Adenosine A\textsubscript{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\textsubscript{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\textsubscript{3}AR is involved in the amelioration of cardiovascular complications by altering the expression of A\textsubscript{1}AR. This review focuses towards the therapeutic potential of A\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{3}AR mRNA, while low levels have been known in the majority of other rat tissues [3]. A high level of A\textsubscript{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\textsubscript{1}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\textsubscript{1}AR protein has been evaluated [3].

In cardiomyocytes, there was no direct evidence of the presence of A\textsubscript{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\textsubscript{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 A\textsubscript{1}, A\textsubscript{2A}, A\textsubscript{2B} and A\textsubscript{3}AR. All subtypes are members of the superfamily of G-protein-coupled receptors (GPCRs). In humans, ARs have 49% sequence similarity between A\textsubscript{1} and A\textsubscript{3} ARs and the A\textsubscript{2A} and A\textsubscript{2B} ARs have 59% sequence similarity [6]. The A\textsubscript{2A} and A\textsubscript{2B} receptors preferably interact with members of the Gs family of G proteins and the A\textsubscript{1} and A\textsubscript{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\textsubscript{3} receptor [2].

The A\textsubscript{1} adenosine receptor (A\textsubscript{1}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\textsubscript{1}.
AR and A2AR subtypes [7] and was similar to the A2AR cloned from rat striatum [8]. Homologs of the rat striatal A2AR have been cloned from sheep and human, disclosing large interspecies differences in A3AR structure. For example, the rat A3AR presents only 74% sequence homology with sheep and human A3AR, while there is 85% homology between sheep and human A3AR. 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 A3AR transcripts like in human and sheep, is shown by sequencing of the cDNA [9].

Human A3AR gene encodes 318 amino acids and is mapped to chromosome 1 p13-p21 [10]. The A3AR 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 A3AR gene is coherent with the task of the receptor in immune function. Bioinformatics analysis has shown that A3AR is present in the nuclear factor kappa B (NF-κB), indicating the function of NF-κB transcription factor in determining A3AR expression level [12]. The main characteristics of A3AR 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 of 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 A2AR 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 (CI-IB-MECA) are the prototypical and most widely used A3AR agonists. Both IB-MECA and CI-IB-MECA are adenosine derivatives carrying a lipophilic substituent (3-iodobenzyl) at the 6-amonogroup and ribose modification in the 5¢ position [19]. The presence of an additional 2-chloro substitutent in CI-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 (A1R), A2A adenosine receptor (A2AR) and A2B adenosine receptor (A2BR), their affinity for the A3AR is minimal. As a result, antagonists for A3AR have been developed by the modification of different molecules with heterocyclic structures. One family of selective A3AR antagonists consists of derivatives of 1,4-dihydropyridine, also known as inhibitors of L-type Ca2+ channels. These molecules bind with high affinity and selectivity for the human A3AR 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 A3ARs, most of the antagonists developed for the human receptor bind with much lower affinity to rat and other rodent A3ARs. Well-known members of this family are MRS1191 (3-ethyl-5-benzyl-2-methyl-4-phenylethyl-6-phenyl-1,4-(z)-dihydropyridine-3,5-dicarboxylate), MRS1334 (1,4-dihydropyridine-2-methyl-6-phenyl-4-(phenylethyl)-3,5-pyridinedicarboxylic acid 3-ethyl-5-[[3-nitropheno]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-pyridinyl)-4-quinazolinyl]-urea) and the triazolouquinazoline MRS1220 are also employed as selective A3AR antagonists [6], both with selectivity only in humans. The flavonoids which are naturally occurring phenolic derivatives also have highly selective antagonists of the human A3AR. MRS1067 (3,6-dichloro-2-¢-isopropylxoy-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, A3AR 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. polylamidomamine dendrimers) of chemically functionalized AR agonists have been introduced as potent polyanvalent activators of the receptors that are qualitatively different in pharmacological characteristics when compared with the monomeric agonists. Several A3AR 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 A3AR 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     | CI-JB-MCEA      | MRS1292         |
| 3     | L3568           | PSB-11          |
| 4     | CP-608,039      | MRS3777         |
| 5     | MRS3558         | MRS1334         |
| 6     | MRS1898         | MRE300-F20      |
| 7     | CP-532,903      |                 |
| 8     | [16]BrMRS5147   |                 |
| 9     | [16]BrMRS3581   |                 |
| 10    | LUF6000         | MRS1220         |
| 11    |                 | MRS1523         |
| 12    |                 |                 |
| 13    |                 | ‘Novartis Compound’ |
| 14    |                 | LJR-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-1H-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 (Ca2+) 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 Gi/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, Ca2+, intracellular calcium, K+ pertussis toxin-sensitive K+ channels, cAMP, adenylyl cyclase.

The typical pathways linked with A3AR activation via Gq 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.](image-url)
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 \(\beta y\) 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\(_3\)AR and A\(_1\)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\(_3\)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 cardioprotection by A\(_3\)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-κB, 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 posts ischemic 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 methamphetamine 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 A1AR 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 A1AR agonist administration at reperfusion protects isolated rabbit hearts by reducing neutrophil activation [55]. After that, other studies also demonstrated a cardioprotective effect after A1AR 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 A1AR have been reported [60].

Ashton et al. [61], in 2003 reported reduced A1AR and increased A2B adenosine receptor (A2B AR) mRNA levels with aging, similar to what happens during ischemia in young hearts [62]. Additionally, a reduction in A1AR has been observed during ischemia in aged hearts. Borea has hypothesised that decreased A1AR and A2AR 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 degradation of mast cells, is completely absent in mice lacking functional A1ARs [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 A1AR-deficient mice, with no significant change in the amount of A1 or A2A ARs, than it is in control mice. The hypotensive effect observed after intravenous adenosine injection in mice lacking the A2AR was notably larger than in control mice [67]. Genetic deletion of the A1AR or antagonism of the A1AR augments coronary flow which is induced either by adenosine or by the A2A AR agonist CGS21680 [68]. However, A2ARs 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 A1ARs are different. The marked species difference, together with the paradoxical protection in A1AR-knockout hearts despite A1AR-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 A1AR 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 A1ARs 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 A1-type adenosine receptors are not active in mediating changes in blood pressure and that they are overpowered by A2AR 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 A1 adenosine receptors mediate degranulation as well as vasconstriction. Interestingly, it was also concluded that adenosine initiates multiple conflicting vasomotor signals, as A1 adenosine receptor-mediated dilatation was competing with constriction (with the A2AR overpowering the A1AR), 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 acceleration of the actions of catecholamines on inward Ca2+ 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 Ca2+ channel activity, e.g., adenosine inhibits Ca2+ 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 Ca2+ concentrations [82].

Focus has been on alterations in cAMP levels in wild-type and in A1 AR knock-out mice, as this is one of the immediate change occurring upon A1AR 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 A$_3$ Receptor: A Promising Therapeutic Target in Cardiovascular Disease

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 potentially be beneficial targets for different aspects during the pathogenesis of cardiovascular disease. Agonists of A$_1$AR and A$_3$AR 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$_3$AR confers protection against ischemic reperfusion injury during myocardial infarction. There is growing evidence for the role of A$_3$AR 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$_3$AR 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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Adenosine A1 Receptor: A Promising Therapeutic Target in Cardiovascular Disease

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