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REVIEW

Purinergic receptors in the endocrine and exocrine pancreas

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Abstract The pancreas is a complex gland performing both endocrine and exocrine functions. In recent years there has been increasing evidence that both endocrine and exocrine cells possess purinergic receptors, which influence processes such as insulin secretion and epithelial ion transport. Most commonly, these processes have been viewed separately. In β cells, stimulation of $P2Y_1$ receptors amplifies secretion of insulin in the presence of glucose. Nucleotides released from secretory granules could also contribute to autocrine/paracrine regulation in pancreatic islets. In addition to $P2Y_1$ receptors, there is also evidence for other P2 and adenosine receptors in β cells ($P2Y_2$, $P2Y_4$, $P2Y_6$, P2X subtypes and A_1 receptors) and in glucagon-secreting α cells ($P2X_7$, A_2 receptors). In the exocrine pancreas, acini release ATP and ATP-hydrolysing and ATP-generating enzymes. P2 receptors are prominent in pancreatic ducts, and several studies indicate that $P2Y_2$, $P2Y_4$, $P2Y_{11}$, $P2X_4$ and $P2X_7$ receptors could regulate secretion, primarily by affecting Cl^- and K^+ channels and intracellular Ca^{2+} signalling. In order to understand the physiology of the whole organ, it is necessary to consider the full complement of purinergic receptors on different cells as well as the structural and functional relation between various cells within the whole organ. In addition to the possible physiological function of purinergic receptors, this review analyses whether the receptors could be potential therapeutic targets for drug design aimed at treatment of pancreatic diseases.

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Abbreviations

AC	adenylate cyclase
BK	big conductance K^+ channel
BzATP	2′&3′-*O*-(4-benzoyl-benzoyl)-ATP
CFTR	cystic fibrosis transmembrane regulator
CX	connexin
DAG	diacylglycerol
ERK	extracellular signal regulated kinase
GLP-1	glucagon-like peptide-1
IK	intermediate conductance K^+ channel
IP_3	inositol 1,4,5 triphosphate
NBC	sodium bicarbonate cotransporter
NECA	adenosine-5′*N*-ethylcarboxamide
NHE	sodium hydrogen exchanger
NO	nitric oxide
NTPDase	nucleoside triphosphate diphosphohydrolase
PKA	protein kinase A
PKC	protein kinase C
PLA_2	protein lipase A_2
PLC	phospholipase C
SK	small conductance K^+ channel
VIP	vasoactive intestinal peptide

Structural and functional basis for pancreatic function

Endocrine cells in the islets of Langerhans constitute only 3-5% of the tissue mass of the pancreas. The islets of Langerhans are dispersed throughout the organ and comprise α, β, δ and PP cells, which secrete the hormones

glucagon, insulin and amylin, somatostatin and pancreatic polypeptide, respectively. The bulk of the gland is exocrine, comprising 70-90% of acinar cells and 5-25% of duct cells, depending on the species [1–3]. Acini secrete fluid containing NaCl and a variety of digestive enzymes including active enzymes (α-amylase, lipase, colipase, carboxyl ester lipase, RNAse, DNAse), zymogens (trypsinogen, chymotrypsinogen, procarboxypeptidases A and B, proelastase and proprotease E) and other proteins (e.g. trypsin inhibitor, pancreatitis-associated protein and lithostathine). This enzyme-rich secretion is led through a series of excurrent ducts, which secrete a $NaHCO_3$-rich fluid. The pancreatic juice aids enzymatic breakdown of various macromolecules in the duodenum and together with the bile and duodenal secretions it contributes to neutralization of acid chyme entering the duodenum.

Both endocrine and exocrine cells are regulated by parasympathetic and sympathetic nerves, hormones, autocrine and paracrine mediators as well as nutrients like glucose. The integrated function of the organ is on the one hand due to the contribution to digestive processes in the gastrointestinal tract, thanks to secretion of pancreatic enzymes, and on the other hand due to contribution to regulation of the body and cellular metabolism, thanks to the pancreatic hormones. In the latter process, the prime function is the production of the energy source, ATP. In recent years it has become accepted that ATP and other nucleotides/nucleosides have extracellular roles. They are (1) released from cells; (2) they act extracellularly via specific purinergic receptors as autocrine, paracrine and neural regulators; and (3) they are hydrolysed by various ectoenzymes. The aim of this review is to address the question of whether such "purinergic signalling" is important for regulation of pancreatic endocrine and exocrine functions—both in health and disease. The focus will be on purinergic receptors, which by virtue of a variety in molecular structures, signalling pathways and distribution could regulate many processes in this complex organ.

Endocrine pancreas

β cells

A peculiar feature of β cells, and also α cells, is that they use ATP in three processes: (1) as an energy source, (2) as an intracellular second messenger acting on K^+ channels and (3) as an extracellular regulator. The latter role was discovered quite early in the purinergic era. In the 1960-1970s it was reported that exogenous ATP stimulated insulin release in various preparations of rat and rabbit pancreas as well as in in vivo experiments performed on man and monkey [4–7]. In 1979 Loubatieres-Mariani et al. [6] proposed that purinergic receptors control the β-cell function. Those early studies established that ATP and ADP were potentiators of insulin secretion, which was initiated by glucose entry. Purines (ATP and ADP) were more effective than pyrimidines, and adenosine had an unusual dose-dependent effect on insulin secretion (see below). The following paragraphs will focus on the effects of ATP/ADP-preferring P2 receptors and adenosine-preferring P1 receptors and extend on the basic model of insulin secretion (Fig. 1).

P2 receptors Purinergic receptors have been studied extensively in in vivo and in vitro preparations of rat, mouse and dog pancreas, as well as on islet preparations of these, and also on the human pancreas and several insulinoma cell lines. In all preparations, except those originating from the mouse pancreas (see below), ATP and ADP induced insulin secretion in the presence of glucose. The identity of purinergic receptors on β cells was deduced from studies using pharmacological tools and monitoring effects on, for example, insulin release and intracellular Ca^{2+} signals. ATP and its analogues (e.g. 2-methylthio ATP, ATPγS) induced insulin secretion, but ADP and its analogues (α,β-methylene ADP, ADPβS, ADPγS) were more potent and they required at least slightly stimulating glucose concentrations (8.3 mM) [8–12]. These findings indicated presence of an ADP-preferring receptor, such as the $P2Y_1$ receptor [13]. Supporting evidence for this receptor is the finding that the $P2Y_1$ inhibitor MRS2179 successfully eliminated the ADP/ATP effect on β cells. Recent studies concentrated on developing thioester nucleotides specific for $P2Y_1$ receptors [11, 14, 15]. The molecular evidence for the $P2Y_1$ receptor was provided by cloning of the receptor from rat and mouse insulinoma cells, i.e. RINm5F and MIN6, respectively [16]. Another P2 receptor was cloned from the human pancreas; this turned out to be the $P2Y_4$ receptor [17, 18], which may originate from pancreatic ducts as well as from β cells (see below).

Although the $P2Y_1$ receptor may be the most important P2Y receptor in regulating insulin secretion, there is also evidence for other P2Y receptors [19–21], and also P2X receptors [12, 22–24], and indeed the $P2X_4$ receptor was cloned from rat pancreatic islets [25]. Evidence for P2X-type receptors was provided by intracellular Ca^{2+} measurements. For example, in the hamster clonal β-cell line HIT, ATP stimulated Ca^{2+} influx rather than Ca^{2+} store release, indicating receptor-operated channels, which could be P2X-type receptors [22]. Petit et al. [12] found on rat β cells and RIN cells that α,β-methylene ATP (P2X receptor agonist) had transient effects on insulin release and inhibited K^+ efflux, and interestingly this happened even at non-stimulating glucose concentrations. A similar finding was made on human islets with α,β-methylene ATP and low

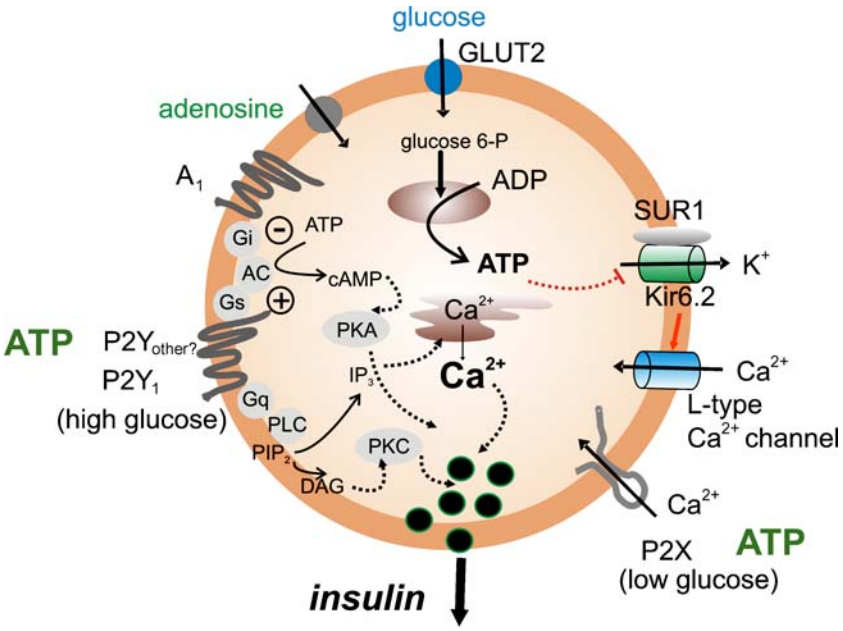

Fig. 1 Role of purinergic receptors in regulation of insulin secretion in the β cell. Glucose enters the cell via the facilitative GLUT-2 transporter. Metabolism of glucose leads to production of ATP, which closes the ATP-sensitive channel, K_{ATP}, comprising four Kir6.2 and SUR1 subunits. Closure of K_{ATP} depolarises the cell membrane and opens voltage-gated L-type Ca^{2+} channels and Ca^{2+} action potentials are generated. Increase in the cellular Ca^{2+} is the triggering event that leads to exocytosis of secretory vesicles containing insulin. Stimulation of P2 receptors can increase triggering and amplification of signals associated with the glucose effect on insulin secretion. The $P2Y_1$ receptors increase cellular Ca^{2+} and activate PKC pathways. In addition, the $P2Y_1$ or other P2Y receptors can activate the cyclic AMP pathway. Putative P2X receptors allow Ca^{2+}/Na^+ influx and membrane depolarisation, and thereby they can elicit some insulin secretion even at low glucose concentrations. Adenosine interacts with the A_1 receptors, which inhibit the cyclic AMP pathways and thereby insulin secretion. At high adenosine concentrations, it is postulated that some adenosine transported into the β cell exerts metabolic effects

glucose concentrations [10]. Notably, ADPβS ($P2Y_1$ receptor agonist) first became effective when the glucose concentration was increased from 3 to 8.3 mM, i.e. from low to slightly stimulating glucose concentrations (Fig. 1) [10, 12].

There are only a few immunohistochemical studies on P2 receptor distribution in the pancreas, and they reveal an unusual pattern [21, 26–28]. $P2Y_1$ receptors were not detected in rat and mouse islets, as one would have expected from functional studies, but instead they were found on capillaries. Clear immunolocalization of $P2X_7$ receptors was found in α cells and transiently in β cells of developing rat pancreas. In early non-obese diabetic mice, $P2X_7$ receptor-labelled cells migrated to the centre of islets, but disappeared at the later stage, and although macrophages and dendritic cells invaded the islets they showed no $P2X_7$ receptor immunoreactivity. Also $P2X_1$ and $P2X_4$ receptors were localised in the islets of adult animals, but they were absent in the neonate animals. $P2Y_4$ receptor immunoreactivity was detected in β cells (and α cells) and staining seemed to increase in the islets of diabetic rats.

Very recently a new report appeared on the molecular characterization of P2 receptors in INS-1 cells [20]. It was shown that INS-1 cells indeed express $P2Y_1$ receptors. In addition, they also express $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptors, which stimulate other Ca^{2+}/PKC signalling, as well as $P2Y_{12}$ receptors, which are coupled to G_i proteins and may have inhibitory function via the cAMP/PKA signalling. Similar molecular characterization of human β cells and other islet cells will be necessary for future elucidation of pharmacological and functional profiles.

What intracellular signals elicited by purinergic receptors in β cells could potentiate Ca^{2+}-dependent exocytosis of insulin? From studies mostly carried out on the insulinoma cell line RINm5F, we learned that extracellular ATP depolarised β cells due to closure of K_{ATP}, and depolarization elicited Ca^{2+} action potentials and Ca^{2+} oscillations [23, 29]. Intracellular Ca^{2+} increase is due to (1) activation of PLC, generation of inositol (1,4,5) triphosphate and Ca^{2+} mobilization from intracellular stores and (2) Ca^{2+} influx via channels that are not L-type voltage-activated channels, perhaps P2X receptors (Fig. 1) [19, 23, 24, 29–31]. Nevertheless, the ATP-induced insulin release can be somewhat independent of extracellular Ca^{2+} [23, 29]. These findings indicate that ATP exerts effect via PLC-dependent and also PLC-independent pathways. Interestingly, when ADPβS was used on β cells and clonal rat insulin-producing INS-1 cells, cyclic AMP and PKA activation enhanced insulin release [32]. Perhaps parallels could be drawn with new studies on INS-1 cells where glucagon and

glucagon-like peptide-1 (GLP-1) elicit interdependent cyclic AMP and Ca^{2+} oscillations [33]. Returning to P2Y receptors, the question is whether one type of receptor, i.e. $P2Y_1$ receptor, stimulates multiple pathways or whether another type of receptor is also involved, e.g. $P2Y_{11}$ receptor, which can signal via different G proteins, G_q and G_s [13]. Taken together, it is well established that P2Y receptors potentiate insulin secretion by a mechanism that involves metabolism of glucose, rise in intracellular Ca^{2+} and possibly cAMP (Fig. 1). The inhibitory effect on K_{ATP} channels is not direct and extracellular, but rather via intracellular events [34].

Let us now consider mouse β cells, which seem a little bit peculiar with respect to P2 receptors and their effect on insulin secretion. In 1989 Petit et al. [9] found that in mouse β cells ADP analogues had inhibitory rather than stimulatory effect on insulin. Poulsen et al. [35] verified the inhibitory effect of ATP on insulin secretion and studied cellular effects. Activity of K_{ATP} channels (measured as whole-cell K^+ currents) and exocytosis (measured as capacitance) were inhibited by 2-methylthio ATP>ATP>ADP>α,β-methylene ATP>UTP, thus indicating participation of $P2Y_1$ receptors and probably also other receptor types. These inhibitory effects on activity K_{ATP} channels and exocytosis involved activation of PLA_2 and calcineurin, respectively. Since the intracellular Ca^{2+} signals and currents were intact, inhibitory action of nucleotides on the secretory machinery lay downstream of Ca^{2+}. Similar inhibitory effects of ATP on insulin release were also observed in the mouse β-cell line MIN6, which has transcripts for $P2Y_4$/$P2Y_6$ receptors [36]. Together, studies to date show that in mouse β cells and MIN6 cells, ATP is more potent than ADP in evoking transient Ca^{2+} spikes [35–37]. In comparison to rat β cells and other preparations, the question is whether mouse β cells have a different complement of P2 receptors or whether $P2Y_1$ receptors have different intracellular coupling to the exocytotic machinery. A recent study on islets isolated from $P2Y_1$-/- mice showed that insulin secretion was increased in the presence of high glucose concentrations. In addition, $P2Y_1$-/- mice exhibited a tendency to glucose intolerance [38]. These findings indicate that $P2Y_1$ receptors do play some physiological role in glucose homeostasis of the mouse.

Adenosine receptors In 1982 Bacher et al. [39] showed on in situ perfused dog pancreas that the adenosine analogue NECA (adenosine-5′*N*-ethylcarboxamide) inhibited insulin release when given in micromolar concentrations, but increased it when given in millimolar concentrations. Similarly, in mouse β cells, low adenosine concentrations inhibited glucose-related electrical activity and insulin release and high adenosine concentrations stimulated it [40]. It is possible that the high-concentration effect was due to transport of adenosine into the cell and subsequent metabolic effects. The inhibitory effect of low-concentration adenosine is exerted via A_1 receptors [7, 19, 40], which via G_i proteins would inhibit adenylate cyclase and thus insulin secretion (Fig. 1). The presence of A_1 receptor could also explain some observations relating to the dual effect of ATP on insulin secretion. That is, the stimulatory effect at low ATP concentrations is exerted via $P2Y_1$ and other receptors as discussed above. In addition, ATP at high concentrations can have inhibitory effects, because it is metabolised to adenosine, which via A_1 receptors would dominate the insulin response [19].

α cells and δ cells

In dog and rat pancreas adenosine and analogues, e.g. NECA, induced dose-dependent glucagon release from α cells, presumably via A_2 receptors [39, 41, 42]. These actions were eliminated by aminophylline and theophylline. Interestingly, Bertrand et al. [43] showed in dog pancreas that 2-methylthio ATP, apart from inducing insulin release, also induced glucagon release. They postulated that since glucagon secretion was delayed with respect to insulin secretion, and since 2-methylthio ATP can be metabolised to adenosine, the effect on glucagon secretion was via adenosine receptors (see Fig. 2). Similarly to β cells, also α cells have K_{ATP} channels, which are more sensitive to ATP, and they play a major role in electrical activity and secretory processes [44, 45].

Regarding δ cells, there are only a few studies available. In low glucose concentrations (4.2 mM), α,β-methylene ADP had no effect on insulin or glucagon secretion, but it induced somatostatin secretion from δ cells—indicating the presence of P2 receptors [43]. A similar finding was made in perfused dog pancreas, where a more stable analogue ADPβS induced release of somatostatin, but not of glucagon [46].

Source of ATP

Pancreatic islets are innervated by both the parasympathetic and sympathetic nerves. From studies of other systems it is known that ATP is released from presynaptic terminals containing other transmitters such as: (1) acetylcholine and vasoactive intestinal peptide (VIP) in some parasympathetic nerves, (2) with noradrenaline and neuropeptide Y in sympathetic nerves and (3) with NO and VIP in enteric inhibitory nerves (Fig. 2) [47–49]. In the pancreas, ATP and acetylcholine have synergistic effects on insulin release [50].

Another source of ATP and nucleotides are insulin-containing granules of β cells [51–54]. Obermuller et al. [55] showed that adenine nucleotides are released with a

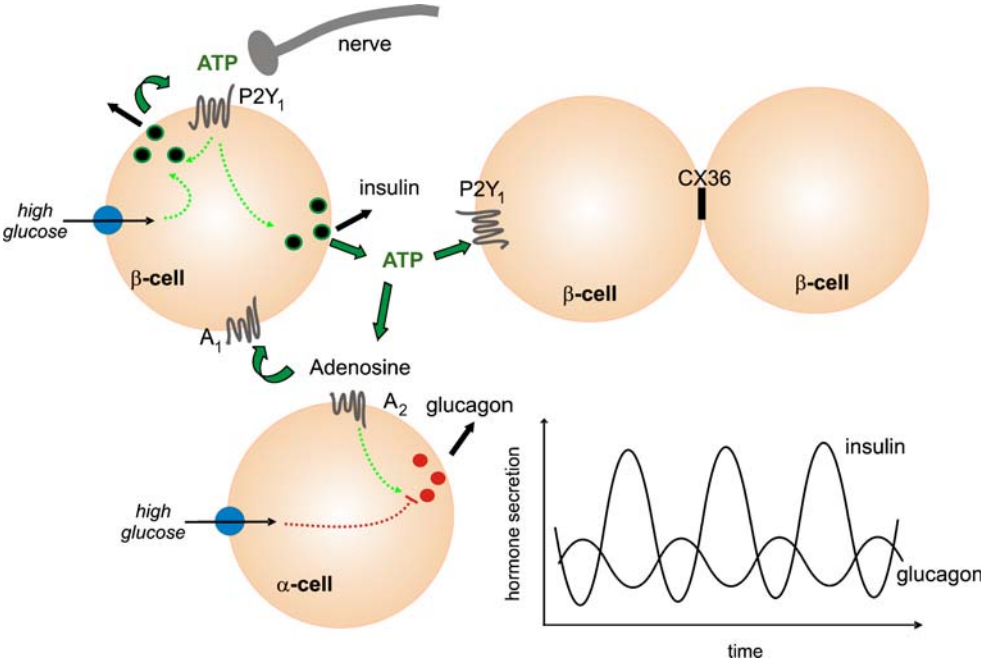

Fig. 2 Sources and roles of nucleotides in a pancreatic islet. Nucleotides are released from nerves or from insulin-containing granules. ATP/ADP can autoregulate insulin release and/or affect coupling between β cells. Extracellular hydrolysis by ectoenzymes leads to production of adenosine that could on the one hand down-regulate insulin secretion from β cells, and on the other hand it could stimulate glucagon release in α cells. High glucose inhibits glucagon release in α cells. The graph shows the theoretical oscillations in insulin and glucagon levels in the presence of high glucose concentrations

very short delay after stimulation, while peptides (insulin) are released later. Using a biosensor technique, Hazama et al. [52] detected ATP release on the cell surface of β cells and estimated the concentration to be about 25 μM. Importantly, glucose alone can cause ATP release, indicating that there is autocrine/paracrine signalling, which could promote insulin release (Fig. 2). Thus, one role for released nucleotides could be potentiation of the glucose effect and perhaps this could explain the biphasic insulin secretion [7] or the oscillatory release of insulin (see below). Another role for released nucleotides could be regulation of exocrine function (see the "Exocrine" section).

Islet as a whole—oscillations and possible role of nucleotides

High glucose induces pulsatile insulin release. This occurs in many species in vivo, in the isolated pancreas and in single islets (see [56]). Insulin oscillations are accompanied by Ca^{2+} oscillations and can be uncoupled in some circumstances, e.g. in *ob/ob* mice, in old animals and with hyperstimulation [57, 58]. Moreover, in type 2 diabetes, the pulsatile pattern of insulin release is impaired [56]. Mechanisms governing oscillations are unclear. A neurogenic component is believed to play a role, but a recent study showed that also coupling between β cells must be important. Loss of connexin, CX36, changes β-cell coupling and islet synchronization of glucose-induced Ca^{2+} and insulin oscillations [59]. In addition, recent studies indicated that also exogenous and endogenous nucleotides may play a role in this rhythmicity. Exogenous ATP (or ADP and 2-methylthio ATP) elicits oscillations in the membrane potential, intracellular Ca^{2+} and PLC [30, 60, 61]. Interestingly, in rat β cells the $P2Y_1$ antagonist MRS2179 inhibited glucose-induced insulin oscillations measured in the portal vein, but strangely it did not inhibit Ca^{2+} oscillations [62].

Glucose is a stimulator of insulin release, but it usually suppresses release of glucagon from α cells in intact islets. Thus, in contrast to β cells, glucagon-secreting α cells presented asynchronous Ca^{2+} oscillatory patterns in response to low glucose concentrations, and this pattern was suppressed in high glucose concentrations [63]. In a recent study on rat preparations, where high frequency sampling of venous effluents was used, it was shown that high glucose induced insulin release and also glucagon release about 180° out of phase, and this was sensitive to the MRS2179 inhibitor [64]. One possible interpretation is that high glucose induced release of insulin and nucleotides, which on the one hand potentiated the effect of glucose on β cells. On the other hand, the hydrolytic product adenosine could then via A_1 receptors on β cells down-regulate release of insulin, and on A_2 receptors of α cells it could stimulate release of glucagon (see model in Fig. 2). Nevertheless, for a physiologically relevant situation, the total amounts of cyclically released insulin and glucagon would have to be coordinated depending on the glucose level: high glucose and ATP should favour insulin secretion; and low glucose and adenosine would favour glucagon release.

Therapeutic potentials for improving insulin secretion

Many drugs can increase insulin secretion in vitro, but only some have a therapeutic potential for treatment of non-insulin-dependent diabetes. The most frequently used drugs to date are sulphonylureas and glinides, which decrease the open probability of K_{ATP} channels, and result in depolarization of the cell membrane, and the increase in cell Ca^{2+} leads to insulin release [61, 65–67]. The action of these drugs is independent of plasma glucose concentrations and therefore it may be difficult to achieve glycaemic control, i.e. they can lead to hypoglycaemia. Since $P2Y_1$ receptor agonists amplify insulin release in the presence of stimulating glucose concentrations, they may potentially reduce the risk of hypoglycaemia. Intravenously and orally administered ADPβS was a potent insulin secretagogue and improved glucose tolerance in the in vivo rat and dog pancreas [46, 68]. P2Y receptor agonists also amplified secretion of insulin on an in vitro pancreas preparation from Zucker diabetic rats [69]. In human islet preparations, P2Y receptor agonists were also successful in inducing glucose-dependent insulin secretion [70]. Nevertheless, it is important to consider possible effects of P2 agonists on blood vessels [71] and also on the exocrine pancreas (see below). Recent efforts have led to the development of C2 substituted ATP analogues [thioester nucleotides such as 2-thioester 5′-*O*-(1-thiotriphosphate) adenosine derivatives], which would be specific for $P2Y_1$ receptors on β cells. Fischer and collaborators found some very effective analogues regarding insulin release (potent in the nanomolar range) and stable regarding degradation by NTPDase, but unfortunately these also had pronounced vasodilatory effects in the rat pancreas [14, 15]. Most recently, a new generation of 2-substituted 5′-*O*-(1-boranotriphosphate) adenosine analogues was developed. One of these, 2-methylthio-ATP-α-B (A isomer) was able to elicit glucose-dependent insulin secretion with high efficacy in rats, and it had minimal effects on pancreatic vascular resistance [11]. Thus, this analogue is a good candidate for future therapeutic developments.

Another approach to increase insulin secretion could be to inhibit A_1 receptors on β cells. Bacher et al. [39] showed that the methylxanthine compound aminophylline attenuated the action of adenosine in the dog pancreas. On human subjects one study showed that aminophylline had no influence on basal insulin secretion, it augmented glucose-induced insulin secretion in healthy subjects, but not in patients with type 2 diabetes [72]. In contrast, another study on a small group of type 2 diabetic patients showed that aminophylline stimulated insulin secretion [73]. Since methylxanthines have many other molecular and systemic effects, future efforts are needed to find β-cell-specific compounds. Apart from the endocrine pancreas, adenosine receptors in other tissues are also targets for diabetes treatment, e.g. stimulation of A_1 receptors in adipocytes inhibits lipolysis and increases glucose metabolism, and inhibition of A_{2B} receptors in hepatocytes would control glucose production [74].

Exocrine pancreas

Pancreatic acini

Pancreatic acini are well studied with respect to mechanism and regulation of enzyme secretion, intracellular Ca^{2+} signalling and ion transporters involved in fluid secretion. Compared to acini of other exocrine glands, knowledge regarding the role of purinergic receptors in pancreatic acini is limited [75, 76]. Reverse transcriptase polymerase chain reaction (RT-PCR) analysis on single acini from the rat pancreas revealed that they contain transcripts for $P2X_1$, $P2X_4$, $P2Y_2$ and $P2Y_4$ receptors [77]. Interestingly, no transcript for $P2X_7$ receptors was found, although these receptors are expressed in ducts (see below) and also in acini of other exocrine glands [78–81]. Clearly, $P2X_7$ receptors, which can form lytic pores under some conditions, would be dangerous in pancreatic acini filled with digestive enzymes. Their untimely activation and release could initiate autodigestive processes, such as occurs in pancreatitis. In this light, it will be important to establish whether human pancreatic acini also lack $P2X_7$ receptors.

Functional studies using intracellular Ca^{2+} measurements and measurement of organic anion uptake on single cells within acini revealed than only about 10-20% of cells responded to extracellular ATP or UTP [77, 82]. Thus, it seems that there are only a few cells within an acinus or a few acini that have functional P2 receptors, and that P2 receptor-induced effects remain contained within single cells and thereby may not have significant effect on secretion processes as a whole. If this assumption holds, it would be tempting to postulate that scarcity of functional P2 receptors in acini is advantageous for the tissue that releases significant amounts of ATP and is prone to autodigestion (see below). However, it cannot be excluded that receptors are not accessible or are down-regulated in the particular rat gland preparation, or that ATP does not reach the receptor due to efficient hydrolytic enzymes (see below). In comparison, about 90% of acini prepared from other exocrine glands (e.g. salivary and lachrymal glands) using similar methods respond to ATP, although there is a functional heterogeneity in $P2X_4$, $P2Y_2$ and $P2Y_1$ receptors, and these acini also express $P2X_7$ receptors [78, 79, 81, 83–88]. It is also established in exocrine gland acini that distribution and function of some P2 receptor is influenced by the developmental state, innervation and duration of cell culture [78, 87–90]. Thus, for pancreatic acini, the possible

role of P2 receptors in short-term and long-term effects on secretory and other processes needs further investigation.

Pancreatic ducts—native tissue

P2 receptors In contrast to pancreatic acini, pancreatic ducts exhibit functional P2 receptors. Detailed analyses of molecular identity and function of P2 receptors were carried out on the rat (and guinea pig) pancreas and therefore this will be the starting and reference point for other numerous studies on human duct lines. In single pancreatic ducts microdissected from the rat pancreas, ATP had a concentration-dependent effect on the membrane potential and it stimulated both Ca^{2+} release and Ca^{2+} influx with the following potency UTP≥ATP>2-methylthio-ATP>α,β-methylene-ATP≫adenosine [91–94]. BzATP, which is an agonist for $P2X_7$ and also other P2X receptors [95], induced fully reversible Ca^{2+} and Na^+ influx. RT-PCR analysis revealed that rat pancreatic ducts have transcripts for $P2Y_2$, $P2Y_4$, $P2X_4$ and $P2X_7$ receptors [94], which agrees with studies of Ca^{2+} transients and other functional assays discussed below. Luo and Muallem [96] found a greater variety of receptors on rat pancreatic tissue, probably reflecting differences in tissue preparation. Using immunohistochemistry, Coutinho-Silva et al. [21, 26] detected $P2Y_2$ receptors on pancreatic ducts, and $P2Y_1$ on neonate ducts, but with given antibodies no $P2X_4$ or $P2X_7$ receptors were detected on the exocrine pancreas, but rather on pancreatic islets. Further experimental approaches are needed to resolve the discrepancy between molecular and functional identity and immunoidentification of the P2 receptors.

Before analysing the possible role of P2 receptors in duct function, let us summarise known secretory and regulatory mechanisms in pancreatic ducts (Fig. 3a). The classic secretagogues secretin and acetylcholine stimulate ductal secretion by primary opening of CFTR (the cystic fibrosis transmembrane regulator) Cl^- channel, regulated by the cAMP pathway, or opening of Ca^{2+}-activated Cl^- channels. Parallel activity of various H^+ and HCO_3^- transporters ensures transport of HCO_3^- into duct lumen [97, 98]. The driving force for anion transport is kept by opened K^+ channels [91, 99–102]. Interestingly, in rat pancreatic ducts UTP (which activates rat $P2Y_2$ and $P2Y_4$ receptors with about equal efficiency) decreased the whole-cell K^+ conductance and no effect on the Cl^- conductance could be detected [94]. Thus, it was predicted that these receptors would down-regulate ductal secretion (Fig. 3b). This was in fact found in isolated guinea pig pancreatic ducts, where the basolateral application of UTP decreased secretion [103]. A similar effect of UTP on secretory processes was also detected in polarised Capan-1 cells, which are epithelial cells derived from pancreatic adenocarcinoma [104]. RT-PCR analysis of the single rat ducts revealed that they express Ca^{2+}-activated K^+ channels of the intermediate conductance (IK also known as SLO, Maxi K and KCNMA1) and big conductance (BK also known as SK4, KCNN4) [105]. Human IK or BK channels were co-expressed with hP2Y$_2$ or hP2Y$_4$ receptors in *Xenopus* oocytes and cell currents were measured. Notably, $P2Y_2$ receptors inhibited BK channels; the other receptor/channel combinations, such as $P2Y_2$ and IK, $P2Y_4$ and BK or IK, were stimulatory. Thus, most likely on the basolateral membrane of pancreatic ducts, the $P2Y_2$ receptor-mediated effect on BK channels dominates the whole-cell conductance and K^+ channel inhibition down-regulates secretion (Fig. 3b). The cellular signalling between the $P2Y_2$ receptor and BK is yet to be established, and recent discoveries of $P2Y_2$ receptor interactions with various intracellular signalling pathways offer a number of possibilities [106, 107]. In theory, the function of this $P2Y_2$ receptor to BK interaction would be the "brake" or "control" point in regulation of secretion in the ductal system, perhaps sensing and preventing distension of the duct. Other mediators, such as substance P and atrial natriuretic factor, have been ascribed similar protective roles since they inhibit duct secretion [108, 109].

In contrast to BK channels, IK channel regulation by $P2Y_4$ or even $P2Y_2$ receptors is stimulatory, as would be required for secretory processes [105]. Therefore, localization of these channels and receptors and conditions that will favour secretory events need to be elucidated. Since exocrine glands, including the pancreas, secrete fluid where K^+ is higher than in plasma, it is predicted that up to about 20% of the total cellular K^+ conductance would increase the secretory rate [110]. In pancreatic ducts, the circuit analysis shows that the luminal K^+ conductance contributes about 10% to the total cell K^+ conductance (Fig. 3b) [99].

Let us turn to the P2X receptors in pancreatic ducts. BzATP had effects on Ca^{2+} influx and membrane voltage/current that could be ascribed to $P2X_7$ or possibly $P2X_4$ receptors, which were detected functionally on the luminal membrane [94, 96]. It was not possible to detect whether BzATP would also open Cl^- channels, or stimulate H^+/HCO_3^- transport [111]. Thus, if $P2X_7$ receptors, or $P2X_4$ receptors, have an effect on secretion, it may be by means of up-regulating secretion rather than initiating it. The intracellular mechanisms stimulated by P2X receptors in pancreatic ducts are unknown, but they may involve Ca^{2+} or Na^+ influx and an independent activation of mitogen-activated protein kinases, ERK1/2 [112]. Interestingly, in guinea pig pancreatic ducts that were cultured overnight injection of ATP into the lumen (or UTP) increased secretion [103]. Possibly, this could be due to stimulation of $P2X_7$ receptors, or other receptors such as $P2Y_2$, $P2Y_4$ or adenosine receptors. Taken together, it is not known whether

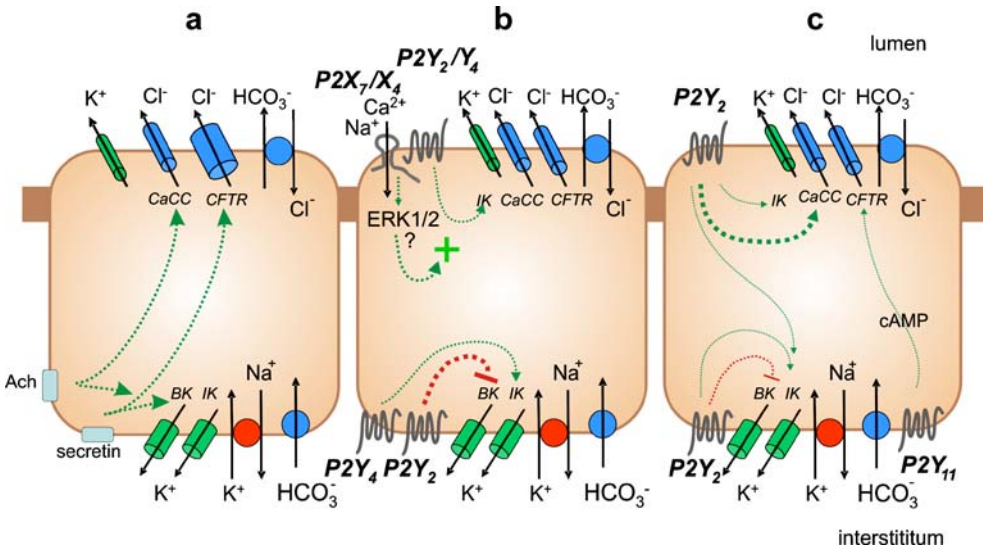

Fig. 3 Purinergic receptors and ion transport in pancreatic ducts. **a** The basic working model for pancreatic duct secretion elicited by secretin or acetylcholine. The primary events are: opening of CFTR or Ca^{2+}-activated Cl^- channels and opening of K^+ channels that keep the driving force on the secretion. The basolateral membrane transporters, such as NBC or NHE (shown as a HCO_3^- import for simplicity), and activity of intracellular carbonic anhydrase lead to accumulation of intracellular HCO_3^-. Exchange of HCO_3^-/Cl^- results in secretion of HCO_3^- into the lumen, and this is accompanied by Na^+ and water transport giving rise to $NaHCO_3$-containing secretion. **b** P2 receptors in native rat pancreatic ducts. $P2Y_2$ receptors have the dominant effect on BK channels, most likely on the basolateral membrane. Some conclusions regarding BK inhibition and IK activation are also in agreement with data on guinea pig ducts and human cell lines. **c** P2Y receptors on human and canine duct epithelium. The size of lines and *arrows* indicates the degree of P2Y receptor and ion channels stimulation most commonly observed. The $P2Y_2$ receptor inhibits BK channels, as indicated by the *dash*

the target effect of putative $P2X_7$ receptors are ion transporters involved in secretion, as may be the case for other exocrine glands [75], or whether effects are long term, involving regulation of cell proliferation or cell death, as described for some cells [95, 113]. The Raf-MEK-ERK signalling cascade plays a crucial role in the regulation of apoptosis, proliferation and metastasis of pancreatic cancer [114]. We already know that other agonists, such as neurotensin and EGF, promote DNA synthesis in pancreatic cancer cells (Panc-1) via ERK activation [115, 116].

One of the well described effects of $P2X_7$ receptors is their ability to form or mediate formation of pores, which would allow permeation of large molecules and eventually cause cell lysis [95, 113]. One of the events precipitating pore formation experimentally is the use of Ca^{2+}- and Mg^{2+}-free solutions. Pancreatic juice normally has free Ca^{2+} concentrations varying between 0.1 and 1 mM. Thus, in a physiological situation pore formation would not be expected on this basis. Recently, other regulators of $P2X_7$ receptors have been proposed. The microbial peptide LL37, from the family of cathelicidins, could also activate $P2X_7$ receptors [117]. Although LL37 is found in salivary glands [118] and its role in $P2X_7$ receptor stimulation has been discussed [119, 120], there are no reports of cathelicidins in the pancreas. It has also been proposed that extracellular Cl^- could regulate the $P2X_7$ receptor in parotid gland ducts [119, 121]. Since pancreatic ducts transport Cl^- and pancreatic secretion varies in concentration of Cl^-, the effect of extracellular Cl^- on luminal $P2X_7$ it is an interesting possibility.

The $P2X_4$ receptors are widely expressed in all exocrine glands—pancreatic acini and ducts, parotid and submandibular acini and ducts [75]. Usually, the function of $P2X_4$ receptors has been difficult to separate from $P2X_7$ receptors, except for a recent study on human parotid acini, where it was shown that cAMP potentiates ATP-evoked Ca^{2+} signalling via $P2X_4$ receptors [81]. Overall, stimulation of P2X receptors in other exocrine glands allows Na^+/Ca^{2+} influx and stimulation of K^+ channels, Na^+/H^+ exchange, Na^+-K^+-Cl^- cotransport, causes volume changes and stimulates amylase release [75, 76]. Therefore, some P2X receptors could be involved in genuine secretory events in the exocrine glands.

Pancreatic ducts—cultured tissue

Numerous studies have been carried out on human pancreatic adenocarcinoma epithelial cell lines—Panc-1, Capan-1, HPAF and CFPAC-1 cells. Only the first two lines express functional CFTR; HPAF cells have low levels of CFTR and CFPAC-1 cells are derived from a CF patient with deletion of Phe-508 in CFTR. CFPAC-1 cells have been of special

interest because it is predicted that P2 receptor stimulation could activate Ca^{2+}-activated Cl^- channels as an alternative secretory pathway to the defective CFTR. Hence, the agonists commonly used are ATP and UTP, and the results are interpreted in connection with the $P2Y_2$ receptor.

In CFPAC-1 cells, ATP or UTP stimulation increased cellular Ca^{2+} and it increased Cl^- efflux measured as halide efflux, short-circuit or whole-cell current, or as a decrease in intracellular Cl^- activity (Fig. 3c) [122–126]. In addition, ATP/UTP also stimulated HCO_3^- efflux, associated with Cl^-/HCO_3^- exchange and monitored by intracellular pH measurements [125, 126]. In some studies it was reported that nucleotides also stimulated K^+ currents [122, 125]. In HPAF cells, which have relatively low expression of CFTR, luminal ATP stimulated Ca^{2+}-activated Cl^- transport, and furthermore transepithelial short-circuit current was dependent on K^+ channel activity [102]. Functionally, the K^+ channels are seen as clotrimazole-sensitive Ca^{2+}-activated K^+ channels and their molecular identity corresponds most likely to IK. Interestingly, this cell line lacks Ba^{2+} sensitivity indicating an absence of BK channels, which are expressed in native duct cells [105]. In most studies on cultured cells, cells were grown on coverslips and nucleotides were added to the apical side. However, in a few studies where nucleotides could be added to either the apical/luminal side as opposed to the basolateral/serosal side, they had a more pronounced effect from the apical side [102, 124, 126]. In two studies nucleotides had the following order of efficacy on stimulation of Cl^- transport: UTP≈ATP>ADP≫α,β-methylene ATP, β,γ-methylene ATP, 2-methylene ATP [124, 127]. These data indicate the presence of UTP-preferring receptors, such as $P2Y_2$ or perhaps $P2Y_4$ [13]. Despite the relevant effects of ATP/UTP on Cl^-, HCO_3^- and K^+ transport described above, the effects are very short lasting—in the order of seconds to minutes. This may be difficult to reconcile with sustained ductal secretion, which should last minutes to hours.

In several studies, CFPAC-1 cells were compared with either cells with functional CFTR, such as Panc-1 or Capan-1 cells, or with CFTR-transfected CFPAC-1 cells. Nucleotides had a similar order of efficacy in stimulating Cl^- efflux from both CFPAC-1 cells and CFTR-corrected CFPAC-1 cells, indicating that P2 receptor expression was not altered [127]. In Panc-1 cells, ATP had a negligible effect on Cl^-/HCO_3^- exchange, while it stimulated Cl^-/HCO_3^- exchange in CFPAC-1 cells [125]. In another study, ATP induced Cl^- efflux in both Capan-1 and CFPAC-1 cells; ATP increased Cl^-/HCO_3^- exchange in Capan-1 cells but in CFPAC-1 cells Cl^-/HCO_3^- exchange was low until the cells were corrected with transfected CFTR [126]. Therefore, it is postulated that P2 receptors could be used to restore some HCO_3^- secretion, but this would not be fully effective until CFTR is also expressed.

Capan-1 cells express functional CFTR and receptors and transporters perhaps closest to a "normal" human duct epithelium. In polarised monolayers, ATP and UTP applied luminally stimulated HCO_3^- secretion (measured as decrease in pH_i), while applied basolaterally they inhibited HCO_3^- secretion [104]. This is in agreement with studies on rat and guinea pig pancreatic ducts (see above). RT-PCR analysis indicates that this epithelium has transcripts for the following receptors: $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_6$ and for $P2X_1$, $P2X_4$ and $P2X_5$, but not $P2X_7$. A recent study on the human pancreas reveals that there are transcripts for $P2X_7$ receptors in the tissue [128], although immunohistochemically receptors could not be localised to exocrine tissue [21]. Nevertheless, our RT-PCR analysis on various human duct cell lines indicates that they have a large repertoire of P2 receptors, including the $P2X_7$ receptor (unpublished data).

Human duct cell lines have some characteristics of large/main ducts, and in addition to ion transport they can also secrete mucins. ATP induced mucin secretion in cell lines expressing functional CFTR: Capan-1 cells, CFTR-corrected CFPAC-1 cells, but not in CFPAC-1 cells [129]. It seems that receptors other than $P2Y_2$ (or $P2Y_4$) are involved in mucin secretion as the sequence of efficacy was different to that for ion transport, i.e. ATP>ADP>AMP>adenosine>UTP, and there was little dependence on extracellular Ca^{2+}. Similarly, in cultured canine main duct epithelium mucin secretion was stimulated by ATP by both Ca^{2+}- and cAMP-dependent pathways [130, 131]. In contrast to ion transport, the ATP effect on mucin secretion was long lasting and seen over 30 min.

Cultured tissue from the canine main ducts was also used for ion transport studies. Although the main duct is a high electrical resistance epithelium compared to low resistance smaller ducts, it nevertheless has a secretory phenotype. Efflux of $^{125}I^-$ was stimulated by ATP, UTP, ATPγS>β,γ-methylene ATP ≫ adenosine and ATP stimulated $^{86}Rb^+$ efflux [132]. Using short-circuit measurements on polarised epithelium, it was shown that both luminal and serosal addition of UTP activated above Cl^- and K^+ conductances, suggesting that $P2Y_2$ receptors are expressed on both sides of the epithelium. Low concentrations of UTP caused Ca^{2+} oscillation and synchronous activity of K^+ currents, presumed to be IK channels, and pH_i measurements indicated HCO_3^- secretion [131]. This finding supports conclusions drawn from studies on rat and human $P2Y_2$ receptors and IK channels [94, 105]. In addition to $P2Y_2$ receptors, there are also $P2Y_{11}$ receptors on the basolateral membrane of canine epithelium that activate large Ca^{2+}-independent Cl^- conductance and cAMP increases (see Fig. 3c) [133].

Taken together studies on cultured duct epithelia indicate that ATP/UTP, possibly via $P2Y_2$ receptors, stimulate ion

transport and signalling pathways, which would be consistent with secretory processes. However, several issues still need to be resolved. First, the ATP/UTP effects on membrane Cl^- and K^+ channels are transient—thus the question is whether full secretion could be supported. Second, molecular identity, pharmacology and immunolocalization of P2 (and adenosine) receptors needs to be resolved. Third, it is not clear why presumed $P2Y_2$ receptors behave differently in human and canine duct epithelia compared to rat, guinea pig ducts and expression system in oocytes (i.e. stimulation of Cl^- channels vs inhibition of BK channels). Possibly, effects of $P2Y_2$ or $P2Y_4$ receptors on Cl^- channels and IK channels dominate in cultured cells with apical exposure. Fourth, duct cell lines originate from adenocarcinomas and/or main ducts and it is not certain whether they have the same phenotype as smaller secretory ducts. Fifth, we need to address the issue of tissue culture and its effect on P2Y receptors and ion channels that they regulate. In studies on salivary glands, it was shown that duct obstruction, inflammation and culture markedly increase $P2Y_2$ receptor expression [87, 134, 135]. Also in a recent study on human pancreas it was shown that the pattern for expression of $P2Y_2$ and $P2X_7$ receptors can change in pathological conditions like chronic pancreatitis, pancreatic cancer and purinergic receptors on other than epithelial cells need to be considered [128]. Lastly, knowledge regarding the expression and physiological function of P2X receptors is lacking for human tissue.

Adenosine receptors There are several studies on the pancreas that have addressed the issue of adenosine receptors. In the intact dog pancreas adenosine modulated secretion evoked by secretin [136, 137]. Since the effects could also be systemic, e.g. by effecting blood flow, it is not easy to conclude whether the effect is exerted on pancreatic ducts and which adenosine receptors are affected. Nevertheless, using agonists and antagonists for adenosine receptors, it was concluded that A_{2A} receptors were involved in HCO_3^- secretory response. On rat pancreatic lobules, adenosine evoked amylase secretion; this was atropine sensitive, therefore indicating that effects may have been mediated by neural acetylcholine release [138]. Indeed, adenosine response was not found on isolated acini.

Regarding human duct cells, there are some controversies especially regarding A_1 receptors. Eidelman et al. [139] demonstrated that A_1 receptor antagonists activated modest Cl^- efflux in CFPAC-1 cells, but had no effect in CFPAC-1 cells transfected with CFTR. This would indicate a role for endogenous adenosine, possibly on Ca^{2+}-activated Cl^- channels. A series of substituted derivatives of 1,3,7-alkylxanthines was synthesised as potential activators for Cl^- efflux in these cells [140]. Among the xanthine derivatives of diverse structure, there was no correlation between potency in Cl^- efflux and adenosine antagonism, suggesting that stimulation of Cl^- efflux was unrelated to adenosine receptors. In fact it was later reported that xanthines could bind to CFTR directly [141]. In addition, no RNA for the human A_1 receptor was found [140]. In a latter study also on CFPAC-1 cells, other results were obtained [127]. Adenosine had no effect on Cl^- efflux in normal CFPAC-1 cells, but after transfection with CFTR, it stimulated Cl^- efflux. Using inhibitors and antagonists for adenosine receptors the results indicate that the effect was due to stimulation of A_1 and A_2 (but not A_{2A}) receptors. Since no significant increase in cyclic AMP was measured, the authors also suggested that the effects of adenosine may be indirect. Nevertheless, a recent study showed that both rat pancreatic ducts and human duct cell lines expressed low levels of adenosine receptors A_1, A_{2A}, A_{2B} and A_3, the most abundant being A_2 receptors, and it seems that the A_{2A} receptor regulates anion transport [142, 143].

Origin and fate of ATP

As mentioned above, the pancreas is supplied by parasympathetic and sympathetic nerves, which could be the source of ATP and other nucleotides. In addition, rat pancreatic acini release significant amounts of ATP. They do this in response to mechanical stimulation and hypotonic shock, but most importantly, they release ATP in response to acetylcholine and cholecystokinin—the two natural acinar secretagogues in the rat pancreas [144, 145]. It appears that secretion is directed towards the lumen and ATP concentrations in the range of 20 μM were detected. Using fluorescent markers for ATP stores, it appears that the secretory granules in acini are a most likely site of ATP, although other stores or other pathways for ATP release are not excluded. Thus acini could provide ATP for the downstream pancreatic ducts expressing P2 receptors on the luminal membrane. In addition to acini, also pancreatic ducts release ATP (unpublished data). They do possess CFTR channels that have been repeatedly in and out of favour as the ATP release channels [146, 147]. Another possibility is that pancreatic ducts distend with oversecretion and release ATP, which then via $P2Y_2$ receptors and BK channels inhibits further secretion and thus overdistension of ducts [94, 105]. In contrast to salivary glands that have myoepithelial cells to hold around ducts, pancreatic ducts do not have such support and may rely more on this self-regulation.

Given the high ATP secretion from rat pancreatic acini, it was perhaps surprising to find that pancreatic juice collected from the main common pancreatic/bile duct of the rat pancreas, and also guinea pig and human pancreas, had a low ATP content [145, 148, 149]. A study on the rat

pancreas showed that CD39 (NTPDase hydrolysing ATP and ADP to AMP) was present luminally on small ducts and basolaterally on larger ducts [148]. In addition, the pancreas secretes ATP-degrading enzymes, CD39 and CD73 (5′-nucleotidase hydrolysing AMP to adenosine), into the juice [145]. CD39 was in fact characterised and purified as one of the first NTPDases from pancreatic tissue (epithelial, endothelial and stromal cells) [150–152]. The origin of secreted enzymes is possibly acinar, as it was shown that CD39 was translocated from plasma membranes and secretory granules towards the lumen and it was secreted into the juice in a particular form, most likely as microvesicles [148]. Along with highly active hydrolytic enzymes, there are also ATP-generating enzymes in pancreatic juice, adenylate kinase and nucleoside diphosphate kinase, capable of sequentially phosphorylating AMP via ADP to ATP [145]. Thus, one would expect that in pancreatic ducts there would be a nucleotide/nucleoside profile favouring ATP and adenosine. Expected concentrations of ADP would be relatively low, which is reflected also by the absence of ADP-preferring receptors [94].

Exocrine pathophysiology and therapeutic potentials

In addition to diabetes, other diseases affecting the pancreas are cystic fibrosis, acute and chronic pancreatitis and pancreatic cancer. Mutations in the CFTR gene lead to aberrant membrane transport and a wide spectrum of diseases affecting epithelia. In the pancreas, faulty Cl^- (and HCO_3^-) and fluid secretion leads to plugging of ducts by mucus and digestive enzymes, which leads to destruction of acini, inflammation and cystic fibrosis. The main defects in airway epithelia are decreased Cl^- and fluid secretion and thus impaired mucociliary clearance and ensuing bacterial infections. One of the treatment strategies for airway epithelia is to develop stable UTP analogues that via $P2Y_2$ receptors would stimulate Ca^{2+}-activated Cl^- channels as an alternative secretory pathway [153]. Clinical trials show some success with increased mucociliary clearance and forced expiratory volume [154]. Studies on human duct cell lines defective in CFTR indicate that apical application of ATP/UTP could also activate Ca^{2+}-sensitive Cl^- channels, although the effect is short lasting. Nevertheless, it would seem difficult to design agonists targeted for the pancreatic duct lumen.

Acute pancreatic injury can lead to a cascade of events beginning with intra-pancreatic activation of zymogens, release of cytokines and other proinflammatory mediators, and ending with systemic inflammatory response and multiple organ failure. The role of adenosine receptors has been investigated in several studies on animal models of pancreatitis. In rats, intraperitoneal administration of a selective A_1 agonist increased serum amylase and induced morphologic changes in the pancreas characterised by interstitial oedema and leucocyte infiltration—reminiscent of pancreatitis changes [155]. In caerulein- and taurocholate-induced pancreatitis, the A_1 receptor antagonist significantly reduced pancreatic oedema, although it did not improve the acinar cell damage of the pancreas or the increase of serum amylase. In two studies on caerulein-induced pancreatitis, A_{2A} receptor stimulation seemed to be protective [156, 157].

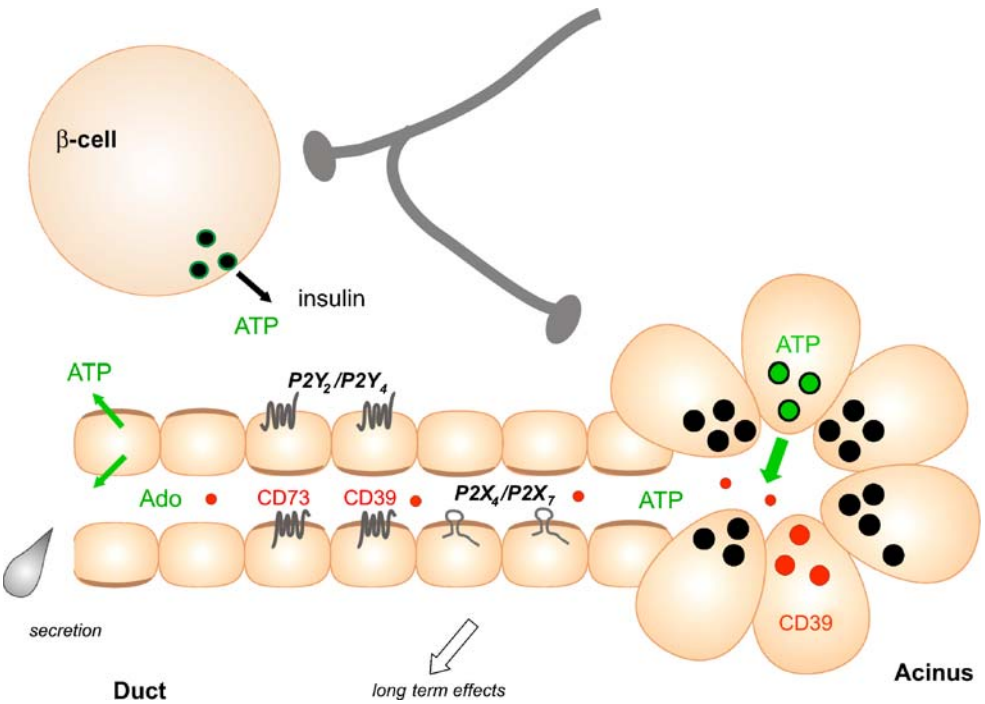

Fig. 4 Model for nucleotide signalling in the pancreas. Pancreatic acini secrete ATP and also hydrolytic enzymes (CD39 and CD73), which would hydrolyse ATP to adenosine (*Ado*). Intraluminal ATP can act on luminal P2X and possibly P2Y receptors, while the postulated ATP release from distended ducts, as well as ATP released from nerves and from β cells, could act on P2Y receptors on the basolateral side of ducts (modified from [148])

There was an attenuation in the activity of serum amylase, pancreatic weight was less affected and there was a reduced degree of pancreatic tissue damage (oedema, leucocyte infiltration, vacuolization of acinar cells), and also pancreatic blood flow was improved. Another type of experiment also indicates that adenosine levels may be important. An adenosine uptake inhibitor (KF24345) ameliorated the severity and mortality of lethal acute pancreatitis, and this effect was abolished by pretreatment with an A_{2A} receptor antagonist (ZM 341385) [158]. Taken together, these results at the organ level indicate that endogenous adenosine might have a cytoprotective role in the pancreas, similar to other organs such as the heart and brain [74, 159]. Whether this can be extended to the whole-body level is unclear, as adenosine is a potential mediator of immunosuppression in multiple organ failure, which can follow acute pancreatitis [160].

Recurrent attacks of acute pancreatitis are believed to result in chronic pancreatitis, which is associated with increased risk of developing pancreatic cancer [161, 162]. Extracellular nucleotides have been implicated as inflammatory and stress mediators in many pathological states and thus it is appropriate to look at this in the human pancreas. In a recent study of human material originating from normal pancreas and pancreas from patients with chronic pancreatitis and pancreatic cancer, it was shown that certain NTPDases and P2 receptors were up-regulated [128]. In chronic pancreatitis, CD39 and $P2X_7$ were up-regulated on the mRNA level and some tendencies were also seen on a protein level. $P2Y_2$ receptors, which were very low in normal pancreas, were up-regulated in pancreatic cancer (both mRNA and protein level). CD39 and CD39L1 were also up-regulated in pancreatic cancer, and patients with high expression of CD39 had longer post-operative survival periods. Immunolocalization with given antibodies showed CD39 distribution in the vasculature and stroma, the $P2X_7$ receptor was in infiltrating leucocytes and $P2Y_2$ in fibroblasts adjacent to tumours. These findings implicate distinct roles of CD39 and purinergic signalling in tissue remodelling, fibrogenesis and perhaps tumour development. Future studies will need to clarify apparent lack and disagreement in immunolocalization of NTPDases [128, 163] and P2 receptors in the parenchyma of the human pancreas and human duct cell lines as well as their molecular identity [104]. At least for P2 receptors, numerous functional studies on human and animal ducts show that they are present on the epithelium (see above).

Integrated pancreatic function

Within the exocrine pancreas, there is evidence that ATP and other components of the purinergic cascade mediate along the acini-duct axis a short-term regulation of secretion. Cholinergic stimulation or cholecystokinin stimulation of acini causes release of ATP, and luminal ATP may lead to up-regulation of secretin-evoked HCO_3^- and fluid secretion of pancreatic ducts (Fig. 4). This scenario would explain the long-standing observation in pancreatic physiology—the fact that in the intact pancreas the stimulation of acini potentiates the effect of secretin on the production of the HCO_3^- -rich juice [164, 165]. Figure 4 shows the working model for how ATP, and/or hydrolytic products as well as the associated enzymes and receptors, could play a role as auto/paracrine regulators in the pancreas. In addition to "within the lumen" signalling, nerves supplying the pancreas could provide nucleotides/nucleosides necessary for activation of purinergic receptors on the basolateral membranes of pancreatic ducts (and to a limited extent on pancreatic acini). In addition, there are two other potential sources of ATP. One is ATP release from the distended ducts, and this may lead to down-regulation of secretion. Another supply may be from endocrine cells. Single endocrine cells are scattered in the epithelium of interlobular ducts. It is now becoming accepted that there is a close anatomical contact between islets and ducts (in addition to islet-acini), and there is an insulo-ductal portal system [166, 167]. Functionally, several islet hormones affect acinar and ductal secretion [166], e.g. insulin potentiates secretin-stimulated secretion in the rat pancreas [165]. Therefore, it is possible that ATP released from β cells could affect closely apposed ductal epithelium. Lastly, it is very likely that pathological increases in extracellular nucleotides/sides levels can occur following ductal hypertension, acinar damage and alcohol exposure, and thus can be one of the factors contributing to development of pancreatitis, and chronic pancreatitis is one of the risk factors for development of pancreatic cancer. From a therapeutic point of view, most advances have been made with respect to finding drugs for modulating insulin secretion. Most promising are $P2Y_1$ receptor analogues that stimulate glucose-dependent insulin secretion in animal models, and thereby have therapeutic potential for treatment of non-insulin-dependent diabetes. This review underpins the complexity and possible interaction in purinergic signalling in the pancreas. Therefore, in order to design selective compounds with good potency and selectivity, molecular subtypes and function of P2 and adenosine receptors on endocrine and exocrine cells as well as on pancreatic blood vessels, immune cells and pancreatic stellate cells must be known.

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