A1 Adenosine Receptor Signaling and Therapeutic Target in Diabetes

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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 A1, A2A, A2B and A3, mediate their effector functions through a G-protein signalling. Among these A1AR, 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 A1AR in diabetes. The insight into the signaling pathway through A1AR 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 A1, A2A, A2B, and A3. The significant advancement has been made in the understanding of the pharmacological and physiological relevance of ARs, but the knowledge of A1 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, A1AR and A3AR are coupled to adenylate cyclase in an inhibitory manner (being coupled to Gi protein) and A2AR and A2BAR in a stimulatory manner (being coupled to Gs 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 A2 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 Gα, βγ 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) \cite{14,15}. On the other hand the antilipolyte effects in adipocytes, the A1AR inhibits adenylate cyclase activity through Gi protein reduces cAMP formation and consequently 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) \cite{16,17}. There are reports which suggest organ damages such as kidney, liver and adipocytes in diabetes \cite{18,19,20}. The direct inhibition of hormone-sensitive lipase by A1AR 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 A1ARs, resulting in the inhibition of hormone-sensitive lipase and or adipose triglyceride lipase.

### Table 1: Agonists and antagonists of adenosine receptors

<table>
<thead>
<tr>
<th>Adenosine receptor subtype</th>
<th>Agonists</th>
<th>Antagonists</th>
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<tbody>
<tr>
<td>A1 AR</td>
<td>CPA</td>
<td>DPCPX</td>
</tr>
<tr>
<td></td>
<td>CCPA</td>
<td>WRC-0571</td>
</tr>
<tr>
<td></td>
<td>CHA</td>
<td>BQ9719</td>
</tr>
<tr>
<td></td>
<td>S-ENBA</td>
<td>KW9302</td>
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<tr>
<td></td>
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<td>FK453</td>
</tr>
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<td></td>
<td></td>
<td>FK194921</td>
</tr>
<tr>
<td>A2 AR</td>
<td>CGS-21680</td>
<td>KW6002</td>
</tr>
<tr>
<td></td>
<td>HE-NECA</td>
<td>SCH58261</td>
</tr>
<tr>
<td></td>
<td>CV-1674</td>
<td>VER6947</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SCH442416</td>
</tr>
<tr>
<td>A2BR</td>
<td>LUF5853</td>
<td>MRS1754</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MRE2029-F20</td>
</tr>
<tr>
<td>A3 AR</td>
<td></td>
<td>MRS1292</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PSB-11</td>
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<td></td>
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<td></td>
<td></td>
<td>MRS1334</td>
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<tr>
<td></td>
<td></td>
<td>MRE3006-F20</td>
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<td></td>
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<td>MRS1523</td>
</tr>
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</table>


**Fig 1.** A1AR signaling in vasculatures

A1AR signaling causes contraction in smooth muscle cells. The β7 subunit of A1AR activates PLC β which causes contraction through MAPK pathway and an increase in intracellular Ca²⁺ as well as ROS. ROS causes phosphorylation in some specific domain of PKC which also plays a role in its activation.

**Fig 2: A1AR cellular signaling in adipocytes**

Indicates inhibition in the signaling pathway.

**A2A Adenosine Receptors:** A2A receptors are highly expressed in neurons and regulate a variety of cardiovascular functions such as vascular conductance, blood platelet aggregation and vascular relaxation \cite{21,22}. These receptors are coupled to Gs proteins. A2A receptor agonists act on endothelium as well as vascular smooth muscles to cause vasodilatation \cite{23}. It has also been reported that A2A AR activation causes vasorelaxation through cytochrome P-450 (CYP) epoxygenases and endothelium-derived hyperpolarizing factors, whereas lack of A2A AR activation promotes vasoconstriction through Cyp4a in the mouse aorta \cite{24}. The activation of A2A receptor, increases MAPK activity and exert a mitogenic effect on endothelial cells by activating ERK1/2 using cAMP-ras-MEK1 pathway \cite{25}. However, the signaling pathways by A2A receptor varies with cell types and signaling machinery possessed by it. A2A receptor activation in some cell lines has been known to activate PKC, Ras and SOS but not Gs, cAMP or PKA. In humans, A2A receptors are present on the GABAergic output neurons in highest abundance \cite{26}. In general, the responses produced by A2A receptors can be classified as anti inflammatory and suppresses the release of inflammatory...
mediators, primarily by inhibiting lymphoid or myeloid 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\(_3\)AR 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\(_1\) 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\(_3\)AR is the receptor subtype that facilitates the degranulation of mast cells\(^{[37]}\). In cardiac cells, A\(_3\)AR agonists activate K\(^{+}\) channels and induce protection. RhoA-phospholipase D1 signaling mediates anti-ischemic effects of A\(_3\)AR \(^{[38]}\). Like other ARs, A\(_3\)AR 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\(_3\)AR 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

<table>
<thead>
<tr>
<th>Adenosine receptor subtype</th>
<th>G protein</th>
<th>Effect of G protein coupling</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(_1)</td>
<td>Gi 1/2/3, Go</td>
<td>↓ cAMP, ↑IP3/DAG,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑arachidonate(PLA2),</td>
</tr>
<tr>
<td>A(_{2A})</td>
<td>Gs, Golf, G15,16</td>
<td>↑cAMP, ↑IP3</td>
</tr>
<tr>
<td>A(_{2B})</td>
<td>Gs, Gq/11</td>
<td>↑cAMP, ↑IP3/DAG (PLC)</td>
</tr>
<tr>
<td>A(_3)</td>
<td>Gi/2,3 Gq/11</td>
<td>↓ cAMP, ↑IP3/DAG</td>
</tr>
</tbody>
</table>

have been found to be mediated by pertussis toxin sensitive G-proteins. However, the first known effector of A1AR 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 A1AR 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+ channel which can be blocked by sulphonylureas. An increase in K+ conductance indirectly decreases Ca2+ entry through voltage-sensitive channels by hyperpolarizing the membrane potential. A1ARs are also directly involved in reducing [Ca2+]i by activating G proteins directly inhibitory to Ca2+ channels.

Experiments conducted on mice have shown that the activation of A1ARs causes contraction through PLC in the A1AR wild-type (A1WT) mice aorta and a decrease in coronary flow in the A1WT mouse heart. The A1AR coupled to Gi/o protein is known to regulate signaling pathways in various tissues, including the modulation of PLC activity, inhibition of PLA2 and adenylate cyclase, activation of K+ channels, and inhibition of Ca2+ channels. Among the various PLC isoforms, PLCβIII is seen to be the predominantly activated isoform. The activation of A1AR in turn activates PKCα which leads to p42/p44 MAPK phosphorylation in CASMCs as well as contraction of vascular smooth muscle. A1 receptor activation can directly activate K+ channels and inhibit Q-, P- and N-type Ca2+ channels. A1AR has been specifically shown to activate p42/44 MAPK (ERK 2) in different cells. It has been seen in COS-7 cells that A1 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 A1AR-mediated contraction in vasculature is still unknown. However, A1AR may be constitutively activated at basal adenosine level of 30-300nM. In general, the activation of A1AR mediates its effect via the following signaling pathway:

A1AR →PLC-βIII →PKC-α → p42/44 MAPK phosphorylation → contraction

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 A1 receptor signalling mechanisms in cells of the immune system are not known.

A1 adenosine receptor and Diabetes

Adenosine A1 receptor (A1-AR) activation can lower plasma glucose in diabetic rats lacking insulin and the change in A1-AR gene expression in diabetic rats has also been reported. They concluded that the gene expression of A1-AR in the liver is increased in insulin deficient diabetic rats. Correction of hyperglycemia by insulin or phlorizin reversed the gene expression of A1-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. 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. Vasorelaxation response to adenosine and its analogues is attenuated in certain pathological conditions affecting the blood vessels, e.g., hypertension and diabetes. 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. 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 acids 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 A1 receptor has been found to attenuate β-adrenergocceptor stimulation [70], delay ischemic contracture [71], and stimulate anaerobic glycolysis [72]. Adenosine receptors are key elements in mediating cardioprotective functions of adenosine. Traditionally, A1 receptor has been found to be the most important of all in cardioprotection. Activation of A1AR reduces the cardiac work and myocardial oxygen consumption. Anti-ischemic effects of adenosine mediated by A1AR have been pointed out in a number of clinical and experimental data [73]. Though, as observed in diabetic rat, the mRNA level for A1AR in whole heart or isolated cardiomyocytes does not change, however, A1AR protein level increase significantly in diabetic cardiomyocytes. Over expression of A1AR however leads to increased protection against ischemia-induced myocyte injury and enhanced preconditioning effect [74]. It has also been reported that A1AR activation following treatment with sildenafil plays an integral role in the signaling cascade responsible for delayed protection against global ischemia reperfusion injury whereas adenosine A1 receptor mediates delayed cardioprotective effect with sildenafil in mouse [75]. A1AR/A2aAR 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 A2aAR counteracts the antiadrenergic effect of A1AR [77]. It can be assumed that an increase in A1AR/A2aAR 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 intum being activated by A1AR [56]. The involvement of the A1-AR in NADPH oxidase activation and in cocaine-induced LV dysfunction and suggesting the A1-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 A1AR in smooth muscle cells in an experimental model. Oxidative stress and adenosine A1 receptor activation differentially modulate subcellular cardiomyocyte mitogen activated protein kinases and A1AR expression by activating nuclear factor kB [83-85].

Under normal conditions, A1 receptor mediated contraction is not evoked. However, in hypertensive aorta, endothelium derived contractile response to adenosine appear to be A1AR mediated, also involving free radicals 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+/K+-ATPases and opening of ATP sensitive potassium channels in vascular smooth muscle cells [87]. Involvement of adenosine has also been reported that A1AR activation negatively modulates coronary vasodilation [88].

**FUTURE PROSPECTUS**

In the present time known that A1AR, 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 A1 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.
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