Adenosine A$_3$ Receptor: A Promising Therapeutic Target in Cardiovascular Disease

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Abstract: Cardiovascular complications are one of the major factors for early mortality in the present worldwide scenario and have become a major challenge in both developing and developed nations. It has thus become of immense importance to look for different therapeutic possibilities and treatments for the growing burden of cardiovascular diseases. Recent advancements in research have opened various means for better understanding of the complication and treatment of the disease. Adenosine receptors have become tool of choice in understanding the signaling mechanism which might lead to the cardiovascular complications. Adenosine A$_3$ receptor is one of the important receptor which is extensively studied as a therapeutic target in cardiovascular disorder. Recent studies have shown that A$_3$AR is involved in the amelioration of cardiovascular complications by altering the expression of A$_3$AR. This review focuses towards the therapeutic potential of A$_3$AR involved in cardiovascular disease and it might help in better understanding of mechanism by which this receptor may prove useful in improving the complications arising due to various cardiovascular diseases. Understanding of A$_3$AR signaling may also help to develop newer agonists and antagonists which might be prove helpful in the treatment of cardiovascular disorder.

Keywords: Adenosine A$_3$ receptor, agonist, antagonist, cardiovascular disorder, signaling.

INTRODUCTION

Adenosine- a purine nucleoside is endogenously produced in response to metabolic stress and cell damage [1]. It is directly or indirectly involved in the regulation of vascular tone [2]. Elevation in extracellular adenosine can be seen in conditions of ischemia, hypoxia, inflammation and trauma [1]. In general extracellular adenosine has a cytoplasmic function in the body [2]. Its effects on tissue protection and repair fall into four categories: (i) increasing the ratio of oxygen supply to demand; (ii) protecting against ischemic damage by cell conditioning; (iii) triggering anti-inflammatory responses; (iv) and promoting angiogenesis.

ADENOSINE RECEPTOR AND ITS SUBTYPES

The A$_3$AR has been extensively distributed, its mRNA being expressed in testis, lung, kidneys, placenta, heart, brain, spleen, liver, uterus, bladder, jejunum, proximal colon and eye of rat, sheep and humans. However, striking differences exist in expression levels within and amongst species. Particularly mast cells and rat testis express high concentrations of A$_3$AR mRNA, while low levels have been known in the majority of other rat tissues [3]. A high level of A$_3$AR mRNA is expressed in the lung and liver in human, while low levels have been found in the aorta and brain. Lung, spleen, pars tuberalis and pineal gland expressed the highest levels of A$_3$AR mRNA in sheep. By means of radio labelled ligand binding, immunoassay or functional assay in a variety of primary cells, tissues and cell lines the presence of A$_3$AR protein has been evaluated [3].

In cardiomyocytes, there was no direct evidence of the presence of A$_3$ARs but studies have reported that it was responsible for cardioprotection in a range of species and models, in addition to isolated myocardial muscle preparations and isolated cardiomyocytes [4]. A$_3$AR was spotted through radio labelled ligand binding and immunohistochemical assays in lung parenchyma and in human lung type II alveolar-like cell line (A549) [5].

There are four known subtypes of adenosine receptors (ARs)-referred to as A1, A$_2$A, A$_2$B and A$_3$AR. All subtypes are members of the superfamily of G-protein-coupled receptors (GPCRs). In humans, ARs have 49% sequence similarity between A$_1$ and A$_3$ ARs and the A$_2$A and A$_2$B ARs have 59% sequence similarity [6]. The A$_2$A and A$_2$B receptors preferably interact with members of the Gs family of G proteins and the A$_1$ and A$_3$ receptors with Gi/o proteins. However, other G protein interactions have also been observed. Adenosine is the preferred endogenous agonist of all these receptors, but inosine can also activate the A$_3$ receptor [2].

The A$_3$ adenosine receptor (A$_3$AR) is the lone adenosine subtype which was cloned before its pharmacological identification. It was initially isolated from rat testis as an orphan receptor, having 40% sequence homology with canine A$_1$

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AR and A_2AR subtypes [7] and was similar to the A_3AR cloned from rat striatum [8]. Homologs of the rat striatal A_3AR have been cloned from sheep and human, disclosing large interspecies differences in A3AR structure. For example, the rat A_3AR presents only 74% sequence homology with sheep and human A_3AR, while there is 85% homology between sheep and human A_3AR. This homology is indicated in the very different pharmacological sketch of the species homologs; particularly in terms of antagonist binding to the receptor which has made characterization of this adenosine subtype difficult. High degree of sequence similarity with that of other mammalian A_3AR transcripts like in human and sheep, is shown by sequencing of the cDNA [9].

Human A_3AR gene encodes 318 amino acids and is mapped to chromosome 1 p13-p21 [10]. The A_3AR gene includes 2 exons which are separated by an intron of approximately 2.2 kb. There is absence of a TATA-like motif in upstream sequence , instead it has a CCAAT sequence and consensus binding sites for transcription factors such as SP1, NF-IL6, GATA1 and GATA3 [11]. The involvement of the SP1, NF-IL6, GATA1 and GATA3 factors in transcriptional organization of A_3AR gene is coherent with the task of the receptor in immune function. Bioinformatics analysis has shown that A_3AR is present in the nuclear factor kappa B (NK-κB), indicating the function of NK-κB transcription factor in determining A_3AR expression level [12]. The main characteristics of A_3AR are that it is a G-protein-coupled receptor (GPCR) having a C-terminal segment in front of the intracellular compartment and 7 transmembrane spanning domains. In contrast to previous adenosine receptors, the C terminal area presents numerous serine and threonine residues, which may operate as possible sites of phosphorylation which are significant for rapid desensitization of the receptor on agonist treatment [13-15]. The high-affinity state phosphorylation leads to a drop in the number of receptors and a decline of agonist potency to inhibit the activity of adenylyl cyclase. At the same time, the receptor trafficking is reversible in an agonist-dependent manner [16].

A₃AR AGONIST AND ANTAGONIST (TABLE 1)

Since the discovery of the hypotensive and bradycardiac properties of adenosine, adenosine receptors have become promising drug targets. Primarily, the reason for this may be the fact that the range of tissues expresses receptors. Particularly, in the central nervous system, in the circulation, on immune cells, and on other tissues the actions of adenosine (or methylxanthine antagonists) can be beneficial in a variety of disorders. Secondly, the presence of a huge number of ligands that have been created by introducing several modifications in the structure of the lead compounds (adenosine and methylxanthine), some of which are highly specific [17].

Currently selective agonists for all four subtypes are available. More than a dozen of these selective agonists are at the present in clinical trials for different conditions, although none has been granted regulatory approval except for the endogenous AR agonist adenosine itself. A range of A_3AR agonists are in clinical testing for the treatment of various disorders such as rheumatoid arthritis and colorectal cancer [18].

N6-(3-iodobenzyl)-adenosine-5¢-N-methylcarboxamide (IB-MECA) and 2-chloro-N6-(3-iodobenzyl)- adenosine-5¢-methylcarboxamide (Cl-IB-MECA) are the prototypical and most widely used A_3AR agonists. Both IB-MECA and Cl-IB-MECA are adenosine derivatives carrying a lipophilic substituent (3-iodobenzyl) at the 6-amino group and ribose modification in the 5¢ position [19]. The presence of an additional 2-chloro substituent in Cl-IB-MECA makes it more selective than IB-MECA [6]. Another highly selective agonist is CP-532,903. Even as the theophylline and methylxanthines caffeine are conventional antagonists for the A1 adenosine receptor (A_1AR), A2A adenosine receptor ($A_{2A}AR$) and A2B adenosine receptor ($A_{2B}AR$), their affinity for the A_3AR is minimal. As a result, antagonists for A_3AR have been developed by the modification of different molecules with heterocyclic structures. One family of selective A_3AR antagonists consists of derivatives of 1,4-dihydropiridine, also known as inhibitors of L-type Ca^{2+} channels. These molecules bind with high affinity and selectivity for the human A_3AR after different modifications which includes the introduction of a 6-phenyl group [20]. As there are significant differences between the sequences of the human and rat A_3ARs, most of the antagonists developed for the human receptor bind with much lower affinity to rat and other rodent A_3ARs. Well-known members of this family are MRS1191 (3-ethyl-5-benzyl-2-methyl-4-phenylethynyl-6-phenyl-1,4-(±)- dihydropyridine-3,5-dicarboxylate), MRS1334 (1,4-dihydro- 2-methyl-6-phenyl-4-(phenylethynyl)-3,5-pyridinedicarboxylic acid 3-ethyl-5-[(3-nitrophenyl)methyl] ester) and MRS1523 (3-propyl-6-ethyl-5-[(ethylthio) carbonyl]-2 phenyl-4-propyl- 3-pyridine carboxylate). The pyridylquinazoline derivative VUF5574 (N-(2-methoxyphenyl)-N'-[2-(3-pyrindinyl)-4-quinazolinyl]-urea) and the triazoloquinazoline MRS1220 are also employed as selective A_3AR antagonists [6], both with selectivity only in humans. The flavonoids which are naturally occurring phenolic derivatives also have highly selective antagonists of the human A_3AR. MRS1067 (3,6-dichloro-2¢-isopropyloxy-4¢-methyl-flavone) is the most important element of this family [21]. A protective effect of the agonists on normal cells was recorded as well, signifying that this unique differential effect of the agonists will contribute to a safety profile of these drug candidates in both pre-clinical and clinical studies. Currently, A_3AR agonists are in clinical trial for the treatment of inflammatory, ophthalmic and liver diseases and exhibit excellent safety and efficacy in Phase 2 clinical studies [22]. Macromolecular conjugates (*e.g.* polyamidoamine dendrimers) of chemically functionalized AR agonists have been introduced as potent polyvalent activators of the receptors that are qualitatively different in pharmacological characteristics when compared with the monomeric agonists. Several A_3AR PET ligands have been introduced for *in vivo* imaging: the antagonist [18F]FE@SUPPY (5-(2-fluoroethyl) 2,4-diethyl-3-(ethylsulfanylcarbonyl)-6-phenylpyridine-5-carboxylate [23], and a pair of nucleosides, e.g. low efficacy agonist [76Br]MRS5147 and full agonist [76Br]MRS3581. The selectivity of A_3AR agonists differs between *in vitro* and *in vivo* models and between species, even though the sequence identity is high (84.4%) within the transmembrane region. The characterization of a given nucleoside derivative as full or partial agonist is very much dependent on the pharmacological system, such that varies

Table 1.   Summarizes the A₃AR and their agonist and antagonists.

S.No.	Agonist	Antagonist
1	IB-MECA	OT-7999
2	Cl-IB-MCEA	MRS1292
3	LJ568	PSB-11
4	CP-608,039	MRS3777
5	MRS3558	MRS1334
6	MRS1898	MRE300-F20
7	CP-532,903	
8	[^{76}Br]MRS5147	
9	[^{76}Br]MRS3581	
10	LUF6000	
11		MRS1220
12		MRS1523
13		'Novartis Compound'
14		LJ-1888

from full agonist to low efficacy partial agonist [24]. A selective positive allosteric modulator of the human A₃AR is LUF6000 (*N*-(3,4-dichloro-phenyl)-2-cyclohexyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine) [25]. Species-dependence of the affinity and selectivity of A₃AR antagonists should be carefully considered in preclinical studies. Functional polymorphism of A₃AR is already known and a high-transcript haplotype of the A₃AR gene was found to be associated with the development of cutaneous hyper-reactivity to aspirin [26].

A₃AR MEDIATED SIGNALING

A₃AR receptor activation inhibits adenylyl cyclase activity via Gi protein, which subsequently results in a decrease in cAMP levels [1, 27]. A₃AR activation can also stimulate the phospholipase C pathway, resulting in the elevation of intracellular inositol 1,4,5-trisphosphate and calcium (Ca^{2+})

levels [3]. The A₃AR can also stimulate mitogen-activated protein kinase (MAPK), such as extracellular signal regulated kinase 1/2 (ERK1/2) and p38 through the upstream activation of phosphoinositide 3-kinase (PI3K) [28]. The A₃AR-associated intracellular signaling pathways are summarized in Fig. (**1**).

Activation of the A₁ and A₃ adenosine receptors (ARs) inhibits adenylyl cyclase activity through activation of pertussis toxin-sensitive G$_{i/o}$ proteins and results in increased activity of phospholipase C (PLC) via Gβγ subunits. Activation of the A₂A and A₂B ARs increases adenylyl cyclase activity through activation of Gs proteins, Ca^{2+}, intracellular calcium, K$^+$ pertussis toxin-sensitive K$^+$ channels, cAMP, adenylyl cyclase.

The typical pathways linked with A₃AR activation via G$_q$ proteins are the inhibition of adenylyl cyclase activity by the coupling with Gi proteins resulting in the stimulation of
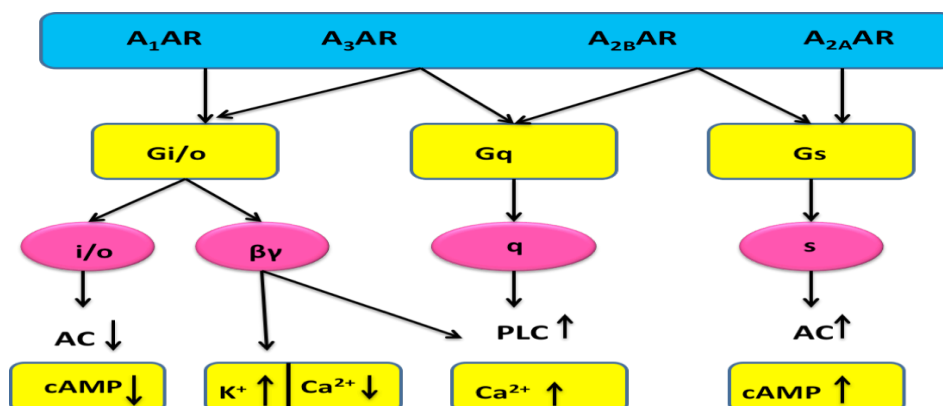
Fig. (1). Adenosine receptor signaling pathways.

phospholipase C (PLC), inositol triphosphate (IP3) and intracellular calcium (Ca^{2+}) [29]. However, some supplementary intracellular pathways have been explained as being significant for A$_3$AR signaling. For example, in the heart, A$_3$AR mediates cardioprotective effects through ATP-sensitive potassium (KATP) channel activation [30]. Anti-ischaemic effect of A$_3$ARs has been demonstrated to mediate by RhoA–phospholipase D1 signaling [31]. Like the other adenosine subtypes, A$_3$AR is engaged in the modulation of mitogen-activated protein kinase (MAPK) activity in addition to various recombinant and native cell lines, [28]. A$_3$AR signaling in Chinese Hamster Ovary cells transfected with human A$_3$AR (CHO-hA3) leads to stimulation of extracellular signal-regulated kinases (ERK1/2). Specifically, A$_3$AR signaling to ERK1/2 depends on the release of βγ subunits from pertussis toxin (PTX)-sensitive G proteins, phosphoinositide 3-kinase (PI3K), Ras and mitogen-activated protein kinase [28]. It has been reported that A$_3$AR activation is capable of decreasing the levels of phosphokinase A (PKA), a downstream effector of cAMP, and of the phosphorylated form of protein kinase B also known as Akt (PKB/Akt) in melanoma cells. This entails the deregulation of the WNT signaling pathway which is normally active in embryogenesis and tumorigenesis to heighten cell cycle progression and cell proliferation [32].

A well-designed study has recently documented the role of A$_3$AR in cell survival signaling in resveratrol preconditioning of the heart. This study gives support to the evidence that A$_1$AR and A$_3$AR gets activated through the preconditioning in the heart by resveratrol, transmitting a survival signal through both the PI3K-Akt-Bcl2 and cAMP response element-binding protein (CREB)-Bcl2 pathways [33]. Consequently, it has been demonstrated that CREB phosphorylation takes place through both Akt-dependent and independent signaling. Recently, in glioblastoma cells, activation of PI3K-Akt-pBAD by A$_3$AR has been detected leading to cell survival in hypoxic conditions [34]. Collectively, these findings demonstrate that numerous intracellular mechanisms are implicated following A$_3$AR stimulation, the understanding of which may be indispensable and crucial for explaining the different facet of its activation.

A$_3$ ADENOSINE RECEPTOR (A$_3$AR) AND ISCHEMIC HEART DISEASE

One of the most important subjects in the field of A$_3$AR-targeted therapy is the protective role of this adenosine receptor subtype in cardiac ischemia. A number of studies have proved that the A$_3$AR is an important player in adenosine induced cardioprotection during and following ischemia-reperfusion [35]. A lot of work has been done that attributes A$_1$AR with a major role in adenosine-mediated effects after the discovery of ischemic preconditioning (IPC) as a mechanism to reduce infarct size [36], and the identification of adenosine as one of the mediators of this phenomenon. Liu *et al.* found that the A$_1$AR antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) was unable to eliminate the anti-infarct effect induced by IPC in rabbit, thus suggesting a possible involvement of another adenosine subtype which subsequently pharmacologically identified as the A$_3$AR [37]. Furthermore, it was demonstrated in rabbit that N6-(3-iodobenzyl)-adenosine-5'-N-methylcarboxamide (IB–

MECA) reproduced IPC, suggesting the involvement of A$_3$AR subtype modulation. Moreover, in dog models there was also a lack of efficacy in reducing IPC-induced cardio-protection by A$_1$AR-selective antagonists [38, 39]. In terms of the timing of cardioprotection, some reports have indicated that pre ischemic A$_3$AR agonism is effective and indispensable, while others studies have suggested that protection occurs post ischemia, and still others have establish that A$_3$AR agonism is able to trigger an anti-infarct response with either pre- or post ischemic treatment [39]. Pre-treatment with an A$_3$AR agonist is responsible for cardioprotection, and it can be categorized into classic or early preconditioning, in which adenosine treatment occurs for 5 min, before exposure to ischemia [30, 40-42] and in which adenosine treatment occurs 24 h before the induction of ischemia also known as delayed or late preconditioning [43, 44].

The mechanism involved in the above effects (shared with the A$_1$AR subtype) was due to the activation of PKC and the regulation of mitochondrial KATP channels [38, 45]. The study of the cardioprotective profile of the A$_3$AR agonist N6-(2,5-dichlorobenzyl)-3-aminoadenosine-5- N-methylcarboxamide (CP-532,903) in an isolated mouse heart model of reperfusion and global ischemia and an in vivo mouse model of infarction, has shown that A$_3$AR activation provides ischemic protection by facilitating the opening of the sarcolemmal isoform of the KATP channel [46]. In addition, roles for MAPK and Akt/PI3 kinase have been acknowledged for early preconditioning [47, 48], whilst for late preconditioning the involvement of NF-kB, synthesis of inducible nitric oxide synthase (NOS) and mitochondrial KATP channels has been suggested [43]. Late preconditioning is more relevant than early preconditioning due to its sustained duration and the possibility of maintaining patients in a protracted, preconditioned, defensive state.

The cardioprotective effects of A$_3$ARs were also discovered in A$_3$AR-over expressing mice, where infarct size was lower than in wild-type mice after in vivo regional ischemia and reperfusion [49]. In these animals, A$_3$ARs overexpression decreased basal heart rate and contractility, preserved ischemic ATP, and decreased postischemic dysfunction [50]. Confirmation obtained by using pharmacological agents and genetic methods suggest that 2-Chloro-N6-(3-iodobenzyl)-adenosine-5′-N-methyluronamide (Cl–IB– MECA) protects against myocardial ischemia/reperfusion injury in mice via A$_3$AR activation. These conclusions were suggested by experiments with a selective A$_3$AR antagonist and by evaluating the A$_3$AR agonist effects on A$_3$AR knockout (KO) mice. Interestingly, in this study, by using congenic (C57BL/6) A$_3$AR KO mice, the deletion of the A$_3$AR gene itself has no effect on ischemic tolerance, suggesting that the previous contradictory results from the same and other [51-53] can probably be explained by differences in the genetic backgrounds of the mice rather than specific deletion of the A$_3$AR gene. Moreover, additional studies using wild-type mice treated with compound 48/80 (a condensation product of p-methoxyphenethyl methylamine with formaldehyde) to deplete mast cell contents, excluding the possibility that Cl–IB-MECA exerts a cardioprotective effect by releasing mediators from mast cells [54] and support the idea that therapeutic strategies focusing on the A$_3$AR subtype are a novel and useful approach to protecting the ischemic myocardium.

However, an important question arises from above data. Preconditioning obtained through adenosine receptor modulation may have clinical relevance (for example in cardiac surgery), but pre treatment is rarely permitted during acute myocardial infarction. Consequently, it would be more practical to achieve a protective effect from ischemia-reperfusion injury when the drug is administered post ischemia or during reperfusion. Literature data indicate that A_3AR agonism is able to protect the heart when given after the onset of ischemia or during reperfusion, suggesting its role in the treatment of acute myocardial infarction. In particular, Vinten-Johansen's group has reported that A_3AR agonist administration at reperfusion protects isolated rabbit hearts by reducing neutrophil activation [55]. After that, other studies also demonstrated a cardioprotective effect after A_3AR activation upon reperfusion in rat [56], guinea pig [57], and dog [39] hearts. As for the molecular mechanism involved in this effect, it has been reported that the opening of mitochondrial permeability transition pore (mPTP) plays a crucial role in myocardial ischemia/reperfusion injury and that blockade of the pore opening is cardioprotective [58, 59]. Interestingly, the inhibition of mPTP opening through the activation of PI3K/Akt and the consequent inhibition of glycogen synthase kinase after the activation of A_3AR have been reported [60].

Ashton *et al.* [61], in 2003 reported reduced A_3AR and increased A_2B adenosine receptor ($A_{2B}AR$) mRNA levels with aging, similar to what happens during ischemia in young hearts [62]. Additionally, a reduction in A_1AR has been observed during ischemia in aged hearts. Borea has hypothesised that decreased A_1AR and A_3AR expression might be responsible for the puzzling results mentioned above [63]. Therefore, it is possible that differences in the modulation of adenosine receptor subtypes occur during aging and, due to the differences and simultaneous involvement of all AR subtypes in cardioprotection [64, 65] it is possible that a better understanding of their interplay and age dependence will provide insights into the treatment of ischemic injuries in the myocardium.

VASODILATION

Cutaneous vasopermeability that is associated with activation and subsequent degranulation of mast cells, is completely absent in mice lacking functional A_3ARs [66]. One of the well-known actions of adenosine is to dilate vascular beds. Interestingly, the concentration of cAMP is higher in the aortae of A_3AR-deficient mice, with no significant change in the amount of A_1 or A_2A ARs, than it is in control mice. The hypotensive effect observed after intravenous adenosine injection in mice lacking the A_3AR was notably larger than in control mice [67]. Genetic deletion of the A_3AR or antagonism of the A_3AR augments coronary flow which is induced either by adenosine or by the $A_{2A}AR$ agonist CGS21680 [68]. However, A_3ARs do not regulate atherogenesis; the development of atherosclerosis and response to injury of the femoral artery were similar to those in wild-type mice [69]. It has been clearly demonstrated that both agonist- and antagonist-binding profiles for the murine and human A_3ARs are different. The marked species difference, together with the paradoxical protection in A_3AR-knockout hearts despite A_3AR-mediated protection in wild-type hearts, could reflect limitations of gene-knockout studies. Also, it should be noted that the selective ligands currently available are only relatively selective for a certain AR subtype. At relatively high concentrations, these ligands may also activate or block other AR subtypes. As such, careful and contemplative interpretation of pharmacological data is essential.

The molecular mechanisms associated with A_3AR mediated cardioprotection has already been described indicating a role for the pro-survival signalling pathways that decrease caspase-3 activity. These observations provide novel insight into the pharmacological effects of A_3ARs in ameliorating myocardial ischaemia/reperfusion injury [70].

HYPERTENSION

Administration of adenosine lowers blood pressure and decreases heart rate [71, 72]. Under regular adenosine concentrations, the A_3-type adenosine receptors are not active in mediating changes in blood pressure and that they are overpowered by A_2AR receptors that promote signals for vasodilation [67].

In a study by Shepherd *et al.*, the vasomotor effects of adenosine were analyzed by following changes in the diameters of micro vessels in hamster cheek pouches [73]. This study led to the conclusion that ligands binding to the mast cell A_3 adenosine receptors mediate degranulation as well as vasoconstriction. Interestingly, it was also concluded that adenosine initiates multiple conflicting vasomotor signals, as A_2 adenosine receptor-mediated dilation was competing with constriction (with the A_2AR overpowering the A_3AR), and thus adenosine analogs, but not adenosine, were able to induce changes in mast cell activation.

Adenosine actions in different systems are essentially of two types: those that are cAMP dependent, and others that are cAMP independent. Activation of sino atrial (SA), atrial, and atrio ventricular (AV) nodal adenosine receptors results in activation of a specific outward potassium current, that is cAMP independent [74, 75]. In ventricular myocytes, adenosine antagonizes the accelerate the actions of catecholamines on inward Ca^{2+} current (ICa) and on the transient inward current [76]. This antagonism by adenosine and its analogs is due to the inhibition of adenylyl cyclase [77, 78]. Several pathways have been proposed to explain the mechanism of action of adenosine in various tissues: (1) modulation of adenylyl cyclase activity, as also observed with the adrenergic receptors [79] ; (2) modulation of Ca^{2+} channel activity, e.g., adenosine inhibits Ca^{2+} uptake in heart [80]; (3) modulation of K^+ conductance, e.g. in pig atria adenosine causes an increase in potassium conductance, which could explain the shortening of the action potential duration and hyperpolarization caused by adenosine [81]; and (4) modulation of phospholipase C activity which may affect intracellular Ca^{2+} concentrations [82].

Focus has been on alterations in cAMP levels in wild-type and in A_3AR knock-out mice, as this is one of the immediate change occurring upon A_3AR receptor activation [67]. In platelets, this receptor is not naturally expressed therefore there is no alteration in cAMP levels as compared to wild-type mice or in platelet aggregation in response to

adenosine. While several studies have directly linked changes in cAMP levels in aorta and heart to blood pressure values, the elevated levels of cAMP is not directly associated with low blood pressure and vice versa [83-89]. It was observed that the A_3AR knock-out mice, the steady-state level of cAMP is elevated in aortas and heart, as compared to wild-type mice, with no change in blood pressure level [67]. Further elevation of cAMP in these tissues from A_3AR knock-out mice treated with adenosine was associated with an increase in blood pressure. An important conclusion can be made that the presence of A_3AR has a significant impact on the steady-state levels of cAMP in the cells, and hence potentially on a range of cAMP-dependent processes, and that adenosine-mediated effects on blood pressure are not directly correlated with changes in the levels of cAMP. As described above, based on the studies by Shepherd *et al.*, which were done with adenosine analogs binding to A_3AR, one may speculate that adenosine treatment of A_3AR knock-out mice will result in a less effective degranulation of mast cells and attenuated release of vasoconstricting substances which may in turn contribute towards vascular changes [73].

Role of A_3AR receptor as a possible cardioprotectant in diabetes has been investigated [90] and studies have verified the involvement of cyclooxygenases (COXs) [91] and NADPH oxidase pathways [92].

The development of cardiovascular disorders, especially, hypertension is a major complication of diabetes. Patients with diabetes show an impairment of endothelium dependent vasodilatation. To some extent this occurs due to the production of reactive oxygen species generated by circulating free fatty acids in diabetics [93].

ATHEROSCLEROSIS

Studies done in mouse models lacking adenosine receptors have shown that the role of adenosine in atherosclerosis, whether protective or deleterious, depends on the ablated receptor. The first glimpse of the role of adenosine in atherosclerosis begins with the elimination of the A_3AR on an Apolipoprotein E (ApoE) null background. ApoE KO mice are a effective model for the study atherosclerosis, as mice do not naturally develop all stages of atherosclerotic lesions along the arterial tree [94-97]. In the case of the A_3AR, there is no effect on the chronic development of atherosclerosis though aortic vascular smooth muscle cells demonstrate decreased proliferation potential upon receptor elimination and A_3AR contributes in the progression of inflammation [69].

The biology of A_3 adenosine receptors is complex and sometimes confusing [3]. Nevertheless, adenosine receptor agonists and antagonists are being worked out for oncology, inflammation, and potentially cardiac indications [17, 18]. A plethora of studies have associated adenosinergic signaling in cardioprotection, a report by Lu *et al.* recommends that A_3AR mediated signaling may be detrimental in the load-stressed myocardium [98].

CONCLUSION

There is no doubt that adenosine has a crucial role in the development of atherosclerosis, myocardial infarction and blood pressure homeostasis. All four receptors can poten-

tially be beneficial targets for different aspects during the pathogenesis of cardiovascular disease. Agonists of A_1AR and A_3AR could be targeted for regulation of ischemia and preconditioning.

Earlier works have shown the cardioprotective role of adenosine receptors especially A_1, A_{2A}, A_{2B} in both hypertension and diabetes. Deletion of A_3AR confers protection against ischemic reperfusion injury during myocardial infarction. There is growing evidence for the role of A_3AR as a cardioprotectant [99] although this has to be investigated further and understanding of the mechanisms involved in cardioprotection might help in the development of various tools which may prove helpful in the amelioration of cardiovascular complications and development of novel antagonist and agonist for the treatment of CVD. Thus, future development of A_3AR agonists would be initial steps towards examining the therapeutic potential of this receptor in humans with respect to atherosclerosis and cardiovascular disease. The availability of genetic information promises to facilitate understanding of the drug-receptor interaction leading to the rational design of a potentially therapeutically important class of drugs. Moreover, molecular modelling may further rationalize observed interactions between the receptors and their ligands.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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