ISSN 0976 – 3333

Available Online at www.ijpba.info.

International Journal of Pharmaceutical & Biological Archives 2011; 2(4):1077-1086

REVIEW ARTICLE

A_1 Adenosine Receptor Signaling and Therapeutic Target in Diabetes

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Received 23 May 2011; Revised 07 Aug 2011; Accepted 11 Aug 2011

ABSTRACT

Diabetes is one of the risk factors to human health which progressively leads to cardiovascular complications such as ischemic heart disease, renal nephropathy, hypertension, endothelial dysfunction, and atherosclerosis. The biochemical and morphological abnormalities in various animal models has been reported in the literatures. These changes may be attributed to altered action of adenosine receptors and these receptors are named as A_1, A_{2A}, A_{2B} and A_3, mediate their effector functions through a G-protein signalling. Among these A_1AR, couples to adenylate cyclase through Gi-protein and leads to vasoconstriction and fatty acid metabolism. Endothelial dysfunction has been known to be one of the factors being responsible for pathogenesis of vascular disease in diabetes. This review will give a general overview of the adenosine receptor and focuses on the role of A_1AR in diabetes. The insight into the signaling pathway through A_1AR could be helpful in developing a novel therapeutic tool to regulate the pathophysiological conditions that arises progressively in diabetes.

Keywords: Adenosine receptors, heart, endothelium, cell signaling pathway, diabetes.

INTRODUCTION

Adenosine is a potent endogenous nucleoside that is released from cells into the extracellular space at sites of inflammation and tissue injury which regulate many physiological functions in mammalian tissues. Its actions are mediated by interaction with specific cell membrane receptors. Four subtypes of adenosine receptors (ARs) have been identified and cloned namely A_1, A_{2A}, A_{2B}, and A_3. The significant advancement has been made in the understanding of the pharmacological and physiological relevance of ARs, but the knowledge of A_1 AR receptor still remains unclear in relation to diabetes in comparison to other receptor subtypes [1]. The intracellular formation of adenosine, increases with increasing cellular workload and this increase is related to oxygen consumption and excitatory transmit release [2,3].

These adenosine receptors belong to a family of G-protein coupled receptors (GPCRs) composed of a hepta-helical structure. All four receptors bind to adenosine with varying affinity and activate various signaling mechanism(s). Among these, A_1AR and A_3AR are coupled to adenylate cyclase in an inhibitory manner (being coupled to G_i protein) and A_2AR and A_{2B}AR in a stimulatory manner (being coupled to G_s protein). Apart from this general concept, it is also known that one adenosine receptor may be coupled to more than one G proteins[4,5]. Genetically engineered mice have played diverse physiological functions mediated by adenosine receptors, modulation of cardiovascular systems [6,7].

The vasodilatory effects of adenosine and its analogues are mediated through adenosine A_2 receptors [8,9]. Vasorelaxant responses to adenosine are partly mediated through adenosine induced release of endothelium derived relaxing factor (EDRF) in some blood vessels [10,11].

Adenosine binds to its receptors; subsequently it initiates signaling cascades, most characterized mechanism being the effect on adenylate cyclase [12]. Adenylate cyclases comprise a family of transmembrane proteins catalysing the formation of cAMP from ATP and exist in nine different isoforms, which are differentially activated by G_α, $\beta\gamma$ subunits and intracellular calcium[13]. Adenosine receptors couple with mitogen activated protein (MAPs) kinases and activate various downstream signaling molecules, mediating their effect of vasoconstriction or vasodilation, each receptor stimulating a specific

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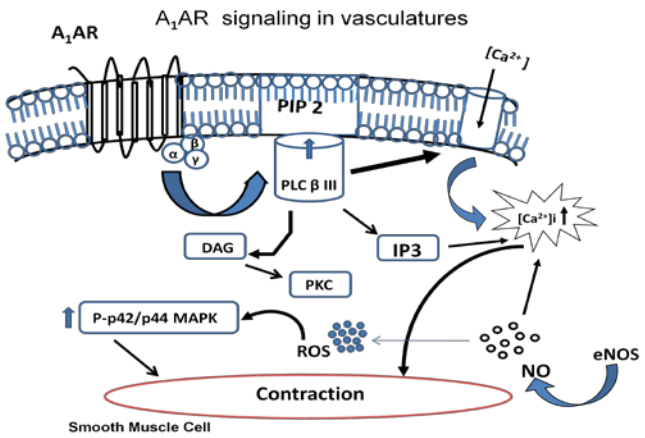
pathway and play major roles in cardiovascular and other systems, (**Fig 1)** [14,15]. On the other hand the antilipolyte effects in adipocytes, the A_1AR inhibits adenylate cyclase activity through Gi protein reduces cAMP formation and consequencely inhibits protein kinase A (PKA) which ultimately reduces the hormone-sensitive lipase and/or adipose triglyceride lipase activity. These result in inhibition of the breakdown of triglycerides to free fatty acids, (**Fig 2**) [16,17]. There are reports which suggest organ damages such as kidney, liver and adipocytes in diabetes [18,19,20]. The direct inhibition of hormone-sensitive lipase by A_1AR agonists has not been demonstrated, because of the well-established role of hormone-sensitive lipase and more recently adipose triglyceride lipase in lipolysis. It is assumed that inhibition of lipolysis by adenosine and its analogs is due to the activation of A_1ARs, resulting in the inhibition of hormone-sensitive lipase and or adipose triglyceride lipase.

Table 1: Agonists and antagonists of adenosine receptors

Adenosine receptor subtype	Agonists	Antagonists
A_1AR	CPA CCPA CHA S-ENBA ADAC	DPCPX WRC-0571 BG9719 KW3902 FK453 FK194921
A_2AR	CGS- 21680 HE-NECA CV-1674	KW6002 SCH58261 VER6947 SCH442416
A_{2B}R	LUF5853	MRS1754 MRE2029-F20
A_3AR	2-Cl-IB-MECA	MRS1292 PSB-11 MRS3777 MRS1334 MRE3008-F20 MRS1523

Fredholm BB. Adenosine,an endogenous distress signal, modulates tissue damage and repair. Cell Death and Differentiation (2007) 14, 1315–1323).

Fig 1. A_1AR signaling in vasculatures



A_1AR signaling causes contraction in smooth muscle cells. The βγ subunit of A_1AR activates PLC β which causes contraction through MAPK pathway and an increase in intracellular Ca^{2+} as well as ROS. ROS causes phosphorylation in some specific domain of PKC which also plays a role in its activation.

Fig. 2: A_1AR cellular signaling in adepocytes

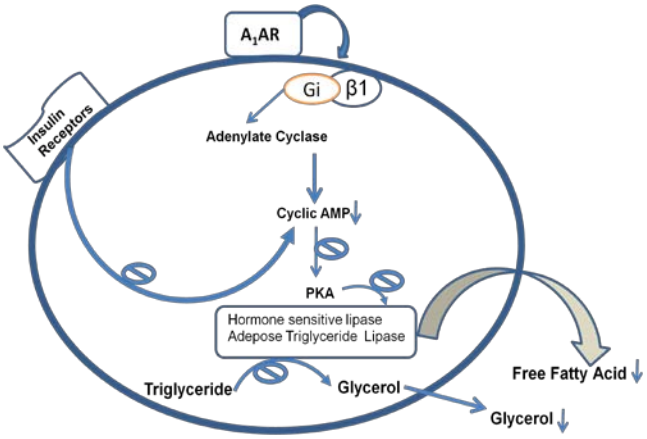


Fig. 2: A_1ARs signaling: The antilipolyte effects in adipocytes. The A_1AR inhibits adenylate cyclase activity through Gi protein reduces cAMP formation and consequencely inhibits protein kinase A (PKA) which ultimately reduces the hormone-sensitive lipase and/or adipose triglyceride lipase activity. These result in inhibition of the breakdown of triglycerides to free fatty acids. Indicates inhibition in the signaling pathway.

A_{2A} Adenosine Receptors: A_{2A} receptors are highly expressed in neurons and regulate a variety of cardiovascular functions such as vascular conductance, blood platelet aggregation and vascular relaxation [21,22]. These receptors are coupled to Gs proteins. A_{2A} receptor agonists act on endothelium as well as vascular smooth muscles to cause vasodilatation [23]. It has also been reported that A_{2A} AR activation causes vasorelaxation through cytochrome P-450 (CYP) epoxygenases and endothelium-derived hyperpolarizing factors, whereas lack of A_{2A} AR activation promotes vasoconstriction through Cyp4a in the mouse aorta [24] The activation of A_{2A} receptor, increases MAPK activity and exert a mitogenic effect on endothelial cells by activating ERK1/2 using cAMP-ras-MEK1 pathway [25]. However, the signaling pathways by A_{2A} receptor varies with cell types and signaling machinery possessed by it. A_{2A} receptor activation in some cell lines has been known to activate PKC, Ras and SOS but not G_s, cAMP or PKA. In humans, A_{2A} receptors are present on the GABAergic output neurons in highest abundance [26]. In general, the responses produced by A_{2A} receptors can be classified as anti inflammatory and suppresses the release of inflammatory

mediators, primarily by inhibiting lymphoid or myloid cells, including neutrophils, macrophages, lymphocytes and platelets [27, 28].

A_{2B} Receptor: A_{2B} receptors are coupled to intracellular pathways different from those of A_{2A} receptors, a finding that may provide the basis for their distinct physiological role. A_{2B} receptors have been implicated in mast cell activation and asthma, vasodilation, regulation of cell growth, intestinal function, and modulation of neurosecretion. The A_{2B} receptor subtype is coupled to both adenylyl cyclase and PLC [29,30]. It is also known to couple to Gs protein, but recent studies have shown that these receptors may couple to G_q and produce Ca^{2+} mobilization and MAPK activation and mediates many of the important functions. In vascular endothelial cells these receptors have been found to cause vasodilatation mediated by Ca^{2+} dependent NO synthase activation [31]. The A_{2B} AR is found to be upregulated by hypoxia and antagonists of this receptor effectively neutralize ATP-elicited reduction in post-hypoxic endothelial permeability [32]. A_{2B} ARs are also important for adenosine-mediated inhibition of cardiac fibroblast functions and the stimulation of NO production during Na^{2+} linked absorption of glucose [33]. It is seen that activation of A_{2B} AR causes an increase in the release of angiogenic factors thus promoting angiogenesis [34].

A_3 Adenosine Receptors: A_3AR is the last member of the adenosine receptor family to have been cloned and has got 40% sequence homology with A_1 and A_{2A} receptor subtypes [35]. This receptor couples to classical second messenger system were adenylate cyclase activity is inhibited and PLC is stimulated through G_i and G_q protein coupling [36]. Activation of PLC is responsible for inositol triphosphate (IP3) and intracellular calcium (Ca^{2+}) elevation in a variety of cellular models. These receptors are susceptible to phosphorylation by G protein coupled receptor kinases (GIRKs) which in turn leads to rapid desensitization of A_3 receptors. A_3AR is the receptor subtype that facilitates the degranulation of mast cells [37]. In cardiac cells, A_3AR agonists activate K^{2+} channels and induce protection. RhoA-phospholipase D1 signaling mediates anti-ischemic effects of A_3AR [38]. Like other ARs, A_3AR also couples to MAPK giving it a role in cell growth, survival, death and differentiation [39]. A number of biological functions have been attributed to A_3AR in ischemic and inflammatory pathologies. It plays a major role in adenosine induced cardioprotection during and following ischemia-reperfusion.

Table 2: G-protein coupling of adenosine receptor subtypes

Adenosine receptor subtype	G protein	Effect of G protein coupling
A_1	Gi 1/2/3, Go	↓ cAMP, ↑IP3/DAG, ↑arachidonate(PLA2),
A_{2A}	Gs, Golf, G15,16	↑cAMP, ↑IP3
A_{2B}	Gs, Gq/11	↑cAMP, ↑IP3/DAG (PLC)
A_3	Gi/2,3 Gq/11	↓ cAMP, ↑IP3/DAG

Ref. #7 Fredholm BB, IJjzerman AP, et al. International Union of Pharmacology. LXXX1. Nomenclature and Classification of Adenosine Receptors- an update. Pharmacol Rev 2011; 63:1-34.

A_1 Adenosine receptors

A_1 AR is a member of P1 family of seven transmembrane adenosine receptors, and is the best characterized of the widely distributed purinergic receptor family, which couples to G_i to decrease the second messenger cAMP, activates K^+ channels or inhibits certain N, P and Q type Ca^{2+} channels in various cells [40]. However, these receptors can stimulate Ca^{2+} mobilization through PLC β with G protein βγ subunit[41]. This signaling pathway is synergistic with other receptors activating PLC via G_q subunit of G protein. A_1 receptors and a heterotrimeric G protein G_o, have been found to be abundant in brain suggesting a possible role of A_1 AR in regulating neurite growth in the CNS [42]. High expression of A_1AR is seen in brain, spinal cord, eye, atria and adrenal gland. It is intermediately expressed in skeletal muscle, liver, kidney, adipose tissue, salivary glands, esophagus, colon, antrum and testis. However, a very low expression is seen in lungs and pancreas [43]. The molecular mass of purified A_1AR characterized from brain is 35-36 kD.

A_1AR Mediated signaling

A_1ARs have been found to produce cellular responses through a number of effector systems such as inhibition of adenylate cyclase [44], enhanced glucose uptake [45], increased ionistol phosphates [46] etc. In general, these responses

have been found to be mediated by pertussis toxin sensitive G-proteins[47]. However, the first known effector of A_1AR was inhibition of adenylate cyclase. Adenosine is found to activate the same K^+ channel in cardiac muscle as well as other tissues, as does acetylcholine and that acetylcholine and adenosine responses are mediated by cholinergic muscarinic acid and A_1AR respectively. However, myocardial adenosine receptors and adenosine receptors in coronary arteries have also been found to be coupled via G proteins to ATP-sensitive K^{2+} channel which can be blocked by sulphonylureas [48]. An increase in K^+ conductance indirectly decreases Ca^{2+} entry through voltage-sensitive channels by hyperpolarizing the membrane potential. A_1ARs are also directly involved in reducing $[Ca^{2+}]_i$ by activating G proteins directly inhibitory to Ca^{2+} channels [49].

Experiments conducted on mice have shown that the activation of A_1ARs causes contraction through PLC in the A_1AR wild-type (A_1WT) mice aorta and a decrease in coronary flow in the A_1WT mouse heart [50]. The A_1AR coupled to Gi/o protein is known to regulate signaling pathways in various tissues, including the modulation of PLC activity, inhibition of PLA_2 and adenylate cyclase, activation of K^+ channels, and inhibition of Ca^{2+} channels[51-54]. Among the various PLC isoforms, PLCβIII is seen to be the predominantly activated isoform. The activation of A_1AR in turn activates PKCα which leads to p42/p44 MAPK phosphorylation in CASMCs as well as contraction of vascular smooth muscle [55]. A_1 receptor activation can directly activate K^+ channels and inhibit Q-, P- and N-type Ca^{2+} channels. A_1AR has been specifically shown to activate p42/44 MAPK (ERK 2) in different cells [56]. It has been seen in COS-7 cells that A_1 receptors activate ERK1/2 via βγ subunits released from pertussis toxin-sensitive G proteins Gi/o. However, the importance of p42/44 MAPK (ERK 1/2) signaling and its relationship with PKC in causing A_1AR-mediated contraction in vasculature is still unknown. However, A_1AR may be constitutively activated at basal adenosine level of 30-300nM. In general, the activation of A_1AR mediates its effect via the following signaling pathway:

A_1AR →PLC -βIII →PKC -α→ p42/p44 MAPK phosphorylation →contraction [57].

Though the cells of the immune system express adenosine receptors and are responsive to the modulatory effects of adenosine in an inflammatory environment, still most of the signalling pathways were uncovered in non-immune cell types, and A_1 receptor signalling mechanisms in cells of the immune system are not known.

A_1 adenosine receptor and Diabetes

Adenosine A_1 receptor (A_1-AR) activation can lower plasma glucose in diabetic rats lacking insulin and the change in A_1-AR gene expression in diabetic rats has also been reported [20]. They concluded that the gene expression of A_1-AR in the liver is increased in insulin deficient diabetic rats. Correction of hyperglycemia by insulin or phlorizin reversed the gene expression of A_1- AR in the liver of diabetic rats, suggesting the major role of hyperglycemia in causing the change in gene expression.

Diabetes is an epidemic of the 21st century. Rare in the past diabetes has grown into an increasingly common disease both in developed countries and in the third world. It has been reported that the most important factor for this unforeseen trend appears to be the increase in body weight around the world attributable to the changes in lifestyle over the last decade. Among other complications of diabetes, cardiovascular and renal vascular diseases are among the most costly in terms of human suffering and national healthcare costs [58-60]. It is likely that the increasing prevalence of diabetes will greatly affect the cardiovascular disease burden in the future. Although the morbidity and mortality of cardiovascular diseases has fallen over the last three decades, this trend may flatten or even reverse. Thus, a better understanding of the consequences of diabetes in the vasculature and the heart is of great importance. Indeed, diabetes markedly affects the function of the cardiovascular system, both in the microcirculation as well as in large conduit arteries supplying vital organs such as the heart, brain and kidney.

Role of adenosine receptors in ameliorating the course of diabetes has been studied. Adenosine was found to increase vascular conductance and flow in nondiabetic and diabetic rats[61]. Vasorelaxation response to adenosine and its analogues is attenuated in certain pathological conditions affecting the blood vessels, e.g., hypertension and diabetes [62]. Development of diabetes leads to dysfunction of many tissues including heart, there being an increased risk of congestive heart failure in patients, structural, functional and biochemical changes as well in diabetic heart [63,64]. One of the most common structural abnormality being noted in diabetic heart is cardiomyopathy arising from

microangiopathic changes in small vessels, a few others being ventricular hypertrophy, microvascular constriction, increased collagen deposition, atherogenesis, etc[65]. Biochemical modifications such as non-enzymatic glycation, sorbitol-myoinositol mediated changes, redox potential alterations, PKC activation and free fatty Acid metabolism have been observed in the cells of endothelium as well as myocytes[66-68]. Among other biochemical changes, an elevated level of adenosine in diabetic heart has also been observed in animal models [69].

In heart, activation of A_1 receptor has been found to attenuate β- adrenoceptor stimulation [70], delay ischemic contracture [71], and stimulate anaerobic glycolysis [72]. Adenosine receptors are key elements in mediating cardioprotective functions of adenosine. Traditionally, A_1 receptor has been found to be the most important of all in cardioprotection. Activation of A_1AR reduces the cardiac work and myocardial oxygen consumption. Anti-ischemic effects of adenosine mediated by A_1AR have been pointed out in a number of clinical and experimental data [73]. Though, as observed in diabetic rat, the mRNA level for A_1AR in whole heart or isolated cardiomyocytes does not change, however, A_1AR protein level increase significantly in diabetic cardiomyocytes. Over expression of A_1AR however leads to increased protection against ischemia-induced myocyte injury and enhanced pre conditioning effect [74]. It has also been reported that A_1AR activation following treatment with sildenafil plays an integral role in the signaling cascade responsible for delayed protection against global ischemia reperfusion injury whereas adenosine A_1 receptor mediates delayed cardioprotective effect with sildenafil in mouse [75]. A_1AR/A_{2a} AR ratio increases in cardiomyocytes of diabetic rat which may have an important physiological consequence, since both receptors exist on the same cell and have similar affinity for adenosine [76], but activation of A_{2A} AR counteracts the anti adrenergic effect of A_1AR [77]. It can be assumed that an increase in $A_1AR/A_{2a}AR$ ratio may alter physiological balance between pro and anti-adrenergic action of adenosine, this may have important consequence for failing heart.

A major complication of diabetes is the development of cardiovascular disorders, especially, hypertension. Patients with diabetes show an impairment of endothelium dependent vasodilatation. This happens partly due to the induction of ROS production by circulating free fatty acids in diabetics [78]. There are various enzyme systems in mammalian cells responsible for ROS generation; however, the major source of ROS generation is NADPH oxidase system [79]. NADPH oxidase in the cells is activated by growth factors, cytokines, stress, hypoxia and G-Protein coupled agonists [80], wherein a role of adenosine receptor can be ruled out. NADPH oxidase complex is known to be activated by PKC via Nox2 [81], PKC inturn being activated by A_1AR [56]. The involvement of the A_1-AR in NADPH oxidase activation and in cocaine-induced LV dysfunction and suggesting the A_1-AR stimulation, at least in part via NADPH oxidase induction, plays a critical role in the events leading to the cardiomyopathy observed after cocaine abuse [82]. Oxidative stress has been seen to upregulate A_1AR in smooth muscle cells in an experimental model. Oxidative stress and adenosine A_1 receptor activation differentially modulate subcellular cardiomyocyte mitogen actiated protein kinases and A_1 AR expression by activating nuclear factor kB [83-85].

Under normal conditions, A_1 receptor mediated contraction is not evoked. However, in hypertensive aorta, endothelium derived contractile response to adenosine appear to be A_1AR mediated, also involving free radicles which are possibly generated through increased release of cyclo oxygenases products from endothelium of hypertensive aorta [86]. Although evidence has been provided in a number of studies for the involvement of nitric oxide pathway in insulin induced vasodilation, alternative pathways may also exist for relaxation of vascular tone in response to insulin. One of the pathways involves Na^{2+}- K^{2+} ATPases and opening of ATP sensitive potassium channels in vascular smooth muscle cells [87]. Involvement of adenosine has also been reported that A_1AR activation negatively modulates coronary vasodilation [88].

FUTURE PROSPECTUS

In the present time known that A_1AR, works through various signaling pathways leads to aortic constriction in mice,diabetes in rats and thus open an area in the cardiovascular research. A better understanding of above pathway will help in its pharmacological modulations by using various agonists and antagonists. This will also allow understanding the pharmacology of A_1 receptor agonists that can be utilized in the development drugs with a number of reported diseases. This may present opportunities for the treatment of cardiovascular disorders and could be therapeutically targeted.

ACKNOWLEDGEMENT

Miss Hiba Shabir, Department of Biosciences acknowledges her Research Fellowship from Jamia Millia Islamia (Central University) for the preparation of this article.

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