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Review

Purinergic signaling in the pancreas and the therapeutic potential of ecto-nucleotidases in diabetes

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It is widely accepted that purinergic signaling is involved in the regulation of functions of all known tissues and organs. Extracellular purines activate two classes of receptors, P1-adenosine receptors and P2-nucleotide receptors, in a concentration-dependent manner. Ecto-enzymes metabolizing nucleotides outside the cell are involved in the termination of the nucleotide signaling pathway through the release of ligands from their receptors. The pancreas is a central organ in nutrient and energy homeostasis with endocrine, exocrine and immunoreactive functions. The disturbances in cellular metabolism in diabetes mellitus lead also to changes in concentrations of intra- and extracellular nucleotides. Purinergic receptors P1 and P2 are present on the pancreatic islet cells as well as on hepatocytes, adipocytes, pancreatic blood vessels and nerves. The ATP-dependent P2X receptor activation on pancreatic β-cells results in a positive autocrine signal and subsequent insulin secretion. Ecto-NTPDases play the key role in regulation of extracellular ATP concentration. These enzymes, in cooperation with 5'-nucleotidase can significantly increase ecto-adenosine concentration. It has been demonstrated that adenosine, through activation of P1 receptors present on adipocytes and pancreatic islets cells, inhibits the release of insulin. Even though we know for 50 years about the regulatory role of nucleotides in the secretion of insulin, an integrated understanding of the involvement of purinergic signaling in pancreas function is still required. This comprehensive review presents our current knowledge about purinergic signaling in physiology and pathology of the pancreas as well as its potential therapeutic relevance in diabetes.

Key words: ATP, adenosine, pancreas, diabetes mellitus, P-type receptors, ecto-nucleotidases

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INTRODUCTION

The concept of purinergic signaling was initially proposed in the year 1972 and at the beginning it referred to the central nervous system. It was based on the conclusion that adenosine 5'-triphosphate (ATP) fulfills the characteristics of classic neurotransmitters. In 1976 purinoreceptors have been defined, and two years later they were divided into P2 receptors activated by adenine (ATP and ADP) and pyrimidine (UTP and UDP) nucleotides and P1 receptors activated exclusively by adenosine. P2 receptors were divided into ionotropic P2X and metabotropic P2Y receptors based on their mechanism of signal transduction. Metabotropic P1 receptors were, in turn, divided into A1, A2A, A2B and A3 subtypes.

Seven P2X receptor subtypes and eight P2Y receptor subtypes have been identified so far in mammals (Burnstock & Novak, 2013). Most of the P2Y receptors couple to Gq/G11 proteins and activate phospholipase C-β (PLC-β) except for P2Y12, P2Y13 and P2Y14 receptors which cooperate with Gi proteins, and thus inhibit adenylate cyclase and P2Y11 receptor that cooperates with Gs and Gq proteins (Burnstock & Novak, 2013). Also, A1 and A3 receptors couple to Gi proteins and inhibit adenylate cyclase activity, whereas A2A and A2B receptors couple to Gs and Go proteins and stimulate the formation of cAMP (cyclic adenosine monophosphate) (Burnstock & Novak, 2013).

In physiological conditions ecto-enzymes, such as NTPDases (ecto-nucleoside triphosphate diphosphohydrolases) degrade ATP and ADP to AMP, while AMP is hydrolyzed by 5'-nucleotidase to adenosine – Fig. 1. Subsequently, adenosine deaminase (ADA) converts adenosine to inosine. Activity of ecto-enzymes plays a pivotal role in the regulation of P1 and P2 receptors agonists concentration. The enzymes involved in the metabolism of nucleotides and nucleosides are present on exocrine and endocrine cells of the pancreas as well as on the pancreatic blood vessels and capillaries. In human and animal cells of the pancreas and blood vessels activity of ecto-enzymes such as NTPDase1, NTPDase2, NTPDase3, 5'- nucleotidase and alkaline phosphatase was demonstrated (Böck, 1989; Lavoie *et al.*, 2010; Burnstock & Novak, 2012).

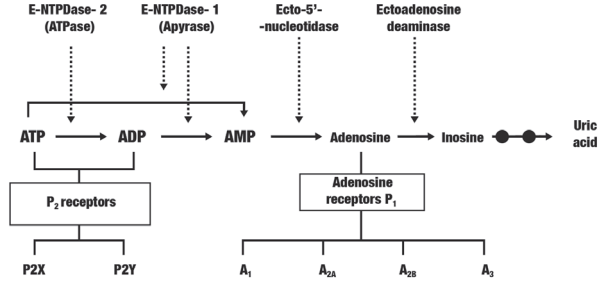

Figure 1. Metabolism of adenine ecto-nucleotides and ecto-nucleosides and types of purinergic receptors (modified from Cieślak, 2012)

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Abbreviations: ADA, adenosine deaminase; FFA, free fatty acids; GLUT4, glucose transporter 4; NTPDase, nucleoside triphosphate diphosphohydrolase; SOCS, suppressor of cytokine signaling; IL-6, interleukin 6.

Diabetes mellitus is characterized by metabolic disorders of the pancreas, liver, skeletal muscles and adipose tissue among others. The essence of these disorders is abnormal glucose metabolism and transport associated with inadequate secretion of insulin. These disturbances lead to hyperglycemia, the formation of free fatty acids (FFA) and release of proinflammatory cytokines. Following these basic changes, metabolic disturbances occur in many other systems and organs: cardiovascular and digestive system, kidney, urinary tract. Moreover, such processes as skin healing, sexual function or muscle strength are affected. Typically, within a few years after diagnosis of diabetes mellitus, and sometimes even before its disclosure, a subset of complications such as micro- and macro-angiopathy, retinopathy and neuropathy occurs (Burnstock & Novak, 2013).

The disturbances in cellular metabolism in diabetes lead also to changes in concentrations of intra- and extracellular nucleotides (ecto-nucleotides). The first reports on the role of ecto-nucleotides in endocrine pancreas appeared more than 50 years ago (Burnstock & Novak, 2012). Many research have been carried out since then, and our present knowledge of purinergic signaling in physiology and pathology of the pancreas is still expanding.

THE ROLE OF PURINERGIC SIGNALING IN ENDOCRINE PANCREATIC ACTIVITY

Pancreatic β-cells and secretion of insulin

In 1963, it was reported that ATP causes an increase in the insulin secretion by the β-cells of rabbit pancreas. We now know, that it is effected through the activation of P2Y and P2X receptors present on pancreatic β-cells, and that the effect triggered by ATP is dependent on blood glucose concentration (Squires *et al*., 1994; Petit *et al*., 1998; Petit *et al*., 2009). It is commonly believed that the P2X receptors are more important for regulation of insulin secretion than P2Y receptors. The study on the isolated pancreatic islets showed that using the P2X receptor antagonists and that way limiting the ATP influence, led to a decrease in insulin secretion by 65% in response to high glucose levels in blood (Jacques-Silva *et al*., 2008, 2010). However, studies on animal models have not given a clear answer about the role of P2 receptor subtypes in the secretion of insulin. On the other hand, the only research of Fernandez-Alvarez and colleagues demonstrated that P2Y receptor agonists (α,β-methylene ATP or ADPβS) amplified insulin secretion by human pancreatic islets (Fernadez-Alvarez *et al*., 2001). These results gave evidence that P2Y receptor agonists are effective in stimulating insulin release in humans. Activation of these receptors results in increase of calcium ions concentration inside the cell.

The granules inside the pancreatic cells contain not only insulin but also ATP and ADP — their release is regulated by the activation of P2X2 receptor present on the β-cells (Karanauskaite *et al*., 2009). Moreover, other molecules as 5-hydroxytryptamine, gamma-aminobutyric acid, glutamate and zinc are released together with ATP and may affect the autocrine secretion of insulin (Karanauskaite *et al*., 2009). In rats, activation of P2X receptors present on pancreatic β-cells results in a transient increase in insulin secretion regardless of low glucose concentration (Petit *et al*., 1998; Petit *et al*., 2009). The pancreatic β-cells of animals demonstrated the presence of the following P2X receptors subtypes: P2X1, P2X2, P2X3, P2X4, P2X6 and P2X7 (Santini *et al*., 2009; Jacques-Silva *et al*., 2010; Burnstock, 2013). There are conflicting reports regarding to the presence of purinergic receptors in humans. The presence of P2X1, P2X2, P2X4 and P2X6 receptors, which are present in rats, was not confirmed on human cells. Undoubtedly, the human β-cells present P2X3 receptors (confirmed by immunocytochemistry) (Silva *et al*., 2008), P2X5 receptors (Burnstock, 2013), P2X7 receptors (Burnstock, 2013) as well as P2Y11 and P2Y12 (confirmed by RT-PCR, Western blot analysis and immunofluorescence) (Lugo-Garcia *et al*., 2008). P2X receptors may occur in human cells as monomers or heteromers in various combinations involving receptors P2X3, P2X5 and P2X7. Only the P2X7 receptor does not form a heteromeric receptor with P2X3 (Jacques-Silva *et al*., 2008). Under physiological conditions, the P2X7 receptor is not involved in the metabolism of β-cells, because activation of this receptor demands high concentrations of ATP in excess of 100 mM. The P2X3 receptor is of particular importance in humans. Activation of P2X3 results in a positive autocrine signal as well as its amplification followed by a secretion of insulin (Jacques-Silva *et al*., 2010). As shown in Fig. 2, ATP released together with insulin from β-cell granules in response to a rapid decrease in blood glucose levels activates P2X3 receptor, that results in the increase of intracellular Ca^{2+} concentration, and further amplification of insulin release. The experiments by Silva and collaborators proved that ATP is involved in an autocrine feedback loop and thereby increases the secretion of insulin from β-cells through activation of human P2X3 receptor (Jacques-Silva *et al*., 2008, 2010).

There are numerous P2Y receptors present on the β-cells of the pancreas and their expression is different between animal species. Studies on pancreatic islet tumor (insulinoma) cells indicated the presence of P2Y receptors, such as P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12 and P2Y13 (Santini *et al*., 2009). In humans, the presence of P2Y11 and P2Y12 receptors on pancreatic β-cells has been demonstrated (Stam *et al*., 1996; Burnstock, 2013). Currently, we do not precisely know the effects of activation of some of these receptors. There are numerous studies reporting that activation of P2Y receptors by adenine nucleotides may increase or decrease insulin secretion, depending on the receptor subtype. However, the prevailed results show that activation of P2Y receptors stimulates glucose-induced insulin secretion, for example the P2Y4 receptor activation stimulates insulin secretion independently of blood glucose level (Santini *et al*., 2009). On the contrary, P2Y1 and P2Y6 receptors activation inhibits insulin secretion despite the high concentration of glucose in blood (Ohtani *et al*., 2008). Other study on the same receptors showed that the activation of P2Y1 and P2Y6 receptors stimulates insulin secretion when the glucose concentration raises over 8 mM (Parandeh *et al*., 2008). It is believed that P2Y1 receptor activated by ADP plays a key role in the secretion of insulin by the β-cells (Leon *et al*., 2005; Lavoie *et al*., 2010). In mice ADP can both stimulate the secretion of insulin by the P2Y13 receptor activation or inhibit the process by P2Y13 receptor activation (Amisten *et al*., 2010). It seems likely that the use of ATP and ADP analogues as therapeutics will trigger an increase in insulin secretion and thereby decrease the glucose concentration in blood. At present, we do not know the effects of activation of other P2Y receptors present on β-cells. However, it is believed that these receptors may also be involved in the regulation of insulin secretion. There are also few reports about the possible involvement of pyrimidine signaling

in insulin secretion. In 2008, Parandeh and colleagues reported that uridine diphosphate (UDP) stimulates insulin secretion through the P2Y6 receptor activation (Parandeh *et al.*, 2008).

Pancreatic α-cells and secretion of glucagon

The ionotropic P2X4 and P2X7 receptors, metabotropic P2Y6 and P2Y1 receptors and the adenosine receptor A1 and A2A subtypes were detected on the surface of pancreatic α-cells (Yang *et al.*, 2012; Burnstock, 2013). There are only few and often conflicting reports about the effects of adenine nucleotides and adenosine on the secretion of glucagon. In humans, ATP resulted in a modest increase in glucagon secretion by affecting the P2X4 receptor (Jacques-Silva *et al.*, 2010). The study of Tuduri and colleagues revealed the presence of P2Y1 receptor and adenosine receptors A1 and A2A on mouse pancreatic α-cells. ATP-mediated activation of P2Y1R resulted in inhibition of Ca^{2+} signaling that was accompanied by a decrease in glucagon secretion. Surprisingly, adenosine was also found to reduce Ca^{2+} signals through activation of A2A receptors (Tuduri *et al.*, 2008).

Pancreatic δ-cells and secretion of somatostatin

P2Y1 receptors and adenosine A1 receptors were also detected on the surface of pancreatic δ-cells (Burnstock, 2013; Burnstock & Novak, 2013). The effects of activation of these receptors and their role in regulation of endocrine secretion are not fully understood.

THE ROLE OF PURINERGIC SIGNALING IN EXOCRINE PANCREATIC ACTIVITY

Pancreatic exocrine dysfunction tends to be a rare cause of diabetes mellitus referred to as type 3c and concerning approximately 10% patients with diabetes (Burnstock & Novak, 2013). It is believed that in this form of the disease the exocrine-endocrine axis is disrupted by dysfunctions of exocrine cells, particularly within pancreatic ducts and Langerhans islets (Burnstock, 2013). ATP is released in the process of exocytosis by pancreatic alveolar cells (acini cells). However, the process of ATP release outside the cell is not fully understood and is the subject of numerous studies (Lazarowski, 2012). Enzymes including nucleotidases (NTPDase1, CD39 and 5'-nucleotidase, CD73) are concentrated in the zymogen granules and released together with ATP (Novak, 2008). Moreover, ecto-NTPDase1 and ecto-NTPDase2 are present on the surface of duct cells and blood vessels (Burnstock, 2013). To date, there has been no convincing evidence that purinergic receptors were present on pancreatic acini cells in humans. Studies in animals and humans have demonstrated that receptors activated by ATP and UDP are present on duct cells (Fong *et al.*, 2003; Burnstock, 2013). Detailed research has identified ionotropic P2X4 and P2X7 receptors, metabotropic P2Y2 and P2Y4 receptors and the adenosine A2A and A2B receptors on duct cells (Novak, 2008; Burnstock, 2013). The fact that ATP and ecto-nucleotidases are secreted by the pancreatic alveolar cells, together with the presence of ATP- and adenosine-activated P2 and P1 receptors on duct cells, suggest that purinergic signaling significantly affects pancreatic exocrine activity.

Another element potentially involved in the purinergic signaling are pancreatic stellate cells. These relatively little known cells are involved in inflammation and fibrosis of the pancreas (Burnstock & Novak, 2013). Studies of Hennings and colleagues showed the presence of mRNA for P2Y1, P2Y2, P2Y6, P2X1, P2X4 and P2X5 receptors in pancreatic stellate cells (Hennings *et al.*, 2011). Research of Künzli and colleagues confirmed the presence of P2X7 receptor (Künzli *et al.*, 2007). To date, the effects of activation of these receptors on pancreatic stellate cells are unknown.

THE ROLE OF ATP AND P2 RECEPTORS IN DIABETES

ATP affects insulin secretion by intracellular mechanisms and by extracellular activation of P2 receptors present on the pancreatic β-cell surface (Wang *et al.*, 2014). Inside the cells, ATP is produced in glycolysis and during mitochondrial oxidative phosphorylation. Mitochondrial dysfunctions cause a decrease in production and release of ATP from β-cells, thus affecting insulin secretion. Furthermore, the decrease in ATP production always leads to an increase in the concentration of free radicals (reactive oxygen species). Therefore, mitochondrial dysfunctions are largely responsible for the progression of diabetes. Many intracellular pathways are involved in the process of insulin secretion from pancreatic β-cells, however, in the first phase all of them are affected by changes in ATP concentration (Chapal *et al.*, 1993). ATP directly closes the ATP-dependent intracellular potassium channels (K_{ATP} channels) and opens the L-type calcium channels (Wang *et al.*, 2014). These processes lead to an increase in the concentration of cytosolic free calcium and reduce potassium efflux from the cell, thus resulting in cell membrane depolarization. The increase in calcium concentration inside the cell is the trigger mechanism of insulin secretion from pancreatic β- cell granules. In 1963, studies on animals have shown that extracellular ATP stimulates insulin secretion from β-cells within Langerhans islets of the pancreas through the activation of ionotropic as well as metabotropic P2 receptors (Rodrigue-Candela *et al.*, 1963). Further studies revealed that the same mechanism regulates insulin secretion in humans (Petit *et al.*, 2009). The main source of ATP outside the cell is both ATP exocytosis from β-cell granules, and its release from the nerve endings of the pancreas (Tahani, 1979; Petit *et al.*, 2009). In 1975, it was found that ATP and insulin are secreted together from pancreatic β-cells (Leitner *et al.*, 1975). A year later, Loubatieres-Mariani and colleagues demonstrated that ATP stimulates secretion of glucagon and insulin, and this process is dependent on the blood glucose level (Loubatieres-Mariani *et al.*, 1976).

Pancreatic islets are innervated by both the sympathetic and parasympathetic nerves and intrapancreatic ganglia are involved in the regulation of insulin secretion (Stagner & Samols, 1985). In 1979, Tahani hypothesized that ATP released from nerve endings regulates insulin secretion (Tahani, 1979). Further studies demonstrated that acetylcholine (ACh) is released with ATP in the parasympathetic intrapancreatic nerve endings and both these compounds act synergistically on insulin secretion (Bertrand *et al.*, 1986; Petit *et al.*, 2009). On the contrary, stimulation of the sympathetic nerve endings inhibits insulin secretion by affecting the α2A adrenoceptor acting through the ATP-dependent K^+-channel (Drews *et al.*, 2010; Burnstock, 2013).

ROLE OF ADENOSINE P1 RECEPTORS IN DIABETES

Adenosine activates four subtypes (A1, A2A, A2B and A3) of G protein-dependent receptors (Friedholm *et al.*,

2011). The A1 receptor (A1R) has been demonstrated to be commonly expressed in the brain and adipose tissue. Moderate expression of the receptor was found in the heart, spinal cord, kidneys, thyroid and adrenergic glands (Dhalla *et al*., 2009). The presence of adenosine receptor A1 and A2B was demonstrated also on pancreatic β-cells, although the precise role of these receptors in insulin secretion remains unclear (Rüsing *et al*., 2006; Johansson *et al*., 2007; Tuduri *et al*., 2008; Salehi *et al*., 2009; Burnstock & Novak, 2012; Yang *et al*., 2012). Increased expression of A1R was shown in pathological states such as oxidative stress, ischemia, inflammation and diabetes (Dhalla *et al*., 2009).

Adenosine inhibits the release of insulin, while, together with ADP and AMP stimulates glucagon secretion (Weir *et al*., 1975; Ismail *et al*., 1977). Exclusive stimulation of glucagon secretion and not insulin secretion by adenosine suggests that α-cells are more sensitive to adenosine rather than β-cells. Research by Töpfer and colleagues have shown that the A1 receptor activation by selective and non-selective agonists impairs insulin secretion (Töpfer *et al*., 2008). In addition, A1 receptor agonists cause an increase in tissue sensitivity to insulin and a decrease in the concentration of free fatty acids and triglycerides (Töpfer *et al*., 2008). Adenosine A1 receptor activation inhibits lipolysis, and thus controls various pathological processes in which free fatty acids play a key role such as diabetes, insulin resistance and dyslipidemia (Dhalla *et al*., 2009). Furthermore, activation of A1R impairs renal function and causes a decrease in heart rate and atrial contractility, and the release of neurotransmitters. Activation of A1 receptors on adipocytes inhibits activity of adenylate cyclase, thus reduces the concentration of cAMP as well as inhibits activity of protein kinase A, and thereby impairs lipolysis. In 1972, it has been found that adenosine and adenosine analogues inhibit adenylate cyclase acting antagonistically to catecholamines, that raise cAMP concentration and therefore induce lipolysis in adipocytes (Fain *et al*., 1972). In 1961, study of Dole showed that in rats adenosine and some of its metabolites inhibit the conversion of triglycerides (TG) to free fatty acids (FFA) (Dole, 1961). It was confirmed by studies of Schwabe and colleagues, in which adenosine deaminase added to the culture of fat cells stimulated lipolysis (Schwabe *et al*., 1973, Schwabe & Ebert, 1974). Recently, Dhalla and collaborators have proposed a putative mechanism of adenosine-mediated lipolysis inhibition (Dhalla *et al*., 2009).

It is believed that inhibition of lipolysis in adipocytes is triggered indirectly by the activation of the A1 receptor, subsequently comes to inhibition of adenylate cyclase, and then to decrease in cAMP concentration. Reduced cAMP concentration inhibits protein kinase A (PKA), and the enzyme exerts an inhibitory effect on hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL). This process directly leads to inhibition of triglycerides metabolism into free fatty acids (Dhalla *et al*., 2009). It is believed that the pharmacological inhibition of lipolysis in order to reduce blood levels of free fatty acids may be an effective treatment for insulin resistance and type 2 diabetes. In the past, such hopes were associated with nicotinic acid and its analogues like acipimox, which were used in dyslipidemia (Carlson, 2005). Unfortunately, after an initial decrease in the concentration of free fatty acids these compounds caused so called rebound effect, which led to an increase in free fatty acids concentration and insulin resistance (Poynten *et al*., 2003). Nicotinic acid may also cause adverse side effects, including hyperglycemia, which obviously would be detrimental in patients with diabetes (Poynten *et al*., 2003). Dipyridamole, a drug used in the treatment of cardiovascular disease, inhibits adenosine uptake as well as reduces blood levels of glucose, free fatty acids and triglycerides (Cheng *et al*., 2000; Cieślak & Komoszyński, 2004). It is thought that the A1 receptor agonists have the potential to become effective drugs for various diseases such as diabetes, insulin resistance and dyslipidemia, in which there is an increase in triglycerides and free fatty acids levels in the blood. In the search for effective antilipolytic drugs, research continues on different A1R agonists, and some of these compounds have been classified as clinical trials. The A1 receptor agonists that have been studied in the past or are currently tested include: SDZ WAG-994 (N-cyclohexyl-2'-O-methyladenosine), GR79236 (N-((1S, 2S)-2-hydroxycyclopentyl)adenosine), and other like ARA and CVT-3619 (Dhalla *et al*., 2009). These compounds inhibit lipolysis in adipocytes, effectively lower blood levels of free fatty acids and glucose. Experimental studies in animals have shown that some of them cause significant side-effects that result from the activation of A1 receptors present especially in the heart and the circulatory system (Dhalla *et al*., 2009). Adverse reactions include in particular a decrease in blood pressure (hypotension) and bradycardia.

Adipose tissue produces proinflammatory agents such as interleukin-6 (IL-6), C-reactive protein (CRP) and plasminogen activator inhibitor 1 (PAI-1), which increase tissue resistance to insulin (Ma *et al*., 2004, Csóka *et al*., 2014). Adenosine through activation of A2B receptors contributes to increased insulin resistance by stimulating production of IL-6 and other cytokines. Animal studies confirmed that the A2B receptor activation causes an increase in IL-6 concentrations in serum (Linden, 2006; Ryzhov *et al*., 2008). The results suggest that IL-6 may participate in both the formation of insulin resistance as well as improve the insulin sensitivity of tissues (Nieto-Vazquez *et al*., 2008; Sarvaš *et al*., 2013). The increase in insulin resistance is explained by the activation of AMP-activated protein kinase (AMPK), and the involvement of such molecules as leptin, SOCS1 (suppressor of cytokine signaling 1) and SOCS3 (Sochocka, 2008; Sarvaš *et al*., 2013). Long-term elevated serum concentration of IL-6 stimulates the expression of SOCS1 and SOCS3

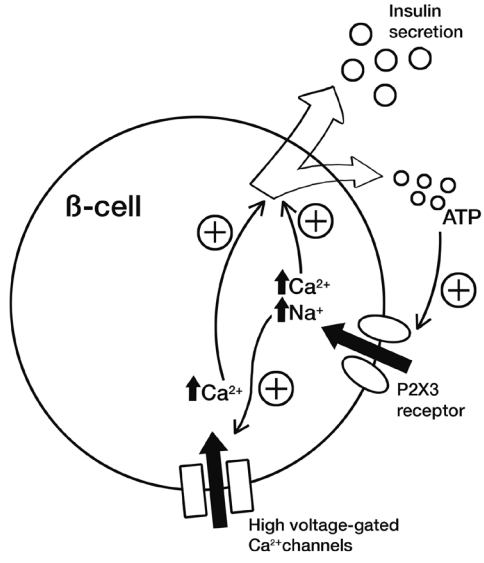

Figure 2. The putative mechanism of P2X3 receptor-mediated regulation of insulin secretion in human β-cells (modified from Jacques-Silva *et al*., 2010 and Burnstock, 2013)

proteins that contribute to the increase in insulin resistance in skeletal muscle, liver and adipose tissue. From the therapeutic point of view, it is desirable to maintain the blood levels of IL-6 less than 5 pg/ml (Kado *et al.*, 1999). Under physiological conditions, e.g. after physical exercises, IL-6 concentration in the blood is significantly increased but quickly returns to basal concentrations. Such a sudden and short increase in IL-6 concentration does not stimulate the expression of SOCS3, thus increases insulin sensitivity (Sarvaš *et al.*, 2013). In contrast, the long-term rise in IL-6 concentration, which occurs in type 2 diabetes and obesity, leads to chronic and sustained up-regulated expression of SOCS3. Therefore, a desirable phenomenon is a short-lived increase in IL-6 concentration to maintain normal sensitivity of peripheral tissues to insulin (Sarvaš *et al.*, 2013).

Depending on the type of cytokines that affect macrophages, these cells are activated either in classical (Th1) or alternative (Th2) way. In the first case macrophages are activated mainly by IFN-γ, while in the second one, by IL-4, IL-10 and IL-13. Because the A2B adenosine receptors are involved in the activation of macrophages, it is believed that their activation initiates adipose tissue inflammation and the development of insulin resistance. Studies recently published by Csóka and colleagues have demonstrated that in transgenic mice with A2B receptor knockout (A2BR-/-), macrophages activated in the alternative way did not express some of the transcription factors as CCAAT/enhancer binding protein β, interferon regulatory factor 4 and peroxisome proliferator-activated receptor γ (Csóka *et al.*, 2014). *In vitro* studies have demonstrated that A2B receptor activation on macrophages inhibited harmful inflammatory and metabolic processes, involving free fatty acids. In addition, activation of adenosine receptors upregulated the interleukin-4-induced expression of CCAAT/enhancer binding protein β, interferon regulatory factor 4, and peroxisome proliferator-activated receptor γ in macrophages (Csóka *et al.*, 2014). It is assumed that in diabetic patients administration of the adenosine receptor antagonists and adenosine deaminase for degradation of adenosine may reduce insulin resistance of skeletal muscles (Budohoski *et al.*, 1984; Challis *et al.*, 1984). Results of Figler and collaborators suggest that blocking of the A2B receptor may be an effective way in the treatment of insulin resistance through decrease in hepatic glucose production (HGP), and in IL-6 and other cytokines synthesis (Figler *et al.*, 2011).

Adenosine in the extracellular space affects the transport of glucose into the cells of skeletal muscles, while in the cardiac muscle cells and adipocytes increases insulin- stimulated glucose transport into the cells. The conversion of adenosine to inosine by adenosine deaminase or blocking the action of adenosine with adenosine receptor antagonists (CPDPX, 8-cyclopentyl-1,3-dipropylxanthine) impairs the insulin-stimulated glucose transport in skeletal muscles (Han *et al.*, 1998). This effect could be due to either a decrease in the number of glucose transporters GLUT4 on the surface of cells or to the reduction of their efficiency. Reducing the number of glucose transporters on the cell surface is also responsible for the reduction in the effectiveness of insulin in glucose transport into skeletal muscle cells and adipocytes, which contributes to the development of insulin resistance (Kuroda *et al.*, 1987; Han *et al.*, 1998). Results obtained by Han and colleagues indicate that adenosine influences the muscle contraction-stimulated and/or insulin-stimulated glucose transport (Han *et al.*, 1998).

A POTENTIAL THERAPEUTIC ROLE OF ECTO-ENZYMES IN DIABETES

Numerous studies have demonstrated the activity of enzymes involved in the metabolism of ecto-nucleotides on pancreatic islet cells, alveolar and duct cells as well as blood vessels. Ecto-NTPDases (ectonucleoside triphosphate diphosphohydrolases) present on the surface of these cells play an essential role in the metabolism of ecto-nucleotides. Four ecto-NTPDases with different biological properties and localization were cloned so far: NTPDase1 (apyrase/CD39), NTPDase2, NTPDase3 and NTPDase8 (Robson *et al.*, 2006; Chia *et al.*, 2012). In humans, the activity of NTPDase1 was found on alveolar cells (acinar cells) and blood vessels and capillaries within the pancreatic islets. Activity of NTPDase2 was found on alveolar cells, on cells surrounding the pancreatic islets and capillaries. NTPDase3 activity has been demonstrated only on Langerhans cells of the pancreas. Furthermore, high NTPDase activity has been demonstrated on platelets of patients with type 2 diabetes (Lunkes *et al.*, 2003). There was no 5'-nucleotidase activity on the pancreatic islets cells, however the activity has been demonstrated in the capillaries of the Langerhans islets (Kittel *et al.*, 2002; Lavoie *et al.*, 2010). NTPDase1 hydrolyses both ATP and ADP, NTPDase2 hydrolyses mainly ADP and NTPDase3 activity has an intermediate hydrolysis profile (Chia *et al.*, 2012). The final product of ATP and ADP hydrolysis is AMP, that is converted to adenosine with the participation of 5'-nucleotidase.

Participation of NTPDase1 (apyrase) in insulin secretion was experimentally confirmed by the results of studies, in which administration of the apyrase inhibitor — ARL67156 caused an increase in insulin secretion (Crack *et al.*, 1995; Westfall *et al.*, 1997; Levesque *et al.*, 2007). NTPDase1 impairs insulin secretion both by hydrolysis of extracellular ATP and ADP as by providing AMP as a substrate for 5'-nucleotidase. Thus, NTPDase1 participates in the formation of adenosine. Adenosine-mediated activation of P1 receptors probably slightly inhibits the secretion of insulin. It can be assumed that a significant reduction in the activity of ecto-5'-nucleotidase should result in decreasing the concentration of adenosine outside the cell, which can affect the secretion of insulin (Thompson *et al.*, 2004). Basic micromolar concentrations of adenosine in isolated pancreatic islets are sufficient to stimulate the secretion of glucagon and to inhibit insulin secretion by the activation of A1 receptor (Chapal *et al.*, 1985; Verspohl *et al.*, 2002). Surprisingly, Jacques-Silva and colleagues showed that the conversion of adenosine to inosine with the participation of adenosine deaminase does not affect the secretion of insulin. These results were confirmed using CGS15943 as P1 receptor antagonist (Jacques- Silva *et al.*, 2010).

NTPDase3 activity in humans has been demonstrated in all Langerhans islet cell types (Lavoie *et al.*, 2010). Presence of NTPDase3 on β-cells suggests that the enzyme may influence the secretion of insulin through the hydrolysis of adenine nucleotides, and thus affecting the activation of P2 receptors. Studies in animals have shown that such a regulatory mechanism of insulin secretion is possible (Lavoie *et al.*, 2010). Studies of Jacques-Silva and colleagues have demonstrated that in humans an ecto-nucleotidase inhibitor — ARL 67156, caused the significant increase in insulin secretion despite of a low concentration of blood glucose (Jacques-Silva *et al.*, 2010). In experimental studies of Munkonda and collaborators, monoclonal antibodies were used for the first time as a specific inhibitor of human NTPDase3 (Mun-

konda *et al*., 2009). These antibodies inhibit recombinant NTPDase3 activity by 60–90%. More importantly, they also inhibit the NTPDase3 expressed efficiently in insulin secreting human pancreatic islet cells *in situ*.

SUMMARY

Diabetes includes the metabolic disorders not only of the pancreas, but also other organs and tissues such as liver, skeletal muscles and adipose tissue. The key pathophysiological disorder is abnormal metabolism and glucose transport associated with inadequate secretion of insulin. This leads to an increase in blood glucose level, the formation of free fatty acids and the release of pro-inflammatory cytokines. In type 2 diabetes these processes lead to the phenomenon of insulin resistance, which is mainly responsible for the progression of the disease. Purinergic signaling plays a key role in the above processes. Purinergic receptors P1 and P2 are present on the pancreatic islet cells as well as on hepatocytes, adipocytes, in the circulatory system and pancreatic nerves. P2X3 receptor is of particular importance in human β-cells. The P2X3R activation results in a positive autocrine signal and its subsequent amplification. Insulin secretion increases as a consequence of the process. ATP participates in this autocrine feedback loop associated with the secretion of insulin. ATP is released together with insulin from β-cell granules in response to the rapid increase in blood glucose concentration. ATP in the extracellular environment, through the P2X3 receptor activation initiates the increase in intracellular calcium ions concentration, and thus amplifies the release of insulin. At present, we do not know the other effects of activation of P2X receptors, and the more P2Y receptors. It can be assumed that in the future the possibility of activation or blockade of P2 receptors, and/or the impact on nucleotides degradation may become an effective way to treat diabetes.

Adenosine and P1 receptors (A1 and A2B), which are present on adipocytes and pancreatic islets cells, play an important role in the pathogenesis of diabetes. It has been demonstrated that adenosine inhibits the release of insulin, while it also stimulates the secretion of glucagon. It confirmes that the pancreatic α-cells are more sensitive to adenosine than β-cells. Through the activation of A1 receptor, adenosine inhibits lipolysis and the activity of adenylate cyclase, leading to the decrease in cAMP concentration. Experimental studies in animals have shown that administration of A1 receptor agonists resulted in stabilization of normal glucose concentration in the blood, decrease in the concentration of free fatty acids and triglycerides and an increase in tissue sensitivity to insulin. Therefore, it can be assumed that adenosine or its analogues may become in the future drugs for dyslipidemia and insulin resistance. Unfortunately, some of the adenosine analogues are endowed with notable side- effects (i. e. hypotension and bradycardia) that result from the activation of A1 receptors present in the heart and the circulatory system.

Adenosine A2B receptors activation contributes to increased insulin resistance by stimulation of IL-6 and other cytokines and regulatory molecules production. Long-term increase in IL-6 concentration, which leads to chronic and sustained increase in the expression of SOCS3 is especially harmful in type 2 diabetes and obesity. Thus, it is desirable to maintain the blood levels of IL-6 less than 5 pg/ml. Furthermore, A2B adenosine receptors are involved in the activation of macrophages, resulting in inflammation and development of adipose tissue insulin resistance. Adenosine affects also glucose transport — this nucleoside lowers the number of glucose transporter GLUT4 molecules on the cell surface, thus reduces the effectiveness of insulin in glucose transport into skeletal muscle cells and adipocytes and contributes to the development of insulin resistance.

It can be assumed, that enzymes involved in the conversion of adenosine (i.e. adenosine deaminase) and A2B receptor antagonists may prove to be effective drugs that increase tissue sensitivity to insulin. The pancreatic islet cells, as well as blood vessels have been shown to express enzymes involved in the metabolism of ecto-nucleotides. Among ecto-NTPDases (ectonucleoside triphosphate diphosphohydrolases) the most important role is assigned to NTPDase3, that activity has only been demonstrated on Langerhans cells of the pancreas. On the contrary, 5'-nucleotidase activity has been demonstrated exclusively on capillaries of Langerhans islets, and not on pancreatic islet cells. NTPDase3 may influence the secretion of insulin by hydrolyzing adenine nucleotides, and thus affects the activation of P2 receptors. Experimental studies have demonstrated that an ecto-nucleotidase inhibitor — ARL 67156 caused a considerable increase in insulin secretion despite a low concentration of glucose in the blood. It can be assumed that a similar effect can be achieved using monoclonal antibodies as a specific inhibitor of human NTPDase3.

The pancreas is a central organ in nutrient and energy homeostasis with endocrine, exocrine and immunoreactive cells, which participate in the complex processes. Even though we know for 50 years about the role of nucleotides in the secretion of insulin, an integrated understanding of the involvement of purinergic signaling in physiology and pathology of pancreas is still required. The diversity of the purinergic system elements can be exploited in drug design for the treatment of diabetes mellitus.

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