Adenosine, Adenosine Receptors and Their Role in Glucose Homeostasis and Lipid Metabolism

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Abstract

Adenosine is an endogenous metabolite that is released from all tissues and cells including liver, pancreas, muscle and fat, particularly under stress, intense exercise, or during cell damage. The role of adenosine in glucose homeostasis has been attributed to its ability to regulate, through its membrane receptors, processes such as insulin secretion, glucose release and clearance, glycogenolysis, and glycogenesis. Additionally, adenosine and its multiple receptors have been connected to lipid metabolism by augmenting insulin-mediated inhibition of lipolysis, and the subsequent increase in free fatty acids and glycerol levels. Furthermore, adenosine was reported to control liver cholesterol synthesis, consequently affecting plasma levels of cholesterol and triglycerides, and the amount of fat tissue. Alterations in the balance of glucose and lipid homeostasis have implications in both cardiovascular disease and diabetes. The ability of different adenosine receptors to activate and inhibit the same signaling cascades has made it challenging to study the influence of adenosine, adenosine analogs and their receptors in health and disease. This review focuses on the role and significance of different adenosine receptors in mediating the effect of adenosine on glucose and lipid homeostasis.

Keywords

Adenosine; glucose; lipids

Introduction

Adenosine is an endogenous purine nucleoside that is released from cells upon injury or inflammation. Adenosine can be generated from adenosine triphosphate (ATP) in the extracellular space through the action of two endonucleotidases: CD39 (ENTPD1; nucleoside triphosphate diphosphorylase 1) and CD73 (NT5E; ecto-5'-nucleotidase) (Hasko et al., 2008; Yegutkin, 2008). In similar fashion, adenosine can be generated intracellularly from ATP and exported to the extracellular space by two transporters called equilibrative nucleoside transporters one and two (ENT1 and ENT2) (Eltzschig et al., 2005). Once in the

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extracellular space, adenosine acts on four different G-protein coupled receptors that are classified as adenylyl cyclase inhibiting (A_1 and A_3) or adenylyl cyclase activating (A_{2a} and A_{2b}) (Tucker and Linden, 1993). Studies have implicated adenosine, signaling through the A2-type adenosine receptors, as an anti-inflammatory autocrine molecule responsible for inhibition of inflammatory cytokine release at baseline (Apasov et al., 2000; Lukashev et al., 2004; Ohta and Sitkovsky, 2001; Ryzhov et al., 2008; Yang et al., 2006b), upon injury (Day et al., 2004; Lukashev et al., 2007; Okusa et al., 2001; Yang et al., 2008), or upon bacterial invasion (Lappas et al., 2005; Yang et al., 2006b). The anti-inflammatory role of adenosine has also been associated with protection of the vasculature in a restenosis like model (Yang et al., 2008) and in improving overall myocardial tone (Guo et al., 2001; Law et al., 1988). It is important to mention that in addition to inflammation, adenosine and adenosine receptors have been associated with regulation of glucose clearance (Han et al., 1998; Johnston-Cox et al., 2012a; Maeda and Koos, 2008), lipolysis (Johansson et al., 2008; Ulrich Schwabe, 1974) and fatty liver prevention post alcohol intake (Peng et al., 2009), or prevention of liver steatosis post high fat diet (Koupenova et al., 2012), regulation of cholesterol synthesis (Koupenova et al., 2012), or fat deposition (Johnston-Cox et al., 2012a).

Glucose homeostasis is achieved by a fine balance among exogenous dietary intake, endogenous release from the liver, and clearance by the muscle and adipose tissues. These processes are precisely regulated by the two major hormones released by the pancreatic alpha and beta cells, glucagon and insulin, respectively (Bansal and Wang, 2008). When blood glucose levels rise after exogenous dietary intake, insulin is released and stimulates glucose uptake by the skeletal muscle and adipose tissue and inhibits glucose production by the liver (Saltiel and Kahn, 2001). At low glucose blood levels, glucagon is released into the circulation. In the liver, glucagon initiates glycogenolysis and gluconeogenesis and the consequent increase in glucose availability, while in adipose tissue it initiates lipolysis and the subsequent release of free fatty acids (FFA) (Bansal and Wang, 2008; Ruan and Lodish, 2003). Lipolysis, in turn, is initiated by Protein kinase A (PKA)-mediated activation of hormone sensitive triacylglycerol lipase, which leads to the increase of triglyceride (TG) break down to glycerol and fatty acids (Allen et al., 1986).

Glucose clearance from the circulation depends on insulin and insulin receptor signaling in adipose and muscle tissue. Under certain physiological conditions such as obesity and/or type II diabetes, insulin is unable to effectively clear blood glucose. The impaired ability of insulin to clear glucose from the circulation is defined as insulin resistance (McGarry, 2002). As a consequence, insulin can no longer inhibit lipolysis in the adipose tissue and levels of free fatty acids and glycerol rise in the plasma. The increase of plasma FFA results in their elevated uptake by the liver which leads to their oxidation and consequent accumulation of acetyl coenzyme A (Acetyl CoA). In turn, the elevated levels of Acetyl CoA in the liver stimulate the rate limiting enzymes for gluconeogenesis (pyruvate carboxylase and phosphoenolpyruvate carboxykinase) and glycogenolysis (glucose-6-phosphatase) to produce more glucose, which in turn results in the production of more insulin by the pancreas (Kovacs and Stumvoll, 2005). On the other hand, accumulation of FFA in the liver gives rise to non-alcoholic fatty liver disease. In that sense, improper glucose homeostasis can cause tissue damage and whole body deterioration (McGarry, 2002).

Under non-pathological conditions, excess blood glucose is cleared by the liver and later stored as glycogen or fatty acids. Fatty acids are stored in the form of triglycerides and esterified cholesterol, which are delivered to the periphery via vesicles called very low density lipoprotein (VLDL). In the circulation VLDL particles ultimately get hydrolyzed to a low density lipoprotein vesicle (LDL) as triglycerides and cholesterol get redistributed (Boullier et al., 2001). Cholesterol from the periphery is delivered back to the liver by high density lipoprotein (HDL) particles, termed the reverse cholesterol transport. Excess

cholesterol in the blood is associated with an increased amount of LDL particles which can progress into pathological conditions such as atherosclerosis (Li and Glass, 2002).

Adenosine has been associated with lipolysis and glucose clearance as well. It has been proposed that adenosine signaling through its receptors in adipose tissue increases insulin sensitivity (Dong et al., 2001b; Green et al., 1997; Vannucci et al., 1992; Vannucci et al., 1989). Consequently, glucose tolerance has also been improved in studies using adenosine-based pharmacological reagents (Crist et al., 1998; Xu et al., 1998); even though studies using adenosine agonists and antagonists seem to exhibit contradictory effects (Crist et al., 1998; Schoelch et al., 2004; Xu et al., 1998). Recent work also associates adenosine with pancreatic insulin synthesis, reverse cholesterol transport and fatty liver prevention (Peng et al., 2009), liver steatosis amelioration (Koupenova et al., 2012), improvement of glucose and insulin clearance (Johnston-Cox et al., 2012a; Yang et al., 2012) .This review will outline the role of the different adenosine receptors in the above mentioned processes.

Adenosine and A_1 Adenosine Receptors

Adenosine has been associated with the regulation of glucose homeostasis for more than thirty years (Dole, 1962; Ismail et al., 1977; Jain and Logothetopoulos, 1978; Loubatieres-Mariani et al., 1979; Schütz et al., 1978; Schütz et al., 1978; Ulrich Schwabe, 1974; Van Calker, 1979). Studies with non-selective adenosine antagonists have shown that adenosine can improve insulin secretion and lower glucose production (Table I) (Arias et al., 2001; Corssmit et al., 1994; Rüsing et al., 2006). Furthermore, adenosine can decrease insulin (Bertrand et al., 1989; Hillaire-Buys et al., 1987) and stimulate glucagon (Chapal et al., 1985; Chapal et al., 1984; Schütz et al., 1978; Schütz et al., 1978) secretion. Adenosine can also increase hepatic glycogenolysis (González-Benítez et al., 2002; Hoffer and Lowenstein, 1986; Oetjen et al., 1990) by activation of A_1 adenosine receptor (A_1AR) in Ca^{2+}-dependent manner (González-Benítez et al., 2002).

The importance of adenosine signaling with respect to glucose homeostasis and insulin sensitivity has been predominantly attributed to its ability to inhibit lipolysis in fat, and glycolysis in muscle. All four adenosine receptors are known to be expressed in muscle and fat, with the expression of A_1AR being the highest (Johansson et al., 2007a; LaNoue and Martin, 1994). Furthermore adenosine can activate A_1AR with EC_{50} values in the range between 10 ηM to 1 μM (Fredholm et al., 2001; Hasko et al., 2008). This is why adenosine signaling in both of these tissues for the longest time has been predominantly attributed to the A_1AR.

Pharmacological Approach

In fat, adenosine signaling results in enhanced insulin sensitivity measured by the ability of insulin to inhibit lipolysis (Table I and II)(Green et al., 1997; Schoelch et al., 2004; Vannucci et al., 1992; Vannucci et al., 1989). The mechanism by which adenosine inhibits lipolysis and possibly enhances insulin sensitivity is mediated by the activation of cAMP-dependent protein kinase (PKA) (Londos et al., 1985; Vannucci et al., 1989). Activation of hormone sensitive lipase by glucagon or norepinephrine is inhibited by insulin. In human adipocytes, insulin inhibits the lypolytic effect of norepinephrine more efficiently when adenosine is present (Heseltine et al., 1995). Similarly to adenosine, activation of rat adipocyte-A_1AR by pharmacological reagents showed the same effect on improving insulin sensitivity and consequent inhibition of lipolysis (Table II). Using pharmacological reagents selective for A_1AR, Green et al, reported a 30% reduction in the responsiveness to insulin in adipocytes, as prolonged treatment impaired insulin dependent lipolysis. Furthermore, a specific, anti-lipolytic A_1AR agonist, ARA ([1S,2R,3R,5R]-3-methoxymethyl-5-[6-(1-[5-trifluoromethyl-pyridin-2-yl]pyrrolidin-3-[S]-ylamino)-purin-9-yl]cyclopentane-1,2-diol), in

Wistar and Zucker rats resulted in reduced lipolysis in visceral and subcutaneous fat, suggestive of increased insulin sensitivity (Schoelch et al., 2004).

The effect of adenosine signaling through the A_1AR also depends on the stage of obesity (Table II). Using A_1AR selective antagonist (8-phenyltheophylline), in primary adipocytes isolated from lean Zucker rats, there was a very low increase in cAMP and virtually no lipolysis observed (Vannucci et al., 1989); however, in adipocytes derived from obese rats, inhibition of A_1AR resulted in high cAMP increase and concomitant lipolysis at almost maximal levels (Vannucci et al., 1989).

On the other hand, in muscle tissue activation of adenosine signaling leads to reduction of insulin sensitivity, measured by the ability of insulin to inhibit glycolysis. Insulin activation in muscle tissue is not only associated with inhibition of glycolysis but with transport of glucose and activation of glycogen synthesis. Reports show that adenosine had an effect on only glycolysis (Budohoski et al., 1984; Challis et al., 1984; Espinal et al., 1983) and glucose transport by stimulation of the insulin sensitive glucose transporters GLUT4 (Vannucci et al., 1992); glycogen synthesis was not affected by adenosine (Challis et al., 1984). In isolated soleus muscles from rats, it was found that depletion of adenosine (by adenosine deaminase) in the surrounding media improved insulin sensitivity. The observed effect on insulin sensitivity was due to decrease in the concentration of insulin necessary to activate glycolysis (Espinal et al., 1983). Using the same model and adenosine analogs, reduction in insulin sensitivity was observed when insulin levels were reduced to half of the maximum dose required to stimulate glycolysis in the muscle (Budohoski et al., 1984). Non-specific adenosine receptor antagonists (methyl xanthines) reversed the inhibitory effect of adenosine on insulin signaling (Budohoski et al., 1984). Interestingly, A_1AR selective agonist (ARA) in the gastrocnemius muscle resulted in the amelioration of insulin sensitivity measured by improvement of glucose infusion rate and reduction of FFA levels in obese rats (Schoelch et al., 2004). The pattern observed does not contradict the earlier mentioned observations (Espinal et al., 1983), as here the authors used an A_1AR specific agonist.

The role of adenosine in glucose clearance, however, has been reported to have contradicting results. On one hand, studies showed that non-selective adenosine receptor antagonists, or removal of adenosine by adenosine deaminase decreases insulin-activated glucose transport (Steinfelder and Pethö-Schramm, 1990). This finding is consistent with the pharmacological studies mentioned above, which indicate improvement of insulin sensitivity (Green et al., 1997; Schoelch et al., 2004; Vannucci et al., 1992; Vannucci et al., 1989). More recent studies, using euglycemic hyperinsulinemic clamp and A_1AR selective antagonist (BWA1433, selective for A_1 at low doses) reported that adenosine signaling through A_1AR improves overall body glucose clearance in obese rats (Crist et al., 1998). In addition, with the help of radioactively labeled glucose, this group determined that there is a tissue specificity of glucose clearance under hyperinsulinemic conditions. In the gastrocnemius (fast and slow twitching fibers) and the soleus muscles (slow twitching) of rats there was an improvement of glucose uptake in obese animals after one week of treatment with A_1AR antagonist. In lean animals, however, glucose uptake under one week of treatment was slightly but significantly lowered (Crist et al., 1998). On the other hand, inhibition of A_1AR by selective antagonist has also been reported to improve overall body glucose tolerance (Xu et al., 1998). This observation contradicts the studies that have shown an improved insulin sensitivity and overall glucose clearance as a result of activation of A_1AR by specific agonists (Crist et al., 1998; Green et al., 1997; Schoelch et al., 2004; Vannucci et al., 1992; Vannucci et al., 1989). It is possible then, that in muscle, these agonists activate the A_2 adenosine receptors, an effect that can oppose the A_1AR signaling. Overall, signaling by adenosine using pharmacological reagents improves glucose clearance

(Crist et al., 1998; Xu et al., 1998), but may result in a tissue specific insulin resistance (Crist et al., 1998).

Mouse model approach

In vivo models have further elicited the role of adenosine signaling through the A_1AR on glucose homeostasis and lipolysis. Consistent with some of the pharmacological data, lack of A_1AR in primary adipocytes, contrary to the wild type, resulted in no observable lipolysis when endogenous adenosine was removed by adenosine deaminase (Johansson et al., 2008; Johansson et al., 2007b). Insulin and adenosine acted additively through cAMP to reduce lipolysis (Johansson et al., 2008). Furthermore, pharmacological activation of the A_1AR was only achievable in wild type adipocytes. The last was tested by inhibition of cAMP induced lipolysis with the hormone norepinephrine. Further, induction of lipogenesis was observed in the presence of a non-selective adenosine analog, but mRNA levels of genes related to fatty acid synthesis were not affected (Johansson et al., 2008). Interestingly, plasma levels of free fatty acids, triglycerides, and glycerol were also increased in the A_1AR knockout mice after administration of the adenosine analog, 2-chloroadenosine (Johansson et al., 2008). This indicates that A_1AR is important in the regulation of lipolysis.

Signaling through the A_1AR is also central to glucose tolerance and insulin clearance. Recent work showed that elimination of A_1AR from mice at young age (8 weeks) on standard diet leads to delayed plasma glucose and insulin clearance. The effect was sustained at older ages (20–29 weeks) on both regular and high fat diet (Faulhaber-Walter et al., 2011). Using a different A_1AR KO model, Johansson et al., showed that there is no difference in non-starved plasma levels of glucose, insulin, and glucagon, or glucose tolerance upon A_1AR elimination when mice were not starved (Johansson et al., 2007a). This same model, however, when staved and challenged with glucose showed an improved glucose and insulin clearance on regular diet (Yang et al., 2012). A short administration of high fat diet also showed an improved course of glucose clearance but it had no effect on insulin tolerance (Yang et al., 2012). After glucose injection, A_1AR knockout mice showed a sustained increase in glucagon and insulin plasma concentrations. Insulin and glucose uptake by skeletal muscle were not significantly different between the wild type and the knockout mice. The null mice showed also an enhanced second phase of pancreatic insulin secretion (Johansson et al., 2007a). A recent study by Maeda et al, suggested that in adult animals endogenous adenosine reduces plasma concentrations of insulin, glucose, and lactate via the selectively inhibiting A_1AR (Maeda and Koos, 2008). These studies demonstrate that in vivo elimination or inhibition of A1AR has an effect on glucose homeostasis and the effect in contingent upon the length of the diet or the level of obesity.

In a similar fashion, genetically engineered-overexpression of adipose A_1AR led to protection against obesity induced insulin resistance during high fat diet (Dong et al., 2001a). In this case, adenosine signaling through A_1AR improved insulin sensitivity measured by glucose tolerance tests and insulin levels on Western diet (Dong et al., 2001b). This model also exhibited lower plasma FFA, indicative of inhibition of lipolysis (Dong et al., 2001b). These observations are in concurrence with the pharmacological studies of adipose tissue where affecting adipose-adenosine signaling improved insulin sensitivity (Green et al., 1997; Schoelch et al., 2004; Vannucci et al., 1992; Vannucci et al., 1989).

In summary, the overall effect of adenosine signaling through A_1AR results in improved insulin sensitivity (Table II) (Dong et al., 2001b; Green et al., 1997; Schoelch et al., 2004; Vannucci et al., 1992; Vannucci et al., 1989), and pronounced reductions of plasma free fatty acids, glycerol, and triglycerides (Johansson et al., 2007a). Overall, A_1AR is important for insulin sensitivity, glucose homeostasis and lipolysis (Dong et al., 2001b; Faulhaber-Walter et al., 2011).

Leptin, Adenosine, and A_1AR

Adenosine and adenosine signaling is associated with regulation of the fat adipokine leptin. Leptin is a chemokine synthesized by adipose tissue and is responsible for control of fat metabolism. It has been associated with control of satiety and balancing caloric intake vs. energy expenditure. Plasma levels of tumor necrosis factor-alpha (TNF-alpha) and insulin have been reported to increase the circulating levels of leptin (Bradley and Cheatham, 1999; Finck et al., 1998; Kirchgessner et al., 1997). In adipocytes, increase in cAMP levels have been associated with a decrease in leptin gene expression and secretion (Deng et al., 1997; Gettys et al., 1996; Slieker et al., 1996) and, as mentioned above, A_1AR inhibits cAMP in the adipose tissue. Rice et al., have reported that direct activation of A_1AR by selective agonist treatment in rats increases secretion, but not gene expression, of serum leptin from isolated adipocytes (Table II) (Rice et al., 2000). Indeed, specific inhibition of adenosine signaling through the A_1AR completely inhibited insulin-stimulated leptin release in isolated white adipocytes from rats. Using pharmacological reagents, it was shown that adenosine released by adipocytes acts to stimulate leptin secretion by insulin in a phospholipase C, protein kinase C (PLC-PKC) dependent manner (Cheng et al., 2000). It has been suggested that leptin induces a novel form of lipolysis by which FFA do not increase in the plasma, contrary to an increase in glycerol (Wang et al., 1999). In turn, endogenous adenosine released by white fat acts additively with insulin to inhibit lipolysis through the A_1AR (Johansson et al., 2008). Leptin-induced lipolysis, in turn, was able to counteract the tonic inhibition of lipolysis by endogenous adenosine only in lean animals (Fruhbeck et al., 2001). In obese animals leptin was not able to oppose the effect of adenosine (Fruhbeck et al., 2001). Consistent with this observation, in high fat treated mice elimination of either the A_1AR (Faulhaber-Walter et al., 2011) or the A_{2b}AR (Johnston-Cox et al., 2012a) caused an increase in circulating leptin. The fact that receptors with opposing effect on cAMP have the same outcome on leptin levels suggests that the effect on leptin under HFD is predominantly due to increased fat mass accumulation.

In conclusion, there is a clearly established relationship between cAMP and leptin levels that can be controlled by adenosine, the strength of which depends on the leanness of the organism. In that sense, there is a possibility that leptin and adenosine may influence the balance between lipolysis and lipogenesis, resulting in effective homeostatic control.

A_3 Adenosine Receptors

The A_3 adenosine receptor (A_3AR) is the other adenylyl cyclase-inhibiting receptor that has an affinity for adenosine with EC_{50} values in the range of 10 ηM- 1 μM (Fredholm, 2007; Fredholm et al., 2000; Hasko et al., 2008). Even though this adenosine receptor inhibits adenylyl cyclase in a similar fashion as A_1AR it has not been identified as a major player in overall body glucose homeostasis or fat metabolism. Signaling through A_3AR and glucose metabolism has been attributed to the ability of this receptor to get activated not only by adenosine but rather by inosine (Gomez and Sitkovsky, 2003; Jin et al., 1997; Tilley et al., 2000). Inosine is an endogenous nucleoside formed by the deamination of adenosine (Barankiewicz and Cohen, 1985) and it can accumulate under ischemic conditions (Wang et al., 1994). Since the liver is the major organ responsible for regulation of glucose homeostasis (Saltiel and Kahn, 2001), it is necessary to mention that in this organ all four adenosine receptors are present (Dixon et al., 1996), with highest levels of A_3AR expression (Salvatore et al., 1993).

Pharmacological and Mouse model approaches

Studies have shown that under ischemic conditions, as a result of tissue damage, inosine is generated from the deamination of adenosine (Linden, 2001; Rubio and Berne, 1980; Silva

et al., 1995). Guinzberg et al., reported that in primary hepatocytes inosine can stimulate gluconeogenesis and glycogenolysis through the A_3AR (Table III) (Guinzberg et al., 2006) . Additionally, inosine is also able to stimulate glucose release from primary rat hepatocytes (Guinzberg et al., 2006). A study from the same group has demonstrated that under baseline conditions, in primary hepatocytes, inosine signaling through A_3AR increases cytosolic Ca^{2+} and reduces cAMP, antagonizing transient signaling by adenosine (Table III). Glycogen metabolism through this receptor was also shown to be affected by adenosine but in small order (González-Benítez et al., 2002). There are no records of A_3AR affecting glucose clearance, or lipid profile in transgenic or A_3AR knockout mice.

In summary, the impact of the A_3AR on glucose homeostasis, by either glucose synthesis or glucose release, may have an increased importance during anaerobic or stress elevating conditions.

A_{2a} Adenosine Receptors

The A_{2a} adenosine receptor (A_{2a}AR) is a receptor with high affinity for adenosine (EC_{50} values in the range of 10 ηM- 1 μM), the activation of which, however, results in the elevation of cAMP (Fredholm, 2007; Fredholm et al., 2000; Hasko et al., 2008). The role of A_{2a}AR in glucose homeostasis and lipid metabolism has not been as clearly identified as that of the A_1AR. In rat hepatocytes it has been reported that adenosine can increase gluconeogenesis by elevating cyclic adenosine monophosphate (cAMP) levels through the A_2AR (Bartrons et al., 1984; González-Benítez et al., 2002; Zentella de Piña et al., 1989).

Pharmacological and Mouse model approach

Studies have shown that exogenous adenosine signaling through A_{2a}AR increases gluconeogenesis and glucose release (González-Benítez et al., 2002; Maeda and Koos, 2008; Yasuda et al., 2003). In fetal sheep, A_{2a}AR activation increases glucose and lactate levels as a result of the increased endogenous adenosine levels under hypoxic conditions (Table IV) (Maeda and Koos, 2008). In rat hepatocytes, gluconeogenesis stimulation was also observed by the activation of A_{2a}AR with a selective agonist (CGS-21680) in cAMP-dependent fashion (González-Benítez et al., 2002).

The role of adenosine in lipid metabolism has been predominantly attributed to the activation of the A_{2a}AR. In vitro studies showed that the A_{2a}AR activated the reverse cholesterol transport (Reiss et al., 2008; Reiss et al., 2004), a process by which cholesterol from the periphery is transferred back to the liver by macrophages. The last is crucial for preventing foam cell formation and atherosclerosis initiation (Reiss et al., 2004). Additionally, pharmacological studies (Table IV) illustrated that activation of A_{2a}AR in human macrophages, as well as cultured primary murine macrophages prevent foam cell formation (Reiss et al., 2008). In support, macrophages lacking A_{2a}AR were not protected from foam cell formation by A_{2a} agonism (Reiss et al., 2004) . The mechanism by which activation of A_{2a}AR is able to initiate this process of cholesterol clearance involves the upregulation of 27-hydroxylase and ATP-binding cassette sub-family A member 1 (ABCA1) transporter, which are proteins involved in the reverse cholesterol transport (Reiss et al., 2008; Reiss et al., 2004).

Murine models with eliminated A_{2a}AR have connected this adenosine receptor with elevation of plasma cholesterol levels under Western and regular chow diet (Wang et al., 2009). This finding was observed in a mouse model that lacks A_{2a}AR on an ApoE null background. The elevation of plasma cholesterol was concentrated in the low density lipoprotein (LDL) particles. Interestingly, when A_{2a}AR is knocked out on just C57BL background there was no difference in the cholesterol or triglycerides levels (Wang et al.,

2009). Additionally, no difference in blood glucose levels was observed when A_{2a}AR was eliminated.

In summary, in vitro murine studies have associated the A_{2a}AR with upregulation of reverse cholesterol transport, as in vivo reports have connected A_{2a}AR with regulation of overall body cholesterol levels under a Western diet. Pharmacological studies have shown that this receptor is also connected to gluconeogenesis and glucose release, although complete elimination of this receptor does not alter basal glucose levels. It is possible that this receptor is more important under stress related conditions. Moreover, its importance can be in tissue specific manner. More exploration is needed to explain the discrepancies between pharmacological and in vivo studies.

A_{2b} Adenosine Receptors

The A_{2b} adenosine receptor (A_{2b}AR) is cAMP elevating and requires high concentration of adenosine in order to be activated. A_{2b}AR has been of little interest due to its low affinity for adenosine (EC50 values are above 10 μM) (Fredholm, 2007; Fredholm et al., 2000; Hasko et al., 2008). The generation of the A_{2b}AR knockout/β-galactosidase knock in (Yang et al., 2006b), and the discovery of the inducibility of this receptor under inflammation, stress or injury (St. Hilaire et al., 2008; Yang et al., 2008), led to increased interest in its biological importance. Looking at the expression of A_{2b}AR in organs related to glucose homeostasis and lipid metabolism, it appears that pancreatic basal expression of A_{2b}AR, as well as, all other adenosine receptors is similar levels (Nemeth et al., 2007). More specifically, A_{2b}AR expression has been found to predominate in the islets of the pancreas (Yang et al., 2006b). Additionally, mRNA of this receptor has been not only detected in the liver (Koupenova et al., 2012; Salvatore et al., 1993) but also in fat and muscle (Dixon et al., 1996; Johansson et al., 2007a; Johnston-Cox et al., 2012b; LaNoue and Martin, 1994).

Pharmacological and Mouse model approach

With respect to glucose homeostasis, the A_{2b}AR has been described as a receptor that has an anti-diabetic potential (Nemeth et al., 2007; Rüsing et al., 2006). Using a nonselective adenosine receptor agonist (5'-N-ethylcarboxamide (NECA)) and different A_{2b}AR specific antagonists, the A_{2b}AR has been associated with an increase in insulin secretion (Rüsing et al., 2006) and amelioration of diabetes (Table V) (Nemeth et al., 2007). It has been suggested that the A_{2b}AR also stimulates glycogenolysis and gluconeogenesis in primary rat hepatocytes in a cAMP-dependent manner (Harada et al., 2001; Yasuda et al., 2003). In a Type I diabetic model, hyperglycemia was reduced predominantly through the activation of A_{2b}AR, which sustained reduction of blood glucose in the circulation for 7 days (Nemeth et al., 2007). Furthermore, in a A_{2a}AR knockout model NECA maintained the suppressive effect on hyperglycemia as the A_1AR and A_3AR were less effective on the containment of the course of diabetes development (Nemeth et al., 2007).

Elimination of A_{2b}AR in mice has an effect on increased fasting glucose and insulin levels as well as impaired glucose tolerance and insulin clearance post high fat diet (Johnston-Cox et al., 2012a). Interestingly, activation of A_{2b}AR by specific agonist (BAY60–6553) in high fat diet induced obesity wild type mice lowered fasting glucose and improved their glucose and insulin tolerance (Johnston-Cox et al., 2012a). The effect of A_{2b}AR was mediated by regulating insulin receptor substrate (IRS) levels, and a similar correlation between obesity, and levels of A_{2b}AR and IRS were found in human adipose tissues (Johnston-Cox et al., 2012a). Interestingly, at young age (8 weeks) mice lacking the A_{2b}AR have a superior glucose clearance (Figler et al., 2011) . This is consistent with the fact that at this age there are almost no detectable levels of A_{2b}AR in fat or liver (Yang et al., 2006a), and one of the other adenosine receptors (such as A_1) is controlling this process. Furthermore,

administration of an antagonist of the A_2ARs (NECA) fed in combination with high fat diet resulted in an improved fasting glucose levels in wild type mice (Figler et al., 2011). The last observation proposes that administration of pharmacological agents in vivo has different effect when administered orally or intraperitoneally, suggesting that the digestive system itself might have an effect on the metabolism of the drugs, or the drugs themselves have an impact on the digestive system before reaching their intra-body targets (such as nutrient absorption). Regardless of the course of administration, A_{2b}AR clearly has an effect on maintaining glucose and insulin homeostasis.

The association of A_{2b}AR with lipid metabolism can be attributed to the role of this receptor in preventing fatty liver formation post alcohol consumption (Peng et al., 2009). It is necessary to note that A_{2b}AR is not the only adenosine receptor involved in the regulation of the fatty liver formation (Table V). Antagonism of A_1AR reduced the expression of genes involved in fatty acid synthesis, and antagonism of A_{2b}AR increased genes involved in fatty acid metabolism, as ethanol ingestion elevated A_1AR, A_{2a}AR, and A_{2b}AR expression in the liver (Peng et al., 2009). A_1AR and A_{2b}AR agonism promoted lipid accumulation in cultured murine hepatocyte cell line (AML-12) and increased TG levels (Peng et al., 2009). The mechanism by which A_{2b}AR and A_1AR induced fatty liver after ethanol stimulation involves increasing lipogenesis (through A_1AR) and decreasing fatty acid oxidation (through A_{2b}AR). A_1 and A_{2b}AR knockout mice models (but not A_{2a}AR) were protected from fatty liver development, consistent with the pharmacological data produced by antagonism of the receptors (Peng et al., 2009). Recent work from our group has demonstrated that A_{2b}AR has a major protective role during high fat diet induced obesity. Indeed, A_{2b}AR regulated liver lipid synthesis, liver steatosis and plasma levels of cholesterol and triglycerides predominantly found in the very low density lipoprotein particles (Koupenova et al., 2012). Liver specific restoration of this receptor by adenovirus as well as specific ligand activation significantly improved plasma lipid levels, and lowered liver lipid synthesis (Koupenova et al., 2012).

In conclusion, the facts that levels of A_{2b}AR dramatically increases in liver (Koupenova et al., 2012) and fat (Johnston-Cox et al., 2012a) with high fat diet, as well as, its ability to improve glucose and insulin clearance and lipid synthesis, make this receptor a favorable drug target.

Summary: significance of adenosine and the adenosine receptors in glucose and lipid homeostasis

Regardless of the controversy through which receptor adenosine conveys its message, it is clear that endogenous adenosine, as a local signaling metabolite, has an important role in major processes such as inflammation, hypoxia, and glucose and lipid metabolism. It is possible that signaling through the adenosine receptors has evolved as a finely tuned buffering mechanism by which all of the four receptors contribute to the same process with opposing effect in order to regulate and prevent the consequences of differences in food intake and inflammatory stimulation (Figure 1). Targeting adenosine receptors becomes more and more promising in prophylaxis against diabetes and cardiovascular diseases. Mouse models are of particular interest in order to address the question of which receptor needs to be activated and/or inhibited in the time course of these disease states. Moreover, these findings need further conformation in human cell models. Future studies could also focus on potential synergizing or opposing signaling by which adenosine receptors exert effects on lipid and glucose homeostasis.

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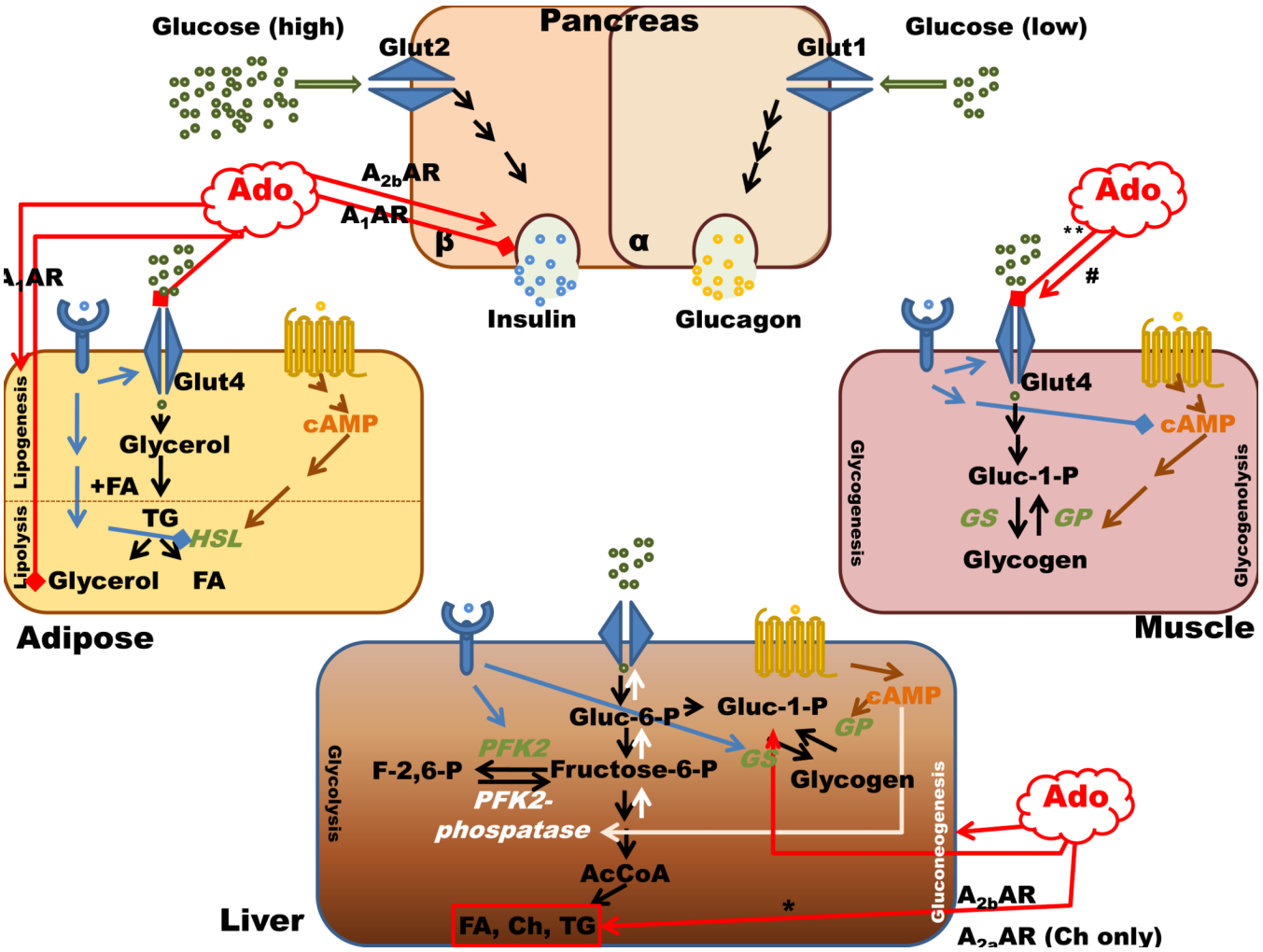

Figure 1. Adenosine and adenosine receptor signaling in major organs involved in glucose and lipid homeostasis

All adenosine receptors in the liver have the ability to activate glucogenolysis and gluconeogenesis with different affinities, possibly through different second messengers. * This elevation of triglycerides is post ethanol consumption. ** This is observed in the soleus muscle, and # is observed in Gastrocnemius muscle. Stimulates (→) Inhibits (—◆). Ado- adenosine, AR- adenosine receptors; GS- glycogen synthase; GP- glycogen phosphorylase; PFK2-phospho frukto kinase 2, HSL-hormone sensitive lipase; FA-fatty acids, Ch-cholesterol, TG-triglycerides.

Table I

Adenosine in glucose and lipid metabolism

Treatment	Effect on ARs	Model system	Physiological effect	References
2-chloroadenosine or N^6-phenylisopropyladenosine	Stimulation	Muscle soleus	reduced insulin sensitivity by increasing the half maximum dose of insulin required to stimulate glycolysis	(Budohoski et al., 1984)
8-phenyltheophylline	Inhibition	Muscle soleus	Increased insulin sensitivity by counteracting the effect of 2-chloroadenosine	(Budohoski et al., 1984)
Adenosine *Micromolar concentration*	Stimulation	Rat islets	Decrease of insulin release	(Bertrand et al., 1989a; Bertrand et al., 1989b; Campbell and Taylor, 1982; Hillaire-Buys et al., 1987; Ismail et al., 1977)
Adenosine *Milimolar concentrations*	Stimulation	Mouse islets Rat pancreas	Increase of insulin release	(Andersson, 1980; Jain and Logothetopoulos, 1978; Loubatieres-Mariani et al., 1979)
Adenosine	Stimulation	Rat hepatocytes	Increases gluconeogenesis	(Bartrons et al., 1984; González- Benítez et al., 2002; Zentella de Piña et al., 1989)
Adenosine	Stimulation	Adipocytes rat	In presence of insulin increases glucose transport activity of GLUT4	(Vannucci et al., 1992)
Adenosine	Stimulation	Rat pancreas	Stimulates glucagon secretion	(Chapal et al., 1985; Chapal et al., 1984)
Adenosine 2-Chloroadenosine	Stimulation	Rat hepatocytes	Increases glycogenolysis	(González-Benítez et al., 2002; Hoffer and Lowenstein, 1986; Oetjen et al., 1990)
Adenosine deaminase	Inhibition by removal	Adipocytes rat	Removal of inhibition of lipolysis in lean>>>obese adipocytes	(Vannucci et al., 1989)
Adenosine deaminase	Inhibition by removal	Muscle soleus	improves insulin sensitivity by decreasing the concentration of insulin necessary to activate the half maximum stimulation of glycolysis	(Espinal et al., 1983)
Aminophylline	Inhibition	Overall body human	Stimulates insulin secretion by inhibition of glucose production	(Arias et al., 2001)
Aminophylline	Inhibition	Overall body Dog	Lowered glucagon release	(Schütz et al., 1978; Schütz et al., 1978)
Pentoxyfylline	Inhibition	Overall body human	Inhibits basal glucose production	(Arias et al., 2001)

Table II

A1AR and its physiological role in glucose and lipid metabolism

Adenosine Receptor	Pharmacological Agent	Activation	Model System	Physiological effect	References
A_1AR	**CPA** (n6-cyclopentyladenosine)	Agonist	Adipocytes Rat	Decreased glucose uptake	(Cheng et al., 2000)
A_1AR	**PIA** N6-(L-2-phenylisopropyl)- Adenosine	Agonist	Adipocytes Rat	Inhibition of lipolysis in presence of β-adrenergic hormone stimulation. Decrease insulin sensitivity measured by reduction in glucose uptake.	(Cheng et al., 2000; Vannucci et al., 1989)
			Perfused rat pancreas	Decreases insulin secretion	(Bertrand et al.,1989b)
			Perfused rat liver	Increase in hepatic glucose release	(Buxton et al., 1987)
A_1AR	**8-PT** (8-phenyltheophylline)	Antagonist	Adipocytes Rat	Lipolysis increased in obese>>>lean (virtually none).	(Vannucci et al., 1989)
			Perfused rat pancreas	Increased insulin secretion	(Bertrand et al., 1989b)
A_1AR	**CPA**	Agonist	Hepatocytes cell line- AML-12	Increased intracellular Triglyceride levels. Increases proteins involved in fatty acid synthesis- SREBP1 and PPARγ and consequently expression of ACL and FAS*	(Peng et al., 2009)
A_1AR	**DPCPX** (8-Cyclopentyl-1,3-dipropylxanthine)	Antagonist	Hepatocytes cell line- AML-12	Reversed the effect of CPA on increased triglyceride levels, SREBP-1, PPARγ, ACL and FAS	(Peng et al., 2009)
A_1AR	**BWA1433** (1,3-dipropyl-8-(4-acrylate)phenylxanthine; 4-(1,2,3,6-tetrahydro-2,6-dioxo-1,3-dipropyl-9H-purin-8-yl)cinnamic acid)	Antagonist	Muscle Gastrocnemius, soleus	Increases glucose uptake in obese animals	(Crist et al., 1998)
			Adipocytes	Decreases glucose uptake in obese and lean animals	(Crist et al., 1998)
A_1AR	**ARA** -[1S,2R,3R,5R]-3- methoxymethyl-5-[6-(1-[5- trifluoromethyl-pyridin-2-yl]pyrrolidin- 3-[S]-ylamino)-purin-9-yl]cyclopentane-1,2-diol	Agonist	Muscle gastrocnemius	Improvement of insulin sensitivity; an increase in glucose infusion rate and reduction of FFA levels in obese rats	(Schoelch et al., 2004)
			Visceral and subcutaneous fat	Reduction in lipolysis	(Schoelch et al., 2004)
A_1AR	**ARA**	Agonist	Overall body	Reduction in FFA, glycerol, TG's; improvement of insulin sensitivity	(Schoelch et al., 2004)
A_1AR	**BWA1433**	Antagonist	Overall body	At lower levels of insulin increase in glucose tolerance by glucose clearance;	(Crist et al., 1998)
A_1AR	**CPA**	Agonist	Overall body and rat adipocytes	Increased leptin secretion	(Rice et al., 2000)
A_1AR	**CHA** (cyclohexyladenosine)	Agonist	Perfused rat liver	Increased hepatic glucose release	(Buxton et al., 1987)

* PPARγ- Peroxisome proliferator-activated receptor gamma; SREBP-1-Sterol Regulatory Element Binding Protein-1; ACL-ATP citrate lyase; FAS-Fatty acid Synthase

Table III

A_3AR and its physiological role in glucose and lipid metabolism

Adenosine Receptor	Pharmacological Agent	Activation	Model System	Physiological effect	References
A_3AR	**Inosine**	Activation	Rat hepatocytes	Stimulates glycogenolysis and gluconeogenesis as	(Guinzberg et al., 2006)
A_3AR	**MRS1220**- N-[9-Chloro-2-(2- furanyl)[1,2,4]-triazolo[1,5-c]quinazo lin-5- yl]benzene acetamide	Antagonist		Specific inhibition of the effect of inosine on glycogenolysis and glyconeogeneis	(Guinzberg et al., 2006)
A_3AR	**IB-MECA** - 1-Deoxy-1-[6-[[(3- iodophenyl)methyl]amino]-9H–purin-9-y l]-N- m ethyl-b-D-ribofuranuronamide	Agonist	Rat hepatocytes	Stimulates glycogenolysis and gluconeogenesis but in minor form compared to A_1, $A_{2a}AR$	(González-Benítez et al., 2002)

Table IV

$A_{2a}AR$ and its physiological role in glucose and lipid metabolism

Adenosine Receptor	Pharmacological Agent	Activation	Model System	Physiological effect	References
$A_{2a}AR$	**CGS-21680** 4-[2-[[6-Amino-9-(N-ethyl-b-D- ribofuranuronamidosyl)-9H -purin-2-yl]amino]ethyl]benzenepropanoic acid hydrochloride	Agonist	Rat hepatocytes	Stimulates gluconeogeneis and glycogenolysis	(González-Benítez et al., 2002)
$A_{2a}AR$	**ZM-241385** 4-(2-[7-Amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]t riazin-5-ylamino]ethyl)phenol	Antagonist	Fetal sheep overall body	Abolished induced hyperglycemia and hyperlactatemia by exogenous administration of adenosine	(Maeda and Koos, 2008)
$A_{2a}AR$	**CGS-21680**	Agonist	THP-1 human macrophages/ murine macrophages	Inhibited foam cell formation in stimulated macrophages, increases the mRNA levels for 27-hydroxylase and ABCA1, proteins involved in the reverse cholesterol transport	(Reiss et al., 2008; Reiss et al., 2004)
$A_{2a}AR$	**MRE-0094** 2- [2-(4-chlorophynyl) ethosy]adenosine	Agonist	THP-1 macrophages	Increases the mRNA levels for 27-hydroxylase and ABCA1*	(Reiss et al., 2004)
$A_{2a}AR$	**ZM-241385**	Antagonist	THP-1 human macrophages/ murine macrophages	Reversed the inhibition of foam cell formation and downregulates 27- hydroxylase and ABCA1 expression	(Reiss et al., 2004)

*ABCA1-ATP-binding cassette, sub-family A

Table V

$A_{2b}AR$ and its physiological role in glucose and lipid metabolism

Adenosine Receptor	Pharmacological Agent	Activation	Place of investigation	Physiological effect	References
$A_{2b}AR$	**PBS-53, PBS-1115** 1-butyl-8-p-carboxyphenylxanthine	Antagonist	INS-1 cells	Increased glucose- stimulated insulin release	(Rüsing et al., 2006)
			Overall body in GotoKakzaki rats	Plasma insulin was increased but blood glucose was unchanged	(Rüsing et al., 2006)
$A_{2b}AR$	**MRS1754** N-(4-Cyanophenyl)-2-[4-(2,3,6,7- -tetrahydro-2,6-dioxo-1, 3-dipropyl-1H- purin-8-yl)phenoxy]-acetamide	Antagonist	CD-1 mice with induced diabeties	Reversed the preventative effect toward diabetes development of the adenosine analog NECA; reversed the suppressive effect on hyperglycemia.	(Nemeth et al., 2007)
$A_{2b}AR$	**NECA** (5'-N-ethylcarboxamidoadenosine) **In concentrations activating $A_{2b}AR$ (10 μM)**	Agonist Non-selective	AML-12	Increased intracellular Triglyceride levels. Reduces proteins involved in fatty acid oxidation- PPARα nuclear levels were decreased and consequently there was reduced expression of ACCα and CPT1. Reduced phosphorylation of AMPK*	(Peng et al., 2009)
	MRS1754 (1 μM)	Antagonist	AML-12	Reversed all the effect of NECA on PPARα, ACCα, CPT1, and AMPK phosphorylation	(Peng et al., 2009)
$A_{2b}AR$	**NECA, CPA, CGS21680**	Non-selective agonist, A1AR agonist, A2aAR agonist	Hepatocytes rat	Increase of hepatic glucose production	(Harada et al., 2001)
	FK453, KF17837, and **L249313**	Selective antagonist for A1, A2aAR, A3AR			
A2bAR	**BAY 60-6853**	Selective A2bAR agonist	Overall body mice	It lowers liver lipid synthesis and plasma levels of cholesterol and triglycerides; it improves liver steatosis	(Koupenova et al., 2012)
				It improves glucose and insulin tolerance and increases leaness	(Johnston-Cox et al., 2012)

*PPARα- Peroxisome proliferator-activated receptor alpha; ACCα- acetyl CoenzymeA carboxylase alpha; CPT1- carnitine palmitoyltransferase 1A; AMPK- AMP-activated protein kinase