADP receptor P2Y₁₃ induce apoptosis in pancreatic β -cells. *Cellular and Molecular Life Sciences* **67** 445–453. (doi:10.1007/s00018-009-0191-3)
- Tang J, Pugh W, Polonsky KS & Zhang H 1996 Preservation of insulin secretory responses to P2 purinoceptor agonists in Zucker diabetic fatty rats. *American Journal of Physiology* **270** E504–E512.
- Töpfer M, Burbiel CE, Müller CE, Knittel J & Verspohl EJ 2008 Modulation of insulin release by adenosine A₁ receptor agonists and antagonists in INS-1 cells: the possible contribution of ⁸⁶Rb⁺ efflux and ⁴⁵Ca²⁺ uptake. *Cell Biochemistry and Function* **26** 833–843. (doi:10.1002/cbf.1514)
- Tudurí E, Filiputti E, Carneiro EM & Quesada I 2008 Inhibition of Ca²⁺ signaling and glucagon secretion in mouse pancreatic alpha-cells by extracellular ATP and purinergic receptors. *American Journal of Physiology. Endocrinology and Metabolism* **294** E952–E960. (doi:10.1152/ajpendo.00641.2007)
- Verspohl EJ, Johannwille B, Waheed A & Neye H 2002 Effect of purinergic agonists and antagonists on insulin secretion from INS-1 cells (insulinoma cell line) and rat pancreatic islets. *Canadian Journal of Physiology and Pharmacology* **80** 562–568. (doi:10.1139/y02-079)
- Wang CZ, Namba N, Gonoi T, Inagaki N & Seino S 1996 Cloning and pharmacological characterization of a fourth P2X receptor subtype widely expressed in brain and peripheral tissues including various endocrine tissues. *Biochemical and Biophysical Research Communications* **220** 196–202. (doi:10.1006/bbrc.1996.0380)
- Wang J, Wan R, Mo Y, Li M, Zhang Q & Chien S 2010 Intracellular delivery of adenosine triphosphate enhanced healing process in full-thickness skin wounds in diabetic rabbits. *American Journal of Surgery* **199** 823–832. (doi:10.1016/j.amjsurg.2009.05.040)
- Weir GC, Knowlton SD & Martin DB 1975 Nucleotide and nucleoside stimulation of glucagon secretion. *Endocrinology* **97** 932–936. (doi:10.1210/endo-97-4-932)
- de Wet H, Proks P, Lafond M, Aittoniemi J, Sansom MS, Flanagan SE, Pearson ER, Hattersley AT & Ashcroft FM 2008 A mutation (R826W) in nucleotide-binding domain 1 of ABCC8 reduces ATPase activity and causes transient neonatal diabetes. *EMBO Reports* **9** 648–654. (doi:10.1038/embor.2008.71)
- Wilschanski M & Novak I 2012 The CF of exocrine pancreas. In *Cystic Fibrosis: Molecular Basis, Physiological Changes, and Therapeutic Strategies*. Eds JR Riordan, RC Boucher & P Quinton. Cold Spring Harbor: Cold Spring Harbor Press.
- Won JH, Zhang Y, Ji B, Logsdon CD & Yule DI 2011 Phenotypic changes in mouse pancreatic stellate cell Ca²⁺ signaling events following activation in culture and in a disease model of pancreatitis. *Molecular Biology of the Cell* **22** 421–436. (doi:10.1091/mbc.E10-10-0807)
- Yabe D & Seino Y 2011 Two incretin hormones GLP-1 and GIP: comparison of their actions in insulin secretion and beta cell preservation. *Progress in Biophysics and Molecular Biology* **107** 248–256. (doi:10.1016/j.pbiomolbio.2011.07.010)

- Yamada T, Okajima F, Akbar M, Tomura H, Narita T, Yamada T, Ohwada S, Morishita Y & Kondo Y 2002 Cell cycle arrest and the induction of apoptosis in pancreatic cancer cells exposed to adenosine triphosphate *in vitro*. *Oncology Reports* **9** 113–117.
- Yamagishi F, Homma N, Haruta K, Iwatsuki K & Chiba S 1985 Adenosine potentiates secretin-stimulated pancreatic exocrine secretion in the dog. *European Journal of Pharmacology* **118** 203–209. (doi:10.1016/0014-2999(85)90130-X)
- Yamagishi F, Homma N, Haruta K, Iwatsuki K & Chiba S 1986 Effects of three purine-related compounds on pancreatic exocrine secretion in the dog. *Clinical and Experimental Pharmacology & Physiology* **13** 425–432. (doi:10.1111/j.1440-1681.1986.tb00921.x)
- Yamano K, Mori K, Nakano R, Kusunoki M, Inoue M & Satoh M 2007 Identification of the functional expression of adenosine A₃ receptor in pancreas using transgenic mice expressing jellyfish apoaeguorin. *Transgenic Research* **16** 429–435. (doi:10.1007/s11248-007-9084-0)
- Yegutkin GG, Samburski SS, Jalkanen S & Novak I 2006 ATP-consuming and ATP-generating enzymes secreted by pancreas. *Journal of Biological Chemistry* **281** 29441–29447. (doi:10.1074/jbc.M602480200)
- Yegutkin GG, Marttila-Ichihara F, Karikoski M, Niemela J, Laurila JP, Elima K, Jalkanen S & Salmi M 2011 Altered purinergic signaling in CD73-deficient mice inhibits tumor progression. *European Journal of Immunology* **41** 1231–1241. (doi:10.1002/eji.201041292)
- Yi E, Smith TG & Love JA 2005 Noradrenergic innervation of rabbit pancreatic ganglia. *Autonomic Neuroscience* **117** 87–96. (doi:10.1016/j.autneu.2004.11.004)
- Zhao Z, Zhao C, Zhang XH, Zheng F, Cai W, Vlassara H & Ma ZA 2009 Advanced glycation end products inhibit glucose-stimulated insulin secretion through nitric oxide-dependent inhibition of cytochrome c oxidase and adenosine triphosphate synthesis. *Endocrinology* **150** 2569–2576. (doi:10.1210/en.2008-1342)
- Zhu JX, Yang N, Zhu H, Chung YW & Chan HC 2007 Effect of NYD-SP27 down-regulation on ATP-induced Ca²⁺-dependent pancreatic duct anion secretion in cystic fibrosis cells. *Cell Biology International* **31** 521–525. (doi:10.1016/j.cellbi.2006.11.021)
- Zsembery A, Strazzabosco M & Graf J 2000 Ca²⁺-activated Cl[−] channels can substitute for CFTR in stimulation of pancreatic duct bicarbonate secretion. *FASEB Journal* **14** 2345–2356. (doi:10.1096/fj.99-0509com)

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