Review
The Adenosinergic System as a Therapeutic Target in the Vasculature: New Ligands and Challenges

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Abstract: Adenosine is an adenine base purine with actions as a modulator of neurotransmission, smooth muscle contraction, and immune response in several systems of the human body, including the cardiovascular system. In the vasculature, four P1-receptors or adenosine receptors—A1, A2A, A2B and A3—have been identified. Adenosine receptors are membrane G-protein receptors that trigger their actions through several signaling pathways and present differential affinity requirements. Adenosine is an endogenous ligand whose extracellular levels can reach concentrations high enough to activate the adenosine receptors. This nucleoside is a product of enzymatic breakdown of extra and intracellular adenine nucleotides and also of S-adenosylhomocysteine. Adenosine availability is also dependent on the activity of nucleoside transporters (NTs). The interplay between NTs and adenosine receptors’ activities are debated and a particular attention is given to the paramount importance of the disruption of this interplay in vascular pathophysiology, namely in hypertension. The integration of important functional aspects of individual adenosine receptor pharmacology (such as in vasoconstriction/vasodilation) and morphological features (within the three vascular layers) in vessels will be discussed, hopefully clarifying the importance of adenosine receptors/NTs for modulating peripheral mesenteric vascular resistance. In recent years, an increase interest in purine physiology/pharmacology has led to the development of new ligands for adenosine receptors. Some of them have been patented as having promising therapeutic activities and some have been chosen to undergo on clinical trials. Increased levels of endogenous adenosine near a specific subtype can lead to its activation, constituting an indirect receptor targeting approach either by inhibition of NT or, alternatively, by increasing the activity of enzymes responsible for ATP breakdown. These findings highlight the putative role of adenosinergic players as attractive therapeutic targets for cardiovascular pathologies, namely hypertension, heart failure or stroke. Nevertheless, several aspects are still to be explored, creating new challenges to be addressed in future studies, particularly the development of strategies able to circumvent the predicted side effects of these therapies.

Keywords: adenosine receptors; nucleoside transporters; vasculature

1. Introduction

Adenosine is an adenine nucleoside involved in nucleic acid assembly that results from ATP degradation in both the intra- and extracellular environment by the action of specific enzymes, and can act as a signaling molecule by interacting with integral membrane proteins, known as adenosine receptors or purinergic P1-receptors [1]. To date four subtypes have been identified, the adenosine A1, A2A, A2B and A3 receptors. It is established that the intracellular segment of each adenosine receptor subtype interacts with the appropriate heterotrimeric guanine (G) nucleotide-binding protein
(G-protein) with subsequent activation of an intracellular signal transduction mechanism. Adenosine receptor subtypes have been grouped into two main categories: (i) subtypes that are coupled to inhibitory G proteins, such as adenosine A\textsubscript{1} and A\textsubscript{3} receptors and (ii) subtypes which are coupled to stimulatory G proteins, like the A\textsubscript{2A} and A\textsubscript{2B} receptors. Evidence has, however, demonstrated that adenosine receptors are in fact pleiotropic since they may couple with several G proteins/transduction mechanisms depending on their degree of activation or cellular/subcellular localization [2]. Adenosine receptors when activated can lead to interactions with the \(\alpha\), \(\beta\) and \(\gamma\) subunits of the G-protein triggering signaling events [3,4].

In addition to the occurrence of adenosine receptors, adenosine availability is also crucial to discriminate which adenosine receptor subtype is activated. Interstitial levels of adenosine are elevated under conditions of increased metabolic demand (such as exercise) and decreased energy supply (such as ischemia), reaching physiologically relevant concentrations. Adenosine is released into the extracellular space signaling to restore the balance between local energy requirements and energy supply [5]. Released adenosine is quickly transported back into cells by an energy-dependent uptake mechanism, which is part of a purine salvage pathway designed to maintain intracellular ATP levels. Adenosine can be transported from inside to outside the cell and interstitial fluid or vice-versa through specific proteins, the nucleoside transporters (NTs). NTs can, thus, modify extracellular adenosine levels [6,7] since they may facilitate the movement of nucleosides and nucleobases across cell membranes. Transport of adenosine across the cellular membrane is crucial since it contributes to regulate extracellular adenosine levels, and subsequently, adenosine receptor subtype activation. Currently, two types of nucleoside transporters have been identified [8,9]: Equilibrative Nucleoside Transporters (ENT: ENT1, ENT2, ENT3 and ENT4) and Concentrative Transporters (CNT: CNT1, CNT2 and CNT3) [10]. It has been speculated that an increase in the activities of ENT1 and CNT2 may reduce the availability of adenosine to its receptors, conditioning their effects. Thus, NTs act as important players in adenosine function by controlling local levels of adenosine in the vicinity of the adenosine receptors. The effectiveness of this adenosine transport system has been demonstrated to be particularly active in humans, and is responsible for the extremely short half-life of adenosine in human blood.

In addition, adenosine availability also results from ATP enzymatic breakdown of both intra- and extracellular adenine nucleotides and intracellular S-adenosylhomocysteine. The reader is referred to Zimmermann et al. [11], who provide an excellent overview on this complex regulatory system. Briefly, ATP present in the cytosol can be sequentially dephosphorylated to ADP, AMP and then to adenosine. Alternatively, ATP can be released from different types of cells (by exocytosis), and then, metabolized by ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1 or CD39) to form ADP, AMP and, finally, by AMP hydrolysis to adenosine (via ecto-5'-nucleotidase, NT5E or CD73) [12] and to inosine by adenosine deaminase.

Extracellular disposition of adenosine availability can also be regulated by the presence of guanosine through an unknown mechanism [13], independent of NTs and ectonucleotidase activities. Guanosine increases adenosine and inosine levels [14,15], and can, therefore, alter adenosinergic system dynamics.

All these players—adenosine, adenosine receptors and nucleoside transporters—constitute together the adenosinergic system that, due to the above features, can exert a “fine-tuning” modulation in multiple physiological and pathophysiological processes.

2. Adenosine Receptor Ligands and Therapeutic Targets

2.1. Adenosine Receptor Structure and Binding “Pocket”

Adenosine receptor structure is crucial for the development of new ligands [16]. Briefly, adenosine receptors present seven transmembrane hydrophobic amino acid domains—TM I-VII—connected by three extracellular and three intracellular hydrophilic loops (with different sizes). These are highly
conserved and their residues are crucial for ligand binding/specificity [17,18]. The C-terminal is intracellularly located, on the cytoplasmatic side of the plasma membrane whereas the N-terminal is extracellularly located. The ligand binding site is formed by 3D-arrangement of the transmembrane domains, similar to a “pocket”. The critical interactions required for ligand recognition occur in TM III, V, VI, VII, where two histidine residues are conserved at TM VI, position 52 among adenosine A₁, A₂A and A₂B receptors, and were described as crucial for ligand recognition [18–20] and contribute to ligand specificity binding within the “pocket”; in adenosine A₃ receptors, these two histidine residues are lacking (TM VI: His52). Other amino acids residues can also be important for ligand recognition, affinity or binding [19,21–23] such as glutamate in TM I of the human adenosine A₂A receptor (critically involved in agonist, but not in antagonist, recognition [24]). Adenosine receptors distal region of the second extracellular loop are also involved in agonists and antagonists binding [17]. Indeed, residues negatively charged in adenosine A₂A receptor seem to be required for agonists and antagonists binding to the A₂A subtype. Additional residues of adenosine A₂A receptors were identified in TM V, VI, VII ligand binding [19]: mutation at histidine residues at TM VI, position 52 and TM VII, position 43 in adenosine A₂A receptor caused a dramatic loss of ligand affinity; in TM VII, position 43 a substitution of histidine for other residues also decreased ligand affinity [20].

The third intracellular loop of the adenosine A₂A receptor seems to be critical for its G-protein selectivity [25]: the cysteine residues forming a disulfide bridge at the third extracellular loop are conserved among the G-protein family and are required for receptor structural integrity and ligand binding [26]. Also the occurrence of mutations in TM IV and of the extracellular loops (both the C-terminal and the N-terminal) of adenosine receptors’ structure have ruled out the importance of this transmembrane domain/loops in ligand recognition/binding [17,18,27].

A structural feature that should also be considered regarding the development of new adenosine receptor ligands is G-protein coupled receptor dimerization. Adenosine receptors have been described to participate in homo- and/or heterodimerization, and also in oligomerization [28,29] phenomena having a major impact on the pharmacological behavior of those ligands.

2.2. New Adenosine Ligands and Their Usefulness

Series of adenosine receptor ligands, both agonists and antagonists [30], have been developed by structure-activity-relationship (SAR) or quantitative-structure-activity-relationship (QSAR) studies, defining both structural and electrostatic requirements for differential ligand affinity of adenosine receptor subtypes. Some of these ligands have been patented and, some are, currently, undergoing clinical evaluation for different therapeutic applications. In Table 1, patents from the last five years related to new adenosine analogues or adenosine receptor antagonists are listed with respective descriptions of the most relevant claimed effects. It is also clear that a recent class of selective adenosine receptor ligands is emerging, the A₂B receptor agonists class. Since these selective ligands have become available it has facilitated research on therapeutic applications and knowledge on adenosine receptors modulation [30]. As shown in Table 1, wide therapeutic applications are presented related to several types of pathological conditions and have involved all subtypes of adenosine receptors.

Another important aspect in the development of putative new selective adenosine receptor ligands, particularly of those with specific clinical applications, relies on the widespread actions of adenosine (due to the ubiquitous presence of adenosine and widespread distribution of adenosine receptors in the body), which may contribute greatly to impair safety delivery and clinical effectiveness of a particular compound. Also, the intricacy of adenosine signaling may explain the innumerable side effects reported and, ultimately, the failure of some of the new compounds in phase I, II or III trials.
| Ligands                        | Claimed Therapeutic Activity                                                                                                                                                                                                 | Patent No.     | Ref. |
|-------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|------|
| Pyridine derivatives          | Treatment of stable and unstable angina pectoris and atrial fibrillation                                                                                                                                                      | CA2442256C     | [31] |
| Imidazoquinoline derivatives  | Therapeutic and/or preventive treatment of dysfunctions of the heart, kidney, respiratory system, central nervous system.                                                                                                      | CA2505910C     | [32] |
|                               | Treatment of B-cell proliferative disorders                                                                                                                                                                                   | EP2178369A4    | [33] |
| Imidazopyridine derivatives   | Treatment, prevention or suppression of diseases and disorders known to be susceptible to improvement by antagonism of the A2B adenosine receptor, such as asthma, chronic obstructive pulmonary disorder, pulmonary fibrosis, emphysema, allergic diseases, inflammation, reperfusion injury, myocardial ischemia, atherosclerosis, hypertension, retinopathy, diabetes mellitus, inflammatory gastrointestinal tract disorders, and/or autoimmune diseases. | US7855202B2    | [34] |
| Pyrazine derivatives          | Treatment, prevention or suppression of diseases and disorders known to be susceptible to improvement by antagonism of the A2B adenosine receptor, such as asthma, chronic obstructive pulmonary disorder, pulmonary fibrosis, emphysema, allergic diseases, inflammation, reperfusion injury, myocardial ischemia, atherosclerosis, hypertension, retinopathy, diabetes mellitus, inflammatory gastrointestinal tract disorders, and/or autoimmune diseases. | US785520B2     | [35] |
| Substituted 2-oxy-3,5-dicyano-4aryl-6-aminopyridines | Prophylaxis and/or treatment of various disorders, in particular disorders of the cardiovascular system (cardiovascular disorders), the substances preferably acting as adenosine-receptor selective ligands. | US7855219B2    | [36] |
| Methanocarbacycloyl nucleoside analogues | Treatment or prevention of various diseases including airway diseases (through A2B, A3, P2Y2 receptors), cancer (through A3, P2 receptors), cardiac arrhythmias (through A1 receptors), cardiac ischemia (through A1, A3 receptors), epilepsy (through A1, P2X receptors), and Huntington’s Disease (through A2A receptors). | CA2397366C    | [37] |
| Substituted 2-thio-3,5-dicyano-4-phenyl-6-aminopyridines | Prophylaxis and/or treatment of various diseases such as, for example, diseases of the cardiovascular system, in particular. Suitable active compounds for use in combination are, in particular, active compounds for treating coronary heart diseases, for example nitrates, betablockers, calcium antagonists and diuretics, in particular. | CA2453747C    | [38] |
| Substituted 2-thio-3,5-dicyano-4-phenyl-6-aminopyridines | Treatment of various disorders, i.e., in particular, for example, disorders of the cardiovascular system (cardiovascular disorders). Active compounds suitable for combinations are in particular active compounds for treating coronary heart disease, such as, for example, in particular nitrates, beta blockers, calcium antagonists or diuretics. | CA2469586C    | [39] |
| Ligands                                      | Claimed Therapeutic Activity                                                                                                                                                                                                                                                                                                                                                   | Patent No.      | Ref.  |
|---------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|-------|
| 8-Pyrazolylxanthine derivatives            | Treatment of conditions and diseases mediated by the adenosine $A_{2B}$ receptor activity. Such conditions include, but are not limited to, chronic and acute inflammatory diseases involving degranulation of mast cells, e.g., asthma, allergic rhinitis and allergic dermatitis; impaired sensitivity to insulin, e.g., type 2 diabetes, pre-diabetic state, and impaired glucose tolerance; diseases in which angiogenesis is a key component of pathogenesis, e.g., solid tumors and angiogenic retinopathies; apnea of preterm infants; etc. | EP2032797A4     | [40]  |
| (N)-Methanocarbaadenine nucleosides        | Prevention, treatment, or amelioration of cancer, inflammation, auto-immune disease, ischemia-reperfusion injury, epilepsy, sepsis, septic shock, neurodegeneration (including Alzheimer’s Disease), muscle fatigue or muscle cramp (particularly athletes’ cramp). | US20110166093   | [41]  |
| Prodrug derivatives of 2-amino-6-         | Treatment a number of diseases, for example, inflammation, cardiac ischemia, stroke, asthma, diabetes, and cardiac arrhythmias. The invention also provides compounds that are agonists of both $A_1$ and $A_3$ adenosine receptors for use in cardioprotection | CN101056879B    | [42]  |
| ($^{13}$sulfanyl)-4-(4-[[2,3-dihydroxypropyl]oxy]phenyl)pyridine-3,5-dicarbonitriles | Treatment and/or prophylaxis of diseases, especially of cardiovascular disorders.                                                                                                                                                                                                                                                                  | EP2379539A1     | [43]  |
| Substituted 2-thio-3,5-dicyano-4-         | Treating mammals for various disease states, such as gastrointestinal disorders, immunological disorders, hypersensitivity disorders, neurological disorders, and cardiovascular diseases due to both cellular hyperproliferation and apoptosis                                                                                                             | US8143249       | [44]  |
| aryl-6-aminopyridines                     | Prophylaxis and/or treatment of various disorders, in particular disorders of the cardiovascular system                                                                                                                                                                                                                                                                   | CA2440218C      | [45]  |
| Xanthine derivatives                      | Treating mammals for various disease states, such as gastrointestinal disorders, immunological disorders, neurological disorders, and cardiovascular diseases due to both cellular hyperproliferation and apoptosis                                                                                                                               | CA2524778C      | [46]  |
| Substituted pyrrolopyridine,             | Treating asthma, inflammatory gastrointestinal tract disorders, cardiovascular diseases, neurological disorders, and diseases related to undesirable angiogenesis                                                                                                                                                           | US20130123280   | [47]  |
| pyrazolopyridine and isoxazolopyridine    | Treating or preventing a cardiovascular disease, a neurological disorder, an ischemic condition, a reperfusion injury, obesity, or wasting disease, or diabetes                                                                                                                                                                                                             | US8609833       | [48]  |
| derivatives                                 | Treatment and/or prevention of diseases and to their use for preparing medicaments for the treatment and/or prevention of diseases, preferably for the treatment and/or prevention of cardiovascular disorders.                                                                                                                                                          | US8609686       | [49]  |
Table 1. Cont.

| Ligands                                      | Claimed Therapeutic Activity                                                                 | Patent No.   | Ref. |
|----------------------------------------------|------------------------------------------------------------------------------------------------|--------------|------|
| 2,4-Disubstituted quinoline derivatives      | Treatment of a condition which is treatable by adenosine or an A<sub>3</sub> agonist            | EP2323661B1  | [50] |
| (N)-Methanocarbaadenine nucleosides          | Treatment a number of diseases, for example, inflammation, cardiac ischemia, stroke, asthma, diabetes, and cardiac arrhythmias | US8518957    | [51] |
|                                              | Preventing, treating, or ameliorating one or more symptoms of glaucoma or ocular hypertension  | EP2611502A1  | [52] |
| 4-Cycloalkyl- and 4-heterocycloalkyl-3,5-dicyano-2-thio-pyridine derivatives | Treatment and/or prophylaxis of diseases, preferably for the treatment and/or prevention of hypertension and other cardiovascular disorders. | EP2099788B1  | [53] |
| Therapeutic method                           | Diagnosis and determining effectiveness of treatment of inflammation and in particular to use therefore of biological markers associated with inflammatory states. | US20130345163| [54] |
| Heteroaryl-substituted dicyanopyridines      | Treatment and/or prophylaxis of diseases, preferably for the treatment and/or prevention of cardiovascular disorders. | US8426602    | [55] |
| 1H-Imidazo-[4,5-c]quinolin-4-amine derivatives | Treatment modulation of A<sub>3</sub> adenosine receptor                                         | US20130197025A1 | [56] |
| Phenylaminothiazole derivatives              | Treatment and/or prophylaxis of diseases, preferably for the treatment and/or prevention of hypertension and other cardiovascular disorders. | US8691850    | [57] |
| Substituted 4-amino-3,5-dicyano-2-thiopyridine derivatives | Treatment and/or prophylaxis of diseases, preferably for the treatment and/or prevention of hypertension and other cardiovascular disorders. | US8703934    | [58] |
| Substituted fused pyrimidine                 | Treating conditions and diseases that are mediated by adenosine receptor activity such as asthma, chronic obstructive pulmonary disorder, angiogenesis, pulmonary fibrosis, emphysema, allergic diseases, inflammation, reperfusion injury, myocardial ischemia, atherosclerosis, hypertension, congestive heart failure, retinopathy, diabetes mellitus, obesity, inflammatory gastrointestinal tract disorders, and/or autoimmune diseases | US8796290B2  | [59] |
| Fused pyrimidine compounds                   | Treating conditions and diseases that are mediated by adenosine receptor activity. These compounds are useful in the treatment, prevention or suppression of diseases and disorders that may be susceptible to improvement by antagonism of the adenosine receptor, such as asthma, chronic obstructive pulmonary disorder, angiogenesis, pulmonary fibrosis, emphysema, allergic diseases, inflammation, reperfusion injury, myocardial ischemia, atherosclerosis, hypertension, congestive heart failure, retinopathy, diabetes mellitus, obesity, inflammatory gastrointestinal tract disorders, and/or autoimmune diseases | CA2718983C   | [60] |
Table 1. Cont.

| Ligands                                                                 | Claimed Therapeutic Activity                                                                 | Patent No.    | Ref.     |
|------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|---------------|---------|
| Substituted 2,4′- and 3,4′-bipyridine derivatives                       | Treatment and/or prophylaxis of diseases, preferably for the treatment and/or prevention of hypertension and other cardiovascular disorders | CA2662728C   | [61]    |
| 2-Alkoxy-substituted dicyanopyridines                                 | Treatment and/or prophylaxis of diseases, preferably for the treatment and/or prevention of cardiovascular disorders. | US9205077    | [62]    |
| 2-amino-6-([(2-(4-chlorophenyl)-1,3-oxazol-4-yl)methylsulfanyl]-4-(4-[2,3-dihydroxypropyl]oxy)phenyl)pyridine-3,5-dicarbonitrile | Treatment and/or prophylaxis of diseases, and their use for the manufacture of medicaments for the treatment and/or prophylaxis of diseases, especially of cardiovascular disorders. | US8741834    | [63]    |
| 2-Amino-6-([(2-(4-chlorophenyl)-1,3-thiazol-4-yl)methyl]thio)-4-[4-(2-hydroxyethoxy)phenyl]pyridine-3,5-dicarbonitrile | Treatment and/or prophylaxis of diseases, and their use for the manufacture of medicaments for the treatment and/or prophylaxis of diseases, especially of cardiovascular disorders. | CA2695036C   | [64]    |
| Substituted aryloxazole derivatives                                   | Treatment and/or prophylaxis of diseases, and their use for the manufacture of medicaments for the treatment and/or prophylaxis of diseases, especially of cardiovascular disorders. | US9095582    | [65]    |
| Substituted 8-[6-carbonylamino-3-pyridyl] xanthines                    | Therapeutic methods are provided herein for treating a pathological condition or symptom in a mammal, such as a human, wherein the activity, e.g., over-activity, of adenosine A2B receptors is implicated in one or more symptoms of the pathology and antagonism (i.e., blocking) is desired to ameliorate such symptoms. | WO2011005871A1 | [66]    |
| 2-Chloro-Ν6-(3-iodobenzyl)-adenosine-5′-N-methyluronamide (CI-B-MECA)    | Treatment of hepatocellular carcinoma                                                        | US20150018299 | [67]    |
| Substituted fused pyrimidine compounds                                | Treatment, prevention or suppression of diseases and disorders that may be susceptible to improvement by antagonism of the adenosine receptor, such as asthma, chronic obstructive pulmonary disorder, angiogenesis, pulmonary fibrosis, emphysema, allergic diseases, inflammation, reperfusion injury, myocardial ischemia, atherosclerosis, hypertension, congestive heart failure, retinopathy, diabetes mellitus, obesity, inflammatory gastrointestinal tract disorders, and/or autoimmune diseases | US9284316    | [68]    |
|                                                                        | Treating conditions and diseases that are mediated by thereof as A2B adenosine receptor antagonists | CN103261200B  | [69]    |
For example, the compound GW493838, an A1 receptor agonist, was tested for its analgesic effect in peripheral nerve injury or neuralgia, and also for its beneficial effects on glaucoma and ocular hypertension, but was discontinued. Other similar examples of clinical trials discontinuation have occurred with the adenosine A1 antagonists BG9928 and KW-3902 [12], all due to their reported side-effects. Another example is illustrated by the recommendation of the U.S. Food and Drug Administration that the use of regadenoson (CVT-3146, Lexiscan) for cardiac nuclear stress tests of patients with signs or symptoms of unstable angina or cardiovascular instability should be avoided because the drug may increase the risk of a fatal heart attack. Nevertheless, and based on growing scientific evidence, several new adenosine receptor ligands are expected to be approved for clinical use and, hopefully, significantly improve the life style and outcome of patients. In Table 2, examples of ongoing or recently completed clinical trials of adenosine receptor ligands are described. Several clinical applications are reported for the cardiovascular system such as cardiac ischemia, chronic heart failure, atrial fibrillation etc. Nevertheless, in the vasculature few clinical studies have been carried out, “Regadenoson Blood Flow in Type 1 Diabetes” is an example, but further physiological/pharmacological studies in this field are needed to clarify the putative use of adenosine ligands as a therapeutic strategy in the treatment of vascular diseases.

2.3. Ligands as Pharmacological Tools

The adenosinergic system has been implicated in several processes such as modulation of neurotransmission, smooth muscle contraction, immune response, both in physiological and pathophysiological conditions. Our knowledge concerning adenosine/adenosine receptor actions/triggering events has improved with the development of ligands, both agonist or antagonists, with individual selectivity for adenosine receptor subtypes. According to the International Union of Pharmacology (IUPHAR), adenosine receptors ligands can be divided into agonists and antagonists depending on their respective adenosine receptor subtype, however there are some studies where some compounds, classified as antagonists, have been described as inverse agonists: caffeine [70] and ZM 241385 [71] as A2A inverse agonists and MRS 1706 [72] as an A2B inverse agonist. A brief summary of the pharmacological ligands currently used for classification of adenosine receptors is presented in Table 3.

Pharmacological studies have revealed that adenosine A1 and A2A receptors are high affinity receptors for adenosine although presenting different Kd (A2A receptors require higher concentrations (1–20 nM) than A1 receptors (0.3–3 nM) [73]). By contrast, adenosine A2B and A3 receptors are low affinity receptors (higher amounts of adenosine are required to activate these subtypes: >1 µM) [74]. An increase in the levels of endogenous adenosine (as a result of NT activity) nearby a specific adenosine receptor subtype can occur leading to its activation. Therefore, the NT may constitute a new target for a different therapeutic approach. Indeed, adenosine mechanisms are the target of commonly used drugs acting by blockade of adenosine reuptake, thus potentiating its actions or antagonizing adenosine receptors. Unfortunately few studies have been carried out and this field of work requires further studies. An approach of an indirect receptor targeting can occur by inhibition of nucleoside transporters. Indeed, nucleoside transporters are a crucial player in adenosine mediated effects by controlling adenosine bioavailability, and subsequently the activation of adenosine receptors [9,75,76]. Evidence also demonstrated that several physiological and pathophysiological conditions [9,75,77–79] and hypoxia can also reduce adenosine uptake [80,81] changing adenosine levels nearby adenosine receptors, therefore, conditioning its activation.

Another example of indirect receptor targeting can be achieved by increasing the activity of enzymes responsible for ATP breakdown. Evidence shows that conditions such as inflammation, hypoxia, and stress lead to an increase in ectonucleotidases expression. Moreover, hypoxia can ultimately stimulate CD73 [82–85], and CD39 [86–89] and, therefore, increase the ability of the tissue to produce adenosine.
Table 2. Clinical trials of adenosine receptor ligands: example of recently completed or ongoing studies.

| Target                              | Ligands                | Clinical Trials: Study                                                                 | C. T. Identifier Code | Ref.  |
|-------------------------------------|------------------------|----------------------------------------------------------------------------------------|-----------------------|-------|
| All adenosine receptor subtypes     | Agonist: adenosine     | Prophylactic Intra-coronary Adenosine to Prevent Post Coronary Artery Stenting Myonecrosis | NCT00612521          | [90]  |
|                                     |                        | Circulating Adenosine Levels Before and After Intravenous (IV) Persantine                | NCT00760708          | [91]  |
| All adenosine receptor subtypes     | Antagonist: caffeine   | Caffeine for Motor Manifestations of Parkinson’s Disease                                 | NCT01190735          | [92]  |
|                                     |                        | Caffeine for Excessive Daytime Somnolence in Parkinson’s Disease                          | NCT00564942          | [93]  |
|                                     |                        | The Impact of Caffeine on Brachial Endothelial Function in Healthy Subjects and in Patients With Ischemic Heart Disease | NCT00564824          | [94]  |
|                                     |                        | Caffeine as a Therapy for Parkinson’s Disease                                           | NCT01738178          | [95]  |
| Adenosine A1 receptor               | Agonist: BAY1067197    | Multiple Dose Study in Heart Failure of BAY 1067197 (PARSiFAL)                          | NCT01945233          | [96]  |
|                                     |                        | Study to Assess the Safety of BAY1067197 in Stable Heart Failure Patients on Standard Therapy Including ß-blocker | NCT01945606          | [97]  |
|                                     |                        | A Trial to Study Neladenoson Bialanate Over 20 Weeks in Patients With Chronic Heart Failure With Reduced Ejection Fraction (PANTHEON) | NCT02992288          | [98]  |
| Adenosine A1 receptor               | Agonist: tecadenoson   | Safety Study of Tecadenoson to Treat Atrial Fibrillation                                 | NCT00713401          | [99]  |
| Adenosine A1 receptor               | Antagonist: PBF-680    | “First-in-human” Study To Assess the Safety and Tolerability of PBF-680 in Male Healthy Volunteers | NCT01845181          | [100] |
|                                     |                        | A Study to Assess the Efficacy of a 5-day, 10-mg PBF-680 Oral Administration on Late Asthmatic Responses (LAR) in Mild to Moderate Asthmatic Patients. | NCT02635945          | [101] |
|                                     |                        | Study to Assess the Efficacy of a Single PBF-680 Oral Administration to Attenuate Adenosine 5′-Monophosphate Challenge-induced Airway Hyperresponsiveness in Mild-to-moderate Asthmatics | NCT01939587          | [102] |
| Adenosine A2A receptor              | Agonist: regadenoson   | Adenosine 2A Agonist Lexiscan in Children and Adults With Sickle Cell Disease            | NCT01085201          | [103] |
| Target                        | Ligands               | Clinical Trials: Study                                                                 | C. T. Identifier Code | Ref. |
|-------------------------------|-----------------------|----------------------------------------------------------------------------------------|----------------------|------|
| Adenosine A2A receptor        | Agonist: binodenoson  | Efficacy and Safety Study of Binodenoson in Assessing Cardiac Ischemia (VISION-305)   | NCT00944970          | [108]|
| Adenosine A2A receptor        | Agonist: MRE0094      | Safety and Efficacy Study of MRE0094 to Treat Chronic, Neuropathic, Diabetic Foot Ulcers | NCT00312364          | [109]|
| Adenosine A2A receptor        | Antagonist: preladenant| A Study to Assess Pharmacokinetics of Preladenant in Participants With Chronic Hepatic Impairment (P06513) | NCT01465412          | [110]|
|                               |                       | Placebo Controlled Study of Preladenant in Participants With Moderate to Severe Parkinson’s Disease (P07037) | NCT01227265          | [111]|
|                               |                       | A Dose Finding Study of Preladenant (SCH 420814) for the Treatment of Parkinson’s Disease (PD) in Japanese Patients (P06402) | NCT01294800          | [112]|
|                               |                       | Study of Preladenant for the Treatment of Antipsychotic Induced Movement Disorders in Participants With Schizophrenia (Study P04628) | NCT00686699          | [113]|
|                               |                       | Study of Preladenant for the Treatment of Neuroleptic Induced Akathisia (Study P05145AM1) (COMPLETE) | NCT00693472          | [114]|
| Adenosine A2A receptor        | Antagonist: istradefylline| Effect of Mild Hepatic Impairment on the Pharmacokinetics of Istradefylline | NCT02256033          | [116]|
|                               |                       | A Placebo- and Active-Controlled Study of Preladenant in Early Parkinson’s Disease (PD) (P05664) (PARADYSE) | NCT01155479          | [115]|
|                               |                       | Study of Preladenant for the Treatment of Neuroleptic Induced Akathisia (Study P05145AM1) (COMPLETE) | NCT00693472          | [114]|
| Adenosine A2A receptor        | Antagonist: istradefylline| Effect of Mild Hepatic Impairment on the Pharmacokinetics of Istradefylline | NCT02256033          | [116]|
|                               |                       | A Placebo- and Active-Controlled Study of Preladenant in Early Parkinson’s Disease (PD) (P05664) (PARADYSE) | NCT01155479          | [115]|

Table 2. Cont.
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| Target | Ligands | Clinical Trials: Study                                                                 | C. T. Identifier Code | Ref. |
|--------|---------|----------------------------------------------------------------------------------------|-----------------------|------|
|        |         | A 12-week Randomized Study to Evaluate Oral Istradefylline in Subjects With Moderate to Severe Parkinson’s Disease (KW-6002) | NCT01968031          | [117] |
|        |         | Long Term Study of Istradefylline in Subjects With Moderate to Severe Parkinson’s Disease | NCT02610231          | [118] |
|        |         | Study of Istradefylline (KW-6002) for the Treatment of Restless Legs Syndrome           | NCT00199446          | [119] |
| Adenosine A3 receptor | Agonist: CF101 | Trial of CF101 to Treat Patients With Psoriasis                                         | NCT00428974          | [120] |
|        |         | Oral CF101 Tablets and Methotrexate Treatment in Rheumatoid Arthritis Patients          | NCT00556894          | [121] |
|        |         | Safety and Efficacy of Daily CF101 Administered Orally in Subjects With Elevated Intraocular Pressure | NCT01033422          | [122] |
|        |         | Trial of CF101 to Treat Patients With Dry Eye Disease                                    | NCT01235234          | [123] |
|        |         | Safety and Efficacy Study of CF101 to Treat Keratoconjunctivitis Sicca                   | NCT00349466          | [124] |
| Adenosine A3 receptor | Agonist: CF102 | A Phase 1–2 Study of CF102 in Patients With Advanced Hepatocellular Carcinoma          | NCT00790218          | [125] |
|        |         | A Phase 1/2 Study of CF102 in Patients With Chronic Hepatitis C Genotype 1              | NCT00790673          | [126] |
|        |         | Phase 2, Randomized, Double-Blind, Placebo-Controlled of the Efficacy and Safety of CF102 in Hepatocellular Carcinoma (HCC) | NCT02128958          | [127] |
| Adenosine A3 receptor | Antagonist: PBF-677 | “First-in-human” Study To Assess the Safety and Tolerability of PBF-677 in Healthy Volunteers | NCT02639975          | [128] |
Table 3. Ligands currently used for adenosine receptors classification.

| Ligand Type | Abbrev. | Ligand | Adenosine Receptor Subtype |
|-------------|---------|--------|---------------------------|
| AGONIST     | ADO     | Adenosine | A₁, A₂A, A₂B, A₃ |
|             | NECA    | 5′-N-Ethylcarboxamidoadenosine | A₁, A₂A, A₂B, A₃ |
|             | CPA     | N⁶-Cyclopentyladenosine | A₁ |
|             | CGS 21680 | 2-(2-((2-Carboxyethyl)phenethylamino)-5′-N-ethylcarboxamidoadenosine hydrochloride | A₂A |
|             | IB-MECA | 1-Deoxy-1-[6-[[3-iodophenyl]methyl]amino]-9H-purin-9-yl]-N-methyl-b-D-ribofuranuronamide | A₃ |
|             | 2Cl-IB-MECA | 2-Chloro-N⁶-(3-iodobenzyl)-5′-(N-methylcarbamoyl)adenosine | A₃ |
| ANTAGONIST  | Teophylline | 3,7-Dihydro-1,3-dimethyl-1H-purine-2,6-dione | A₁, A₂A, A₂B, A₃ |
|             | Caffeine | 1,3,7-Trimethylpurine-2,6-dione | A₁, A₂A |
|             | DPCPX   | 1,3-Dipropyl-8-cyclopentylxanthine | A₁ |
|             | SCH 58261 | 5-Amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo-[4,3-c]-1,2,4-triazolo[1,5-c]pyrimidine | A₂A |
|             | ZM 241385 | 4-[2-[7-Amino-2-[2-furyl]-[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-yl-amino]ethyl]phenol | A₂A |
|             | MRS 1754 | N-(4-Cyanophenyl)-2-[4-(2,6-dioxo-1,3-dipropyl-2,3,4,5,6,7-hexahydro-1H-purin-8-yl)-phenoxy]acetamide | A₂B |
|             | MRS 1706 | N-(4-Acetylphenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl)phenoxy]acetamide | A₂B |
|             | MRS 1220 | N-[9-Chloro-2-(2-furanyl)[1,2,4]triazolo[1,5-c]quinazolin-5-yl]benzene acetamide | A₃ |
|             | MRS 1523 | 2,3-Diethyl-4,5-dipropyl-6-phenylpyridine-3-thiocarboxylate-5-carboxylate | A₃ |
Indirect receptor targeting can be, therefore, an alternative therapeutic strategy using enzymes involved in adenosine production or compounds that modify nucleoside transporter activity as promising therapeutic targets in the cardiovascular disorders. Thus, clinical application of nucleoside transporters can be extended, as at present they have been used successfully in anticancer and antiviral therapy [77,78,129,130].

3. Adenosinergic System in the Vasculature

A considerable body of evidence has been gathered in the past years concerning the actions of adenosine in several systems including the cardiovascular system. Indeed, intense research in this field revealed some favorable conditions in which adenosine actions are more relevant. Pathophysiological or hypoxic/ischemic conditions are examples of such, since they favor an augmentation of extracellular adenosine levels with subsequent activation of adenosine receptors.

3.1. Vascular Smooth Muscle

Adenosine is able to regulate cardiac functions such as heart rate, contractility and can also influence the coronary flow. Cardiac electrophysiological effects mediated by adenosine occur mostly through direct activation of adenosine A1 receptor or, indirectly, by opposing the β-adrenoceptor-mediated effects. A2A receptors have been considered the main receptor subtype involved in coronary blood flow regulation, causing vasodilation in coronary arteries (see reviews [131,132] for further details). Indeed, in vascular tissues, adenosine is known to induce vasodilation, an effect classically ascribed to A2 receptors on vascular smooth muscle cells, leading to an increase in blood flow and oxygenation [133]. Nevertheless, evidence has also demonstrated that adenosine A2 receptor subtype can mediate vasodilation [134] in an endothelium- and nitric oxide-dependent [131] fashion. Indeed, in more recent studies, it was demonstrated that A1 and A2A receptor activation in endothelial cells promotes NO production and, consequently, NO-mediated vasodilation. Adenosine A2B receptors have been described to be involved in the inhibition of vascular smooth muscle cell proliferation and vasodilation in vessels such as aorta and saphenous vein [135–140]. On the other hand, A3 receptor activation has also been linked to producing relaxation/vasodilation of blood vessels [141].

A broad number of studies reported antimitogenic effects to adenosine, via activation of adenosine A2B receptors in pulmonary [142], aorta [135,143] and glomerular [136] artery smooth muscle cells. Vascular smooth muscle proliferation can be inhibited after adenosine A2B receptor activation through cAMP/Epac (exchange protein directly activated by cAMP) pathway [138]. More recently and, by contrast, adenosine A1 receptor was found to promote coronary smooth muscle cells proliferation [139]. These opposite effects ascribed to adenosine in the media layer of arteries, mediated by different adenosine receptor subtypes, evidence the importance of adenosine levels, a crucial factor determining the protective or promoter role of adenosine. Therefore, depending on the subtype of adenosine receptor that is activated, inhibition or stimulation of smooth muscle cells hypertrophy may occur.

3.2. Vascular Endothelium

Adenosine may not only promote cell proliferation but can also selectively influence vascular cell death, in a process involving endothelial apoptosis. This process is inhibited by A2A [144] and A1 receptor pathways [145]. In addition to endothelial apoptosis, smooth muscle cell apoptosis can also occur due to the action of adenosine via activation of A2B receptor dependent pathways [146].

It is well established that endothelium may influence vascular responsiveness by producing vasoactive substances such as NO, ROS, endothelins and adenosine. For example, in endothelium, it has been described that adenosine induces NO production through adenosine A1 and A2A receptor activation pathway which ends with activation of endothelial nitric oxide synthase [147]. Therefore, adenosine can stimulate endothelial NO synthase activity, which in turn, generates higher amounts of NO, a well-known vasodilator [148,149]. Adenosine A2B receptor subtype, in endothelial cells,
was implicated in cell proliferation, [150] suggesting that pharmacological or molecular activation of this receptor subtype may be useful in modulating vascular remodeling. Adenosine \( A_2B \) receptor is, therefore, a protective effector against hyperplasia. Moreover, adenosine \( A_2B \) receptors were found to be highly expressed in macrophages and vascular smooth muscle cells presenting an important role in the regulation of inflammation and vascular adhesion: deficiency in adenosine \( A_2B \) receptors was shown to promote lesions or thickness of the neointima after vascular injury [151] revealing its protective role in atherosclerosis. Some studies also identified adenosine \( A_2A \) [152] and \( A_3 \) [153] receptors as being protective against endothelial injury induced by the inflammatory processes. In vascular tissues, recent studies concerning the role of endothelium in hypertension, had suggested that the main mechanism regulating extracellular adenosine levels involves nucleoside uptake to endothelial cells with the subsequent impairment of adenosine \( A_1 \) receptor activation [154].

3.3. Vascular Adventitia

In addition to the adenosine mediated effects, ascribed to receptors/signaling pathways located in smooth muscle cells or endothelium, adenosine actions on sympathetic nerves (or even at central nucleus of the brain) are also of paramount importance in the regulation of vascular tonus. Animal and human studies have demonstrated sustained increases in sympathetic activation and, as a consequence, a direct induction of vascular remodeling. Indeed, sympathetic activation leads to systemic vasoconstriction, increases blood pressure and improves the perfusion pressure. This systemic vasoconstriction could be deleterious to the ischemic organ if not for the simultaneous local inhibitory actions of adenosine, which produces vasodilation and inhibition of noradrenaline release. These actions are, for the most part, circumscribed to the local ischemic tissue so that it is protected from sympathetically mediated vasoconstriction while it benefits from the improved perfusion pressure. Thus, adenosine seems to provide a link between local mechanisms of blood flow autoregulation and systemic mechanisms of autonomic cardiovascular regulation.

Several studies described the occurrence of a neuromodulatory role ascribed to adenosine receptor subtypes activation in sympathetic nerve fibers located in the adventitia layer of pulmonary [155], mesenteric [156–158], aorta [159], tail [3,4,154,160–162] and renal [163,164] arteries as well as in veins such as mesenteric veins [165–167]. For example, adenosine \( A_2 \) receptors, known to facilitate noradrenaline release, may have a profound impact in vascular remodeling, by enhancing noradrenaline levels in the synaptic cleft. On the other hand, the idea that endothelium could influence neurotransmission [155] was recently supported by findings where endogenous adenosine (derived from endothelium) altered neurotransmission (mesenteric and tail arteries) [161]. Endothelium-derived adenosine was also described to activate prejunctional adenosine receptors, mainly \( A_1 \) and \( A_2A \), which modulate neurotransmission influencing vascular tonus [154]. Taken together, correlated morphological and functional data allowed advances into the insights of neurovascular sympathetic modulation mediated by adenosine receptors, particularly in pathological conditions such as hypertension.

Many studies report greater circulating levels of noradrenaline in patients with hypertension than in normotensive control subjects. In normotensive subjects, increased levels of circulating noradrenaline generally induce a down-regulation of noradrenergic receptors. However, in subjects with hypertension, such down-regulation appears to be missing, resulting in an enhanced sensitivity to noradrenaline. The combination of enhanced sensitivity to and increased circulating levels of noradrenaline likely contributes significantly to sympathetic nervous system activity-related hypertension. In fact, some studies have demonstrated that in hypertensive arteries and veins there are impairment in the neuromodulatory effects mediated by adenosine \( A_1 \) receptors, while the adenosine \( A_2A \) receptor-mediated facilitation of noradrenaline release is preserved [166–169]. Adenosine \( A_2B \) and \( A_3 \) receptors in vessels seem to have an important role in conditions where the amounts of adenosine are higher, i.e., in pathological conditions such as in hypertension [158,170] and
diabetes [171–173]. Additionally, adenosine A₂B receptors increase noradrenaline release [162] while adenosine A₃ receptors have the opposite effect, inhibiting the release of this neurotransmitter.

An important effect mediated by adenosine in the adventitia layer of vessels is also related with the role of adenosine receptors in inflammation. Indeed, adventitial tissue present several cells involved in inflammatory processes: macrophages, lymphocytes, fibrocytes, cells where adenosine receptor subtypes were found mediating anti or pro-inflammatory effects [174–176]. Other type of insights is the interplay between adenosine receptors and signaling molecules involved in inflammation and oxidative stress such as reactive oxygen species (ROS) and NO. Indeed, data indicates that adenosine receptor (A₂A subtype) activation promote the increase of ROS generation [177] having a role in oxidative stress and, consequently, in a large number of pathologies where oxidative stress/inflammation is a promotor of the disease. Moreover, adenosine receptors (adenosine A₁ or A₂A receptors) may also activate eNOS leading to an increase of NO production [147], which may impair the deleterious effects mediated by ROS and oxidative stress.

3.4. Adenosine Receptors and Angiogenesis

Multiple mechanisms mediated by adenosine lead to the promotion of vessel growth, through stimulation of vascular endothelial cell proliferation, migration and tube formation [178,179]. Adenosine can, thus contribute to angiogenesis and vasculogenesis. The reader is referred to a Carmeliet and Jain article [180] that provides an excellent overview of the angiogenesis process. Numerous studies have shown that adenosine or nucleoside transporter inhibitors can stimulate blood vessel growth [178,181]. Indeed, elevated levels of adenosine can promote the production of pro-angiogenic factors (particularly, vascular endothelial growth factor, VEGF, angiopeiotin-1, ANG-1, etc.), key factors to stimulate angiogenesis initiation in several type of cells including endothelial and mesenchymal cells such as monocytes/macrophages. Adenosine has a mitogenic effect on endothelial cells through activation of A₁, A₂A and A₂B subtypes [150,179,182]. Hypoxia increases adenosine levels favoring activation of A₂A and A₂B receptors [183] in parenchymal cells and of A₁ receptors located in circulating monocytes [184,185], lead to VEGF production. VEGF is, then, able to activate VEGFR2 receptors located in endothelial cells (Tip cell) promoting endothelial cell proliferation, migration and tube formation (key steps of angiogenesis). Additionally, in hypoxic conditions, the expression of adenosine receptor subtypes, A₂A and A₂B is upregulated, contributing for a favorable environment to the angiogenic process [180]. It is important to notice, however, that adenosine can also modulate the production of anti-angiogenic substances in vascular and immune cells. Adenosine can mediate opposite effects in angiogenesis, by promoting pro-angiogenic or anti-angiogenic factors production. Adenosine can stimulate the release of pro-angiogenic factors such as IL-8, and VEGF, by A₂B receptor activation, or can inhibit thrombospondin-1 (anti-angiogenic factor) release by involving A₂A receptor subtype dependent pathways [186,187].

3.5. Distribution Profile of Adenosine Receptors and NT

The presence of adenosine receptors/nucleoside transporters is, therefore, crucial to predict the impact of adenosinergic system modulation in a particular location. The presence of adenosine receptor subtypes in vascular beds (intima, media and adventitia), both in arteries [3,4,156,161,162,168,169,188–196] and veins [197–199] has been documented. From immunohistochemical studies it was possible to identify the presence of adenosine A₁ (tail artery [159], mesenteric artery and vein [166–168]), A₂A and A₂B (tail and mesenteric artery and vein [162,166] and A3 (mesenteric artery and vein [166]) receptors. Recent studies allowed the visualization of adenosine receptor subtypes (A₁, A₂A, A₂B and A₃) in sympathetic nerve fibers [167–169]. In the endothelium of several arteries such as tail artery [159,162] and aorta [153,159] identification of all adenosine receptors (A₁, A₂A, A₂B and A₃) was carried out. Nevertheless, few studies have characterized the presence of adenosine receptors in veins [197–199].

Nucleoside transporters presence is also relevant to predict and understand adenosinergic dynamics. Evidence have revealed that CNTs are most likely expressed in a tissue-specific fashion
with CNT transport process occurring primarily in specialized epithelia while ENTs present a wide distribution, possibly in all cell types [200]. Nevertheless, studies demonstrated that ENT1 and ENT2, can be found in cell basolateral membranes. ENT2 are also abundantly found in skeletal muscle. ENT3 and ENT4 are widely distributed, but in the heart ENT3 is the most abundant ENT while in the vessels, particularly in endothelium, evidence indicates ENT4 as the most relevant ENT.

In the vasculature, studies on the role of endothelium in hypertension have raised the possibility that the main mechanism regulating extracellular adenosine levels is related with adenosine uptake to endothelial cells, thus, causing a subsequent impairment of adenosine A1 receptor activation [161].

The possibility that an increase in the activities of ENT1 and CNT2 may reduce the availability of adenosine to its receptors, conditioning adenosine-mediated effects, have been raised by several authors: For example, King and co-workers [201] have described that ENTs can modulate adenosine-mediated effects in the sinoatrial node of the heart, since dipyridamole potentiates A1 receptor-mediated chronotropic effects (via inhibition of adenosine uptake [75]; ENT1/ENT2 modulated adenosine-mediated effects of K+ channels and also of the cystic fibrosis transmembrane conductance regulator (CFTR). Other evidence was described in Slc29a1-null mice studies where authors revealed an important role of ENT1 in anxiety-related behavior [202,203] in ethanol preference and consumption [76,204,205] as well as in cardioprotection during ischemia [206]. The later alterations can be ascribed by altered ENT1-mediated modulation of adenosine levels with a subsequently differential adenosine receptor activation and signaling. Consistent with this possibility was the evidence that Slc29a1-null mice have elevated plasma levels of adenosine 131.

Nucleoside transporters are relevant players in adenosine functions since they regulate by “fine-tuning” local levels of adenosine in the vicinity of adenosine receptors.

3.6. Adenosine Receptors Interaction with P2 Receptors

Evidence has clearly demonstrated that interactions between GPCRs can modulate their activity, by inhibiting or facilitating it. It was also demonstrated that this type of interactions can occur due to receptor dimerization (formation of a physical complex), or due to the occurrence of cross-talk, when second messengers integrate coincident signals from multiple receptors [207,208]. In this regard, purinergic receptors (both P1 and P2) evidence interactions, such as duration, magnitude, and/or direction of the signals triggered by purines or pyrimidines. For instance, adenosine A1, A2A receptors or P2X1,3,4,7, or P2Y1,2,4,6,12 subtypes are receptors where such interactions have been reported in several organs (brain [209], kidney [210], oviduct [211], epididymis [212]). In addition, reciprocal influences can also be critical for the effect that each single ligand has on a variety of short- and long term physiological functions [213].

In vascular beds few studies have been done to address the putative interaction between P1 and P2 receptors. For example, regulation of vascular smooth muscle and endothelial cell proliferation by A2 receptors and P2Y1 and P2Y2 receptors acting by triggering MAPK pathways has been described [214]; P2X7 and P1 receptors have been linked to apoptosis [215,216]; facilitation of noradrenaline release mediated by A2A receptors is favored by activation of release inhibitory receptors such as P2 but also a2-adrenoceptors and A1 receptors in tail artery [160]. In arteries and veins, future studies are needed to completely understand the interactions occurring between P1 and P2 receptors, particularly of receptors present in the different vascular layers and of their impact on vascular pathologies.

4. Conclusions

In the past years intense research on adenosinergic system dynamics has occurred, enhancing our current knowledge about the interplay between adenosine, adenosine receptors, nucleoside transporters and other signaling molecules and heteroreceptors. The way these interactions are orchestrated in the vasculature, particularly under conditions such as inflammation or oxidative stress, has highlighted the putative role of adenosinergic players as attractive therapeutic targets for several cardiovascular pathologies, namely hypertension, heart failure, stroke, etc.
A renewed interest in this field has led to the development of new adenosine receptor ligands, which is reflected by an increased number of recent patents related to the adenosinergic system. As a consequence, at present several clinical trials are underway, reviewing the potential pharmacotherapy of adenosinergic ligands. In this respect, particularly relevant is the knowledge concerning the presence of adenosine receptors/nucleoside transporters in specific tissue locations since it creates new challenges that can be explored in future studies, namely by elaborating strategies able to circumvent the predicted side effects of these ligands by, for instance, regarding the putative implementation of site/target specific therapies.

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References
1. Burnstock, G. Purinergic signalling—An overview. In Novartis Foundation Symposium; John Wiley: Chichester, UK, 2006; Volume 276, pp. 26–48.
2. Queiroz, G.; Talaia, C.; Goncalves, J. Adenosine A2A receptor-mediated facilitation of noradrenaline release involves protein kinase C activation and attenuation of presynaptic inhibitory receptor-mediated effects in the rat vas deferens. J. Neurochem. 2003, 85, 740–748. [CrossRef] [PubMed]
3. Fresco, P.; Diniz, C.; Goncalves, J. Facilitation of noradrenaline release by activation of adenosine A(2A) receptors triggers both phospholipase C and adenylate cyclase pathways in rat tail artery. Cardiovasc. Res. 2004, 63, 739–746. [CrossRef] [PubMed]
4. Fresco, P.; Oliveira, J.M.; Kunc, F.; Soares, A.S.; Rocha-Pereira, C.; Goncalves, J.; Diniz, C. A2A adenosine-receptor-mediated facilitation of noradrenaline release in rat tail artery involves protein kinase C activation and betagamma subunits formed after alpha2-adrenoceptor activation. Neurochem. Int. 2007, 51, 47–56. [CrossRef] [PubMed]
5. Londos, C.; Cooper, D.M.; Wolff, J. Subclasses of external adenosine receptors. Proc. Natl. Acad. Sci. USA 1980, 77, 2551–2554. [CrossRef] [PubMed]
6. Conlon, B.A.; Ross, J.D.; Law, W.R. Advances in understanding adenosine as a plurisystem modulator in sepsis and the systemic inflammatory response syndrome (SIRS). Front. Biosci. 2005, 10, 2548–2565. [CrossRef] [PubMed]
7. Fredholm, B.B. Adenosine receptors as targets for drug development. Drug News Perspect. 2003, 16, 283–289. [CrossRef] [PubMed]
8. Baldwin, S.A.; Yao, S.Y.; Hyde, R.J.; Ng, A.M.; Foppolo, S.; Barnes, K.; Ritzel, M.W.; Cass, C.E.; Young, J.D. Functional characterization of novel human and mouse equilibrative nucleoside transporters (hENT3 and mENT3) located in intracellular membranes. J. Biol. Chem. 2005, 280, 15880–15887. [CrossRef] [PubMed]
9. Baldwin, S.A.; Beal, P.R.; Yao, S.Y.; King, A.E.; Cass, C.E.; Young, J.D. The equilibrative nucleoside transporter family, SLC29. Pflugers Arch. 2004, 447, 735–743. [PubMed]
10. Young, J.D.; Yao, S.Y.; Baldwin, J.M.; Cass, C.E.; Baldwin, S.A. The human concentrative and equilibrative nucleoside transporter families, SLC28 and SLC29. Mol. Aspects Med. 2013, 34, 529–547. [CrossRef] [PubMed]
11. Zimmermann, H.; Zebisch, M.; Strater, N. Cellular function and molecular structure of ecto-nucleotidases. Purinergic Signal. 2012, 8, 437–502. [PubMed]
12. Chen, J.F.; Eltzschig, H.K.; Fredholm, B.B. Adenosine receptors as drug targets—What are the challenges? Nat. Rev. Drug Discov. 2013, 12, 265–286. [CrossRef] [PubMed]
13. Rathbone, M.; Piliutti, L.; Caciagli, F.; Jiang, S. Neurotrophic effects of extracellular guanosine. Nucleosides Nucleotides Nucleic Acids 2008, 27, 666–672. [CrossRef] [PubMed]
14. Jackson, E.K.; Mi, Z. The guanosine-adenosine interaction exists in vivo. J. Pharmacol. Exp. Ther. 2014, 350, 719–726. [CrossRef] [PubMed]
15. Lanznaster, D.; Dal-Cim, T.; Permartiri, T.C.; Tasca, C.I. Guanosine: A Neuromodulator with Therapeutic Potential in Brain Disorders. Aging Dis. 2016, 7, 657–679. [CrossRef] [PubMed]
16. Diniz, C.; Borges, F.; Santana, L.; Uriarte, E.; Oliveira, J.M.; Goncalves, J.; Fresco, P. Ligands and therapeutic perspectives of adenosine A2A receptors. Curr. Pharm. Des. 2008, 14, 1698–1722. [CrossRef] [PubMed]

17. Olah, M.E.; Jacobson, K.A.; Stiles, G.L. Identification of an adenosine receptor domain specifically involved in binding of 5'-substituted adenosine agonists. J. Biol. Chem. 1994, 269, 18016–18020. [PubMed]

18. Olah, M.E.; Ren, H.; Ostrowski, J.; Jacobson, K.A.; Stiles, G.L. Cloning, expression, and characterization of the unique bovine A1 adenosine receptor. Studies on the ligand binding site by site-directed mutagenesis. J. Biol. Chem. 1992, 267, 10764–10770. [PubMed]

19. Townsend-Nicholson, A.; Schofield, P.R. A threonine residue in the seventh transmembrane domain of the human A1 adenosine receptor mediates specific agonist binding. J. Biol. Chem. 1994, 269, 2373–2376. [PubMed]

20. Beukers, M.W.; den Dulk, H.; van Tilburg, E.W.; Brouwer, J.; Ijzerman, A.P. Why are A(2B) receptors low-affinity adenosine receptors? Mutation of Asn273 to Tyr increases affinity of human A(2B) receptor for 2-(1-Hexynyl)adenosine. Mol. Pharmacol. 2000, 58, 1349–1356. [PubMed]

21. Jiang, Q.; Van Rhee, A.M.; Kim, J.; Yehle, S.; Wess, J.; Jacobson, K.A. Hydrophilic side chains in the third and seventh transmembrane helical domains of human A2A adenosine receptors are required for ligand recognition. Mol. Pharmacol. 1996, 50, 512–521. [PubMed]

22. Tucker, A.L.; Robeva, A.S.; Taylor, H.E.; Holeton, D.; Bockner, M.; Lynch, K.R.; Linden, J. A1 adenosine receptors. Two amino acids are responsible for species differences in ligand recognition. J. Biol. Chem. 1994, 269, 27900–27906. [PubMed]

23. AP, I.J.; Von Frijtag Drabbe Kunzel, J.K.; Kim, J.; Jiang, Q.; Jacobson, K.A. Site-directed mutagenesis of the human adenosine A2A receptor. Critical involvement of Glu13 in agonist recognition. Eur. J. Pharmacol. 1996, 310, 269–272.

24. Fredholm, B.B.; AP, I.J.; Jacobson, K.A.; Klotz, K.N.; Linden, J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. Pharmacol. Rev. 2001, 53, 527–552. [PubMed]

25. Rebola, N.; Sebastiao, A.M.; de Mendonca, A.; Oliveira, C.R.; Ribeiro, J.A.; Cunha, R.A. Enhanced adenosine A2A receptor facilitation of synaptic transmission in the hippocampus of aged rats. J. Neurophysiol. 2003, 90, 1295–1303. [CrossRef] [PubMed]

26. Olah, M.E. Identification of A2a adenosine receptor domains involved in selective coupling to Gs. Analysis of chimeric A1/A2a adenosine receptors. J. Biol. Chem. 1997, 272, 337–344. [PubMed]

27. Cunha, R.A. Neuroprotection by adenosine in the brain: From A(1) receptor activation to A (2A) receptor blockade. Purinergic Signal. 2005, I, 111–134. [CrossRef] [PubMed]

28. Franco, R.; Casado, V.; Cortes, A.; Perez-Capote, K.; Mallol, J.; Canela, E.; Ferre, S.; Lluis, C. Novel pharmacological targets based on receptor heteromers. Brain Res. Rev. 2008, 58, 475–482. [CrossRef] [PubMed]

29. Canals, M.; Marcellino, D.; Fanelli, F.; Ciruela, F.; de Benedetti, P.; Goldberg, S.R.; Neve, K.; Fuxe, K.; Agnati, L.F.; Woods, A.S.; Ferre, S.; Lluis, C.; Bouvier, M.; Franco, R. Adenosine A2A-dopamine D2 receptor-receptor heteromerization: Qualitative and quantitative assessment by fluorescence and bioluminescence energy transfer. J. Biol. Chem. 2003, 278, 46741–46749. [CrossRef] [PubMed]

30. Muller, C.E.; Jacobson, K.A. Recent developments in adenosine receptor ligands and their potential as novel drugs. Biochim. Biophys. Acta 2011, 1808, 1290–1308. [CrossRef] [PubMed]

31. Rosentreter, U.; Krämer, T.; Vaupel, A.; Hübsch, W.; Diedrichs, N.; Krahn, T.; Dembowsky, K.; Stasch, J.P.; Shimada, M. Substituted 2-thio-3,5-dicyano-4-phenyl-6-aminopyridines with Adenosine Receptor-Binding Activity and Their Use as Cardiovascular Preparations. U.S. Patent 7,078,417, 18 July 2006.

32. Aranyi, P.; Balazs, L.; Balogh, M.; Batori, S.; Nagy, L.T.; Timari, G.; Boer, K.; Kapui, Z.; Mikus, E.; Gerber, K.; et al. Imidazoquinoline Derivatives as Adenosine A3 Receptor Ligands. U.S. Patent 7,419,977, 2 September 2008.

33. Rickles, R.; Lee, M.S. Treatments of b-Cell Proliferative Disorders. W.O. Patent 2,009,011,893, 22 January 2009.

34. Juan, B.V.; Trias, C.E.; Pueyo, L.S.; Eastwood, P.R. Pyrazine Derivatives Useful as Adenosine Receptor Antagonists. U.S. Patent 7,790,728, 7 September 2010.
36. Rosentreter, U.; Krämer, T.; Vaupel, V.; Hübsch, W.; Diedrichs, N.; Krahn, T.; Dembowsky, K.; Stasch, J.P. Substituted 2-oxo-3,5-dicyano-4-aryl-6-aminopyridines and Use Thereof. U.S. Patent 7,855,219, 21 December 2010.
37. Jacobson, K.A.; Marquez, V. Methanocarba Cycloakyl Nucleoside Analogues. U.S. Patent 20,030,216,412, 20 November 2003.
38. Rosentreter, U.; Kramer, T.; Shimada, M.; Hubsch, W.; Diedrichs, N.; Krahn, T.; Henninger, K.; Stasch, J.P. Substituted 2-thio-3,5-dicyano-4-phenyl-6-aminopyridines and Their Use as Adenosine Receptor-Selective Ligands. U.S. Patent 7,045,631, 16 May 2006.
39. Rosentreter, U.; Krämer, T.; Shimada, M.; Hübsch, W.; Diedrichs, N.; Krahn, T.; Henninger, K.; Stasch, J.P. Substituted 2-thio-3,5-dicyano-4-phenyl-6-aminopyridines and the Use of the Same. U.S. Patent 7,109,218, 19 September 2006.
40. Baraldi, P.G.; Borea, P.A.; Moorman, A.; Tabrizi, M.A. Adenosine A2B Receptor Antagonists. U.S. Patent 7,767,685, 3 August 2010.
41. Richardson, P. Use of Adenosine Receptor Agonists in Therapy. U.S. Patent 7,790,698, 7 September 2010.
42. Jacobson, K.A.; Joshi, B.V.; Tchilibon, S. Purine Derivatives as A3 and A1 Adenosine Receptor Agonists. U.S. Patent 7,825,126, 2 November 2010.
43. Meibom, D.; Lerchen, H.-G.; Vakalopoulos, A.; Albrecht-Küpper, B.; Nell, P.; Keldenich, J.; Zimmermann, K.; Krenz, U. Amino Acid Ester Prodrugs and the Use Thereof. U.S. Patent 20,110,237,629, 29 September 2011.
44. Koltun, D.; Zablocki, J.; Kalla, R. Prodrugs of a2b Adenosine Receptor Antagonists. W.O. Patent 2,006,138,376, 28 December 2006.
45. Rosentreter, U.; Krämer, T.; Vaupel, A.; Hübsch, W.; Diedrichs, N.; Krahn, T.; Dembowsky, K.; Stasch, J.P. Adenosine Receptor Selective Modulators. U.S. Patent 7,825,255, 2 November 2010.
46. Kalla, R.; Perry, T.; Elzein, E.; Varkhedkar, V.; Li, X.; Ibrahim, P.; Palle, V.; Xiao, D.; Zablocki, J. Xanthine Derivatives as a2b Adenosine Receptor Antagonists. W.O. Patent 2,004,106,337, 9 December 2004.
47. Kalla, R.; Perry, T.; Elzein, E.; Varkhedkar, V.; Li, X.; Ibrahim, P.; Palle, V.; Xiao, D.; Zablocki, J. A2B Adenosine Receptor Antagonists. U.S. Patent 7,317,017, 8 January 2008.
48. Jagtap, P.; Szabo, C.; Salzman, A.L. Purine Derivatives as Adenosine A1 Receptor Agonists and Methods of Use Thereof. E.P. Patent 2,221,307, 25 August 2010.
49. Nell, P.; Vakalopoulos, A.; Süßmeier, F.; Albrecht-Küpper, B.; Zimmermann, K.; Keldenich, J.; Meibom, D. Substituted Azabicyclic Compounds and the Use Thereof. U.S. Patent 20,110,003,845, 6 January 2011.
50. Ijzerman, A.P.; Goblyos, A.; Brussee, J. A3 Adenosine Receptor Allosteric Modulators. U.S. Patent 20,110,144,156, 16 June 2011.
51. Jacobson, J.A.; Tosh, D.K. Methanocarba Adenosine Derivatives and Dendrimer Conjugates Thereof. W.O. Patent 2,011,068,978, 9 June 2011.
52. Armstrong, R.C.; Belli, B.A.; Holladay, M.W.; Rowbottom, M.W. Adenosine a3 Receptor Modulating Compounds and Methods of Use Thereof. W.O. Patent 2,012,030,918, 8 March 2012.
53. Nell, P.; Hübsch, W.; Albrecht-Küpper, B.; Vakalopoulos, A.; Süßmeier, F.; Keldenich, J.; Telser, J. Cyclically Substituted 3,5-dicyano-2-thiopyridines and Use Thereof. U.S. Patent 8,304,412, 6 November 2012.
54. Fishman, P.; Bar-Yehuda, S.; Madi, L. Biological Marker for Inflammation. U.S. Patent 8,541,182, 24 September 2013.
55. Meibom, D.; Vakalopoulos, A.; Albrecht-Küpper, B.; Zimmermann, K.; Nell, P.; Süßmeier, F. Heteroaryl-Substituted Dicyanopyridines and Their Use. U.S. Patent 20,110,207,698, 25 August 2011.
56. Goblyos, A.; Brussee, J.; Ijzerman, A.P.; Gao, Z.-G.; Jacobson, K.A. A3 Adenosine Receptor Allosteric Modulators. U.S. Patent 8,420,664, 16 April 2013.
57. Erguden, J.-K.; Karig, G.; Rosentreter, U.; Albrecht, B.; Henninger, K.; Hutter, J.; Diedrichs, N.; Nell, P.; Arndt, S.; Hübsch, W.; et al. Substituted Phenylaminothiazoles and Use Thereof. U.S. Patent 20,080,269,300, 30 October 2008.
58. Nell, P.; Diedrichs, N.; Albrecht-Küpper, B.; Vakalopoulos, A.; Süßmeier, F.; Keldenich, J. Substituted 4-amino-3,5-dicyano-2-thiopyridines and Use Thereof. U.S. Patent 20,100,069,363, 18 March 2010.
59. Ramdas, V.; Koul, S.; Basu, S.; Waman, Y.; Shejul, Y.; Barawkar, D.; Palle, V.P. Substituted Fused Pyrimidine Compounds, Its Preparation and Uses Thereof. U.S. Patent 8,796,290, 5 August 2014.
60. Palle, V.; Basu, S.; Waman, Y.; Ramdas, V.; Barawkar, D. Heterocyclic Compounds as Adenosine Receptor Antagonists. W.O. Patent 2,009,118,759, 1 October 2009.
61. Nell, P.; Hübsch, W.; Albrecht-Küpper, B.; Keldenich, J.; Knorr, A. Novel Substituted Bipyridine Derivatives and Their Use as Adenosine Receptor Ligands. U.S. Patent 20,100,093,728, 15 April 2010.

62. Hübsch, W.; Meibom, D.; Vakalopoulos, A.; Albrecht-Küpper, B.; Nell, P.; Zimmermann, K.; Süßmeier, F.; Keldenich, J. 2-alkoxy-substituted dicyanopyridines and their use. U.S. Patent 9,203,077, 22 April 2014.

63. Lerchen, H.-G.; Meibom, D.; Vakalopoulos, A.; Albrecht-Küpper, B.; Keldenich, J.; Zimmermann, K.; Nell, P.; Krenz, U. Dipeptoid Prodrugs and the Use Thereof. U.S. Patent 8,741,834, 3 June 2014.

64. Lerchen, H.-G.; Krenz, U.; Keldenich, J.; Diedrichs, N.; Krahn, T.; Hirth-Dietrich, C.; Albrecht-Küpper, B. Dipeptoid Prodrugs and the Use Thereof. U.S. Patent 8,703,696, 4 August 2015.

65. Nell, P.; Hübsch, W.; Albrecht-Küpper, B.; Keldenich, J.; Vakalopoulos, A.; Süßmeier, F.; Zimmermann, K.; Lang, D.; Meibom, D. Substituted Aryloxazoles and Their Use. U.S. Patent 9,095,582, 4 August 2015.

66. Wang, G.; Rieger, J.M.; Thompson, R.D. Substituted 8-[6-carbonylamine-3-pyridyl] Xanthines as Adenosine A2B Antagonist. W.O. Patent 2,011,005,871, 13 January 2011.

67. Fishman, P.; Cohen, S.; Bar-Yehuda, S. Treatment of Liver Conditions. U.S. Patent 20,150,018,299, 15 January 2015.

68. Palle, V.; Ramdas, V.; Barawkar, D.; Basu, S.; Koul, S.; Waman, Y.; Patel, M.; Panmand, A. Substituted Fused Pyrimidine Compounds. W.O. Patent 2,010,103,547, 16 September 2010.

69. Barawkar, D.; Basu, S.; Ramdas, V.; Palle, V.P.; Waman, Y.; Patel, M.; Panmand, A. Purine Compounds as Prodrugs of A2B Adenosine Receptor Antagonists, Their Process and Medicinal Applications. U.S. Patent 8,940,751, 27 January 2015.

70. Fernandez-Duenas, V.; Gomez-Soler, M.; Lopez-Can, M.; Taura, J.J.; Ledent, C.; Watanabe, M.; Jacobson, K.A.; Ciruela, F. Uncovering caffeine’s adenosine A2A receptor inverse agonism in experimental parkinsonism. ACS Chem. Biol. 2014, 9, 2496–2501. [CrossRef] [PubMed]

71. Lebon, G.; Warne, T.; Edwards, P.C.; Bennett, K.; Langmead, C.J.; Leslie, A.G.; Tate, C.G. Agonist-bound adenosine A2A receptor structures reveal common features of GPCR activation. Nature 2011, 474, 521–525. [CrossRef] [PubMed]

72. Li, Q.; Ye, K.; Blad, C.C.; den Dulk, H.; Brouwer, J.; Ijzerman, A.P.; Beukers, M.W. ZM241385, DPCPX, MRS1706 are inverse agonists with different relative intrinsic efficacies on constitutively active mutants of the human adenosine A2B receptor. J. Pharmacol. Exp. Ther. 2007, 320, 637–645. [CrossRef] [PubMed]

73. Dunwiddie, T.V.; Masino, S.A. The role and regulation of adenosine in the central nervous system. Annu. Rev. Neurosci. 2001, 24, 31–55. [CrossRef] [PubMed]

74. Cieslak, M.; Komoszynski, M.; Wojtczak, A. Adenosine A(2A) receptors in Parkinson’s disease treatment. Purinergic Signal. 2008, 4, 305–312. [CrossRef] [PubMed]

75. King, A.E.; Ackley, M.A.; Cass, C.E.; Young, J.D.; Baldwin, S.A. Nucleoside transporters: From scavengers to novel therapeutic targets. Trends Pharmacol. Sci. 2006, 27, 416–425. [CrossRef] [PubMed]

76. Choi, D.S.; Cascini, M.G.; Mailliard, W.; Young, H.; Paredes, P.; McMahon, T.; Diamond, I.; Bonci, A.; Messing, R.O. The type 1 equilibrative nucleoside transporter regulates ethanol intoxication and preference. Nat. Neurosci. 2004, 7, 855–861. [CrossRef] [PubMed]

77. Molina-Arcas, M.; Marce, S.; Villamor, N.; Huber-Ruano, I.; Casado, F.; Bellosillo, B.; Montserrat, E.; Gil, J.; Colomer, D.; Pastor-Anglada, M. Equilibrative nucleoside transporter-2 (hENT2) protein expression correlates with ex vivo sensitivity to fludarabine in chronic lymphocytic leukemia (CLL) cells. Leukemia 2005, 19, 64–68. [CrossRef] [PubMed]

78. Lu, H.; Chen, C.; Klaassen, C. Tissue distribution of concentrative and equilibrative nucleoside transporters in male and female rats and mice. Drug Metab. Dispos. 2004, 32, 1455–1461. [CrossRef] [PubMed]

79. Zhang, J.; Visser, F.; King, K.M.; Baldwin, S.A.; Young, J.D.; Cass, C.E. The role of nucleoside transporters in cancer chemotherapy with nucleoside drugs. Cancer Metastasis Rev. 2007, 26, 85–110. [CrossRef] [PubMed]

80. Eltzschig, H.K.; Abdulla, P.; Hoffman, E.; Hamilton, K.E.; Daniels, D.; Schonfeld, C.; Loffler, M.; Reyes, G.; Duszenko, M.; Karhausen, J.; et al. HIF-1-dependent repression of equilibrative nucleoside transporter (ENT) in hypoxia. J. Exp. Med. 2005, 202, 1493–1505. [CrossRef] [PubMed]

81. Morote-Garcia, J.C.; Rosenberger, P.; Nivillac, N.M.; Coe, I.R.; Eltzschig, H.K. Hypoxia-inducible factor-dependent repression of equilibrative nucleoside transporter 2 attenuates mucosal inflammation during intestinal hypoxia. Gastroenterology 2009, 136, 607–618. [CrossRef] [PubMed]
82. Hart, M.L.; Grenz, A.; Gorzolla, I.C.; Schittenhelm, J.; Dalton, J.H.; Eltzschig, H.K. Hypoxia-inducible factor-1alpha-dependent protection from intestinal ischemia/reperfusion injury involves ecto-5'-nucleotidase (CD73) and the A2B adenosine receptor. *J. Immunol.*, 2011, 186, 4367–4374. [CrossRef] [PubMed]

83. Eckle, T.; Krahn, T.; Grenz, A.; Kohler, D.; Mittelbronn, M.; Ledent, C.; Jacobson, M.A.; Osswald, H.; Thompson, L.F.; Unertl, K.; et al. Cardioprotection by ecto-5'-nucleotidase (CD73) and A2B adenosine receptors. *Circulation* 2007, 115, 1581–1590. [CrossRef] [PubMed]

84. Thompson, L.F.; Eltzschig, H.K.; Ibla, J.C.; Van De Wiele, C.J.; Resta, R.; Morote-Garcia, J.C.; Colgan, S.P. Crucial role for ecto-5'-nucleotidase (CD73) in vascular leakage during hypoxia. *J. Exp. Med.* 2004, 200, 1395–1405. [CrossRef] [PubMed]

85. Eckle, T.; Fullbier, L.; Wehrmann, M.; Khoury, J.; Mittelbronn, M.; Ibla, J.; Rosenberger, P.; Eltzschig, H.K. Identification of ectonucleotidases CD39 and CD73 in innate protection during acute lung injury. *J. Immunol.* 2007, 178, 8127–8137. [CrossRef] [PubMed]

86. Eltzschig, H.K.; Ibla, J.C.; Furuta, G.T.; Leonard, M.O.; Jacobson, K.A.; Enjoji, K.; Robson, S.C.; Colgan, S.P. Coordinated adenine nucleotide phosphohydrolysis and nucleoside signaling in posthypoxic endothelium: Role of ectonucleotidases and adenosine A2B receptors. *J. Exp. Med.* 2003, 198, 783–796. [CrossRef] [PubMed]

87. Eltzschig, H.K.; Kohler, D.; Ecke, T.; Kong, T.; Robson, S.C.; Colgan, S.P. Central role of Sp1-regulated CD39 in hypoxia/ischemia protection. *Blood* 2009, 113, 224–232. [CrossRef] [PubMed]

88. Hart, M.L.; Gorzolla, I.C.; Schittenhelm, J.; Robson, S.C.; Eltzschig, H.K. SP1-dependent induction of CD39 facilitates hepatic ischemic preconditioning. *J. Immunol.* 2010, 184, 4017–4024. [CrossRef] [PubMed]

89. Reutershan, J.; Vollmer, I.; Stark, S.; Wagner, R.; Ngamsri, K.C.; Eltzschig, H.K. Adenosine and inflammation: CD39 and CD73 are critical mediators in LPS-induced PMN trafficking into the lungs. *FASEB J.* 2009, 23, 473–482. [CrossRef] [PubMed]

90. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT00612521?term=NCT00612521&rank=1 (accessed on 22 February 2017).

91. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT00760708?term=NCT00760708&rank=1 (accessed on 22 February 2017).

92. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT00459420?term=NCT00459420&rank=1 (accessed on 22 February 2017).

93. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT01939587?term=NCT01939587&rank=1 (accessed on 22 February 2017).

94. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT01085201?term=NCT01085201&rank=1 (accessed on 23 February 2017).

95. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT00208312?term=NCT00208312&rank=1 (accessed on 23 February 2017).
Molecules 2017, 22, 752

105. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT00881218?term=NCT00881218&rank=1 (accessed on 23 February 2017).

106. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT01019486?term=NCT01019486&rank=1 (accessed on 23 February 2017).

107. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT01788631?term=regadenoson&rank=8 (accessed on 24 February 2017).

108. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT00944970?term=NCT00944970&rank=1 (accessed on 24 February 2017).

109. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT00312364?term=NCT00312364&rank=1 (accessed on 24 February 2017).

110. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT01465412?term=preladenant&rank=1 (accessed on 24 February 2017).

111. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT01227265?term=preladenant&rank=3 (accessed on 24 February 2017).

112. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT01294800?term=preladenant&rank=4 (accessed on 24 February 2017).

113. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT00686699?term=preladenant&rank=6 (accessed on 24 February 2017).

114. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT00693472?term=preladenant&rank=8 (accessed on 24 February 2017).

115. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT01155479?term=preladenant&rank=7 (accessed on 24 February 2017).

116. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT02256033?term=istradefylline&rank=1 (accessed on 24 February 2017).

117. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT01968031?term=istradefylline&rank=3 (accessed on 24 February 2017).

118. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT00349466?term=istradefylline&rank=6 (accessed on 24 February 2017).

119. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT00199446?term=istradefylline&rank=17 (accessed on 24 February 2017).

120. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT00428974?term=NCT00428974&rank=1 (accessed on 24 February 2017).

121. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT00556894?term=NCT00556894&rank=1 (accessed on 24 February 2017).

122. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT01033422?term=CF101&rank=3 (accessed on 25 February 2017).

123. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT01235234?term=CF101&rank=4 (accessed on 25 February 2017).

124. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT00790218?term=CF101&rank=6 (accessed on 25 February 2017).

125. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT02639975?term=a3+receptor&rank=4 (accessed on 25 February 2017).

126. Huber-Ruano, I.; Pastor-Anglada, M. Transport of nucleoside analogs across the plasma membrane: A clue to understanding drug-induced cytotoxicity. Curr. Drug Metab. 2009, 10, 347–358. [CrossRef] [PubMed]
transporters I and Chimeric constructs reveal a role for the ENT2 helix 5–6 region in nucleobase translocation. J. Biol. Chem. 2002, 277, 24938–24948. [CrossRef] [PubMed]

131. Mustafa, S.J.; Morrison, R.R.; Teng, B.; Pelleg, A. Adenosine receptors and the heart: Role in regulation of coronary blood flow and cardiac electrophysiology. Handb. Exp. Pharmacol. 2009, 161–188. [CrossRef]

132. Headrick, J.P.; Peart, J.N.; Reichelt, M.E.; Haseler, L.J. Adenosine and its receptors in the heart: Regulation, retaliation and adaptation. Biochim. Biophys. Acta 2011, 1808, 1413–1428. [CrossRef] [PubMed]

133. Bryan, P.T.; Marshall, J.M. Adenosine receptor subtypes and vasodilatation in rat skeletal muscle during systemic hypoxia: A role for A1 receptors. J. Physiol. 1999, 514 (Pt 1), 151–162. [CrossRef] [PubMed]

134. Tabrizchi, R.; Bedi, S. Pharmacology of adenosine receptors in the vasculature. Pharmacol. Ther. 2001, 91, 133–147. [CrossRef]

135. Dubey, R.K.; Gillespie, D.G.; Osaka, K.; Suzuki, F.; Jackson, E.K. Adenosine inhibits growth of rat aortic smooth muscle cells. Possible role of A2B receptor. Hypertension 1996, 27, 786–793. [CrossRef] [PubMed]

136. Jackson, E.K.; Gillespie, D.G.; Dubey, R.K. 2′-AMP and 3′-AMP inhibit proliferation of preglomerular vascular smooth muscle cells and glomerular mesangial cells via A2B receptors. J. Pharmacol. Exp. Ther. 2011, 337, 445–450. [CrossRef] [PubMed]

137. Mayer, P.; Hinze, A.V.; Harst, A.; von Kugelgen, I. A(2)B receptors mediate the induction of early genes and inhibition of arterial smooth muscle cell proliferation via Epac. Cardiovasc. Res. 2011, 90, 148–156. [CrossRef] [PubMed]

138. St Hilaire, C.; Yang, D.; Schreiber, B.M.; Ravid, K. B-Myb regulates the A(2B) adenosine receptor in vascular smooth muscle cells. J. Cell. Biochem. 2008, 103, 1962–1974. [CrossRef] [PubMed]

139. Shen, J.; Halenda, S.P.; Sturek, M.; Wilden, P.A. Novel mitogenic effect of adenosine on coronary artery smooth muscle cells: Role for the A1 adenosine receptor. Circ. Res. 2005, 96, 982–990. [CrossRef] [PubMed]

140. Dubey, R.K.; Gillespie, D.G.; Shue, H.; Jackson, E.K. A(2B) receptors mediate antimitogenesis in vascular smooth muscle cells. Hypertension 2000, 35, 267–272. [CrossRef] [PubMed]

141. Ho, M.F.; Low, L.M.; Rose'Meyer, R.B. Pharmacology of the Adenosine A3 Receptor in the Vasculature and Essential Hypertension. PLoS ONE 2016, 11, e0150021. [CrossRef] [PubMed]

142. Wiklund, N.P.; Cedervist, B.; Gustafsson, L.E. Adenosine enhancement of adrenergic neuroeffector transmission in guinea-pig pulmonary artery. Br. J. Pharmacol. 1989, 96, 425–433. [CrossRef] [PubMed]

143. Dubey, R.K.; Gillespie, D.G.; Mi, Z.; Jackson, E.K. Adenosine inhibits growth of human aortic smooth muscle cells via A2B receptors. Hypertension 1998, 31, 516–521. [CrossRef] [PubMed]

144. Delikouras, A.; Fairbanks, L.D.; Simmonds, A.H.; Lechler, R.I.; Dorling, A. Endothelial cell cytoprotection induced in vitro by allo- or xenoreactive antibodies is mediated by signaling through adenosine A2 receptors. Eur. J. Immunol. 2003, 33, 3127–3135. [CrossRef] [PubMed]

145. Liu, J.; Tian, Z.; Gao, B.; Kunos, G. Dose-dependent activation of antiapoptotic and proapoptotic pathways by ethanol treatment in human vascular endothelial cells: Differential involvement of adenosine. J. Biol. Chem. 2002, 277, 20927–20933. [CrossRef] [PubMed]

146. Dawicki, D.D.; Chatterjee, D.; Wyche, J.; Rounds, S. Extracellular ATP and adenosine cause apoptosis of isolated working hearts and the role of endothelium and NO. J. Pharm. Pharmacol. 2002, 54, 859–867. [CrossRef] [PubMed]

147. Ray, C.J.; Marshall, J.M. The cellular mechanisms by which adenosine evokes release of nitric oxide from rat arterial endothelium. J. Physiol. 2006, 570 (Pt 1), 85–96. [CrossRef] [PubMed]

148. Maddock, H.L.; Broadley, K.J.; Bril, A.; Khandoudi, N. Effects of adenosine receptor agonists on guinea-pig isolated working hearts and the role of endothelium and NO. J. Pharm. Pharmacol. 2002, 54, 859–867. [CrossRef] [PubMed]

149. Wyatt, A.W.; Steinert, J.R.; Wheeler-Jones, C.P.; Morgan, A.J.; Sugden, D.; Pearson, J.D.; Sobrevia, L.; Mann, G.E. Early activation of the p42/p44MAPK pathway mediates adenosine-induced nitric oxide production in human endothelial cells: A novel calcium-insensitive mechanism. FASEB J. 2002, 16, 1584–1594. [CrossRef] [PubMed]

150. Bot, I.; de Vries, H.; Korporaal, S.J.; Foks, A.C.; Bot, M.; van Veldhoven, J.; Ter Borg, M.N.; van Santbrink, P.J.; van Berkel, T.J.; Kuiper, J.; Ijzerman, A.P. Adenosine A(2)B receptor agonism inhibits neointimal lesion development after arterial injury in apolipoprotein E-deficient mice. Arterioscler. Thromb. Vasc. Biol. 2012, 32, 2197–2205. [CrossRef] [PubMed]
152. Escudero, C.; Bertoglia, P.; Hernadez, M.; Celis, C.; Gonzalez, M.; Aguayo, C.; Acurio, J. Impaired A2A adenosine receptor/nitric oxide/VEGF signaling pathway in fetal endothelium during late- and early-onset preeclampsia. *Purinergic Signal.* 2013, 9, 215–226. [CrossRef] [PubMed]

153. Ansari, H.R.; Nadeem, A.; Tilley, S.L.; Mustafa, S.J. Involvement of COX-1 in A3 adenosine receptor-mediated contraction through endothelium in mice aorta. *Am. J. Physiol. Heart Circ. Physiol.* 2007, 293, H3448–H3455. [CrossRef] [PubMed]

154. Diniz, C.; Fresco, P.; Leal, S.; Goncalves, J. Adenosine receptors involved in modulation of noradrenaline release mediated by inhibitory A(1)-adenosine receptors in the mesenteric vein and artery. *Eur. J. Pharmacol.* 2011, 652, 5–9. [CrossRef] [PubMed]

155. Persson, P.; Hansell, P.; Palm, F. Adenosine A2 receptor-mediated regulation of renal hemodynamics and glomerular filtration rate is abolished in diabetes. *Adv. Exp. Med. Biol.* 2013, 765, 225–230. [PubMed]

156. Zhang, W.; Zhang, Y.; Wang, W.; Dai, Y.; Ning, C.; Luo, R.; Sun, K.; Glover, L.; Grenz, A.; Sun, H.; et al. Elevated ecto-5′-nucleotidase-mediated increased renal adenosine signaling via A2B adenosine receptor contributes to chronic hypertension. *Circ. Res.* 2013, 112, 1466–1478. [CrossRef] [PubMed]

157. Zhang, G.L.; Miyahara, H.; Suzuki, H. Inhibitory actions of adenosine differ between ear and mesenteric arteries in the rabbit. *Pflugers Arch.* 1989, 415, 56–62. [CrossRef] [PubMed]

158. Zhang, W.; Zhang, Y.; Wang, W.; Dai, Y.; Ning, C.; Luo, R.; Sun, K.; Glover, L.; Grenz, A.; Sun, H.; et al. Elevated ecto-5′-nucleotidase-mediated increased renal adenosine signaling via A2B adenosine receptor contributes to chronic hypertension. *Circ. Res.* 2013, 112, 1466–1478. [CrossRef] [PubMed]

159. Leal, S.; Sa, C.; Goncalves, J.; Fresco, P.; Diniz, C. Immunochemical characterization of adenosine receptors in rat aorta and tail arteries. *Microsc. Res. Tech.* 2008, 71, 703–709. [CrossRef] [PubMed]

160. Escudero, C.; Bertoglia, P.; Hernadez, M.; Celis, C.; Gonzalez, M.; Aguayo, C.; Acurio, J. Impaired A2A adenosine receptor/nitric oxide/VEGF signaling pathway in fetal endothelium during late- and early-onset preeclampsia. *Purinergic Signal.* 2013, 9, 215–226. [CrossRef] [PubMed]

161. Sousa, J.B.; Vieira-Rocha, M.S.; Arribas, S.M.; Gonzalez, M.C.; Fresco, P.; Diniz, C. Endothelial dysfunction impairs vascular neurotransmission in tail arteries. *PLOS ONE.* 2015, 10, e0129224. [CrossRef] [PubMed]

162. Persson, P.; Hansell, P.; Palm, F. Adenosine A2 receptor-mediated regulation of renal hemodynamics and glomerular filtration rate is abolished in diabetes. *Adv. Exp. Med. Biol.* 2013, 765, 225–230. [CrossRef] [PubMed]

163. Ansari, H.R.; Nadeem, A.; Tilley, S.L.; Mustafa, S.J. Involvement of COX-1 in A3 adenosine receptor-mediated contraction through endothelium in mice aorta. *Am. J. Physiol. Heart Circ. Physiol.* 2007, 293, H3448–H3455. [CrossRef] [PubMed]

164. Jackson, E.K.; Cheng, D.; Tofovic, S.P.; Mi, Z. Endogenous adenosine contributes to renal sympathetic neurotransmission via postjunctional A1 receptor-mediated coincident signaling. *Neurochem. Int.* 2012, 62, 399–405. [CrossRef] [PubMed]

165. Talaia, C.; Morato, M.; Quintas, C.; Goncalves, J.; Acurio, J. Impaired A2A adenosine receptor/nitric oxide/VEGF signaling pathway in fetal endothelium during late- and early-onset preeclampsia. *Purinergic Signal.* 2013, 9, 215–226. [CrossRef] [PubMed]

166. Rocha-Pereira, C.; Sousa, J.B.; Vieira-Rocha, M.S.; Fresco, P.; Goncalves, J.; Diniz, C. Differential inhibition of noradrenaline release mediated by inhibitory A(1)-adenosine receptors in the mesenteric vein and artery from normotensive and hypertensive rats. *Neurochem. Int.* 2013, 62, 399–405. [CrossRef] [PubMed]

167. Ansari, H.R.; Nadeem, A.; Tilley, S.L.; Mustafa, S.J. Involvement of COX-1 in A3 adenosine receptor-mediated contraction through endothelium in mice aorta. *Am. J. Physiol. Heart Circ. Physiol.* 2007, 293, H3448–H3455. [CrossRef] [PubMed]

168. Sousa, J.B.; Vieira-Rocha, M.S.; Sa, C.; Ferreirinha, F.; Correia-de-Sa, P.; Fresco, P.; Diniz, C. Lack of endogenous adenosine tonus on sympathetic neurotransmission in spontaneously hypertensive rat mesenteric artery. *PLOS ONE.* 2014, 9, e105540. [CrossRef] [PubMed]

169. Rocha-Pereira, C.; Arribas, S.M.; Fresco, P.; Gonzalez, M.C.; Goncalves, J.; Diniz, C. Impaired inhibitory function of presynaptic A1-adenosine receptors in SHR mesenteric arteries. *J. Pharmacol. Sci.* 2013, 112, 59–70. [CrossRef] [PubMed]

170. Carney, E.F. Chronic kidney disease: Renal adenosine in hypertensive CKD. *Nat. Rev. Nephrol.* 2013, 9, 309. [CrossRef] [PubMed]

171. Sakowicz, M.; Pawelczyk, T. Insulin restores expression of adenosine kinase in streptozotocin-induced diabetes mellitus rats. *Mol. Cell. Biochem.* 2002, 236, 163–171. [CrossRef] [PubMed]
172. Cardenas, A.; Toledo, C.; Oyarzun, C.; Sepulveda, A.; Quezada, C.; Guillen-Gomez, E.; Diaz-Encarnacion, M.M.; Pastor-Anglada, M.; San Martin, R. Adenosine A(2B) receptor-mediated VEGF induction promotes diabetic glomerulopathy. *Lab. Investig.* 2013, 93, 135–144. [CrossRef] [PubMed]

173. Figler, R.A.; Wang, G.; Srinivasan, S.; Jung, D.Y.; Zhang, Z.; Pankow, J.S.; Ravid, K.; Fredholm, B.; Hedrick, C.C.; Rich, S.S.; et al. Links between insulin resistance, adenosine A2B receptors, and inflammatory markers in mice and humans. *Diabetes* 2011, 60, 669–679. [CrossRef] [PubMed]

174. Fishman, P.; Bar-Yehuda, S. Pharmacology and therapeutic applications of A3 receptor subtype. *Curr. Top. Med. Chem.* 2003, 3, 463–469. [CrossRef] [PubMed]

175. Ohta, A.; Sitkovsky, M. Extracellular adenosine-mediated modulation of regulatory T cells. *Front. Immunol.* 2014, 5, 304. [CrossRef] [PubMed]

176. Duro, E.; Pallai, A.; Koroskenyi, K.; Sarang, Z.; Szondy, Z. Adenosine A3 receptors negatively regulate the engulfment-dependent apoptotic cell suppression of inflammation. *Immunol. Lett.* 2014, 162 (Pt B), 292–301. [CrossRef] [PubMed]

177. Thakur, S.; Du, J.; Hourani, S.; Ledent, C.; Li, J.M. Inactivation of adenosine A2A receptor attenuates basal and angiotensin II-induced ROS production by Nox2 in endothelial cells. *J. Biol. Chem.* 2010, 285, 40104–40113. [CrossRef] [PubMed]

178. Adair, T.H. Growth regulation of the vascular system: An emerging role for adenosine. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2005, 289, R283–R296. [CrossRef] [PubMed]

179. Ahmad, A.; Ahmad, S.; Glover, L.; Miller, S.M.; Shannon, J.M.; Guo, X.; Franklin, W.A.; Bridges, J.P.; Schaack, J.B.; Colgan, S.P.; White, C.W. Adenosine A2A receptor is a unique angiogenic target of HIF-2alpha in pulmonary endothelial cells. *Proc. Natl. Acad. Sci. USA* 2009, 106, 10684–10689. [CrossRef] [PubMed]

180. Carmeliet, P.; Jain, R.K. Molecular mechanisms and clinical applications of angiogenesis. *Nature* 2011, 473, 298–307. [CrossRef] [PubMed]

181. Adolfsson, J. The time dependence of training-induced increase in skeletal muscle capillarization and the spatial capillary to fibre relationship in normal and neovascularized skeletal muscle of rats. *Acta Physiol. Scand.* 1986, 128, 259–266. [CrossRef] [PubMed]

182. Shen, J.; Halenda, S.P.; Sturek, M.; Wilden, P.A. Cell-signaling evidence for adenosine stimulation of coronary smooth muscle proliferation via the A1 adenosine receptor. *Circ. Res.* 2005, 97, 574–582. [CrossRef] [PubMed]

183. Gessi, S.; Fogli, E.; Sacchetto, V.; Merighi, S.; Varani, K.; Preti, D.; Leung, E.; Maclennan, S.; Borea, P.A. Adenosine modulates HIF-1(alpha), VEGF, IL-8, and foam cell formation in a human model of hypoxic foam cells. *Arterioscler. Thromb. Vasc. Biol.* 2010, 30, 90–97. [CrossRef] [PubMed]

184. Auchampach, J.A. Adenosine receptors and angiogenesis. *Circ. Res.* 2007, 101, 1075–1077. [CrossRef] [PubMed]

185. Clark, A.N.; Youkey, R.; Liu, X.; Jia, L.; Blatt, R.; Day, Y.J.; Sullivan, G.W.; Linden, J.; Tucker, A.L. A1 adenosine receptor activation promotes angiogenesis and release of VEGF from monocytes. *Circ. Res.* 2007, 101, 1130–1138. [CrossRef] [PubMed]

186. Feoktistov, I.; Goldstein, A.E.; Ryzhov, S.; Zeng, D.; Belardinelli, L.; Voyno-Yasenetskaya, T.; Biaggioni, I. Differential expression of adenosine receptors in human endothelial cells: Role of A2B receptors in angiogenic factor regulation. *Circ. Res.* 2002, 90, 531–538. [CrossRef] [PubMed]

187. Desai, A.; Victor-Vega, C.; Gadangi, S.; Montesinos, M.C.; Chu, C.C.; Cronstein, B.N. Adenosine A2A receptor stimulation increases angiogenesis by down-regulating production of the antiangiogenic matrix protein thrombospondin 1. *Mol. Pharmacol.* 2005, 67, 1406–1413. [CrossRef] [PubMed]

188. Headrick, J.P.; Ashton, K.J.; Rose’meyer, R.B.; Peart, J.N. Cardiovascular adenosine receptors: Expression, actions and interactions. *Pharmacol. Ther.* 2013, 140, 92–111. [CrossRef] [PubMed]

189. Teng, B.; Ledent, C.; Mustafa, S.J. Up-regulation of A 2B adenosine receptor in A 2A adenosine receptor knockout mouse coronary artery. *J. Mol. Cell. Cardiol.* 2008, 44, 905–914. [CrossRef] [PubMed]

190. Ansari, H.R.; Teng, B.; Nadeem, A.; Roush, K.P.; Martin, K.H.; Schnermann, J.; Mustafa, S.J. A(1) adenosine receptor-mediated PKC and p42/p44 MAPK signaling in mouse coronary artery smooth muscle cells. *Am. J. Physiol. Heart Circ. Physiol.* 2009, 297, H1032–H1039. [CrossRef] [PubMed]

191. Hinze, A.V.; Mayer, P.; Harst, A.; von Kugelgen, I. Adenosine A(3) receptor-induced proliferation of primary human coronary smooth muscle cells involving the induction of early growth response genes. *J. Mol. Cell. Cardiol.* 2012, 53, 639–645. [CrossRef] [PubMed]
192. Qian, G.; Cao, J.; Chen, C.; Wang, L.; Huang, X.; Ding, C.; Cai, X.; Yin, F.; Chu, J.; Li, G.; Ye, J. Paeoniflorin inhibits pulmonary artery smooth muscle cells proliferation via upregulating A2B adenosine receptor in rat. *PLoS ONE* **2013**, *8*, e69141. [CrossRef] [PubMed]

193. Umapathy, S.N.; Kaczmarek, E.; Fatteh, N.; Burns, N.; Lucas, R.; Stenmark, K.R.; Verin, A.D.; Gerasimovskaya, E.V. Adenosine A1 receptors promote vasa vasorum endothelial cell barrier integrity via Gi and Akt-dependent actin cytoskeleton remodeling. *PLoS ONE* **2013**, *8*, e59733.

194. Alencar, A.K.; Pereira, S.L.; Montagnoli, T.L.; Maia, R.C.; Kummerer, A.E.; Landgraf, S.S.; Caruso-Neves, C.; Ferraz, E.B.; Tesch, R.; Nascimento, J.H.; et al. Beneficial effects of a novel agonist of the adenosine A2A receptor on monocrotaline-induced pulmonary hypertension in rats. *Br. J. Pharmacol.* **2013**, *169*, 953–962. [CrossRef] [PubMed]

195. Zhu, Y.; Liu, L.; Peng, X.; Ding, X.; Yang, G.; Li, T. Role of adenosine A2A receptor in organ-specific vascular reactivity following hemorrhagic shock in rats. *J. Surg. Res.* **2013**, *184*, 951–958. [CrossRef] [PubMed]

196. Booth, L.C.; Tummers, L.; Jensen, E.C.; Barrett, C.J.; Malpas, S.C.; Gunn, A.J.; Bennet, L. Differential effects of the adenosine A1 receptor agonist adenosine amine congener on renal, femoral and carotid vascular conductance in preterm fetal sheep. *Clin. Exp. Pharmacol. Physiol.* **2008**, *35*, 1316–1320. [CrossRef] [PubMed]

197. Hassanian, S.M.; Dinarvand, P.; Rezaie, A.R. Adenosine regulates the proinflammatory signaling function of thrombin in endothelial cells. *J. Cell. Physiol.* **2014**, *229*, 1292–1300. [CrossRef] [PubMed]

198. Acuñio, J.; Troncoso, F.; Bertoglia, P.; Salomon, C.; Aguayo, C.; Sobrevia, L.; Escudero, C. Potential role of A2B adenosine receptors on proliferation/migration of fetal endothelium derived from preeclamptic pregnancies. *Biomed. Res. Int.* **2014**, *2014*, 274507. [CrossRef] [PubMed]

199. Guzman-Gutierrez, E.; Westermeier, F.; Salomon, C.; Gonzalez, M.; Pardo, F.; Leiva, A.; Sobrevia, L. Insulin-increased L-arginine transport requires A(2A) adenosine receptors activation in human umbilical vein endothelium. *PLoS ONE* **2012**, *7*, e41705. [CrossRef] [PubMed]

200. Cass, C.E.; Young, J.D.; Baldwin, S.A. Recent advances in the molecular biology of nucleoside transporters of mammalian cells. *Biochem. Cell Biol.* **1998**, *76*, 761–770. [CrossRef] [PubMed]

201. King, K.M.; Damaraju, V.L.; Vickers, M.F.; Yao, S.Y.; Lang, T.; Tackaberry, T.E.; Mowles, D.A.; Ng, A.M.; Young, J.D.; Cass, C.E. A comparison of the transportability, and its role in cytotoxicity, of cladribine, and fludarabine by recombinant human nucleoside transporters produced in three model mammalian cells. *Mol. Pharmacol.* **2006**, *69*, 346–353. [CrossRef] [PubMed]

202. Chen, J.; Rinaldo, L.; Lim, S.J.; Young, H.; Messing, R.O.; Choi, D.S. The type 1 equilibrative nucleoside transporter regulates anxiety-like behavior in mice. *Genes Brain Behav.* **2007**, *6*, 776–783. [CrossRef] [PubMed]

203. Kost, S.; Sun, C.; Xiong, W.; Graham, K.; Cass, C.E.; Young, J.D.; Albensi, B.C.; Parkinson, F.E. Behavioral effects of elevated expression of human equilibrative nucleoside transporter 1 in mice. *Behav. Brain Res.* **2011**, *224*, 44–49. [CrossRef] [PubMed]

204. Chen, J.; Nam, H.W.; Lee, M.R.; Hinton, D.J.; Choi, S.; Kim, T.; Kawamura, T.; Janak, P.H.; Choi, D.S. Altered glutamatergic neurotransmission in the striatum regulates ethanol sensitivity and intake in mice lacking EN1. *Behav. Brain Res.* **2010**, *208*, 636–642. [CrossRef] [PubMed]

205. Nam, H.W.; Lee, M.R.; Zhu, Y.; Wu, J.; Hinton, D.J.; Choi, S.; Kim, T.; Hammack, N.; Yin, J.C.; Choi, D.S. Type 1 equilibrative nucleoside transporter regulates ethanol drinking through accumbal N-methyl-D-aspartate receptor signaling. *Biol. Psychiatry* **2011**, *69*, 1043–1051. [CrossRef] [PubMed]

206. Rose, J.B.; Naydenova, Z.; Bang, A.; Eguchi, M.; Sweeney, G.; Choi, D.S.; Hammond, J.R.; Coe, I.R. Equilibrative nucleoside transporter 1 plays an essential role in cardioprotection. *Am. J. Physiol. Heart Circ. Physiol.* **2010**, *298*, H771–H777. [CrossRef] [PubMed]

207. Abbracchio, M.P.; Burnstock, G.; Boeynaems, J.M.; Barnard, E.A.; Boyer, J.L.; Kennedy, C.; Knight, G.E.; Fumagalli, M.; Gachet, C.; Jacobson, K.A.; Weisman, G.A. International Union of Pharmacology LVIII: Update on the P2Y G protein-coupled nucleotide receptors: From molecular mechanisms and pathophysiology to therapy. *Pharmacol. Rev.* **2006**, *58*, 281–341. [CrossRef] [PubMed]

208. Burnstock, G. Purine and pyrimidine receptors. *Cell. Mol. Life Sci.* **2007**, *64*, 1471–1483. [CrossRef] [PubMed]

209. Tonazzini, I.; Trincavelli, M.L.; Storm-Mathisen, J.; Martini, C.; Bergersen, L.H. Co-localization and functional cross-talk between A1 and P2Y1 purine receptors in rat hippocampus. *Eur. J. Neurosci.* **2007**, *26*, 890–902. [CrossRef] [PubMed]
210. Gao, Z.; Chen, T.; Weber, M.J.; Linden, J. A2B adenosine and P2Y2 receptors stimulate mitogen-activated protein kinase in human embryonic kidney-293 cells. cross-talk between cyclic AMP and protein kinase c pathways. *J. Biol. Chem.* **1999**, *274*, 5972–5980. [CrossRef] [PubMed]

211. Morales, B.; Barrera, N.; Uribe, P.; Mora, C.; Villalon, M. Functional cross talk after activation of P2 and P1 receptors in oviductal ciliated cells. *Am. J. Physiol. Cell Physiol.* **2000**, *279*, C658–C669. [PubMed]

212. Shum, W.W.; Ruan, Y.C.; Da Silva, N.; Breton, S. Establishment of cell-cell cross talk in the epididymis: Control of luminal acidification. *J. Androl.* **2011**, *32*, 576–586. [CrossRef] [PubMed]

213. Amadio, S.; Apolloni, S.; D’Ambrosi, N.; Volonte, C. Purinergic signalling at the plasma membrane: A multipurpose and multidirectional mode to deal with amyotrophic lateral sclerosis and multiple sclerosis. *J. Neurochem.* **2011**, *116*, 796–805. [CrossRef] [PubMed]

214. Burnstock, G. Purinergic signaling and vascular cell proliferation and death. *Arterioscler. Thromb. Vasc. Biol.* **2002**, *22*, 364–373. [CrossRef] [PubMed]

215. Zheng, L.M.; Zychlinsky, A.; Liu, C.C.; Ojcius, D.M.; Young, J.D. Extracellular ATP as a trigger for apoptosis or programmed cell death. *J. Cell Biol.* **1991**, *112*, 279–288. [CrossRef] [PubMed]

216. Jacobson, K.A.; Hoffmann, C.; Cattabeni, F.; Abbracchio, M.P. Adenosine-induced cell death: Evidence for receptor-mediated signalling. *Apoptosis* **1999**, *4*, 197–211. [CrossRef] [PubMed]