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## Purinergic signaling in diabetes and metabolism

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### Abstract

Purinergic signaling, a concept originally formulated by the late Geoffrey Burnstock (1929–2020), was found to modulate pathways in every physiological system. In metabolic disorders there is a role for both adenosine receptors and P2 (nucleotide) receptors, of which there are two classes, i.e. P2Y metabotropic and P2X ionotropic receptors. The individual roles of the 19 receptors encompassed by this family have been dissected - and in many cases the effects associated with specific cell types, including adipocytes, skeletal muscle, liver cells and immune cells. It is suggested that ligands selective for each of the four adenosine receptors (A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub>), and several of the P2 subtypes (e.g. P2Y<sub>6</sub> or P2X<sub>7</sub> antagonists) might have therapeutic potential for treating diabetes and obesity. This is a developing story with some conflicting conclusions relevant to drug discovery, which we summarize here.

### Keywords

adenosine receptors; P2Y receptors; P2X receptors; diabetes; obesity

### 1. Introduction

Type 2 diabetes (T2D) or diabetes mellitus is a cluster of metabolic disorders that are characterized by dysregulation of glucose metabolism due to decreased insulin sensitivity in peripheral metabolically active tissues and defects in insulin secretion from pancreatic  $\beta$  cells. An epidemic of T2D continues to rise due to a major shift towards physical inactivity and unhealthy eating habits increasing obesity in people worldwide. According to a national diabetes statistics report in 2020 (Centers for Disease Control, <https://www.cdc.gov/diabetes/data/statistics-report/index.html>, accessed Oct. 26, 2020), 34.2 million people, or 10.5% of the U.S. population, have diabetes. By 2035, approx. 600 million people worldwide are predicted to suffer from T2D. T2D also leads to severe pathophysiological complications

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such as coronary artery disease, stroke, atherosclerosis, diabetic nephropathy, diabetic neuropathy, diabetic retinopathy and certain types of cancer. Therefore, identifying promising pharmacological approaches to contain the onset and progression of T2D and associated complications is of utmost importance.

The purine nucleoside adenosine functions as a modulatory extracellular signaling molecule mediating a range of physiological and patho-physiological responses through its four target adenosine receptors (ARs). Caffeine and other alkylxanthines are the prototypical AR antagonists, and their effect in the human diet can be used to indicate the possible role of these receptors in diabetes. These four ARs constitute part of the larger purinergic signalome, which includes eight P2Y metabotropic and seven P2X ionotropic receptors that are activated by extracellular ATP and other nucleotides. Numerous studies [1–3] have indicated that purinergic tuning of metabolic processes has the potential to provide new treatments for T2D and obesity.

Adenosine, ATP and other nucleotides are released from cells as autocrine and paracrine mediators, in response to metabolic stress, inflammation and injury [4]. ARs, P2YRs and P2XRs are widely expressed in metabolically active tissues such as adipose tissue, skeletal muscle (SKM), liver, pancreas, brain and immune cells. Growing evidence highlights the critical role of purinergic signaling in the regulation of pathophysiological processes associated with T2D and associated co-morbidities [5]. Targeting purinergic receptors selectively for therapeutics of T2D and associated complications has been challenging due to the ubiquitous distribution and expression of the receptors and variable levels of the endogenous agonists. Furthermore, the intricate nature of purinergic signaling can contribute to unacceptable side effects and to pharmacological and genetic discrepancies between cellular models, animal models and human pathology. However, it is to be noted that each purinergic receptor might have different effects when activated tonically or clonically, and changes of purine receptor function often occur in pathological conditions [2]. In this review, we provide a background on the roles of purine nucleotide and nucleoside signaling in the pathophysiological mechanisms underlying T2D in different metabolic tissues regulating glucose and energy homeostasis (Tables 1 and 2).

### 1.1. Overview of purinergic signalome system

The term ‘purinergic’ was introduced by Geoffrey Burnstock in 1972 when he first proposed adenosine 5'-triphosphate (ATP) as a purinergic neurotransmitter in the gut and bladder [6, 7]. ATP can be released from diverse cell types by various mechanisms including release from the lysosome via exocytosis, co-release with other hormones, uncontrolled release from necrotic cells, controlled release through pannexin hemichannels and release through connexin hemichannels and P2X7 ion channels [8–13]. The mammalian genome codes for seven different ionotropic ligand-gated ion channel P2X receptors (P2X1–7) that are principally activated by ATP. The P2Y metabotropic G protein-coupled receptors (GPCRs) (P2Y1,2,4,6,11,12,13,14) are identified and characterized in mammals and are activated by a group of nucleotides and nucleotide sugars (ATP, ADP, UTP, UDP, UDP-glucose). P2Y receptors couple to different G-proteins, activating different intracellular signaling pathways,

either the five members of the P2Y<sub>1</sub>-like subfamily (P2Y<sub>1,2,4,6</sub> - G<sub>q/11</sub>, G<sub>o</sub>, G<sub>12/13</sub>; P2Y<sub>11</sub> - G<sub>q/11</sub> and G<sub>s</sub>) or the three P2Y<sub>12</sub>-like receptors (P2Y<sub>12,13,14</sub> - G<sub>i/o</sub>).

ATP is dephosphorylated into adenosine in the extracellular space mainly by a two-step enzymatic sequence, beginning with CD39 (ectonucleoside triphosphate diphosphohydrolase 1: ENTPD1, NTPDase1) and three other NTPDases that convert ATP or ADP to AMP, followed by AMP hydrolysis by CD73 (ecto-5'-nucleotidase: NT5E) to adenosine. Other cell-surface associated enzymes such as pyrophosphatases, alkaline phosphatases and phosphodiesterases can also generate extracellular adenosine [14]. Adenosine can also be generated intracellularly by hydrolysis of AMP or S-adenosylhomocysteine and transported across cell membranes by equilibrative nucleoside transporters (ENTs) and concentrative nucleoside transporters (CNTs) that help to maintain extracellular adenosine levels. Reuptake of adenosine into cells is mediated by nucleoside transporters followed by rapid phosphorylation to AMP by adenosine kinase or deamination to inosine by adenosine deaminase (ADA).

Adenosine bind to and activates four purinergic (P1) receptors namely, A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub>ARs. A<sub>1</sub> and A<sub>2A</sub>ARs have high affinity for adenosine, whereas A<sub>3</sub>AR and especially A<sub>2B</sub>AR have lower affinity [15]. A<sub>1</sub> and A<sub>3</sub>ARs couple to pertussis toxin-sensitive G<sub>i</sub> and G<sub>o</sub> proteins, and their activation decreases intracellular cAMP levels. Activation of G<sub>s</sub>- and G<sub>oif</sub>-coupled A<sub>2A</sub> and A<sub>2B</sub>ARs increases cAMP levels (Fig. 1). ARs can activate various signaling pathways such as mitogen activated protein kinase (MAPK) pathways comprising of extracellular signal-regulated kinase 1 and 2 (ERK1 and ERK2), c-Jun-N-terminal kinase (JNK) and p38 MAPK (Fig. 1).

## 2. Adenosine receptors and diabetes

A fine balance between dietary intake, endogenous glucose release from liver, glucose uptake and utilization by SKM and adipose tissues is required to maintain glucose homeostasis in an organism. These processes are regulated by two main hormones released by pancreatic cells, i.e. insulin and glucagon. Other hormones of the pancreas such as somatostatin and amylin also affect blood glucose levels. Exogenous dietary intake stimulates insulin release from pancreatic β-cells enhancing glucose uptake by muscle and adipose tissues and inhibiting glucose production by the liver cells. Under conditions of obesity and T2D, insulin lacks its ability to clear plasma glucose due to the development of insulin resistance in peripheral tissues. Inhibition of lipolysis by insulin action on adipose tissue is also abolished, increasing plasma free fatty acid (FFA) levels that can accumulate in liver to cause non-alcoholic fatty liver disease (NAFLD). Infiltration of immune cells in different metabolic tissues and production of pro-inflammatory cytokines result in chronic low-grade inflammation associated with obesity and T2D. Genetic and pharmacological approaches undertaken to understand the contribution of ARs in the regulation of these processes in metabolically active organs are described below.

### Coffee and caffeine in diabetes

Caffeine is a prototypical nonselective AR antagonist, and coffee is one of the most widely consumed beverages worldwide. Accumulating evidence suggest that persistent

consumption of coffee is inversely correlated to the incidence of T2D [16–19], but little is known about the mechanisms responsible for this beneficial effect. Caffeine is one of the main components in coffee, but contrary to coffee's beneficial effects, caffeine has been shown to have a deleterious effect on glucose metabolism in humans. A recent study demonstrated that acute caffeine ingestion impaired insulin sensitivity in healthy subjects [20]. In diabetic patients, acute administration of caffeine impaired postprandial glucose metabolism and insulin sensitivity [21]. In a crossover randomized clinical trial (RCT) in healthy individuals, caffeinated coffee consumption resulted in a higher area under curve (AUC) during 2 h for glucose and insulin compared to decaffeinated coffee [22]. In addition, insulin sensitivity was reduced by 40% in the caffeinated coffee group compared to decaffeinated coffee [22]. In overweight individuals, ingestion of caffeinated coffee lowered insulin levels and elevated glucose concentrations [23]. In a crossover RCT in subjects with T2D, consumption of caffeinated coffee resulted in a higher AUC for glucose compared to groups consuming water and decaffeinated coffee [24]. The subjects showed no difference in insulin sensitivity in this study [24]. Some studies did not find significant differences in glucose or insulin concentration following caffeinated coffee consumption [25]. On the contrary, long term trials to assess the effects of caffeine on glucose metabolism showed that caffeinated coffee ingestion may improve glycaemic metabolism by decreasing glucose levels and increasing insulin response [26–28]. Long term trials corroborate the epidemiologic studies showing a reduction of T2DM risk due to coffee consumption. These long-term beneficial effects on glucose metabolism may be due to anti-inflammatory/anti-oxidant effects of other constituents of coffee [29] or due to development of tolerance to the acute effects of caffeine consumption [27].

**2.1. Liver**—The liver plays a key role in the systemic regulation of glucose and lipid metabolism in both fasting and post-prandial states [30]. Circulating insulin's action on liver controls blood glucose levels by activating glycogen synthesis and inhibiting gluconeogenesis. In pathological conditions of obesity and T2D, aberrant insulin action fails to decrease glucose production and lipid synthesis leading to hepatic insulin resistance [30]. Excessive triglyceride storage leads to the development of NAFLD, which may progress to nonalcoholic steatohepatitis (NASH). In studies of liver fibrosis and particularly the protective effects of A<sub>2A</sub> and A<sub>2B</sub> adenosine antagonists, the mRNA of all four ARs has been detected in various liver cells such as hepatocytes, hepatic stellate cells and Kupffer cells (resident macrophages) [31]. Current studies employing different pharmacological approaches and genetic animal models show contradictory effects of AR activation on liver function.

In isolated rat hepatocytes, A<sub>1</sub>AR activation enhanced glucose output mainly via Ca<sup>2+</sup>-mediated glycogenolysis [32]. A<sub>1</sub>AR was also shown to regulate hepatic lipogenesis and steatosis. A study by Peng et al., showed that A<sub>1</sub>AR activation enhanced hepatic lipogenesis and hence contributed to liver steatosis in an alcohol-induced fatty liver mouse model [33]. Mice lacking the A<sub>1</sub>AR were protected from developing fatty liver in this model [33]. However, a study reported that hyperglycemia increased A<sub>1</sub>AR gene expression in the liver of insulin-deficient diabetic rats [34]. This increased A<sub>1</sub>AR enhanced glycogen

incorporation in hepatocytes from diabetic rats, leading to better blood glucose clearance [34].

Enhanced cAMP-mediated gluconeogenesis in rat hepatocytes was shown to be mediated by activation of the A<sub>2A</sub>AR [32]. Activation of A<sub>2A</sub>AR with a selective agonist (CGS21680) promoted glucose release from rat hepatocytes [32]. A<sub>2A</sub>AR activation resulted in anti-inflammatory effects [35, 36], whereas A<sub>2A</sub>AR deficiency enhanced pro-inflammatory responses [37]. Global A<sub>2A</sub>AR deficient mice displayed an increase in HFD-induced NAFLD and hepatic inflammation [38]. Lack of A<sub>2A</sub>AR in both hepatocytes and macrophages contributed to the enhanced HFD-induced inflammation [38].

Furthermore, A<sub>2A</sub>AR deficiency increased the abundance and the activity of sterol regulatory element-binding protein 1c (SREBP1c), elevating lipogenic events in mouse hepatocytes [38]. A<sub>2A</sub>AR deficient mice fed a methionine- and choline-deficient (MCD) diet displayed greater liver weight, increased severity of hepatic steatosis and liver inflammation compared to the controls [39]. Furthermore, enhanced MCD-induced NASH in A<sub>2A</sub>AR deficient mice was largely due to the increased inflammatory responses in macrophages [39]. The protective role of A<sub>2A</sub>AR for the development of NASH was largely attributed to the receptor's effect on suppressing inflammation caused by lipotoxicity [40, 41].

However, the A<sub>2B</sub>AR has been gaining attention with respect to its role in regulating liver function. The A<sub>2B</sub>AR is associated with regulation of hepatic lipid and glucose metabolism. Lack of A<sub>2B</sub>AR protected mice from hepatic triglyceride accumulation and development of alcohol-induced fatty liver [33]. Antagonism of A<sub>2B</sub>AR increased expression of genes required for fatty acid metabolism [33]. Furthermore, A<sub>2B</sub>AR activation enhanced lipid accumulation in a cultured mouse hepatocyte cell line (AML-12) [33]. A<sub>2B</sub>AR inhibition by selective antagonist ATL-801 in diabetic KKA(Y) mice resulted in reduced glucose production during hyperinsulinemic-euglycemic clamp [42]. In macrophages from KKA(Y) mice, another A<sub>2B</sub>AR-selective antagonist, ATL-692, blocked the increase of IL-6 induced by non-selective agonist NECA, which did not occur with A<sub>1</sub>, A<sub>2A</sub> or A<sub>3</sub> agonists. Thus, some of the beneficial effects of caffeine ingestion in KKA(Y) mice, and potentially in human T2D, may occur due to its blockade of the A<sub>2B</sub>AR [42]. Accordingly, a single nucleotide polymorphism (SNP) in A<sub>2B</sub>AR (1976T > C) has been identified to modulate the physiological effects of caffeine. The individuals with SNP-1976T > C displayed a higher postprandial glucose response after consuming caffeine and carbohydrate compared to carbohydrate alone [43].

A contrasting study showed that A<sub>2B</sub>AR activation inhibited hepatic lipogenesis via inhibiting sterol regulatory element binding protein-1 (SREBP-1). Lack of A<sub>2B</sub>AR led to the development of hepatic steatosis with elevated plasma triglyceride and cholesterol levels in high fat diet (HFD) fed mice [44]. However, adenovirus-mediated expression of hepatic A<sub>2B</sub>AR and its activation improved metabolism by reducing hepatic lipid synthesis [44]. A<sub>2B</sub>AR knockout (KO) mice on a HFD displayed impaired glucose tolerance and insulin sensitivity [45]. Treatment of wild-type (WT) mice on a HFD with an A<sub>2B</sub>AR agonist/partial agonist (BAY60-6553) improved glucose and insulin tolerance and decreased fasting blood glucose levels [45]. The impact of the A<sub>2B</sub>AR on lipid metabolism renders it a good drug

target for the treatment of liver diseases, although both A<sub>2B</sub>AR agonists and antagonists have been under consideration for T2D treatment.

Although in mouse liver the level of A<sub>3</sub>AR mRNA expressed in stellate cells greatly exceeds that in hepatocytes [46], very few studies have been conducted to study the effect of A<sub>3</sub>AR on liver metabolism. A recent study showed that administration of an A<sub>3</sub>AR agonist in the form of a solubilizing prodrug (MRS7476, 5 mg/kg, p.o., b.i.d.) protected mice against NASH in the STAM mouse model [46]. The prodrug contained two succinyl ester groups that were cleaved in vivo to release the potent and highly selective A<sub>3</sub>AR agonist MRS5698. mRNA levels of human A<sub>3</sub>AR were 1.9-fold decreased in liver tissue from NAFLD patients compared to the control samples, indicating a possible role of A<sub>3</sub>AR in NAFLD pathophysiology [46]. Likewise, lack of A<sub>3</sub>AR in HFD fed mice increased gene expression of hepatic inflammation and steatosis markers [46]. This study was further supported by clinical studies that show A<sub>3</sub>AR agonists have anti-inflammatory effects in diseases such as hepatocellular carcinoma (HCC) and hepatitis [47]. An A<sub>3</sub>AR agonist, CI-IB-MECA (namodenoson), has shown efficacy in a NASH model in the mouse [48] and is currently in a Phase 2 clinical trial for treating NASH.

**2.2. Skeletal muscle**—SKM is the largest insulin-sensitive organ as it comprises ~40% of total body mass in a healthy individual [49]. Due to its large mass, SKM accounts for ~30% of resting energy consumption and close to 100 % of enhanced energy consumption during exercise [49, 50]. High requirements for nutrients and fuel consumption render SKM a key contributor to systemic energy homeostasis. Following a meal, SKM is responsible for ~80% of the insulin-stimulated glucose uptake and disposal [51]. Glucose transported into myocytes via a GLUT4 transporter is immediately phosphorylated to a form that either gets stored as glycogen or enters the glycolytic pathway for oxidation. In insulin-resistant conditions, such as obesity and T2D, insulin-stimulated glucose uptake in SKM is greatly impaired [52, 53].

Contradictory studies have been reported with respect to the regulation of SKM glucose metabolism by adenosine signaling. Treatment of muscle preparations with ADA to degrade endogenous adenosine enhanced the insulin-induced glucose uptake and utilization [54, 55]. This inhibitory action of adenosine was observed to be mediated by A<sub>1</sub>AR in SKM [56]. By contrast, a study showed that activation of A<sub>1</sub>AR increased insulin-stimulated glucose uptake in rat soleus muscle [57]. AR antagonism by caffeine or a moderately A<sub>1</sub>AR-selective antagonist DPCPX (8-cyclopentyl-1,3-dipropylxanthine) resulted in a reduced rate of glucose uptake during muscle contractions [58]. Among the other ARs, few studies were conducted on A<sub>2B</sub>AR's role in SKM metabolism. A recent study showed that A<sub>2B</sub>AR activation elevated glucose uptake in primary human myocytes and had an additive effect on insulin-mediated glucose uptake [59]. The authors also reported that SKM lacking A<sub>2B</sub>AR showed decreased expression of genes involved in mitochondrial biogenesis, function, and oxidative metabolism. However, pharmacological activation of A<sub>2B</sub>AR increased oxidative capacity [59]. A<sub>2B</sub>AR also plays a key role in muscle hypertrophy that may regulate whole body metabolism. Muscle mass was reduced in mice lacking A<sub>2B</sub>AR [59]. Pharmacological treatment of these mice with A<sub>2B</sub>AR agonist BAY60–6553 significantly increased muscle mass [59]. Interestingly, treatment of aged mice for 4 weeks with the same A<sub>2B</sub>AR agonist

restored muscle mass to the elevated levels observed in young mice, protecting aging mice from muscle loss [59]. However, antagonism of the A<sub>2B</sub>AR during hyperinsulinemic-euglycemic clamp of diabetic KKA(y) mice increased glucose uptake in SKM [42]. Treatment of control animals with an A<sub>2B</sub>AR antagonist decreased insulin sensitivity, whereas an A<sub>2B</sub>AR antagonist rescued the insulin resistance phenotype in high sucrose-fed, insulin-resistant Wistar rats [60]. No detailed studies have been conducted to ascertain the role of A<sub>2A</sub>AR and A<sub>3</sub>AR in the regulation of SKM metabolism. Detailed *in vivo* studies with tissue-specific KO of ARs are required to better understand the role of adenosine signaling in modulation of SKM metabolism.

**2.3. Adipose tissue**—Adipose tissue plays a very significant role in the pathophysiology of obesity and T2D, as it is a major organ that stores energy in the form of lipids and contributes to non-shivering thermogenesis [30]. During obesity and T2D conditions, dysfunction in adipose tissue is marked by increased lipolysis which leads to increased plasma FFAs. This causes increased uptake and storage of FFA by liver and SKM causing secondary insulin resistance [30]. Multiple studies have been conducted to understand the role of ARs in adipose tissue-metabolic processes. Activation of the A<sub>1</sub>AR has long been known to inhibit lipolysis in adipose tissue [61–63]. In Zucker rats, a genetic model of obesity, pharmacological stimulation of A<sub>1</sub>AR decreased circulating FFAs and improved insulin-stimulated glucose uptake by metabolically active peripheral tissues [64, 65]. Mechanistically, it has been demonstrated that activation of A<sub>1</sub>AR reduced cAMP levels in adipocytes that caused reduction in hormone-sensitive lipase and adipose triglyceride lipase activities inhibiting breakdown of triglycerides to FFAs [62]. These studies point toward beneficial effects of A<sub>1</sub>AR on whole body metabolism, and various A<sub>1</sub>AR agonists and partial agonists have been studied clinically for treatment of diabetes [155]. However, a study by Baraket et al., proposed that excessive A<sub>1</sub>AR activation may lead to development of obesity due to its inhibition of lipolysis [66]. A<sub>1</sub>AR KO mouse models have been used to decipher the receptor's role in the development of obesity and regulation of whole-body metabolism. A<sub>1</sub>AR KO and WT mice kept on regular chow did not show any difference in body weight gain [67]. Similar results were found in another study where the authors demonstrated no difference in body weight in young (8 – 9 weeks of age) A<sub>1</sub>AR KO and WT mice on regular diet or HFD [68]. However, the authors reported higher body weight of aged A<sub>1</sub>AR KO (20 – 29 weeks of age) compared to WT mice fed either a regular or HFD due to higher food intake [68]. Similar changes in body weight were reported by a study, where old A<sub>1</sub>AR KO mice gained more weight than the controls [63]. However, Yang et al. demonstrated that lack of A<sub>1</sub>AR mitigated age-associated visceral adipose tissue accumulation due to reduced oxidative stress and inflammatory responses [69]. Consistent with the study, chronic treatment of Zucker obese rats with an A<sub>1</sub>AR antagonist (BW-1433) led to improved glucose tolerance [70]. These contrasting data highlight the need for the generation of adipose tissue-specific A<sub>1</sub>AR KO mouse models that can help to better understand the receptor's role in regulation of lipolysis and its secondary effects on tissues such as liver and SKM.

A recent study showed A<sub>2A</sub>AR to be most abundant AR in mouse and human brown adipose tissue (BAT), the site of non-shivering thermogenesis upon cold exposure, suggesting the

receptor's role in regulation of thermogenesis [71]. The authors used pharmacological stimulation of the receptor and genetic approaches to show that  $A_{2A}AR$  contributes to the browning of white fat. The activation of  $A_{2A}AR$  also protected mice from development of diet-induced obesity by improving whole body metabolism [71].  $A_{2A}AR$ -deficient mice displayed enhanced adipose tissue inflammation and insulin resistance on HFD [72]. Disruption of  $A_{2A}AR$  also resulted in enhanced palmitate-induced macrophage pro-inflammatory activation, suggesting a protective role of  $A_{2A}AR$  in obesity-associated inflammation [72]. However, there are contrasting studies suggesting that  $A_{2A}AR$  activation enhanced food intake and inhibited thermogenesis [73–75]. As with the  $A_1AR$ , adipose-specific KO mouse models of  $A_{2A}AR$  are warranted to understand the receptor's role in the above-mentioned processes.

Similar to  $A_{2A}AR$ , the  $A_{2B}AR$  is also highly expressed in BAT.  $A_{2B}AR$  activation was found to reduce adipocyte inflammation, as well as insulin resistance and islet destruction [76]. However, some beneficial results were also observed with  $A_{2B}AR$  antagonists in a diabetic mouse strain [76].

Adipose-specific ablation of  $A_{2B}AR$  reduced BAT-dependent energy expenditure, while  $A_{2B}AR$  activation protected mice from diet-induced obesity [59]. The authors also reported high expression of  $A_{2B}AR$  in BAT samples from lean compared to obese patients, and the receptor expression positively correlated with uncoupling protein 1 (UCP1) expression [59]. Consistent with this study,  $A_{2B}AR$  KO mice gained more weight than the WT mice and developed insulin resistance on a HFD [45]. Increased accumulation of visceral adipose tissue was demonstrated in aged  $A_{2B}AR$  KO mice [77, 78]. Pharmacological activation of  $A_{2B}AR$  reduced weight, due to regulation of immune cells preventing cell death, and decreased adipogenesis, leading to improved insulin resistance [78, 79]. These studies conclude that an  $A_{2B}AR$  agonist could potentially be used for therapeutic treatment of obesity and T2D (Fig. 1).

The role of  $A_3AR$  in the regulation of adipose tissue metabolism and development of obesity and T2D is unknown.

**2.4. Pancreas**—T2D is characterized by a progressive decrease in  $\beta$ -cell function including impaired insulin secretion mainly due to rising lipotoxicity and hyperglycemia [80]. Several studies have demonstrated the importance of adenosine signaling in the regulation of  $\beta$ -cell function.

All four ARs are known to be expressed in mouse pancreatic tissue [81]. Cell lines derived from rat (INS-1) and mouse ( $\beta$ -TC6) insulin producing  $\beta$ -cells also express all four ARs [82, 83]. Earlier studies demonstrated the role of adenosine in stimulating insulin release from pancreatic islets. Treatment of rat pancreatic islets with adenosine decreased glucose-stimulated insulin release in a dose-dependent manner [84, 85]. Treatment with a high concentration of adenosine (100  $\mu$ M) also decreased insulin secretion by mouse pancreatic islets in the presence of normal or a high glucose concentration [83]. Genetic ablation of  $A_1AR$  in mice led to enhanced glucose-stimulated insulin release [67, 86]. Pharmacological antagonism of  $A_1AR$  in rats showed similar effects by increasing insulin release by  $\beta$ -cells



[87]. Mechanistically, increased cAMP levels due to inhibition of A<sub>1</sub>AR resulted in insulin release from β-cells [67, 86]. These studies were further confirmed by Yang et al., who showed enhanced secretion of insulin from islets of A<sub>1</sub>AR KO than the control mice [69]. Improved β-cell function in A<sub>1</sub>AR KO mice was attributed to reduced oxidative stress [69]. These studies strongly suggest that antagonism of A<sub>1</sub>AR could improve β-cell function in T2D conditions.

Treatment of mouse islets with adenosine also increased insulin secretion through A<sub>2A</sub>AR, and its antagonism by A<sub>2A</sub>AR-selective SCH58261 decreased insulin release [83]. By contrast, activation of A<sub>2A</sub>AR in INS-1 cells suppressed insulin release [88]. Thus, in vivo studies are required to understand the role of A<sub>2A</sub>AR in the regulation of β-cell function. Adenosine signaling can also affect β cell proliferation and survival, associated with the development of type 1 diabetes (T1D) [83, 89, 90]. Using a zebrafish model of T1D, Andersson et al. identified that NECA increased β-cell proliferation and restored normoglycemia via A<sub>2A</sub>AR activation [90]. Another study showed that A<sub>2A</sub>AR activation in mice ameliorated T1D by increasing insulin levels in pancreas as well as in circulation [91]. A<sub>2B</sub> receptor antagonists improve insulin secretion [2,21]. Potential association of A<sub>3</sub>AR with T1D was also suggested; treatment of the β-TC6 cell line with A<sub>3</sub>AR agonist CI-IB-MECA, at very high concentrations (10 μM), reduced cell proliferation and viability [83]. A<sub>3</sub>AR agonists at low concentrations are associated with attenuating apoptosis. However, CI-IB-MECA at high concentrations triggered β-TC6 cell death that could be partially prevented by antagonizing the receptor [83].

Increasing adenosine accumulation by inhibiting adenosine kinase can enhance β-cell proliferation [89]. Increased adenosine intracellular levels resulted in activation of mammalian target of rapamycin (mTOR) to promote β cell proliferation [89]. ADA inhibition can also increase adenosine levels, and elevated ADA activity in patients with T2D has been reported, suggesting its inhibition may provide therapeutic benefits [92–94].

In conclusion, available scientific data suggest that A<sub>1</sub>AR inhibition and A<sub>2A</sub>AR activation may prove useful as therapeutic approaches for increasing insulin secretion and treatment of diabetes (Fig. 1). However, the effects of A<sub>2B</sub>AR and A<sub>3</sub>AR modulators on the pancreas with respect to T2D require further validation.

**2.5 Hypothalamus**—Adenosine is a prototypic neuromodulator that can regulate neuronal activity including sleep, memory, anxiety, aggression, pain, locomotion, cardiac and immune functions, and neurodegenerative diseases [95–100]. However, few studies have been conducted to understand the role of neuronal ARs in regulation of energy homeostasis. A study by Wu et al. showed that HFD feeding increased adenosine levels in plasma, cerebrospinal fluid and hypothalamus of obese mice [101]. Among all ARs, expression levels of only A<sub>1</sub>AR was upregulated in the hypothalami of obese mice, suggesting A<sub>1</sub>AR's role in the development of obesity [101]. A<sub>1</sub>AR activation or its over-expression in the paraventricular nucleus (PVN) region of the hypothalamus increased food intake and decreased energy expenditure in mice. Moreover, intracerebroventricular (i.c.v.) administration of caffeine reduced appetite in mice. The i.c.v. administration of caffeine for

7 days decreased obesity and plasma triglyceride levels to improve glucose tolerance in these mice [101].

### 3. P2 purinergic receptors and diabetes

Extracellular ATP activates two structurally and pharmacologically distinct families of P2 purinergic receptors - P2X and P2Y receptors. Various P2X and P2Y receptors are expressed in metabolically active tissues in human and mouse, suggesting their role in the regulation of glucose and energy homeostasis (Fig. 2).

#### 3.1. Liver

P2 receptors have been shown to regulate glucose and lipid metabolism in liver [102]. In isolated rat hepatocytes, P2Y<sub>1</sub>R activation stimulates glycogen phosphorylase by increasing cytoplasmic Ca<sup>2+</sup> levels [103] and may contribute to hepatic glucose output. Purinergic signaling also modulates beneficial reverse cholesterol transport in liver. High density lipoprotein (HDL)-cholesterol levels in plasma were significantly decreased and their liver uptake increased after chronic P2Y<sub>13</sub>R activation by a partial agonist [104]. This conclusion was supported by Fabre et al. using P2Y<sub>13</sub>R KO mice [105]. Cangrelor (AR-C69931MX), a P2Y<sub>12</sub>R antagonist and approved antithrombotic agent that also acts as a partial P2Y<sub>13</sub> agonist, stimulated hepatic HDL uptake in control mice and mice lacking the HDL-selective cholesteryl ester uptake system, but not in P2Y<sub>13</sub>R KO mice [73]. Knowledge of P2R function in the liver tissue associated with the development of diabetes and obesity is lacking. Liver-specific KO of these receptors should be generated to decipher their role in the liver pathophysiology and development of T2D.

#### 3.2. Skeletal muscle

Purinergic receptors are expressed in human SKM fibers [106]. Enhanced glucose uptake in the C2C12 mouse SKM cell line was demonstrated after ATP stimulation, while nonselective P2R antagonist (suramin) ablated the effect, suggesting involvement of P2 receptors [107]. Balasubramanian et al. showed that stimulation of C2C12 cells with a P2Y<sub>6</sub>R dinucleotide agonist (MRS2957) significantly increased glucose uptake, and this stimulation was decreased by a selective P2Y<sub>6</sub>R antagonist (MRS2578) [108]. The enhancement of glucose uptake was mediated by AMP-activated protein kinase (AMPK) activation as treatment with an AMPK inhibitor abolished the effect [108]. These results were supported by a recent study where P2Y<sub>6</sub>R was KO specifically in SKM, and its effect on glucose metabolism was studied. Ablation of P2Y<sub>6</sub>R impaired glucose tolerance and insulin sensitivity and reduced insulin stimulated glucose uptake by mouse SKM (gastrocnemius) [109]. Thus, P2Y<sub>6</sub>R activation emerged as a potential means for improving SKM glucose uptake in diabetic subjects.

#### 3.3. Pancreas

Insulin secretory vesicles contain adenine nucleotides, which are co-released with insulin during exocytosis [110–114]. Secreted ATP and ADP act in an autocrine manner in pancreatic  $\beta$ -cells by stimulating P2 receptors [115, 116]. ATP was proposed to act via the P2X<sub>3</sub>R by increasing intracellular calcium concentration [117]. Another study showed that

ATP activates P2Y<sub>1</sub>R on human  $\beta$ -cells, stimulating electrical activity and coupling Ca<sup>2+</sup> influx to Ca<sup>2+</sup> release from endoplasmic reticulum stores [115, 118]. The P2Y<sub>1</sub>R acts on  $\beta$ -cells by activating protein kinase C [119]. Short ATP pulses stimulate, whereas longer exposure of  $\beta$ -cells to high concentrations of ATP can have negative feedback on the insulin secretion [117, 120–122]. This dual effect of ATP coordinates with Ca<sup>2+</sup> oscillations [123]. Some earlier studies reported similar insulin responses to P2R agonist treatment in the perfused pancreas from T1D and T2D compared to control mouse groups. ADP $\beta$ S, a P2Y<sub>1</sub>R agonist, induced a similar insulin response in the perfused pancreas of streptozotocin-treated (STZ) rats as in controls [124, 125]. These studies suggest that P2YR agonists might be useful for enhancing insulin release from glucose-unresponsive islets from diabetic animals. However, another study by Leon et al. indicated negative regulation of insulin secretion by P2Y<sub>1</sub>R activation. The authors observed a higher insulin response to a stimulatory glucose concentration in islets isolated from P2Y<sub>1</sub>R KO compared to control mice [126]. Similarly, Ohtani et al. demonstrated significant inhibition of insulin release at high glucose concentration by P2Y<sub>1</sub>R activation [127]. Inhibition of insulin release was also observed after P2Y<sub>6</sub>R activation in mouse islets [127]. By contrast, activation of mouse MIN6 cells by a P2Y<sub>1</sub>R agonist (2-MeSADP) and a P2Y<sub>6</sub>R agonist (Up<sub>3</sub>U) stimulated calcium-mediated insulin release [128]. The insulin secretion was blocked by P2Y<sub>1</sub>R and P2Y<sub>6</sub>R antagonists, respectively [128]. Mechanistically, P2Y<sub>6</sub> agonists induced AMPK activation, which enhanced insulin release by MIN6 cells [129].

### 3.4. Adipocytes

P2X and P2Y receptors are expressed in various adipose depots and are involved in regulation of adipose tissue processes such as differentiation, browning, glucose uptake and thermogenesis. P2Y<sub>1</sub>, P2Y<sub>2</sub>, P2Y<sub>4</sub> and P2Y<sub>11</sub> receptor activation promoted adipogenic differentiation of bone marrow or adipose tissue derived stem cells [130–132]. P2Y<sub>1</sub> and P2Y<sub>4</sub> receptor activation by ADP and UTP, respectively, stimulated adipogenesis in human bone marrow-derived mesenchymal stem cells though activation of PPAR $\gamma$ , while the action of ectonucleotidases leading to increased AR activation resulted in osteogenic differentiation [133].

Human adipocytes display constitutive P2Y<sub>2</sub>R activity that suppresses basal lipolysis [134]. Pharmacological inhibition or knockdown of the P2Y<sub>2</sub>R increases cAMP levels and hence basal lipolysis [134]. P2Y<sub>2</sub>KO mice on a HFD were protected from diet-induced obesity, as the preadipocytes from KO mice differentiated less robustly than the WT cells [135]. Protection from obesity also resulted in reduced inflammation and improved glucose tolerance in P2Y<sub>2</sub>KO mice on a HFD [135]. Anti-adipogenic effects were exhibited upon P2Y<sub>13</sub>, P2Y<sub>14</sub> and P2X<sub>7</sub> receptor activation [131, 136]. Supporting an anti-adipogenic effect of the P2X<sub>7</sub>R, P2X<sub>7</sub>R KO mice exhibited increased body weight, adipocyte hyperplasia, ectopic lipid accumulation in pancreas [137]. Some studies indicate that P2X<sub>7</sub>R activation resulted in differentiation of stem cells into an osteoclast lineage rather than adipocytes [138]. P2X<sub>7</sub>R expression is enhanced in human adipose tissues from patients suffering from metabolic syndrome [139]. The P2X<sub>7</sub>R and NLRP3 inflammasome expression and IL-1 $\beta$  secretion were higher in obese subjects [140]. In metabolically unhealthy obese humans, a P2X<sub>7</sub>R antagonist blocked the adaptive Th17 polarization of T<sub>eff</sub> cells in visceral adipose

tissue [141]. P2X7R activates and its antagonists suppress the NLRP3 inflammasome, which is a step leading to the pathology of diabetes [142]. Thus, P2X7R antagonists might be useful in treating diabetes [142]. In contrast, another study showed that P2X7 KO mice are not protected from diet-induced obesity, adipose tissue inflammation and associated metabolic dysfunction [143].

Interestingly, lack of P2Y<sub>14</sub>R in the whole body did not protect mice from diet-induced obesity [144]. However, P2Y<sub>14</sub> KO mice were protected from HFD-induced insulin resistance in metabolic tissues such as liver, SKM and adipose tissue. Furthermore, the authors showed that lack of P2Y<sub>14</sub>R protected mice from macrophage infiltration in liver, thereby reducing obesity-associated inflammation [144]. Lack of P2Y<sub>14</sub>R in other metabolically active tissues and immune cells may contribute to the observed phenotype. Hence, an adipose tissue-specific P2Y<sub>14</sub> KO mouse model may provide better understanding of the receptor's role in the regulation of adipose tissue metabolism.

P2 receptors have been studied to decipher their role in glucose uptake in adipocytes. Stimulation of rat adipocytes with low concentrations of ATP inhibited insulin-stimulated glucose transport [145, 146]. A study showed that activation of the P2Y<sub>6</sub>R-AMPK pathway caused GLUT-4 translocation thereby enhancing glucose uptake in mouse white adipocytes [108]. A recent study showed that KO of P2Y<sub>6</sub>R in the whole body or in an adipocyte-specific manner protected mice from diet-induced obesity to improve glucose tolerance, insulin sensitivity and to reduce systemic inflammation. This improvement was associated with reduced JNK signaling with consequent increase of peroxisome proliferator-activated receptor (PPAR)- $\alpha$  and beiging of white fat [109]. This suggests that P2Y<sub>6</sub>R antagonists could be examined as a potential treatment of diabetes and obesity.

Purinergic signaling can also modulate production and secretion of adipokines critical for regulating metabolism in an organism. P2Y<sub>1</sub>R inhibition by highly selective antagonist MRS2500 decreased leptin production in isolated mouse white adipocytes [147]. Circulating leptin levels were reduced in P2Y<sub>1</sub>R KO mice, indicating that P2Y<sub>1</sub>R controls leptin production and secretion in white adipocytes [147]. Adiponectin expression and secretion in cardiac adipocytes was inhibited by a P2Y<sub>4</sub>R agonist (MRS4062), while adiponectin levels were increased in P2Y<sub>4</sub>R KO mice [148].

Mouse brown adipocytes express P2X and P2Y receptors [149]. P2X5R mRNA expression level is significantly higher in BAT compared to WAT and other mouse tissues [150]. Therefore, P2X5R is proposed as a novel cell surface marker for brown and beige adipocytes [150]. Furthermore, chronic cold exposure increased P2X5R expression in BAT and subcutaneous WAT, suggesting its role in thermogenesis [150, 151]. However, the mechanism of P2X5R action in different adipose tissues is largely unknown.

### 3.5. Hypothalamus

Over the last decade, interest in understanding the role of central nervous system (CNS) in the regulation of energy balance in mammals has been growing rapidly. Arcuate nucleus of the hypothalamus (ARH) in brain can respond to different nutrient signals from gut and adipose tissues to influence energy homeostasis. Activation of agouti-related peptide (AgRP)

neurons in ARH regulates appetite by enhancing food intake [152]. A comprehensive study showed that P2Y<sub>6</sub>R is expressed in AgRP neurons and that P2Y<sub>6</sub>R activation by UDP increased AgRP firing rate and feeding in mice [153]. Pharmacological inhibition of P2Y<sub>6</sub>R in AgRP neurons by MRS2578 decreased food intake in mice [153]. Moreover, AgRP-P2Y<sub>6</sub>R KO mice are protected from obesity and insulin resistance on an obesogenic diet [154]. These studies show that P2Y<sub>6</sub>R in the CNS can be targeted to inhibit excessive feeding and to overcome development of obesity and T2D.

#### 4. Conclusion

Present preclinical studies have highlighted the significance of adenosine signaling in the regulation of pathophysiological processes underlying diabetes. Among the G<sub>s</sub>-coupled ARs, A<sub>2B</sub>AR stands out as a potential therapeutic target, as the receptor activation improves function of SKM and brown fat in aged as well as obese mice (Fig. 1). However, future studies are required to address A<sub>2B</sub>AR's role in other metabolically active tissues such as liver and brain. A<sub>3</sub>AR has also emerged as a potential target providing protection against the development of NASH. However, its role in other metabolic tissues is largely unknown. Among P2Y and P2X receptors, inhibiting P2Y<sub>6</sub>R and P2X<sub>7</sub>R may provide therapeutic benefits against diet-induced obesity and diabetes (Fig. 2). A majority of the present studies utilized pharmacological manipulation or whole-body KO mouse models, which makes understanding of a receptor's role in a tissue-specific manner highly complicated. Future studies are warranted to address this uncertainty. Numerous agonists and antagonists for purinergic receptors and inhibitors of enzymes regulating adenosine levels have been synthesized with the potential for use in preclinical and clinical studies targeting diabetes and associated complications [155–159].

Combining tissue-specific genetic approaches with pharmacological interventions may foster the development of novel drugs targeting purinergic signaling that may mitigate the development of diabetes and associated metabolic disorders.

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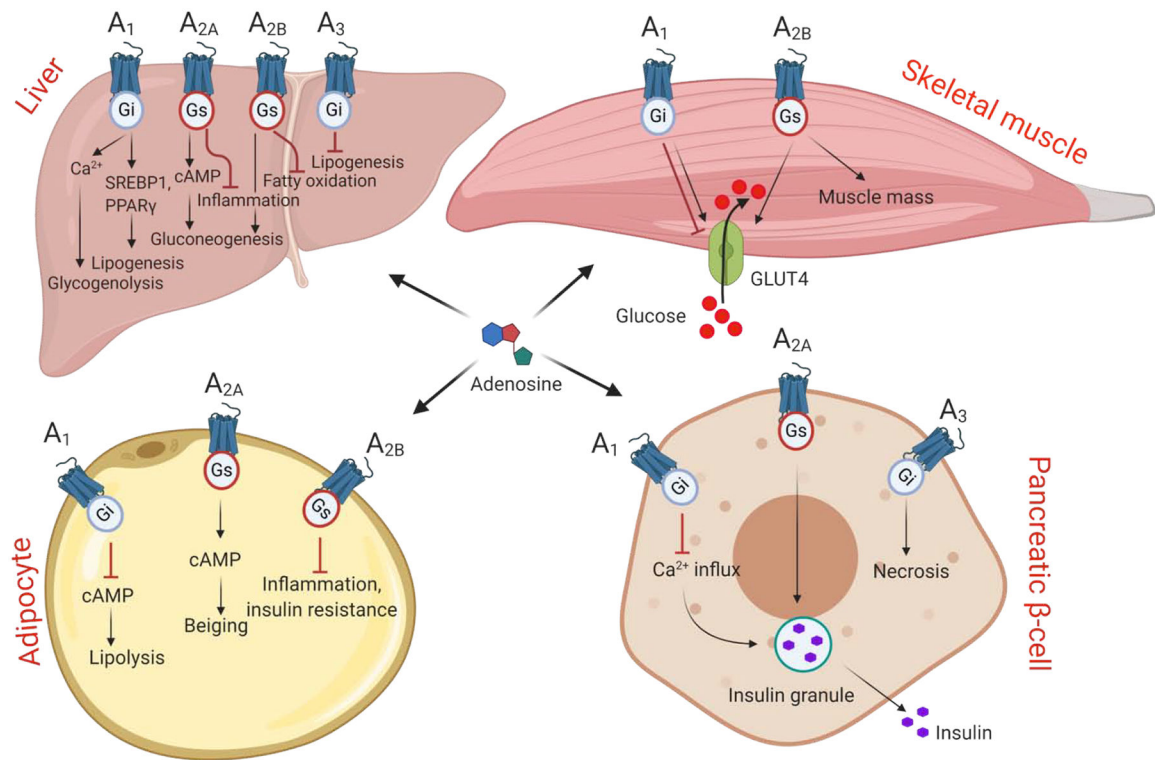


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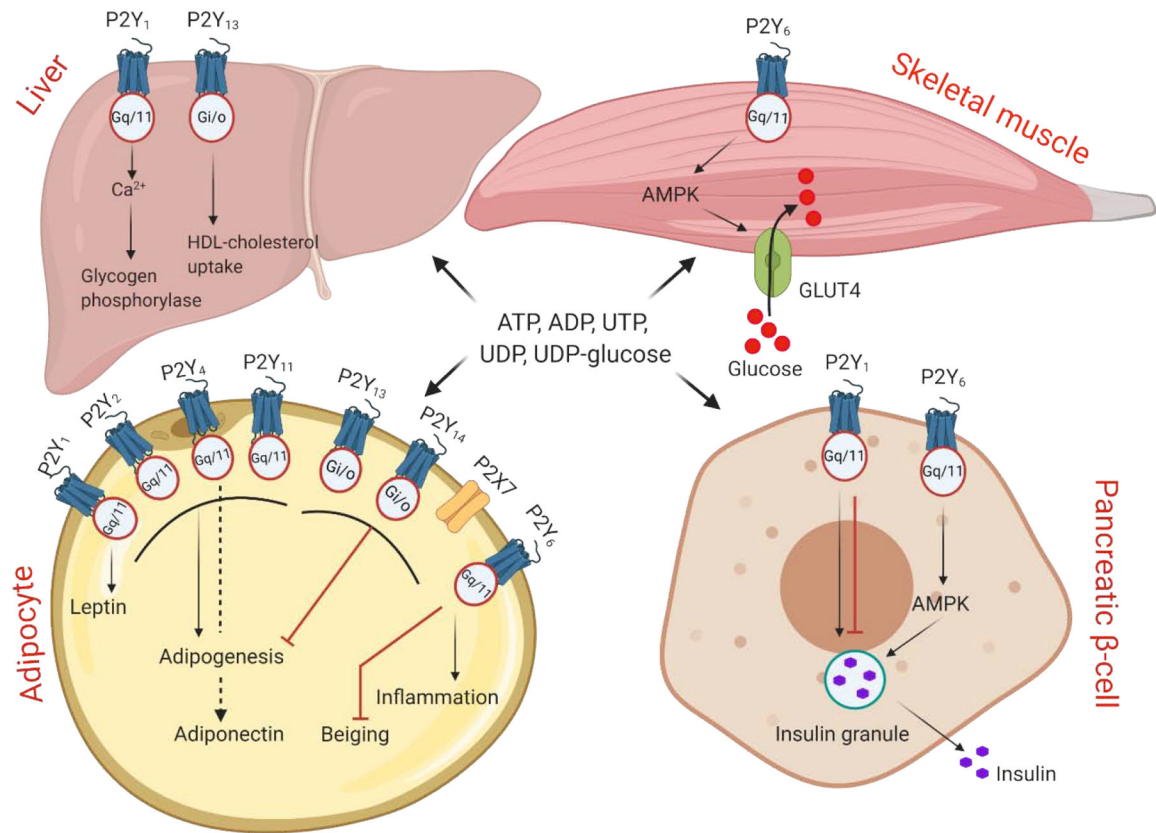
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**Figure 1.** Modulation of metabolic processes by adenosine signaling. Four adenosine receptors couple to G<sub>i</sub> or G<sub>s</sub> proteins and modulate the level of secondary messengers such as cAMP/Ca<sup>2+</sup> to activate different signaling proteins. Metabolic processes regulated by adenosine receptors in liver, skeletal muscle, adipose tissue and pancreas. See Section 2 for details.



**Figure 2.**

Regulation of metabolically active tissues functions by P2Y and P2X receptors.

Eight P2Y receptors are activated by receptor-specific nucleotide or nucleotide sugars. The receptors couple to different G-protein as indicated. Seven P2X receptors are membrane ion channels that are solely activated by ATP. Metabolic processes regulated by P2 receptors in liver, skeletal muscle, adipose tissue and pancreas. See Section 3 for details.

**Table 1.**

Location and effects of adenosine receptors in diabetes and obesity.

Receptor subtype	Location	Effect
<b><u>Liver</u></b>		
A <sub>1</sub>	Rat hepatocytes	Enhanced glycogenolysis [32]
A <sub>1</sub>	Mouse liver	KO protected from ethanol induced fatty liver [33]
A <sub>1</sub>	Rat hepatocytes	Enhanced glycogen incorporation and glucose clearance [34]
A <sub>2A</sub>	Rat hepatocytes	Enhanced gluconeogenesis [32]
A <sub>2A</sub>	Mouse whole body	KO increased NAFLD, hepatic inflammation [38]
A <sub>2A</sub>	Mouse whole body	KO mice on MCD diet increased NASH [39]
A <sub>2A</sub>	Mouse liver	Activation suppress inflammation cause by lipotoxicity [40, 41]
A <sub>2B</sub>	Mouse liver	KO protected from ethanol-induced fatty liver [33]
A <sub>2B</sub>	Mouse liver	Antagonism reduced glucose production during clamp studies [42]
A <sub>2B</sub>	Mouse liver	Activation inhibited hepatic lipogenesis [44] KO enhanced hepatic steatosis in HFD mice [44]
A <sub>2B</sub>	Mouse liver	KO impaired glucose tolerance and insulin sensitivity [45] Treatment with agonist improved metabolism [45]
A <sub>3</sub>	Mouse STAM model	Activation by a prodrug protects against NASH [46]
A <sub>3</sub>	Human studies	Agonist imparts anti-inflammatory effects in HCC and hepatitis [47]
A <sub>3</sub>	Mouse NASH model	Agonist protects against NASH [48]
<b><u>Skeletal muscle</u></b>		
A <sub>1</sub>	Mouse skeletal muscle	Inhibited glucose uptake [56]
A <sub>1</sub>	Rat soleus muscle	Enhanced insulin-mediated-glucose uptake [57]
A <sub>1</sub>	Rat skeletal muscle	Antagonism reduced glucose uptake during muscle contractions [58]
A <sub>2B</sub>	Human myocytes	Enhanced glucose uptake [59]
A <sub>2B</sub>	Mouse skeletal muscle	Increased muscle mass and oxidative capacity [59]
A <sub>2B</sub>	Mouse skeletal muscle	Antagonism increased glucose uptake [42]
A <sub>2B</sub>	Mouse skeletal muscle	Antagonism decreased insulin sensitivity [60]
A <sub>2B</sub>	Mouse skeletal muscle	Increased insulin sensitivity in high sucrose fed insulin-resistant mice[60]
<b><u>Adipose tissue</u></b>		
A <sub>1</sub>	Rat adipocytes	Activation inhibits lipolysis [61–63]
A <sub>1</sub>	Zucker rat whole body	Stimulation decreased plasma FFA and enhanced insulin stimulated glucose uptake [64, 65]
A <sub>1</sub>	Mouse whole body	Excessive activation causes obesity [66]
A <sub>1</sub>	Mouse whole body	Aged KO mice gained more weight on a HFD, higher food intake [68]
A <sub>1</sub>	Mouse whole body	KO showed reduced age-associated fat accumulation [69]
A <sub>1</sub>	Zucker rat whole body	Antagonist treatment improved glucose tolerance [70]
A <sub>2A</sub>	Mouse adipocytes	Enhanced browning of fat, protected from HFD induced obesity, improved whole body metabolism [71]
A <sub>2A</sub>	Mouse whole body	KO increased inflammation and insulin resistance [72].
A <sub>2A</sub>	Mouse adipocytes	Activation enhanced food intake and inhibited thermogenesis [73–75]
A <sub>2B</sub>	Mouse whole body	Activation reduced inflammation and insulin resistance [76]

Receptor subtype	Location	Effect
A <sub>2B</sub>	Mouse adipocytes	Activation protected against HFD-induced obesity [59]
A <sub>2B</sub>	Mouse adipocytes	KO increased weight gain and insulin resistance on a HFD [45]
A <sub>2B</sub>	Mouse adipocytes	Aged KO mice accumulated more visceral fat on a HFD [77, 78]
A <sub>2B</sub>	Mouse adipocytes	Activation reduced weight, improved insulin resistance [78, 79]
<b><u>Pancreas</u></b>		
A <sub>1</sub>	Mouse whole body	KO enhanced glucose-stimulated insulin release [67,86]
A <sub>1</sub>	Rat islets	Antagonism increased insulin release [87]
A <sub>1</sub>	Mouse islets	Enhanced secretion of insulin from islets of KO [69]
A <sub>2A</sub>	Mouse islets	Antagonism decreased insulin release [83]
A <sub>2A</sub>	INS-1 cells	Activation suppressed insulin release [88]
A <sub>2A</sub>	Zebrafish model of T1D	Activation increased P-cell proliferation [90]
A <sub>2A</sub>	Mouse-T1D model	Activation increased insulin release and ameliorated T1D [91]
A <sub>3</sub>	P-TC6 cell line	Activation reduced cell proliferation and viability [83]
<b><u>Hypothalamus</u></b>		
A <sub>1</sub>	Mouse hypothalamus	Activation increased food intake, decreased energy expenditure [101]



**Table 2.**

Location and effects of P2 receptors in diabetes and obesity.

Receptor subtype	Location	Effect
<b><u>Liver</u></b>		
P2Y <sub>1</sub>	Rat hepatocytes	Stimulates glycogen phosphorylase [103]
P2Y <sub>13</sub>	Mouse liver	Activation increased reverse cholesterol transport [104]
P2Y <sub>13</sub>	Mouse liver	KO decreases hepatic HDL cholesterol uptake [105]
<b><u>Skeletal muscle</u></b>		
P2Y <sub>6</sub>	C2C12 cell line	Activation increased glucose uptake [108]
P2Y <sub>6</sub>	Mouse skeletal muscle	KO decreased insulin-stimulated glucose uptake in obese mice, impaired glucose tolerance and insulin sensitivity [109]
<b><u>Pancreas</u></b>		
P2X <sub>3</sub>	Human beta cells	ATP increases calcium concentration [117]
P2Y <sub>1</sub>	Human beta cells	ATP stimulate electrical activity, regulating calcium flux [115]
P2Y <sub>1</sub>	STZ rats-perfused pancreas	Activation caused insulin response same as control [124, 125]
P2Y <sub>1</sub>	Mouse islets	KO showed higher glucose-stimulated insulin response [126]
P2Y <sub>1</sub>	Mouse islets	Activation decreased glucose-stimulated insulin response [127]
P2Y <sub>1</sub>	MIN6 cell line	Activation increased insulin response [128]
P2Y <sub>6</sub>	MIN6 cell line	Activation increased insulin response [129]
<b><u>Adipose tissue</u></b>		
P2Y <sub>1</sub>	Mesenchymal stem cells	Promotes adipogenic differentiation [130]
P2Y <sub>1</sub>	Mouse whole body	KO decreased leptin production [147]
P2Y <sub>2</sub>	Human adipocytes	Suppress basal lipolysis [134]
P2Y <sub>2</sub>	Mouse whole body	KO protected from HFD-induced obesity, improved glucose tolerance, impaired adipocyte differentiation [135]
P2Y <sub>4</sub>	Mesenchymal stem cells	Promotes adipogenic differentiation [130]
P2Y <sub>4</sub>	Mouse whole body	KO increased adiponectin levels [147]
P2Y <sub>6</sub>	Mouse adipocytes	Activation increased glucose uptake [108]
P2Y <sub>6</sub>	Mouse adipose tissue	KO protected from diet induced obesity/inflammation [109]
P2Y <sub>13</sub>	Mesenchymal stem cells	Promotes anti-adipogenic effect [136]
P2Y <sub>14</sub>	Mouse whole body	KO protected from HFD induced insulin resistance, reduced macrophage infiltration in the liver [144]
P2Y <sub>14</sub>	Mesenchymal stem cells	Promotes anti-adipogenic effect [131]
P2X <sub>7</sub>	Mouse whole body	KO gained more weight, adipocyte hyperplasia, activation caused anti-adipogenic effect [137, 138, 131]
P2X <sub>7</sub>	Mouse adipocytes	Antagonism suppresses NLRP3 inflammasome [141, 142]
P2X <sub>7</sub>	Mouse whole body	KO protected from diet induced obesity, inflammation [143]
<b><u>Hypothalamus</u></b>		
P2Y <sub>6</sub>	Mouse AgRP neurons	Activation promoted feeding [153]
P2Y <sub>6</sub>	Mouse AgRP neurons	Antagonist inhibited feeding in obesity [154]
P2Y <sub>6</sub>	Mouse AgRP neurons	KO protected from diet-induced insulin resistance [154]