U.PORTO

MESTRADO INTEGRADO EM MEDICINA

Pharmacological tuning of adenosine signal nuances underlying heart failure with preserved ejection fraction

Alexandrina Campos Martins

M

2021

Pharmacological tuning of adenosine signal nuances underlying heart failure with preserved ejection fraction

Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto

Mestrado Integrado em Medicina

Junho de 2021

Autora:

Alexandrina Campos Martins

Sexto ano do Mestrado Integrado em Medicina no Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto (ICBAS-UP), Porto, Portugal

Endereço eletrónico: up201504506@icbas.up.pt

Orientadora:

Prof. Doutora Ana Patrícia Nunes Fontes de Sousa

Prof. Auxiliar, DVM, PhD, Laboratório de Farmacologia e Neurobiologia, Centro de investigação Farmacológica e Inovação Medicamentosa (MedInUP), Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto (ICBAS-UP), Porto, Portugal

Coorientador:

Professor Doutor Paulo Jorge da Silva Correia de Sá

Prof. Catedrático, Título de Agregado, Laboratório de Farmacologia e Neurobiologia, Centro de investigação Farmacológica e Inovação Medicamentosa (MedInUP), Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto (ICBAS-UP), Porto, Portugal

Pharmacological tuning of adenosine signal nuances underlying heart failure with preserved ejection fraction

Porto, junho de 2021

Autor: Alexandrina Campos Martins

Orientadora: Ana Patrícia Fonseca dos Santos

Coorientador: Assinado por : **Paulo Jorge da Silva Correia de Sá**
Num. de Identificação: 07980455
Data: 2021.06.03 16:19:14+01'00'

À minha tia

Acknowledgements | Agradecimentos

Uma primeira palavra de profunda gratidão aos meus orientadores, Prof. Doutora Ana Patrícia Fontes de Sousa e Professor Doutor Paulo Correia de Sá. O rigor e espírito de crítica que me foram incutidos, desde o primeiro momento, foram a base para a realização deste trabalho. Sem dúvida que estes são valores que pretendo espelhar em futuros desafios. Uma palavra de agradecimento ao Mestre Bruno Bragança, pelo apoio constante à realização deste projeto. Não posso deixar de agradecer ao Laboratório de Farmacologia e Neurobiologia, do Instituto de Ciências Biomédicas Abel Salazar, pela forma como me integrou e proporcionou o meu crescimento a nível científico e pessoal. A todos, o meu sincero agradecimento!

Aos meus pais e à minha irmã, obrigada por estarem sempre presentes, nesta tão desafiante e bonita caminhada. São a minha força e inspiração! À minha querida família, muito obrigada por tudo.

Aos meus amigos, agradeço-vos do fundo do coração toda a amizade, apoio e companheirismo. Recordo com carinho todos os momentos partilhados e laços construídos!

Esta dissertação contou com o apoio da Fundação para a Ciência e Tecnologia (FCT) através do fundo número UIDB/04308/2020.

This work was supported by Portuguese Foundation for Science and Technology (FCT) through Grant numbers UIDB/04308/2020.

As figuras 1, 2 e 3 usam modelos da Servier Medical Art.

Figures 1, 2 and 3 use templates from Servier Medical Art.

Anseio por cumprir uma grande e nobre tarefa,

mas o meu principal dever é cumprir pequenas tarefas

como se fossem grandes e nobres.

Helen Keller

Highlights

- The 'systemic microvascular paradigm' has been proposed to explain the pathophysiological complexities of heterogeneous heart failure with preserved ejection fraction.
- Extracellular adenosine levels dramatically increase following cardiovascular stress and pathological insults.
- The nucleoside acts as a 'retaliatory metabolite' through the activation of P1 receptors, which are abundantly expressed in the cardiovascular system.
- Re-admission of adenosine actions may revert countless cardiovascular events, namely the often-neglected heart failure with preserved ejection fraction.

Resumo

A Insuficiência cardíaca com fração de ejeção preservada (ICpFE) representa, aproximadamente, metade dos casos de insuficiência cardíaca nos países desenvolvidos. O paradigma da inflamação microvascular foi proposto no sentido de explicar a heterogeneidade associada à apresentação desta síndrome. A inexistência de terapêuticas eficazes, aliada à falta de tratamentos modificadores de prognóstico, fazem da ICpFE, na atualidade, um dos principais desafios clínicos. Os níveis endógenos do nucleósido purinérgico, adenosina, aumentam, de forma significativa, em resposta a eventos cardiovasculares. Através da ativação dos recetores P1, a adenosina exerce efeitos cardioprotetores, neuromoduladores e imunossupressores. As suas ações benéficas têm sido demonstradas em estudos pré-clínicos. Neste sentido, o presente trabalho providencia uma revisão crítica, abrangente e atual das principais vantagens em manipular as vias de sinalização ativadas pela adenosina na ICpFE, sem descurar os seus efeitos laterais e a forma como estes podem ser minimizados.

Abstract

Heart failure with preserved ejection fraction (HFpEF) roughly represents half of cardiac failure events in developed countries. The proposed 'systemic microvascular paradigm' has been used to explain HFpHF presentation heterogeneity. The lack of effective treatments with few evidence-based therapeutic recommendations makes HFpEF one of the greatest unmet clinical necessities worldwide. The endogenous levels of the purine nucleoside, adenosine, increase significantly following cardiovascular events. Adenosine exerts cardioprotective, neuromodulatory and immunosuppressive effects through the activation of plasma membrane-bound P1 receptors abundantly expressed in the cardiovascular system. Its proven benefits have been demonstrated in preclinical animal tests. Here, we provide a comprehensive and up-to-date critical review about the main therapeutic advantages of tuning adenosine signalling pathways in HFpEF, without discounting their side effects and how these can be seized.

Metodologia

A base de dados MEDLINE-Pubmed foi a ferramenta utilizada para a seleção das referências bibliográficas. Na sua maioria, a bibliografia é constituída por artigos originais. Alguns artigos de revisão foram incluídos por compilarem informações necessárias à compreensão do tema ou por formularem hipóteses originais em relação à função da adenosina no sistema cardiovascular. Os artigos 9, 30 e 75 continham figuras que serviram de base à realização das presentes ilustrações, no sentido de sintetizar e elucidar a informação apresentada. Quanto às tabelas I e II, estas compilam informação relevante acerca do papel da adenosina no sistema cardiopulmonar e comorbilidades metabólicas associadas, respetivamente. A tabela III resume o papel benéfico e deletério da ativação dos recetores da adenosina, assim como das enzimas e vias de sinalização intracelular envolvidas no seu metabolismo, na insuficiência cardíaca com fração de ejeção preservada. As figuras 1, 2 e 3 foram elaboradas com recurso ao banco de imagens online Servier Medical Art (http://smart.servier.com).

No motor de busca foram introduzidas as seguintes palavras-chave, isoladas ou em combinação: *adenosine, adenosine receptor, heart failure*, *preserved ejection fraction*, *endothelial dysfunction*, *cardiac fibrosis*, *cardiac hypertrophy* e *cardiac comorbidities*. Todos os artigos considerados relevantes foram incluídos para a realização da revisão bibliográfica proposta. A pesquisa bibliográfica decorreu entre os meses de Agosto de 2020 e Abril de 2021. Os artigos selecionados, escritos no idioma inglês, foram publicados entre 1929 e 2021.

A organização do trabalho respeita a seguinte estrutura: *highlights* (conjunto de frases com o propósito de sintetizar os pontos-chave do artigo), *abstract* (texto que intenta captar a atenção e demonstrar a relevância do tema), introdução, desenvolvimento e conclusão. Pretendeu-se abordar, de forma pragmática, mas com a devida fundamentação, os avanços recentes sobre a adenosina na área das Ciências Cardiovasculares, com particular enfoque na síndrome clínica da insuficiência cardíaca com fração de ejeção preservada.

Abbreviation list | Lista de abreviaturas

AC – Adenylate cyclase

ADA – Adenosine deaminase

ADK – Adenosine kinase

ADP – Adenosine 5'-diphosphate

Akt – Protein kinase B

AMP – Adenosine 5'-monophosphate

Ang II – Angiotensin II

AR – Adenosine receptor

ATP – Adenosine 5'-triphosphate

Ca^{2+} – Calcium

CADO – 2-chloroadenosine

cAMP – Cyclic AMP

CAP – Capadesonon

CCPA – 2-cholor-N6-cyclopentyladenosine

CKD – Chronic kidney disease

CMD – Coronary microvascular dysfunction

CNT – Concentrative nucleoside transport

COPD – Chronic pulmonary obstructive disease

CPA – N6-cyclopentyladenosine

DAG – Diacylglycerol

DM – Diabetes *mellitus*

DOCA – Deoxycorticosterone acetate

E-NPP – Ecto-nucleotide pyrophosphatases/phosphodiesterases

ENT – Equilibrative nucleoside transporter

E-NTPDase – Ecto-nucleoside triphosphate diphosphohydrolase

Epac – Exchange factor directly activated by cAMP

Erk – Extracellular signal-regulated kinase

GLUT – Glucose transporter

GSK-3β – Glycogen syntheses kinase 3 beta

HF – Heart failure

HFD – High fat diet

HFpEF – Heart failure with preserved ejection fraction

HSu – High-sucrose

IFN-γ – Interferon-gamma

IL – Interleukin

I/R – Ischemia/ reperfusion

K^+ – Potassium

KO – Knockout

LHD – Left heart disease

LV – Left ventricle

MAPK – Mitogen-activated protein kinase

MCT – Monocrotaline

MI – Myocardial infarction

MIP – Macrophage inflammatory protein

mTOR – Mechanistic target of rapamycin

NO – Nitric oxide

NOS – Nitric oxide synthase

PAH – Pulmonary arterial hypertension

PH – Pulmonary hypertension

PI3K – Phosphatidylinositol 3-kinase

PKA – Protein kinase A

PKC – Protein kinase C

PLC-β – Phospholipase C beta

ROS – Reactive oxidative species

SAH – S-adenosylhomocysteine

SERCA2a – sarco/endoplasmic reticulum Ca^{2+}-ATPase 2a

SGLT2i – Sodium-glucose cotransporter-2 inhibitor

SHR – Spontaneously hypertensive rat

STZ – Streptozotocin

TAC – Transaortic constriction

TGF- β – Transforming growth factor beta

TNF-α – Tumour necrosis factor- alpha

VCAM – Vascular cell adhesion molecule

VSMC – Vascular smooth muscle cell

Index | Índice

Table list | Lista de tabelas

Figure list | Lista de figuras

1. Introduction

Heart failure (HF) is a clinical syndrome characterized by alterations in the cardiac structure and/or function; it is associated with a poor quality of life, high rates of hospitalizations and significant mortality[1]. HF with preserved ejection fraction (HFpEF) is a subclass of HF defined by left ventricular ejection fraction equal or above 50%[1]. Nowadays, it represents up to half of all cases of HF in the developed world, which at least in part may be explained by the actual lifestyle and/or prolongation of the life expectancy[2]. HFpEF has a 'heterogeneous' presentation often coursing with unspecific clinical symptoms (e.g. exercise intolerance and dyspnoea) mostly because this clinical condition is associated with a wide range of age-related cardiac and non-cardiac abnormalities; these features challenge its diagnosis and clinical management[1]. Notwithstanding the pathophysiological complexities related to HFpEF, associated systemic and cardiac comorbidities drive most of the molecular and haemodynamic mechanisms implicated in this disease, with emerging data supporting systemic microvascular inflammation as an enduring aggravation factor[3]. These findings contrast with HF with reduced ejection fraction (left ventricular ejection fraction under 40%), where cardiomyocytes loss and their replacement by fibrotic tissue is the most frequent pathological hallmark[4]. In this case, the systolic dysfunction results in neurohormonal activation of renin-angiotensin-aldosterone system along with sympathetic stimulation, giving the basis for using angiotensin converting enzyme inhibitors, mineralocorticoid receptors antagonists and β-blockers as disease-modifying drugs[1]. Conversely, there is still no proven therapy to impact the course of HFpEF, thus representing one of the biggest current challenges of cardiovascular medicine[5]. Thus, unravelling the dysfunctional signalling pathways associated with HFpEF may shed some light to propose novel pharmacological to treat this unmet clinical condition.

The purine nucleoside, adenosine, was first identified in 1929 when Drury and Szent-Gyorgyi successfully extracted a rhythm-influencing adenylic substance from the mammalian heart and other tissues[6]. Only in 1972, Geoff Burnstock (born: 10 May 1929, died: 2 June 2020) coined the term purinergic signalling referring to the extracellular effects of adenosine 5'-triphosphate (ATP). Following the Burnstock's pioneering work on the role of ATP-sensitive P2 purinoceptors, also its metabolite, adenosine, has been recognized as an extracellular signalling molecule through the activation of plasma membrane-bound P1 receptors family expressed in all organs in the body. In the cardiovascular system, adenosine is considered a 'retaliatory metabolite' because it originates from the catabolism of ATP in stressed cells and normally decreases cellular energy consumption, while increasing the blood tissue supply[7]. In view of this, adenosine-mediated signals have been implicated in many pathophysiological processes. Nowadays, adenosine receptor ligands are being extensively investigated as promising

druggable compounds, some of which are undergoing clinical trials [8]. However, besides the native nucleoside, only a limited number of adenosine-related drugs are approved for clinical use[8].

Current knowledge points towards adenosine as a ubiquitous signalling mediator of countless physiological processes throughout the body via the activation of four G protein-coupled receptors known as P1 receptors [adenosine receptors (ARs): A_1AR, A_{2A}AR, A_{2B}AR, A_3AR][9]. The A_1AR and A_3AR were first characterized as being negatively coupled to adenylate cyclase (AC) through G_i and G_o proteins binding, which normally results in decreased intracellular cyclic AMP (cAMP) levels. Contrariwise, both high affinity A_{2A}R and low affinity A_{2B}AR couple to G_s proteins and stimulate AC leading to intracellular cAMP accumulation. Despite this too simplistic view of the canonical coupling of P1 receptors to the AC/cAMP pathway, evidence has been gathered demonstrating that A_1AR, A_{2B}AR and A_3AR are also able to activate the phospholipase C beta (PLC-β) isoform, resulting in increases in inositol 1,4,5-trisphosphate and intracellular calcium (Ca^{2+}) mobilization; both intracellular Ca^{2+} recruitment and diacylglycerol (DAG) production synergize to stimulate Ca^{2+}-dependent protein kinase C (PKC) and/or other Ca^{2+}-associated downstream pathways (Figure 1)[9]. Interestingly, the cardiovascular (and many other) effects of adenosine are mediated by a multiplicity of more recently discovered intracellular signalling cascades, like exchange protein directly activated by cAMP (EPAC), phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt), mitogen activated protein kinase (MAPK)/extracellular signal-regulated kinases (Erk), glycogen syntheses kinase 3 beta (GSK-3β), that only marginally intersect with the canonical G-protein coupled modulation of AC and/or PLC (see Section 3.b. and Figure 2). Due to their relevance to the current knowledge, involvement of these pathways to adenosine-mediated effects will be further discussed in the following sections.

2. Generation, transport, and metabolism of adenosine

Adenosine is a purine nucleoside continuously generated from the catabolism of adenine nucleotides via a cascade of nucleotidases, including the rate limiting enzyme 5´-nucleotidase, which dephosphorylates 5'-adenosine monophosphate (AMP) to adenosine both intra- and extracellularly (Figure 1)[10]. Regarding the fate of intracellular adenosine, it may (preferentially) re-enter the purines savage pathway by intracellular phosphorylation via adenosine kinase (ADK) (K_m= 2 μM) or may be, alternatively, inactivated to inosine by adenosine deaminase (ADA) (K_m= 17–45 μM) when the phosphorylation pathway is overloaded or inhibited[9]. Another possible source of adenosine results from the hydrolysis of S-

adenosylhomocysteine (SAH) by SAH hydrolase, being this the main pathway responsible for the intracellular levels of adenosine in conditions of adequate oxygen supply[11]. Under low oxygen supply conditions and/or during high energy working loads, the ATP hydrolysis increases resulting in intracellular adenosine accumulation, which drives its diffusion to the extracellular medium via equilibrative nucleoside transporters (ENTs), mostly ENT1 and ENT2 encoded by SLC29A1 and SLC29A2 genes, respectively[12,13]. NBTI-sensitive (ENT1) and insensitive (ENT2) transporters are expressed in the vascular endothelium, erythrocytes, inflammatory cells, and cardiomyocytes[14,15]. The sensitivity of both transporters to dipyridamole, allowed this drug to be introduced in the market as coronary vasodilator more than half a century ago, which is still used nowadays as antithrombotic and vasodilator with promising anti-oxidant properties[16]. Transport of adenosine across the plasma membrane can also occur through concentrative nucleoside transporters (CNTs) encoded by SLC28A1, SLC28A2 and SLC28A3 genes; these proteins concentrate adenosine (and other purines and pyrimidines) inside the cells via a Na^+-nucleoside cotransporter[17]. While ENTs may have a major role in maintaining nucleoside homeostasis, CNTs may contribute to adenosine sensing and signal transduction inside the cells (transceptor function).

As aforementioned, extracellular adenosine may result from the release of the nucleoside as such via ENTs and/or through the extracellular breakdown of released adenine nucleotides, namely ATP, 5'adenosine diphosphate (ADP) and AMP, by a cascade of ecto-nucleotidases bound to the plasma membrane. Besides originating from damaged cells, adenine nucleotides may be released from intact cells by vesicular exocytosis, as well as via other mechanisms involving plasma membrane "pores", namely ionotropic P2 purinoceptors, ABC proteins and hemichannels containing connexins and/or pannexins[18].

Four members of the ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase) family, namely, NTPDase1, NTPDase2, NTPDase3, and NTPDase8 and two members of the ecto-nucleotide pyrophosphatases/phosphodiesterases (E-NPP) family, NPP1 and NPP3, are located at the plasma membrane and hydrolyse extracellular nucleotides[19]. NTPDase1 (CD39 or apyrase) dephosphorylates ATP directly into AMP, with minimal accumulation of ADP. NTPDase2 (ATPase) is a preferential nucleoside triphosphatase that hydrolysis ADP 10 to 15 times less efficiently than ATP, leading to minimal AMP accumulation. NTPDase3 and NTPDase8 are functional intermediates between NTPDase1 and NTPDase2. In fact, NTPDases are considered a potential therapeutic target due to their role in coagulation, immune responses, vascular inflammation, and cancer[20]. Interestingly, NPP1 and NPP3 phosphorylated product (e.g. AMP) has a higher binding affinity to this enzyme than substrates do, leading to the inhibition of nucleoside 5'-monophosphate release[20]. The rate limiting enzyme of the ecto-nucleotidase

cascade is ecto-5'-nucleotidase/CD73, which dephosphorylates AMP to adenosine and inorganic phosphate. This enzyme is abundantly expressed in immune (e.g. T and B lymphocytes) and mesenchymal originated cells[21].

Extracellular adenosine levels are tightly regulated by cellular uptake via ENTs and/or by deamination into inosine by ADA, which is found on the cell surface (ecto-ADA) or as a soluble form after cleavage of its anchor to the plasma membrane (exo-ADA). Low levels of adenosine are normally found in biological fluids, but the extracellular concentration of the nucleoside may substantially increase in stressed cells following ischemia/reperfusion, inflammation, neuronal activation, and tissue damage[22-24]. Adenosine in the plasma has a short half-life mainly because of its rapid inactivation both by blood cells and the vascular endothelium[25]. Notwithstanding this, high adenosine levels have been detected in the plasma of patients with heart failure, both humans[26,27] and animals[28]. Moreover, the ecto-5'-nucleotidase/CD73 activity increases in the plasma and ventricular myocardium of chronic HF patients[29]. Together, plasma adenosine and ecto-5'-nucleotidase/CD73 activity may be reliable markers for the diagnosis of severity grade and follow-up of HF, even though adenosine levels are normally underestimated due to its extensive inactivation in the blood stream.

3. Adenosine in the cardiovascular system

a. Distribution of adenosine receptors in the cardiovascular system

The A_1AR is the most expressed adenosine receptor subtype found in the intact myocardium and in isolated cardiomyocytes, being particularly abundant in atria. The second most abundant receptor in the heart is the A_{2A}AR, followed by A_{2B}AR and A_3AR, which exhibit low expression levels in the heart[30]. Nevertheless, one should remember that this pattern is mostly influenced by receptors expressed in cardiomyocytes, which occupy up to 85% of the mammalian heart volume[31]. Looking more deeply into the distribution of adenosine receptor in cardiac cells, this scenario may change with A_{2B}AR and A_{2A}AR being more abundant in endothelial cells and cardiac fibroblasts[30]. Other tissues and organs influencing the cardiac function are also enriched in adenosine receptors subtypes. Large amounts of A_1AR are found in kidneys, adipose tissue, pancreas, and brain. The A_{2A}AR is highly expressed in peripheral immune cells (particularly in leucocytes), platelets, smooth muscle fibres and endothelial cells[30].

The activity of the low affinity A_{2B}AR has been physiologically neglected mostly because higher amounts of adenosine are required to activate this receptor even in cells where its expression is high, like fibroblasts, smooth muscle fibres, endothelial cells, immunocytes, and

platelets[32] . However, this assumption is changing given to the fact that evidence showing A_{2B}AR overfunctioning has been gathered in several pathological conditions, including hypoxia, inflammation, and cell stress, thereby providing support for a meaningful role of this receptor in health and disease[33]. Considering the retaliatory nature of adenosine, the A_{2B}AR can be seen as a 'dormant' receptor that 'wakes up' because of cells and tissue injury[33]. The presence of the A_3AR in cardiomyocytes, vascular smooth muscle cells (VSMCs) and immune cells have been implicated in its cardioprotective role against ischemia, as well as in the control of blood vessels tone and remodelling[30].

b. From cardiovascular effects of adenosine to cardioprotection

In the cardiovascular system, adenosine exerts additional protective effects to control neuronal output and inflammation, while decreasing cardiac metabolism, myocardial contractility, impulse generation and conduction, and the coronary tone, as well as adrenergic responsiveness and blood pressure (Figure 2)[30].

Stimulation of A_1AR induces negative chronotropism and dromotropism, as this receptor is highly expressed in the heart conduction system (sinoatrial and atrioventricular nodes, and the His-Purkinje network)[30]. In this regard, while adenosine exerts direct inhibitory effects on chronotropism and dromotropism in atrial cardiomyocytes, it also counteracts β-adrenergic effects on impulse generation and contractility. The A_1AR-induced hyperpolarization of the supraventricular tissue is accomplished by favouring outward potassium (K^+) currents through opening G protein-coupled inwardly rectifying K^+ channels (GIRK or KIR3.1/3.4), while counteracting adrenergic effects through inhibition of Ca^{2+} influx and/or attenuation of hyperpolarization-activated cyclic nucleotide-gated channel 4 mediating the pacemaker "funny" (I_f) currents[34,35]. These effects lead to bradycardia and atrial hypocontractility. In this respect, our group demonstrated that adenosine-induced negative atrial inotropism may be partially counteracted by multiple downstream intracellular pathways ending up to increase the time available for Ca^{2+} influx through Ca_v1 (L-type) channels[36]. Taken together, the electrophysiological properties of the A_1AR justify the use of adenosine in the treatment of supraventricular tachycardia and to control the rate of ventricular contractions during atrial fibrillation[37,38].

Adenosine counteracts the sympathetic drive operated by catecholamines on cardiac β-adrenoceptors by decreasing impulse generation and contractility via a mechanism involving both pre- and post-junctional effects. The positive inotropic action of β-adrenoceptors may be directly controlled by adenosine via inhibition of cAMP production and protein kinase A (PKA)

activation[39,40]. The A_1AR-mediated cardioprotection extends to intracellular organelles, namely mitochondria through a PKC-mediated reduction of the permeability of mitochondrial transition pores, stabilization of mitochondrial membrane potential and inhibition of hypoxia-induced production of reactive oxygen species (ROS), which ends up in the opening of K_{ATP} mitochondrial channels and cytoprotection[41]. Activation of A_1AR also increases epidermal growth factor receptor activation through the action of myocardial survival kinases Erk 1/2, Aκt[42]. Overall, these properties confer myocardial protection under ischemic pre-conditioning and during ischemia/reperfusion pathological conditions[43-45].

While adenosine cardiac actions are mostly focused on activation of the most abundant A_1AR, increasing data suggest the involvement of the A_{2A}AR subtype on myocardial contractile performance of the nucleoside[46] via PKA-dependent increases in intracellular Ca^{2+} [47]. However, activation of A_{2A}AR counteracts the antiadrenergic effects of the A_1AR, which is attributable to the formation of A_1AR:A_{2A}AR heteromers leading to a predominance of a A_{2A}AR pharmacology[48]. Both A_{2A}AR[49,50] and A_{2B}AR[51] have vasodilatory effects mediated by cAMP generation and PKA activation in VSMCs[50]. Involvement of K_v and K_{ATP} channels[52], nitric oxide (NO)[53,54] and prostanoids[55] production, have also been demonstrated. Coupling of A_{2A}AR and A_{2B}AR to such downstream signalling pathways may entitle adenosine to regulate coronary blood flow and to protect against chronic ischemia secondary to endothelial dysfunction. Long term stimulation of these receptors also fosters mitogenic signalling pathways leading to vascular overgrowth and angiogenesis; these pathways include A_{2A}AR and A_{2B}AR mediated-PI3K/Aκt activation and MAPK/ Erk activation by A_{2A}AR[56,57].

Mounting evidence has been gathered suggesting that A_{2A}AR activation is cardioprotective and immunosuppressant during early reperfusion after myocardial infarction (MI). The selective A_{2A}AR agonist, CGS 21680, decreased neutrophils adhesion to the endothelium and, subsequent, myocardial infiltration, together with a reduction of superoxide production, in a canine model of ischemia and reperfusion[58]. Moreover, other A_{2A}AR agonist, ATL146e, attenuated resident mast cells degranulation in a mouse model of myocardial ischemia[59]. Cardioprotection is also accomplished because activation of A_{2A}AR prevents the infarct-boosting effect of interferon-gamma (IFN-γ) produced by CD4+ T cells, as this cytokine favours reperfusion injury by activating pro-inflammatory macrophages[60]. Although preconditioning is hardly feasible in clinical settings, clinical trials are ongoing to test whether A_{2A}AR agonists can be used in coronary artery disease and MI[8]. In this context, it is worth noting that both adenosine and the selective A_{2A}AR agonist, regadenoson, have been clinically approved for myocardial perfusion imaging[61].

Current knowledge suggest that the A_{2B}AR is the only adenosine receptor subtype which levels are upregulated in ischemic hearts of both mice and humans[62]. Activation of the A_{2B}AR controls Period 2 protein, a metabolic master-switch of myocardial adaptation to ischemia, consequently stabilizing the hypoxia-inducible factor 1α and, thereby, the transduction of glycolytic enzymes[63] to provide optimization of oxygen consumption and to protect the myocardium from infarction reperfusion[62]. Moreover, both A_{2B}AR and A_{2A}AR are associated to inhibition of mitochondrial GSK-3β phosphorylation, thus preventing the mitochondrial permeability transition pore opening, which is critical step to afford cardioprotection during myocardial reperfusion[64]. Inhibition of ROS production by mitochondria broadens the cardioprotective effects of the A_{2B}AR in cardiac ischemia by involving multiple PKC-mediated signalling pathways affecting nitric oxide synthase (NOS), PI3K/Aκt and Erk 1/2 enzymatic activities[65-67].

Only a few studies have been conducted to elucidate the role of the A_3AR in cardiac pathophysiology, most probably because this receptor subtype has low expression levels in cardiac tissues. This situation may also occur, because the A_3AR has unpredictable effects in rodent models as it behaves like a low affinity receptor for adenosine contrary to the high affinity for the nucleoside in human tissues[30]. Current data suggest that the A_3AR limits injury processes occurring in the ischemic myocardium, while exerting an anti-inflammatory action during cardiac reperfusion[68]. Surprisingly, A_3AR agonists cause a biphasic haemodynamic response that is characterized, initially, by indirect activation of high affinity A_{2A}AR followed by subsequent A_3AR-mediated effects, which prevail after A_{2A}AR become desensitized[69]. Both in cardiomyocytes and in intact hearts of rats subjected to ischemia and reperfusion, A_3AR agonists reduced the infarct size by upregulating pro-survival signalling pathways, such as mitogen-activated protein kinase kinase 1/2- Erk 1/2 and PI3K/Aκt, which are known to decrease the activity of caspase-3 used as a biomarker of cellular apoptosis[70]. The cardioprotection associated with the A_3AR activation may also be due to the induction of human coronary smooth muscle cells proliferation via a mechanism involving PLC activation and downstream transcriptional factors activation, like the early growth response element 2/3[71].

4. Impact of adenosine in the molecular pathways underlying HFpEF

a. Systemic and cardiac inflammatory breakthrough to cardiac fibrosis

HFpEF is a complex clinical syndrome, which is characterized by a systemic inflammation background that is evidenced by increased levels of circulating pro-inflammatory markers, such

as interleukin-6 (IL-6) and tumour necrosis-alpha (TNF-α)[72]. Paulus et al. hypothesized the existence of a 'systemic microvascular paradigm' regarding HFpEF in which comorbidity-induced systemic inflammation predisposes and perpetuates systemic microvascular inflammation (Figure 3)[3]. The most important comorbidities associated with this systemic inflammatory state are diabetes mellitus (DM), overweight/obesity, hypertension, chronic obstructive pulmonary disease (COPD) and chronic kidney disease (CKD)[3]. Thus, the paradigm of this disease has been shifted from the traditional 'overload model' to the revolutionary 'microvascular hypothesis', where patients are more easily identified by elevated body mass index rather than elevated blood arterial pressure[73].

It, thus, can be argued that systemic and cardiac comorbidities predispose to cardiac inflammation. Indeed, left ventricular endomyocardial biopsies from patients with HFpEF show increases in the expression of biomarkers of inflammation and fibrosis, such as vascular cell adhesion molecule 1 (VCAM-1), CD3, CD11, and CD45-positive myocardial leucocytes, transforming growth factor-beta (TGF-β), types I and III collagen species and extracellular matrix deposition[74]. Cardiac inflammation leads to several molecular mechanisms involved in HFpEF, such as endothelial dysfunction, fibrosis, concentric hypertrophy and cardiometabolic functional abnormalities[75]. These alterations lead to left ventricular diastolic dysfunction, a key defining feature of HFpEF[74], which ultimately may cause unspecified clinical manifestations related to abnormal haemodynamic (e.g. dyspnoea and fatigue) in association with cardiovascular structural and function worsening[75].

The pleiotropic actions of the purine nucleoside, adenosine, in several organic systems put this retaliatory modulator in good position to participate in most (if not all) the pathophysiological mechanisms associated with HFpEF, which include systemic microvascular inflammation and related multi-organ comorbidities, in parallel to the nucleoside effects on cardiovascular function and myocardial remodelling. Adenosine regulates the activity of the immune system via self-contained signalling pathways aimed at promoting tissues integrity by resolving inflammatory insults (Figure 3), although a pro-inflammatory activity has also been described (reviewed in [76,77]).

Activation of the A_{2A}AR is paramount to the anti-inflammatory effect of adenosine; the coupling of the A_{2A}AR to the canonical AC/cAMP/PKA pathway is downstream associated to decreases of nuclear factor-kappa B signalling and inhibition of the release of pro-inflammatory cytokines[78]. Adenosine signalling in neutrophils either stimulates the expression of adhesion molecules via A_1AR or decreases adhesion of these cells to the vascular endothelium, as well as the production of superoxide radicals by A_{2A}AR, A_{2B}AR and A_3AR activation. Anti-inflammatory (M2) macrophages overexpress A_{2A}AR, A_{2B}AR and A_3AR, which activation attenuates the

production of pro-inflammatory mediators (TNF-α, IL-6, IL-12, NO, and macrophage inflammatory protein (MIP)-1α). Activation of A_{2A}AR and A_{2B}AR also favours (1) the release of the anti-inflammatory cytokine IL-10 by monocytes and macrophages[76,77], and (2) the switch from M1 (pro-inflammatory) to M2 (anti-inflammatory) macrophage phenotypes[79]. On adaptive immunity, adenosine modulates lymphocytes functions, mainly through A_{2A}AR activation and related suppressive effects, as it inhibits both IL-4 and IFN-γ production by naïve CD4+ T cells and Th1 and Th2 cells[76,77]. Taken together, increased extracellular adenosine levels caused by inflammatory processes[22,23] emerges as a putative therapeutic target against inflammatory diseases with encouraging results in preclinical settings[76] as well as by analogy to clinical data concerning rheumatoid arthritis[80] and anticancer immunotherapy[81]. In this context, adenosine mediates the anti-inflammatory effects of methotrexate, a gold standard treatment for patients with rheumatic diseases. By inhibiting certain enzymatic pathways, methotrexate induces adenosine release allowing the nucleoside to exert its anti-inflammatory effects via A_{2A}AR and A_3AR activation[82].

Mice submitted to transverse aortic constriction (TAC), a model of HFpEF, show significant cardiac inflammation that is initiated by transient myeloid cells infiltration followed by chronic increase of myocardial T cells (Table I)[83]. Noteworthy, chemotaxis and activation of myocardial T cells are under the immunosuppressive control of adenosine formed by ecto-5'-nucleotidase/CD73[84] and A_{2A}AR activation[85]. Indeed, the production of inflammatory cytokines (IL-3, IL-6, IL-13, IL-17, MIP-1α and MIP-1β) associated with cardiac fibrosis and decreased contractility is exaggerated by TAC in mice lacking CD73 on T cells, as well as in global CD73 mutants[83]. These animals exhibit compensatory increases in the enzymatic machinery participating in adenosine formation (e.g. CD39, pyrophosphatases ENPP1 and ENPP3, and CD38) and overexpress A_{2A}AR in activated T cells infiltrating the injured heart[83]. Additionally, overexpression of A_{2A}AR attenuates cardiac inflammation and remodelling, thereby attenuating cardiac dysfunction (Table I)[85]. These data provide compelling evidence that deficient adenosine formation by T cells lacking CD73 worsens the prognosis of HFpEF, raising the hypothesis that increasing extracellular adenosine formation from the extracellular catabolism of released adenine nucleotides and/or direct activation of the A_{2A}AR subtype may overcome HFpEF by reducing myocardial damage caused by pro-inflammatory cytokines and maladaptive cardiac remodelling[83,85]. Thus, maintenance of ecto-5'-nucleotidase/CD73 activity may be crucial to define the pattern of extracellular ATP-derived adenosine formation favouring A_{2A}AR activation to prevent the pathological changes observed in HFpEF, as also predicted in other cell systems[86].

b. Microvascular dysfunction

The current trend is that coronary microvascular dysfunction (CMD) may be a cornerstone of the complex molecular pathways driving the clinical manifestations of HFpEF[87]. CMD is inevitably associated with a compromised vasodilatory response and consequently to the existence of myocardial focal ischemic lesions contributing to diastolic dysfunction of the left ventricle (LV)[88]. This feature is further compromised by systemic comorbidities and local inflammatory states ending up into structural and functional abnormalities of the heart and blood vessels[87]. Diastolic dysfunction, the hallmark of HFpEF, is related to CMD, independently from the presence of coronary artery disease[87].

Experimental models of diabetes, obesity and metabolic syndromes predict that systemic inflammation unbalances the equilibrium between endothelium derived relaxing factors (e.g. NO) and endothelium-derived vasoconstrictors (e.g. endothelin-1), thus increasing stiffness and vasoconstriction of large vessels at rest[3]. Endothelial activation favours the expression of adhesion molecules, trans-endothelial migration of circulating leucocytes, and release of pro-inflammatory cytokines and ROS, which contribute to perpetuate local inflammation[74]. Moreover, vascular inflammation decreases NO bioavailability[74,89] resulting in downstream reduction of soluble guanylate cyclase activity, low levels of cyclic GMP and decreased activation of protein kinase G, which is normally associated with titin hypophosphorylation, increased rigidity and myocardial diastolic dysfunction (Figure 3)[3].

The vascular endothelium is a key player linking metabolic disorders and HFpEF. Excessive production of ROS by endothelial Nox2 activation has been associated with other cardiovascular diseases, such as hypertension and atherosclerosis, which are common comorbidities of HFpEF[90]. *In vitro* blockage of the A_{2A}AR effectively inhibits ROS production by endothelial Nox2 and attenuates angiotensin II (AngII)-induced oxidative stress and endothelial dysfunction, by inhibiting MAPK activation and, consequently, reducing $p47^{phox}$ phosphorylation and binding to Nox2[91]. Taking together, these findings suggest a potential for A_{2A}AR antagonism to counteract AngII-induced Nox2 activation in endothelial cells[91]. However, contradictory results urge depending on the vascular territory considered; the A_{2A}AR inactivation displays a protective role in reducing oxidative damage in neurodegenerative diseases[92] and in the heart[93], whereas its inactivation increased tracheal ROS production from Nox2[94].

In fact, the A_{2A}AR activation is reported to play a major role in endothelium homeostasis, culminating in increased production of NO by the endothelium, and subsequent vasodilation in most vascular beds (Figure 3)[54]. Nevertheless, a previous study showed that the A_{2A}AR inactivation preserved the endothelium-dependent vasodilation to acetylcholine while reducing ROS production. This might be explained due to the very low Nox2 expression in the VSMCs, the

predominant cellular component in the vessel wall[91]. Notwithstanding, the fact that the A_{2A}AR is expressed in endothelial cells, its activation operates both endothelium-dependent and -independent relaxation of blood vessels. Controversy still exists on the receptor subtype (A_{2A}AR and/or A_{2B}AR) predominating on endothelial cells[50,54,95]. Endothelial dysfunction in small penile vessels underlying erectile dysfunction may be taken as an early surrogate of cardiovascular disease severity[96]. In this context, adenosine regulates smooth muscle tone in human corpora cavernosa through the activation of high-affinity A_{2A}AR and low-affinity A_{2B}AR located on smooth muscle fibres and on endothelial cells, respectively[55]. Interestingly, corpora cavernosa from men with vasculogenic impotence is partially resistant to adenosine relaxation due to endothelial A_{2B}AR dysfunction, while keeping almost unaltered relaxation of cavernosal vessels via A_{2A}AR on the smooth muscle layer[55]. Insufficient adenosine formation linked to deficits in CD73 enzymatic activity has been associated with age-dependent endothelial dysfunction and NO production deficits in mice[97], which may strengthen the production of the pro-inflammatory cytokine, IL-6, and endothelial adhesion molecules, like intercellular adhesion molecule-1 and VCAM-1[97].

Another hallmark of CMD in patients with HFpEF is coronary microvascular rarefaction[98]. Thus, adenosine-induced angiogenesis via A_{2A}AR, A_{2B}AR and A_3AR activation may be relevant in this scenario[56,57,71]. In addition, it has been reported that activation of the A_1AR subtype increases capillary density and oxygen diffusion distance in dogs submitted to TAC (Table I)[99]. Interestingly, increased intracellular adenosine levels in human endothelial cells lacking ADK lead to hypomethylation in DNA promotor regions of pro-angiogenic genes[100]. Epigenetic upregulation of pro-angiogenic genes favour endothelial proliferation *in vitro* and ischemia induced-angiogenesis *in vivo*[100].

Besides its antithrombotic properties, the $P2Y_{12}$R antagonist, ticagrelor, has been increasingly used in cardiometabolic diseases like DM, most probably because of its pleiotropic/anti-inflammatory effects, which at least some of them are shared with adenosine[101]. Interestingly, ticagrelor restores defective ATP release and inhibits adenosine uptake by red blood cells directly impacting the actions of these two purines (or its metabolites) on immunocytes and endothelial cells[101], which might be useful to counteract the development of atherosclerosis and related metabolic syndrome. In this regard, adenosine receptors (namely the A_{2A}AR subtype) counteract the development of atherosclerosis by providing endothelial / vascular protection due to the nucleoside anti-inflammatory properties and by modulating the cholesterol transport in macrophages, as well as the hepatic metabolism of lipids (reviewed in [102]). Consistent with these findings, the THEMIS-PCI (The Effect of Ticagrelor on Health Outcomes in Diabetes Mellitus Patients-Percutaneous Coronary Intervention) sub-study revealed a reduction of major adverse cardiac events in diabetic patients submitted to percutaneous

coronary intervention and treated with ticagrelor plus aspirin compared to aspirin monotherapy[103]. A more comprehensive about the mechanisms underlying ticagrelor-induced endothelial dysfunction improvement is needed to support indication of this drug for cardiometabolic diseases[101].

c. Interstitial fibrosis and loss of myocardial compliance

Structural abnormalities in HFpEF involve cardiomyocyte hypertrophy and interstitial fibrosis[3,4,75]. Under pathological stressful conditions, unopposed actions of pro-fibrotic and pro-hypertrophic paracrine and neurohormonal signals further aggravate cardiac chambers stiffness and impaired relaxation. Diastolic dysfunction and inherent increased LV filling pressure fosters secondary left atria remodelling and dysfunction, further aggravating HFpEF symptoms and long-term prognosis[3,4,75]. Cardiac fibroblasts are the predominant interstitial cell type in the adult mammalian heart[104]. Thus, it is not surprising that cardiac fibrosis and associated decrease in myocardial compliance represents a common pathological hallmark in the failing heart[3,4].

Among all adenosine receptors, A_{2A}AR emerges by its cyclic AMP-dependent anti-fibrotic action[105]. Separating the contribution of both A_{2A}R and A_{2B}AR to fibrotic processes has been difficult due to the lack of selective drugs used in the past[106]. As aforementioned, the cardiac anti-inflammatory/anti-fibrotic role of A_{2A}AR has been translated into disease models of HFpEF [83,85] and ischemia/reperfusion (I/R)[58-60], with evidence suggesting that this receptor might have an anti-fibrotic role[105]. Activation of the A_{2A}AR decreased the expression of fibrosis-related genes and cardiac fibrosis, while improving cardiac remodelling and function, in the deoxycorticosterone acetate (DOCA)-salt induced hypertensive mice model of cardiac remodelling (Table I)[107]. One may, therefore, hypothesize that the anti-fibrotic effect of adenosine via A_{2A}AR activation is operated either, directly, by inhibiting growth and/or differentiation of cardiac fibroblast, or, indirectly, through nucleoside-induced immunosuppressive effects resulting in prevention of pro-fibrotic cells chemotaxis and inflammatory mediators release. In addition to these possibilities, adenosine deamination to inosine by third party ADA cell providers (e.g. inflammatory cells) may also play a role in the anti-fibrotic effect of adenosine, as we recently demonstrated in human subcutaneous fibroblasts[108]. Thus, clarification of the mechanism and receptor involved in the anti-fibrotic role of adenosine in myocardial fibrosis is warranted given to the fact it may exert pro-fibrotic effects in other organs, such as the liver[109] and the skin [108], while the opposite is verified with the A_{2B}R which inhibits fibrosis in the heart (see below) and promotes fibrosis of the lung (reviewed in [33]).

The low affinity A_{2B}AR is the most abundant receptor in rat cardiac fibroblasts[110]. Therefore, it is not surprisingly that the anti-fibrotic effect of the A_{2B}AR is rather consensual at least *in vitro*[111], while questions may arise about the endogenous amounts of the nucleoside required to activate this low affinity receptor under both normal and pathological conditions. Nevertheless, it has been shown that intracellular cyclic AMP accumulation prevents TGF-β-, Ang II-, and endothelin-1-induced collagen synthesis and α-smooth muscle actin expression by activated cardiac myofibroblasts via EPAC and PI3K dependent pathways[112]. Likewise, fibroblast proliferation, collagen synthesis, α-smooth muscle actin expression and myofibroblast differentiation were all attenuated by cyclic AMP-coupled A_{2B}AR activation, thus counteracting pro-fibrotic stimuli induced by endothelin-1 and Ang II (Figure 3)[112] via a mechanism involving cAMP and EPAC downstream pathways[113,114]. The cyclicAMP/EPAC/PI3K/Akt signalling pathway was also involved in A_{2B}AR-mediated suppression of fibroblasts growth and α-SMA-expressing myofibroblast differentiation induced by endothelin-1[115]. In a canine *in vivo* model of HF it was found that the EPAC1 content in fibroblasts of left atria exhibiting fibrotic remodelling was significantly downregulated[116]. Taking together, data suggest A_{2B}AR coupling to the cyclic AMP/EPAC pathway may provide potential targets to prevent/overcome cardiac fibrosis that may be required to rescue myocardial compliance.

Notwithstanding the fact that A_{2B}AR agonists exhibit cardiac anti-fibrotic effects *in vitro*, these findings were not consistently translated into *in vivo* data, most probably due to the dual action of this receptor in cardiac inflammation[106]. Available data suggests that A_{2A}AR activation before ischaemia followed by reperfusion may have a cardioprotective effect, most likely because of the inhibition of pro-inflammatory/pro-fibrotic cytokines release via PI3K/Akt pathway[117-119]. Moreover, complex interplay between A_1AR and A_{2B}AR subtypes is also possible, given to the fact that apparently the A_1AR-operated cardiac protection relies on A_{2B}AR overexpression by a PKC-dependent pathway during ischemia and preconditioning[66], Nevertheless, in contrast to the A_{2A}AR, there is no consensus about the putative beneficial roles of the A_{2B}AR on the remodelling phase after MI[120]; with studies demonstrating a protective role of either A_{2B}AR blockage[121,122] or activation[123] after MI. Blockage of A_{2B}AR after MI occurrence seems to attenuate PKC-δ/p38-MAPK-mediated secretion of pro-inflammatory and pro-fibrotic mediators, like TGF-β, TNF-α and IL-6[121,122,124]. On the other hand, deletion of A_{2B}AR did not change fibrosis in the remodelling phase after MI, probably due to opposing pro- and anti-inflammatory stimulus[125]. Specifically, deletion of A_{2B}AR increased cardiac immune cells infiltration, while downregulated the expression of IL-6 further counteracting inflammation. The downregulation of IL-6 transcripts supports the preponderant role of A_{2B}AR under these conditions[125]. In fact, the injured heart depends mostly on the low-affinity A_{2B}AR probably due

to functional inactivation of the A_{2A}AR through dimerization[126]. It is worth noting that A_{2B}AR may couple to both G_s and G_q proteins, as well as to other G proteins; its preferential coupling to a certain G protein dependents on multiple factors, including the cell type, receptor expression levels, and chemical nature of the agonist - 'biased receptor' function (reviewed in [33]). Thus, the A_{2B}AR may be functionally polymorphic exerting either pro-inflammatory or anti-inflammatory roles depending on organ systems. As such activation of the A_{2B}AR by high endogenous levels of adenosine promotes chronic inflammation in asthma[127] and COPD[128]. Thus, clarification of the precise mechanisms involved in pro- *vs.* anti-fibrotic effects of the A_{2B}AR in the heart is still needed to correctly infer any benefit derived from the manipulation of the activity of this receptor in patients with HFpEF.

d. Cellular structural changes

Overload pressure, oxidative stress and tissue injury, as well as the influence of neurohormonal and inflammatory mediators, like Ang II, endothelin-1, catecholamines, growth factors and TNF-α, foster ventricular hypertrophy (reviewed in [129]). While cardiac hypertrophy may be considered a physiologically adaptive response to external insults, over time it becomes pathological resulting in progression to HF[4,129].

The myocardial adenosine content highly increases in hypertrophied hearts[26,27]. Given that the nucleoside counteracts the activity of many neurohumoral factors involved in cardiac hypertrophy, namely endothelin-1[130], renin-angiotensin-aldosterone system[131] and TNF-α[132], and it inhibits noradrenaline release from overactivated sympathetic nerves [133], it was hypothesized that adenosine may exert an anti-hypertrophic action and, in this sense, counteract cardiac dysfunction[134]. Indeed, inhibition of adenosine uptake with dipyridamole increased myocardial adenosine levels, which significantly ameliorated LV filling and pulmonary congestion, while attenuating β-adrenergic dysfunction, in rats with pressure overload cardiac hypertrophy due to abdominal aortic constriction (Table I)[135]. Conversely, inhibition of adenosine formation from released adenine nucleotides in CD73 deficient mice promoted myocardial hypertrophy, LV dilation and LV dysfunction (Table I) after aortic constriction, but not in healthy animals[136]. These findings strengthen the theory that adenosine may exert a cardioprotective role in conditions of chronic systolic overload through activation of the mechanistic target of rapamycin (mTOR)/ p70S6K pathway[136].

It, thus, seems that A_1AR play a chief role in the putative anti-hypertrophic action of adenosine in the heart[99,134,137]. The enzymatically stable A_1AR agonists, 2-chloroadenosine (CADO) and N^6-cyclopentyladenosine (CPA), significantly reduced the plasma concentrations of

noradrenaline, renin, and brain natriuretic peptic (a molecular marker of cardiac hypertrophy) in a mice model of TAC-induced HF (Table I)[134]. When used *in vivo*, these drugs also effectively attenuated cardiomyocytes hypertrophy and fibrosis in interstitial and perivascular spaces through A_1AR activation, resulting in improved cardiac function and reduced pulmonary congestion[134]. Both CADO and CPA attenuated protein synthesis induced by activation of G_q-protein-coupled receptors for AngII, endothelin-1, and noradrenaline, as well as the PKA-mediated hypertrophic actions of isoproterenol in neonatal rat cardiomyocytes[134]. Interestingly, *in vivo* stimulation of α_1-adrenoceptor with phenylephrine upregulated A_1ARs expression. Activation of overexpressed A_1AR with CPA counteracted the hypertrophic phenotype caused by phenylephrine, but the same was not verified concerning cardiac hypertrophy/ fibrosis caused by AngII and IGF1 (Table I)[137]. These findings suggest that different signalling pathways operate maladaptive cardiac responses to distinct neurohumoral agents, namely AngII and phenylephrine[137].

Adenosine counteraction of cyclic AMP accumulation induced by β-adrenoceptors stimulation (anti-adrenergic effect), has been proposed to explain the anti-hypertrophic effect of the nucleoside (Figure 3)[138], while regulation of G protein signalling 4 which attenuates G_q induced hypertrophy could explain its opposing effect regarding cardiac hypertrophy caused by the α_1-adrenoceptor agonist[134]. A_1AR-receptor-mediated interference on intracellular Ca^{2+} handling may also play a role against myocytes hypertrophy probably by downregulating the calcineurin pathway[138]. As a matter of fact, sustained β-adrenergic activation increases interstitial fibrosis and myocyte hypertrophy, two features of myocardial remodelling and HF[139]. Upon β-adrenergic stimulation, cardiomyocytes let intracellular cAMP flow to the extracellular space via the ATP-binding cassette sub-family C member 4 transporter where it may act as an important paracrine cardioprotective signalling molecule. Once in the extracellular space, cAMP may be enzymatically converted to AMP by ecto-phosphodiesterases, which will then be dephosphorylated to adenosine by ecto-5'nucleotidase/CD73. In this sense, one may hypothesize that myocardial hypertrophy due to β-adrenoceptors overactivation may be partially counteracted by extracellular adenosine yields through activation of A_1AR negatively coupled to the AC while delivering anti-fibrotic signals to cardiac fibroblasts through A_2AR activation (Figure 3)[105]. Thus, the anti-adrenergic effect of adenosine may serve as an endogenous local β-blocker by limiting intracellular cAMP formation with inherent beneficial cardiac effects. As a matter of fact, capadesonon (CAP), a partial A_1AR agonist, decreased myocardial hypertrophy and fibrosis, while increasing capillary density and oxygen supply, which significantly improved LV function in dogs with advanced HF (Table I)[99]. All together these

evidences suggest that A_1AR may be a potential target to prevent transition from compensated myocardial hypertrophy to irreversible HF.

In addition to the A_1AR-mediated anti-hypertrophic effect of adenosine, A_{2A}AR overexpression or agonist activation attenuated cardiomyocyte hypertrophy[85,107] and decreased hypertrophy-associated genes encoding β-myosin heavy chain and atrial natriuretic peptide along with their transcription activator protein GATA-4 in mice with pressure-induced HF secondary to TAC[85]. These cardioprotective A_{2A}AR-mediated effects are associated with attenuation of the inflammatory response via MAPK- and PKC-operated pathways independently of PKA activation[85].

Considering the prominent cardioprotective role to adenosine against chronic cardiac pressure overload, unexpected results were obtained using A_3AR knockout (KO) mice as these animals exhibited partial resistance to TAC-induced LV hypertrophy, fibrosis, and oxidative stress (Table I)[140]. Hemodynamically, A_3AR KO mice exhibited higher ejection fractions and smaller LV end-systolic diameters compared to wild type animals; the plasma levels of the atrial natriuretic peptide detected in the A_3AR KOs were also lower compared to controls confirming less cardiac hypertrophy. Authors interpreted their findings by suggesting that A_3AR activation might potentiate the inflammatory response triggered by cardiac pressure overload, thus favouring LV hypertrophy and cardiac dysfunction[140], in line with the paradigm recently advocated to explain HFpEF. In this context, it was interesting to verify that selective blockage of A_3AR with MRS1911 potentiated the anti-hypertrophic effect of CADO in cardiomyocytes treated with phenylephrine, strengthening the idea that activation of the A_3AR might have a deleterious effect in cardiac remodelling following chronic pressure overload[140]. The effects obtained with the A_3AR antagonist were remarkably similar to those verified upon reducing the activation of MAPK and PI3K-Aκt signalling pathways[140], which are often activated in response to extracellular stress, such as oxidative stress and inflammation[129]. While confirmation of the protective role of A_3AR blockage on the inflammatory component attenuating pressure overload cardiac remodelling requires the use of more potent and selective drugs, it is also worth noting that the adenosine deamination metabolite, inosine, may exert opposite effects to adenosine and is a potent A_3AR agonist, which may accumulate in tissues infiltrated by ADA-bound inflammatory cells[108]. This concept raises new lines of research using multi-target drugs designed to increase local adenosine levels while decreasing inosine formation (e.g. ADA inhibitors also blocking adenosine cellular uptake, like the lymphocytotoxic drug 2'-deoxycoformycin or pentostatin) applied in combination with enzymatically stable adenosine receptor agonists and/or antagonists.

There is evidence that adenosine may also exert cardioprotective actions in the heart via non-receptor-mediated mechanisms. In support of a role for intracellular adenosine metabolism in regulating hypertrophy, it has been demonstrated that ADK inhibitors, such as iodotubercidin and ABT-702, completely reversed the anti-hypertrophic actions of adenosine and its enzymatically stable analogue, CADO, in phenylephrine-induced hypertrophic neonatal rat cardiomyocytes[141]; data identified ADK as an important mediator of adenosine attenuation of cardiomyocyte hypertrophy acting, at least in part, through inhibition of Raf signalling to mTOR/p70S6k. The role of ADK in the anti-hypertrophic effect of adenosine has also been demonstrated *in vivo* in a KO mice model of cardiac pressure overload induced by TAC (Table I)[142]. ADK enzymatic activity dampened cardiac growth signalling (by mTOR complex 1 and Erk), as well as the excessive microtubule stabilization/detyrosination[142]. Overall, these data points towards a novel ADK-dependent adenosine receptor-independent mechanism to protect against adverse hypertrophy remodelling and excessive cardiomyocyte microtubule stabilization.

e. Cardiometabolic dysfunction

Mechanisms underlying impaired myocardial energetics in HF can be divided into three subgroups: 1) abnormal mitochondrial structure and function; 2) change in substrate utilization; and 3) intracellular Ca^{2+} overload[143].

The failing heart is characterized by abnormal mitochondrial structure and function including hyperplasia and reduced organelle size, poor organelle respiration, reduced mitochondrial membrane potential and opening membrane permeability pores. Therefore, ATP synthesis is reduced because of impaired electron transport chain and excessive ROS generation by mitochondria is also observed[143]. Association between A_1AR activity and mitochondrial dysfunction has been observed in HF (Figure 3)[99]. Treatment with the partial A_1AR agonist, CAP, caused a near normalization of mitochondrial dysfunction in animal models of HF (Table I)[99]. CAP decreased the opening rate of mitochondrial permeability transition pores resulting in reduced apoptosis and normalization of citrate synthase expression, used as a marker of unaltered mitochondrial function[99]. Likewise, the A_1AR agonist, 2-choloro-N^6-cyclopentyladenosine (CCPA), reduced the permeability of mitochondrial transition pores in rat cardiomyocytes submitted to hypoxia through a mechanism involving PKC activation, stabilization of mitochondrial membrane potential and inhibition of ROS production, culminating with the opening of K_{ATP} mitochondrial channels[144]. The low affinity A_{2B}AR has also been associated with

the reduction of the production of mitochondrial ROS via a mechanism involving PI3K, Erk and NOS in adult rabbit cardiomyocytes[145].

Due to abnormal mitochondrial structure and function with consequent impairment of the electron transport chain, there is a switch in the cellular energetics in the failing heart from a preferential metabolism of fatty acids to glucose. This shift in the cellular energetics to anaerobiosis in order to spare oxygen consumption is potentiated by A_1AR activation, as these receptors have been shown to restore the plasma membrane expression of glucose transporters 1 (GLUT)-1 and 4 (GLUT-4) to normal levels (Table I)[99] and to decrease levels of free fatty acids[146,147].

Intracellular Ca^{2+} overload in HF results, among other mechanism, from an abnormal function of the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) 2a, which normally pumps Ca^{2+} from the cytosol into the sarcoplasmic reticulum during diastole; as a consequence, active muscle relaxation is impaired and ventricular cardiomyocyte stiffness becomes an issue[75]. Activation of A_1AR with CAP ameliorated SERCA2a function, which by restoring the buffering Ca^{2+} capacity of the sarcoplasmatic reticulum indirectly facilitates the diastolic relaxation of cardiomyocytes, while also restraining intracellular Ca^{2+} overload before the next contraction (Table I)[99]. On the other hand, although the activation of the A_{2A}AR increases intracellular Ca^{2+} transients during systolic activity, they rapidly subside during diastole in a mouse model of cardiac pressure overload due to TAC[85], through a PKA dependent mechanism (Table I)[85].

5. Important comorbidities in HFpEF

a. Diabetes mellitus and metabolic syndrome

Diabetes mellitus (DM) is frequently associated to the occurrence and severity of HF in clinical practice; roughly 30-40% of patients with HFpEF are also affected by this endocrinopathy[148]. According to the clinical trial RELAX (Phosphodiesterase-5 Inhibition to Improve Clinical Status and Exercise Capacity in Diastolic Heart Failure), diabetic HFpEF patients were younger, mostly males, and affected by other comorbidities, namely obesity, hypertension, renal dysfunction, pulmonary disease and vascular disease[149]. HFpEF patients with DM have worse prognosis concerning morbidity and mortality, which may be related to a more severe disease phenotype associated with higher filling pressures[149].

The role of adenosine in insulin resistance and obesity has been recently reviewed by our group[150]. Adenosine, via A_1AR activation, supresses lipolysis and plasma free fatty acids; increases lipogenesis and adipogenesis; it also regulates insulin sensitivity and glucose tolerance,

indirectly interfering with myocardial substrate handling[151,152]. Moreover, it has been recently shown that adenosine, via ADA acting on RNA 1, may control appetite signalling and modulation along with limiting obesity and insulin resistance (Table II)[147]. ADA acting on RNA 1-deficient heterozygous mice fed with a high fat diet (HFD) for 12 weeks exhibited a lean phenotype compared to wild type controls which became obese within the same time period; the lean phenotype was associated with reduced fat mass, together with less severe dyslipidaemia and insulin resistance parameters[147]. Under the same experimental conditions, ADA acting on RNA 1-deficient heterozygous mice submitted to HFD showed decreased food intake, decreased gastric ghrelin expression, and attenuated the reduction of serum peptide YY levels[147]. Altogether these findings suggest that ADA acting on RNA 1 may contribute to diet-induced obesity by modulating genes related to appetite control, such as ghrelin ("hunger hormone") and peptide YY ("satiety hormone")[147].

In mice with streptozotocin (STZ)-induced diabetes, activation of the A_1AR with CPA decreases plasma glucose, cholesterol, and triglyceride levels, and improves glucose utilization in peripheral tissues by increasing its uptake and glycogen synthesis by target cells (Table II)[153]. In this context, A_1AR agonists have been proposed for the treatment of type 2 DM and obesity. Several A_1AR agonists have been evaluated in clinical trials for type 2 DM with proven ability to increase insulin sensitivity. The first A_1AR full agonists undergoing clinical trials, ARA and GR79236, were successful in improving insulin sensitivity, but failed their goals due to undesirable cardiovascular side effects[154]. GS-9667 (previously known as CVT-3619), a highly selective partial A_1AR agonist, reduced plasma free fatty acids and was well-tolerated both in healthy non-obese and obese subjects, without showing any signs of receptor desensitization or rebound problems on suspension (Table II)[146]. This is not surprising given to the fact that A_1AR are highly much more abundant in adipocytes than in the atrioventricular node[155,156].

Activation of the low affinity A_{2B}AR has been given a special attention as a putative therapeutic target for obesity due to its ability to inhibit adipogenesis, increase lipolysis, improve insulin signalling and decrease white adipose tissue mass, which are key pathological features underlying obesity development[157,158]. Activation of A_{2A}AR also increases lipolysis; this receptor is by far more expressed in the brown adipose tissue compared to the white adipose tissue, which might explain why it favours thermogenesis and browning of the white adipose tissue[150]. These findings together with the anti-inflammatory potential of A_{2A}AR activation, put this receptor in good position to counteract insulin resistance[150]. Previous results using the DOCA-salt induced hypertensive mice model of cardiac remodelling showed that A_{2A}AR activation promotes the release of fibroblast growth factor 21 by brown adipocytes, which presence seems to be crucial to combat hypertensive cardiac remodelling (Table I)[107]. Confounding data,

however, emerged from studies where the chronic consumption of caffeine reduced the risk of insulin resistance and type 2 DM[159]. It is worth noting that caffeine is the most widely psychoactive substance consumed worldwide and it acts mainly through the antagonism of adenosine receptors under moderate consumption of caffeine-containing beverages. A recent study carried out in Wistar rats of both gender fed either with a normalized chow or with a high-sucrose (HSu) diet showed for the first time that chronic adenosine receptor blockage with caffeine favoured insulin resistance in control animals, while restored insulin sensitivity in animals treated with the HSu diet (Table II)[160]. These authors also made clear that whole-body insulin sensitivity is under the control of both A_{2A}AR and/or A_{2B}AR given to the fact that selective blockage of each one of these receptors rescued impaired insulin sensitivity in skeletal muscles, both in males and females. Concerning the adipose tissue, chronic A_1AR antagonism decreased fat accumulation, whereas the opposite was observed upon blockage of A_{2A}AR and/or A_{2B}AR; in this respect, gender differences were found with A_1AR blockage being more effective in females, while antagonism of the A_{2A}R was more powerful in males. Overall, these findings indicate that the effect of adenosine against insulin resistance via A_{2A}AR and/or A_{2B}AR is more prominent in skeletal muscles rather than in the adipose tissue where the A_1AR seems to predominate[160].

Accumulating evidence suggest a role for adenosine in endothelial homeostasis maintenance. Due to its high affinity for the nucleoside, ADK is a key intracellular enzyme that is responsible for maintaining low intracellular adenosine levels while consistently driving the nucleoside to the purine nucleotides salvage pathway[161]. Deletion of ADK, leading to increased intracellular adenosine accumulation and subsequent outflow of the nucleoside from cells, was found to increase β-cell replication, thereby protecting against HFD-induced glucose intolerance[162]. Endothelial ADK was significantly upregulated in mice submitted to HFD; conversely, endothelial-specific ADK deficiency protected mice from HFD-induced insulin resistance and metabolic syndrome probably by increasing adenosine translocation to the extracellular milieu where it can activate plasma-membrane bound receptors, including endothelial A_{2B}AR, which downstream contributes to phosphorylate NOS 3 fostering NO production and NO-related protective anti-inflammatory effects due to vasodilation and angiogenesis (Table II)[163]. The anti-inflammatory potential of ADK inhibition is also associated with reduced leucocyte adhesion to the endothelium, further contributing to regulation of glucose homeostasis and insulin sensitivity[164].

b. Renal dysfunction

Upon population aging and incorrect lifestyle habits the prevalence of HFpEF and CKD is dramatically increasing[165,166]. HFpEF has been considered a "vicious cycle" disorder of kidney and cardiovascular function disequilibrium, as the presence of either HFpEF or CKD may accelerate the pathological progression of each other. Both disease conditions have overlapping comorbidities, including association with hypertension, diabetes and obesity, and share common underlying features, like hypervolemia, systemic inflammation and endothelial dysfunction[165].

Adenosine has been reported to reduce urinary protein excretion[167], an earlier marker of underlying kidney injury causing renal dysfunction[168], by acting at the kidney level (reviewed in [169]). Specifically, adenosine controls renin release, glomerular filtration rate, tubuloglomerular feedback mechanism and vascular tone through all four P1 receptor subtypes.

The A_1AR plays an important role in renal physiology, as it controls afferent arteriole vasoconstriction, reabsorption of sodium in the proximal tubule and the tubuloglomerular feedback; through inhibition of renin release from justaglomerular cells along with renal arteriole vasodilation, adenosine reduces renal blood flow lowering the glomerular filtration pressure [169]. Reduction of the glomerular filtration pressure by A_1AR activation is protective on its own against kidney damage caused by metabolic diseases, such as diabetes and hypertension, which are common comorbidities of HFpF[166,169]. The promising favourable effects of sodium-glucose cotransporter-2 inhibitors (SGLT2i), like empagliflozin, on diabetes-related hyperglycaemia, obesity and hypertension may be at least in part mediated by the reduction of glomerular hyperfiltration via activation of A_1AR receptors and TGF-mediated afferent arteriolar vasoconstriction, independently of their ability to lower the plasma glucose levels[170]. SGLT2i use provide a new perspective for the cardio- and reno-protective role of A_1AR activation as it decreases the intraglomerular pressure and prevents hyperfiltration, which may counteract CKD progression and impaired renal function indirectly ameliorating the cardiovascular outcome [170]. Moreover, renal activation of A_1AR reduces the metabolic demand, tubular cell apoptosis and inflammatory reactions in mice models of I/R (Table II)[171,172], strengthening the reno-protective effects of A_1AR agonists.

The ability of A_1AR antagonists to induce natriuresis without compromising the glomerular filtration rate suggests that this approach may be beneficial to control volume overload disorders, such as HF[173]. The selective A_1AR antagonist, rolofylline, increased diuresis in patients with acute HF and enhanced diuresis and renal plasma flow in patients with chronic HF (Table II)[174,175]. However, in the large phase III clinical trial PROTECT, rolofylline reduced fluid retention, but did not prevent the worsening of renal dysfunction in acute HF patients[176]. In fact, despite the putative benefits in controlling hypertension and volume overload, inhibition of the

A_1AR activity may be detrimental for glomerular architecture and function, as it increases intraglomerular pressure by antagonizing preglomerular vasoconstriction operated by A_1AR[177].

Notwithstanding this, long-term dual blockage of A_1AR / A_{2B}AR with the orally-available drug tonapofylline (BG9928) reduced glomerulosclerosis in ZSF_1 rats used as an animal model of HFpEF related to metabolic syndrome[177]. Diabetic nephropathy is associated with reduced nitrergic vascular innervation, NO bioavailability and VEGF-NO uncoupling leading to excessive endothelial cell proliferation, stimulation of macrophage chemotaxis and VSMCs growth, which ultimately causes glomerular hypertrophy favouring proteinuria[178]. Taking these findings together, it may well be that the renoprotective effect of BG9928 in preventing glomerulosclerosis may be undertaken by the A_{2B}AR antagonism[177]. Indeed, isolated renal fibroblasts treated with the A_{2B}AR agonist, BAY-606583, increased the release of pro-inflammatory and pro-fibrotic mediators[179]. On the other hand, blockage of the A_{2B}AR with MRS1754 significantly decreased albuminuria and improved the renal function in STZ-induced diabetic mice; the antagonism of overexpressed A_{2B}AR normalized VEGF-A upregulation in these animals, while enhancing NO production and decreasing the occurrence of glomerulosclerosis lesions (Table II)[180].

In a recent study performed in a rat model of HF secondary to renal injury the mineralocorticoid receptor antagonist, spironolactone, significantly up-regulated the A_{2A}AR expression, inhibited endothelial-to-mesenchymal transition and attenuated cardiorenal fibrosis, both *in vivo* and *in vitro* (Table II)[181]. These outcomes suggest the A_{2A}AR as a potential therapeutic target for the cardiorenal syndrome[181]. In addition to the anti-fibrotic role of the A_{2A}AR, its ability to dilate the efferent arteriole may be renoprotective role by reducing the glomerular pressure[169]. Long-term administration of the selective A_{2A}AR agonist, CGS21680, reduces diabetes-induced glomerular hyperfiltration in STZ-induced diabetic mice through a mechanism depending on NO production (Table II)[182]. The anti-inflammatory role of A_{2A}AR activation was reflected by a decreased number of infiltrating immune cells and in the reduction of urinary pro-inflammatory cytokines along with reduced albuminuria[183]. These results were corroborated by a different research group using the same animal model, who proved that selective A_{2A}AR activation with CGS21680 prevents proteinuria and glomerular damage by activating T regulatory cells resulting in reduced macrophage infiltration and TNF-α secretion (Table II)[184]. Likewise, inhibition of ADK leading to adenosine overflow to the extracellular milieu had a protective role against renal failure in STZ-induced diabetic mice by decreasing renal inflammation and oxidative stress lesions and by restoring glomerular filtration and permeability (Table II)[185]. These effects were consistent with the reduction in renal macrophage infiltration,

nuclear factor-κB phosphorylation and monocyte chemoattractant protein-1 excretion, along with increased endothelial NOS expression and MAPK phosphorylation[185].

In a model of hypertensive-diabetic nephropathy using spontaneously hypertensive (SHR) rats treated with STZ, the enzymatically-stable adenosine analogue, CADO, improved glucose metabolism (decreased hyperglycaemia and glycosuria), renal function (decreased proteinuria), and renal fibrosis (decreased glomerular collagen deposition), along with decreased renal oxidative stress (Table II)[186]; authors interpreted these findings as being mediated by renal A_{2A}AR activation, given that this receptor, but not A_1AR nor A_{2B}AR, immunoreactivity was upregulated in superficial glomeruli, proximal tubules, and distal tubules of the kidneys of this animal model[186]. The use of the hypertension-inducing adenosine receptor antagonist, 1,3-dipropyl-8-sulfophenylxanthine aggravated renal fibrosis, but did not alter the global metabolic status and renal function, most probably due to selective preservation of tonic activation of A_3AR, given that downregulation of this receptor by long term activation is considered protective against renal fibrosis[186]. Taken together, these results emphasize the protective role of adenosine in hypertensive-diabetic nephropathy through dynamic expression of its receptors, which may be fine-tuned by A_{2A}AR upregulation and A_3AR downregulation, thus prompting for a novel therapeutic target for this disease conditions[186].

As a matter of fact, A_3AR overexpression may be a therapeutic target to abrogate progression of diabetic nephropathy in db/db leptin receptor deficient mice, the most widely used animal model of type 2 DM exhibiting increased body weight, impaired glucose tolerance, and LV hypertrophy leading to HFpEF, while showing no changes in blood pressure. Chronic administration with LJ-2698 (a novel orally-active and highly selective A_3AR antagonist) to these animals was more efficient in preventing diabetic nephropathy progression than the widely used AT_1 receptor antagonist, losartan (Table II)[187]. Moreover, inhibition of renal lipid accumulation with increases in PGC1α, a key regulator of mitochondrial biogenesis, were obtained upon treating these animals with either LJ-2698 or losartan, further supporting the renoprotective effects of selective A_3AR antagonism[187]. Whether the combination of A_3AR and AT_1 receptor antagonists exerts synergic renoprotective effects requires investigation in future studies[187].

c. Pulmonary disease

Left heart disease (LHD)-induced pulmonary hypertension (PH) (PH-LHD), the WHO group 2 of PH, tend to be more symptomatic and to have worse prognosis when compared to patients with LHD alone[188]. Specifically, PH associated with diastolic dysfunction, also referred as PH secondary to HFpEF (PH-HFpEF) is the most common endophenotype attributable to

LHD[188]. Diastolic dysfunction coupled with LA changes in HFpEF result in a passive backward transmission of increased LV filling pressure with consequent increases in pulmonary congestion[188]. As aforementioned, studies in animal models of HFpEF induced by cardiac pressure overload revealed a putative beneficial role of adenosine in improving cardiac structure and function alterations and, thereby, attenuating cardiac dysfunction[134,189] while decreasing pulmonary congestion[134]. Major benefits of adenosine in this endeavour result from A_1AR and A_{2A}AR activation[83,85,107] associated or not with A_3AR antagonism[140], which may also ameliorate symptoms and prognosis of HFpEF caused by cardiac pressure overload.

Adenosine levels in the pulmonary circulation are pathologically low in patients with WHO group 1 pulmonary arterial hypertension (PAH) as a consequence of local endothelial dysfunction and increased inactivation by ADA, possibly indicating that adenosine A_1AR and A_{2A}AR activation deficits unbalanced by inosine-mediated A_3AR tone[108] may contribute to maladaptive lung disease[190]. In contrast to other vascular beds where adenosine has potent vasodilatory actions, in the pulmonary circulation the nucleoside exerts dual opposing effects depending on the vascular tone[191]. Under low pressure conditions, adenosine fosters vasoconstriction through A_1AR activation, whereas upon increasing the vascular tone, as observed in PH, the A_2AR subtypes acquire more relevance to promote vasodilatation.

Among all adenosine receptor subtypes, the A_{2A}AR gathered the most attention concerning PAH treatment given to the fact that A_{2A}AR KO mice exhibit structural and functional abnormalities similar to PAH[192,193] and overexpress RhoA and ROCK at mRNA and protein levels, which are known to be involved in pulmonary vascular remodelling and PAH pathophysiology (Table II)[193]. In mice with PAH induced by monocrotaline (MCT), activation of A_{2A}AR reduced pulmonary endothelial dysfunction and vascular remodelling, while increasing pulmonary vasodilation and the LV stroke volume (Table I)[194,195]. The way adenosine builds its positive influence on RV hypertrophy and dysfunction may be indirectly related to the counteracting effects of the A_{2A}AR on increased pulmonary resistance (Table I)[195]. Synergism between the A_{2A}AR agonist, LASSBio-1359, and the phosphodiesterase type 5 inhibitor, sildenafil, has been observed in the treatment of rats with PAH; combination of low doses of these two compounds decreased pulmonary endothelial dysfunction and RV overload, hypertrophy and dysfunction (Table I)[196], opening a new avenue for the use of LASSBio-1359 in combination with sildenafil in PAH patients refractory to monotherapy with the phosphodiesterase type 5 inhibitor[196]. Additionally, the anti-inflammatory actions of A_{2A}AR agonists may improve cardiopulmonary homeostasis as discussed above. Although stress tests with the A_{2A}AR agonist, regadenoson, showed no major adverse effects in PAH patients, clinical trials to evaluate its therapeutic potential are still needed in these patients[197].

Immunolocalization studies demonstrated infiltration of the RV myocardium by A_{2B}AR -positive fibroblasts and macrophages in rats with MCT-induced PAH[198]. This result is compatible with adenosine being able to downregulate pro-inflammatory and pro-fibrotic stimuli, thus contributing to mechanical adaptation of RV in response to cardiac pressure overload[198]. COPD, a highly prevalent pulmonary comorbidity in patients with HFpEF, is also linked to PH development. Interestingly, COPD patients with PH exhibit increased pulmonary vascular remodelling and mastocyte-induced airway hyperactivity associated with A_{2B}AR overexpression (Table I)[199]. Notwithstanding this, the role of the low affinity A_{2B}AR in cardiopulmonary pathophysiology lacks comprehensive studies before inferring any beneficial effects from targeting this receptor to improve RV failure secondary to PAH (reviewed in [33]). Besides controversial studies claiming deleterious effects of A_{2B}AR in cardiopulmonary pathophysiology others (fewer) emphasize some beneficial effects from A_{2B}AR activation[33].

Considering that most studies available so far about the role of the A_{2B}AR in cardiopulmonary function rely on animal models of PAH, care must be taken before extrapolating the therapeutic effects of this receptor in PH-HFpEF. While the vast majority of PH-HFpEF patients develop HF secondary to pulmonary venous congestion (isolated post-capillary PH)[188], studies have shown that many patients with HFpEF also present with coexisting pulmonary vascular disease due to a metabolic syndrome-induced low grade inflammatory status[200]. Together with the control of actual lifestyle trend leading to increased prevalence of cardiometabolic diseases, development of novel therapeutic strategies towards combined pre- and post-capillary PH is an unmet clinical need[200]. Through its cardiovascular protective effects and role in metabolic diseases, together with aforementioned effects in PAH, adenosine signalling stands as a putative novel target to manage PH-HFpEF, providing that data from preclinical studies translate correctly to clinical trials in humans.

6. Targeting adenosine signal nuances in the treatment of HFpEF

Adenosine handling, metabolism and signalling is implicated in cardiac remodelling and progression to HF (Table III). Indeed, increased cardiac and plasma adenosine levels are hallmarks of severity in patients with HF, which might result from decreased cellular energy charge, shift to anaerobic metabolism, plasma membrane damage and decrease ADA activity[26,27]. Excessive adenosine endogenous tone may lead to downregulation of adenosine receptor genes, namely A_{2A}AR, A_{2B}AR and A_3AR, in the failing heart[26,27]. Alterations in adenosine signalling might contribute to the pathological changes detected in failing hearts; given that adenosine receptors regulate numerous molecular pathways implicated in the pathophysiology

of HF, increased endogenous adenosine levels may reflect an adaptive mechanism to counteract adenosine signalling impairment[26,27]. This is confirmed because increases in the myocardial concentration of adenosine obtained by inhibiting the nucleoside cellular uptake is protective against cardiac remodelling and β-adrenergic dysfunction, thereby counteracting progression to HF[135].

As already outlined, all adenosine receptor subtypes may be involved, in one way or another, in the pathophysiology of HFpEF and related comorbidities (Figures 2 and 3). Data indicate that A_1AR provides cardioprotective effects due to reversal of cardiac hypertrophy/remodelling, improvement of mitochondrial function, enhancement of SERCA2a activity and Ca^{2+} handling, increases in capillary density and oxygen diffusion distance, modifications in substrate utilization, and enhancements in skeletal muscle performance. In addition, A_1AR activation has been associated to anti-ischemic properties by decreasing catecholamine release and β-adrenergic overactivation[43,201]. Activation of A_1AR also improves the metabolic profile[150] and attenuates renal metabolic demand and glomerular filtration pressure[169]. It is, therefore, not surprising that the most expressed adenosine receptor in the heart, the A_1AR, has been the most widely studied receptor in cardiovascular, renal and metabolic diseases, both at preclinical and clinical levels[8,43,154,202]. However, the anti-adrenergic cardioprotective effects of the A_1AR may be partially counteracted by A_{2A}AR activation[203], as detected in cardiac pressure overload[138] and hypertensive animals[204].

However, clinical application of A_1AR full agonists may present some limitations related to off-target side effects, either cardiac (bradycardia up to atrioventricular block, negative dromotropy and inotropy) and extra-cardiac (functional depression of the central nervous system, inhibition of diuresis by reduction in glomerular filtration rate and related vasoconstriction of afferent arteriole, lipolysis and decreased glucose tolerance)[43,201]. Another potential limitation of chronic administration of A_1AR full agonists is receptor desensitization[201]. Mounting preclinical evidence now suggest that partial A_1AR agonists may have a more favourable haemodynamic profile with minimal side effects in terms of heart rate, atrioventricular conduction, blood pressure and renal function, along with no evidence of adenosine-mediated negative inotropism[43,201], agreeing with our prediction that adenosine acting via the A_1AR is a chronoselective atrial depressant[36]. These findings prompted for the development of clinical trials with A_1AR partial agonists for HF either with reduced ejection fraction or HFpEF patients[205]. Data from two small pilot studies demonstrate that the novel orally-available partial A_1AR agonist, neladesonon, seems to be safe with no atrioventricular, neurological, and renal side effects in patients with HF with reduced ejection fraction[206], but no beneficial early changes in the cardiac function were observed (Table I)[206]. Data from a phase II

double-blinded placebo-controlled clinical trial (PANACHE), designed to explore the effects of neladesonon on exercise capacity among 300 patients with HFpEF, has been recently provided (Table I)[202]. Neladesonon did not had a significantly impact on exercise capacity, which was interpreted given to the fact that enrolled HFpEF patients had lower than normal prevalence of coronary artery disease together with a higher prevalence of patients using β-blockers that may abrogate the antiadrenergic effects of the A_1AR partial agonist[202]. Although the results were disappointing, a low incidence of major adverse effects were observed in patients under neladesonon treatment[202], except a dose-dependent decline in renal function and heart rate, reflecting the previously mentioned vulnerable balance between adenosine receptor agonists and antagonists in HF[202].

Data from preclinical studies also ascribed cardioprotective roles to the A_{2A}AR subtype. Activation of A_{2A}AR attenuate cardiac, renal and pulmonary damage due to its ability to counteract inflammation, in addition to its vasodilation and angiogenic properties, while increasing cardiac inotropism without interfering with Ca^{2+} homeostasis[30]. Positive cardiac repercussions against hypertensive remodelling resulting from A_{2A}AR activation may be owe to amelioration of insulin resistance associated with its effects on brown adipocytes and inflammation[107,150]. Controversy still exists regarding the effect of the A_{2A}AR on the whole body insulin sensitivity, since antagonism this receptor in skeletal muscles attenuated whole-body insulin resistance[160]. Like that observed for the A_1AR subtype, the anti-adrenergic effect of the A_{2A}AR is also impaired in hypertensive animals[204]. The chronic use of selective A_{2A}AR agonists are also not free of cardiovascular side effects, like arterial hypotension and tachycardia. Additionally, these compounds may have residual actions on other adenosine receptor subtypes. This pushed forward a new era of nucleoside-5'-monophosphates as prodrugs of A_{2A}AR activators, which require ecto-5'-nucleotidase/CD73 activation in close proximity to receptor sites[207,208], providing that the adenosine forming enzyme is available and functional.

Conflicting evidence exists regarding A_{2B}AR-mediated cardioprotection, as well as its pulmonary effects (Table III)[185]. While activation of A_{2B}AR may counteract fibrosis[112-114] and ischemic preconditioning[209] in the heart, other studies suggest that this low affinity adenosine receptor may be deleterious in ischemic cardiac remodelling[121,122], as well as in renal fibrosis[177,180]. Like A_{2A}AR, the action of the A_{2B}AR in DM and obesity is also controversial[160]. The same occurs regarding the least expressed A_3AR in the heart (Table III)[9]. The A_3AR may be cardioprotective against reperfusion[68-70] by promoting angiogenesis[71], but it may aggravate pathological structural changes in the heart[140] and kidney[186].

Manipulation of endogenous adenosine inactivation, through cellular uptake and/or ADA, may be a valuable strategy to increase the extracellular concentration of the nucleoside

and, thereby, its receptors activation. Inhibition of ENT by dipyridamole attenuates cardiac remodelling and LV dysfunction, prevents β-adrenergic dysfunction and induces coronary vasodilation[135,210]. Downregulation of ADA activity counteracts diet-induced obesity and insulin resistance through decreases in food intake[147]. The adenosine deamination metabolite, inosine, may exert opposite effects to adenosine in the heart via A_3AR receptors, which activation is strengthen when tissues become infiltrated by ADA-bearing inflammatory cells[108]. This concept raises new lines of research using multi-target drugs designed to increase local adenosine levels while decreasing inosine formation (e.g. ADA inhibitors also blocking adenosine cellular uptake, like the lymphocytotoxic drug 2'-deoxycoformycin or pentostatin) applied in combination with enzymatically stable adenosine receptor agonists and/or antagonists[108].

Regulating intracellular adenosine concentration by manipulating ADK, a key intracellular enzyme that is responsible for maintaining low intracellular adenosine levels, thus favouring extracellular adenosine uptake by the cells, may also be a putative strategy to increase the extracellular levels of the nucleoside (Table III). As a matter of fact, inhibition of ADK may be cardioprotective due to increases in the extracellular levels of the adenosine which stimulates angiogenesis, promotes insulin sensitivity, and attenuates inflammation and oxidative stress[100,163,185]; contrariwise, when this enzyme is fully operative reduction of the extracellular levels of the nucleoside may be deleterious as it abrogates the anti-hypertrophic effect of adenosine[141,142].

Aforementioned data suggest that before translating the putative preclinical benefits of manipulating the adenosinergic signals to the therapeutic armamentarium, one must keep in mind that adenosine production and/or inactivation, receptor subtype expression dynamics, and downstream intracellular messenger pathways might change in a moment-to-moment basis according to the tissue, pathological situation and stage of each disease condition. Another pitfall in our current knowledge is due to the relatively complex pathophysiology of HFpEF owing to a limited number of animal models that reflect entire features of the human pathology, thus further challenging translation of preclinical data to clinical settings[211]. Nevertheless, the beauty of targeting the adenosinergic system is that it can differentially control not only heart structure and function abnormalities, but also most of the co-morbidities concurring with HFpEF in terms of precipitating causes and/or aggravating factors.

7. Conclusion

Taken together, these preclinical and clinical evidences shed some lights on the adenosine role in molecular mechanisms and comorbidities related to HFpEF. Adenosine was found to counteract cardiac pathophysiological mechanisms implicated in this clinical syndrome, namely cardiac inflammation and microvascular dysfunction, especially via A_2ARs, in addition to extracellular and cellular structural abnormalities, and energy metabolism and Ca^{2+} handling, being these effects more under A_1AR and A_2AR control. Consequently, adenosine also counteracts cardiac remodelling and related diastolic dysfunction, the pathological hallmark of this clinical syndrome. Considering the new paradigm of a systemic microvascular dysfunction as a perpetuating factor for myocardial dysfunction, adenosine receptors, enzymatic machinery and downstream intracellular messenger pathways ***s***urge as a promising treatment target, in part because it is also implicated in a wide range of pathophysiological process related to HF comorbidities, such as diabetes and metabolic syndrome, along with kidney and pulmonary dysfunction.

Quando fazes o bem, nasce dentro de ti um maravilhoso sentimento

(…) sim, é a isto mesmo que aspiro sentir.

Rabi Harold Kushner

Appendix | Apêndice

Table I: Results of adenosine receptors agonism/antagonism in heart failure with preserved ejection fraction and pulmonary hypertension. Different agonists and antagonists were used to assess adenosine effects on cardiovascular diseases. The grey area corresponds to studies carried out in human patients.

	Adenosine receptors	Adenosine receptor manipulation			Model and species	Outcome	Comments	Refs
		Pharmacological tool						
		Agonist	Antagonist	Other				
Heart failure with preserved ejection fraction				Dipyridamole (Adenosine uptake blocker)	Sprague–Dawley rats (abdominal aortic banding)	↑ Myocardial adenosine levels ↓ Chamber dilatation, LV[a] filling abnormalities and pulmonary congestion Prevented β-adrenergic desensitization	A_1AR[b] desensitization is in line with an increased exposition to adenosine *in vivo*	135
				CD73 KO[c] and CD4 CD73 KO	Mice (transverse aortic constriction)	↑ CD73 exclusively on T cells ↑ T cell enzymatic machinery for formation of AMP[d] ↓ Cardiac function and ↑cardiac fibrosis in both CD73 KO and CD4 CD73 KO mice ↑ Proinflammatory cytokines in CD73 KO mice ↑$A_{2A}AR$ on T cells	Transient ↑ in infiltration of monocytes, granulocytes, and B cells and persistent ↑ of cytotoxic T cells, T-helper cells, and regulatory T cells	83
				CD73-KO	Mice (transaortic constriction)	↑ Myocardial hypertrophy ↑ LV dilation and LV dysfunction	Cardiac dysfunction was not present in healthy animals. Attenuation of myocardial hypertrophy is due to mTOR[e]/p70S6K activation	136
				ADK[f]-KO	Mice (transaortic constriction)	↑ LV hypertrophy and dysfunction ↑ Pulmonary congestion	In rat neonatal cardiomyocytes, ADK activity dampened cardiac growth signalling and excessive microtubule stabilization/detyrosation	142
	A_1AR	CADO[g] (non selective)			C57BL/6 mice (transaortic constriction)	↓ Plasma concentrations of noradrenaline, renin and BNP[h] ↓ Cardiac hypertrophy ↓ Interstitial and perivascular fibrosis ↑ Gene expression of RGS[i]-4 ↓ Myocardial dysfunction and pulmonary congestion	RGS-4 is an inhibitory factor of hypertrophy due to attenuation of G-protein mediating signalling	134
		CPA[j] (selective)				↓ Cardiac hypertrophy ↓ Myocardial dysfunction	These effects were abolished by DPCPX[k]. Studies with CPA and DPCPX to evaluate the role of A_1AR on cardiac fibrosis were not performed	
		CPA (selective)			C57BL/6 mice (phenylephrine)	Upregulation of A_1AR ↓ Cardiac remodelling ↓ Oxidative stress	CPA did not attenuate AngII[l] or IGF[m]-1-induced cardiac hypertrophy	137

		CAP[n] (partial)			Dogs (microembolization-induced HF[o])	↓ Cardiac remodelling ↑ LV function ↑ Capillary density and oxygen diffusion distance ↓ Rate of MPTP[p] opening Normalized SERCA[q]-2a activity and expression of UCP[r]-2 and -3 and GLUT[s]-1 and -4		99
		Neladenoson (selective partial)			11 patients with HFrEF[t] treated on β-blockers therapy	No 2nd or 3rd degree atrioventricular block occurred on 48-hours Holter monitoring	Significant early changes in cardiac function were not detected. No significant change in renal function and neurological side effects were detected	206
					31 patients with HFrEF[u] on β-blockers therapy	No 2nd or 3rd degree atrioventricular -block occurred on 48-hours Holter monitoring. No effects were observed in heart rate and blood pressure		
		Neladenoson (selective partial)			261 patients with HFpEF	No significant dose-response regarding to the change in exercise capacity. No significant improvement in markers of cardiac structure and function related to HFpEF progression. Dose-dependent declines in renal function and heart rate were observed		202
	$A_{2A}AR$			Overexpressed $A_{2A}AR$	Mice (transverse aortic constriction)	Attenuation of ↓ cardiac function ↓ Inflammatory factors genes expression ↓ β-MHC[v], ANP[w], and GATA-4 gene expression ↑ Intracellular calcium homeostasis	GATA-4 controls several genes that are upregulated in cardiac hypertrophy, including β-MHC, cardiac troponin-C, atrial natriuretic factor, NCX[x] and A_1AR	85
		CGS21680 (selective)			C57BJ/6J mice (DOCA[y]- salt)	↓ Cardiac inflammation, fibrosis, hypertrophy, and dysfunction ↑ iBAT[z]-derived FGF21[i]	iBAT surgery depletion induces significantly cardiac remodelling refractory to the cardioprotective function of CGS21680	107
	A_3AR	-	-	A_3AR KO	Mice (transaortic constriction)	↓ Cardiac remodelling ↓ Oxidative stress ↓ANP and LV dysfunction	Attenuation of LV hypertrophy and dysfunction by reducing activation of the MAPK[ii] and PI3K-Akt[iii] signalling pathways	140
Pulmonary hypertension	$A_{2A}AR$	-	-	$A_{2A}AR$ KO	Mice (hypoxia)	↑ RVSP[iv] and Fulton index ↑ Thickness in pulmonary resistance vessels ↑ RhoA and ROCK expression	RhoA and ROCK are signalling pathways related to pulmonary vascular remodelling and PAH[v] generation	193
		LASSBio-1359 (selective)			Wistar rats (MCT[vi]-IPHM[vii])	↑ PA[viii] vasorelaxation and flow ↓ Pulmonary vascular remodelling ↓ RV[ix] remodelling and RVSP ↑ LV stroke volume and cardiac output		194

		LASSBio-1386 (selective)			Wistar rats (MCT-IPHM)	↑ Exercise tolerance ↑ PA vasorelaxation and flow ↓ Pulmonary vascular remodelling ↓ RV remodelling and RVSP ↑ LV stroke volume and cardiac output ↑ eNOSx[x] and A_{2A}AR expression in lungs ↑ A_{2A}AR and SERCA2a expression in RV ↑ Ca^{2+}-ATPase activity in RV	A_{2A}AR agonism attenuates RV dysfunction indirectly by counteracting structural and functional changes in pulmonary vasculature	195
		LASSBio-1359+	Selective A_{2A}AR agonist	Sildenafil (PDE5i[xi])	Wistar rats (MCT-IPHM)	↓Endothelial dysfunction ↓ RV overload, hypertrophy, and dysfunction	Monotherapy with low doses of LASSBio-1359 or Sildenafil did not have an impact in these outcomes	196
	A_{2B}AR	-	-		13 COPD[xii] with or without PH[xiii]patients	↑ Pulmonary vascular remodelling in COPD-PH patients ↑ A_{2B}AR gene and protein expression		199

Abbreviations: LV[a] – Left ventricle; AR[b] – Adenosine receptor; KO[c] – Knockout; AMP[d] – 5'-adenosine monophosphate; mTOR[e] – Mechanistic target of rapamycin; ADK[f] – Adenosine kinase; CADO[g] – 2-chloroadenosine; BNP[h] – B-type natriuretic peptide; RGS[i] – Regulator of G protein signalling; CPA[j] – N6-cyclopentyladenosine; DPCPX[k] – 8-Cyclopentyl-1,3-dipropylxanthine; AngII[l] – Angiotensin II; IGF[m] – Insulin growth factor; CAP[n] – Capadesonon; HF[o] – Heart failure; MPTP[p] – Membrane permeability pores; SERCA[q] – sarco/endoplasmic reticulum Ca2+-ATPase; UCP[r]- Uncoupling protein; GLUT[s] – Glucose transporter type; HFrEF[t]– Heart failure with reduced ejection fraction; HFpEF[u]– Heart failure with preserved ejection fraction; β-MHC[v] – myosin heavy chain-beta; ANP[w] – Atrial natriuretic peptide; NCX[x]– Sodium-calcium exchanger; DOCA[y]– Deoxycorticosterone acetate; iBAT[z]– interscapular brown adipose tissue;FGF21[z] – Fibroblast growth factor 21; MAPK[ii] – mitogen-activated protein kinase; PI3K-Akt[i] – phosphoinositol-3 kinase- protein kinase B; RVSP[iv]- Right ventricle systolic pressure; PAH[v] – Pulmonary arterial hypertension; MCT[vi] – Monocrotaline; IPHM[vii] –Induced pulmonary hypertension model; PA[viii] – Pulmonary artery; RV[ix] – Right ventricle; eNOS[x] – Endothelial nitric oxide synthase; PDE5i[xi] – Phosphodiesterase type 5 inhibitor; COPD[xii] – Chronic obstructive pulmonary disease; PH [xiii] – Pulmonary hypertension.

Table II: Results of adenosine receptors agonism/antagonism in diabetes mellitus/obesity and chronic kidney disease. Different agonists/antagonists were used to assess its effects on HFpEF comorbidities. The grey area corresponds to studies carried out in human patients.

	Adenosine receptors	Adenosine receptor manipulation			Model and species	Outcome	Comments	Refs
		Pharmacological tool						
		Agonist	Antagonist	Other				
Diabetes mellitus/ Obesity				ADAR1[a] deficiency	Mice (HFD[b])	↓ Fat mass, dyslipidemia, and insulin resistance parameters ↓ Food intake and gastric ghrelin ↑ Serum peptide YY	No difference was shown on fat mass in ADAR1 deficient mice under chow diet compared to HFD	147
	A_1AR[c]	CPA[d] (selective)			Wistar rats (STZ[e] injection)	↓Plasma glucose, cholesterol, and triglyceride ↑ Glucose uptake into peripheral tissues ↑ Uptake and glycogen synthesis	Plasma glucose lowering effect mediated by endogenous insulin was negligible	153
	A_1AR	GS-9667 (selective, partial)			55 non-obese and 23 overweight/ obese patients	↓ Plasma FFA[f] Well-tolerated No desensitization or rebound	Partial A_1AR agonism was aimed to reduce side effects	146
	A_1AR $A_{2A}AR$ $A_{2B}AR$ (respectively)		DPCPX[g] SCH58261 MRS1754 (all selective)		Wistar rats (HSu[h] diet)	SCH58261 and MRS1754 decreased insulin sensitivity in control animals and improved insulin sensitivity in the skeletal muscle of HSu animals. DPCPX reverted the increase in total and visceral fat induced by HSu diet.	Adenosine exerts opposite effects on insulin sensitivity under control or resistant states Under context of insulin resistance, A_2AR in the skeletal muscle, rather than on adipose tissue, is the main mediator of whole-body insulin sensitivity	160
	$A_{2B}AR$		MRS1754 (selective)	ADK[i] deficiency	Mice (HFD)	↑Glucose tolerance and insulin sensitivity ↓ Metabolic syndrome ↑ Intracellular adenosine ↑ NO[j] production due to ↑ NOS3[k] activity and modulation of NOS3 by $A_{2B}AR$	Feeding mice with a HFD enhanced expression of endothelial ADK. $A_{2B}AR$ antagonism abolished NOS3 expression, but was not associated with increased phosphorylation of NOS3	163
Renal disease				BT702 (ADK inhibition)	C57BL/6 mice (STZ injection)	↓ Hyperglycemia ↓ Albuminuria and markers of glomerular injury ↓ Renal oxidative stress and inflammatory parameters Restored glomerular permeability and filtration function	The reduced renal inflammation is consistent with the reduction in renal macrophage infiltration, NF-κB[l] phosphorylation and MCP-1[m] excretion The increased eNOS[n] expression counteracts renal oxidative stress	185

							Attenuated glomerular damage is attributed to increased MAPK[o] phosphorylation	
	A_1AR	CCPA[p] (selective)			C57BL/6 mice (renal I/R injury)	↑ Renal function ↓ inflammatory markers, necrosis, and apoptosis		171
			DPCPX (selective)			↓ Renal function ↑ Inflammatory markers, necrosis, and apoptosis		
		CCPA (selective)			C57BL/6 mice (renal I/R injury)	↑ HSP27[q] expression ↑ Renal function ↓ Pro-inflammatory cytokines ↓ Caspase 3 activity	HSP27 is an anti-apoptotic molecule as it is associated with an increased resistance to stressful conditions, such as oxidative stress and exposure to toxic drugs A_1AR- mediated renal protection, anti-inflammatory and anti-apoptotic effects were removed by inhibition of HSP27	172
			DPCPX (selective)			↑ Natriuresis and diuresis ↓ HSP27 expression		
			KW-3902 (rolofylline)		32 patients with congestive HF[s] and renal impairment	↑ GFR[t] and renal plasma flow	The increased in GFR persisted more time than the predicted by pharmacokinetics	174
			KW-3902 (rolofylline)		146 patients with volume overload and an estimated CrCl[r] of 20-80 mL/min	↑ Urine output	A_1AR antagonism enhances the response to loop diuretics	175
	$A_{2B}AR$		MRS1754 (selective)		C75BL/6 mice (STZ injection)	↓ Albuminuria ↑ Renal function ↓ VEGF-A[u] expression ↑ NO production ↓ Glomerulosclerosis	In diabetic mice kidney, there was a significant increase in VEGF-A and $A_{2B}AR$ gene expression	180
	$A_{2A}AR$		ZM241385 (selective)	Spironolactone (mineralocorticoid receptor antagonist)	Sprague-Dawley rats (isoprenaline)	↑ $A_{2A}AR$ ↓ EndMT[v] process ↓ Cardiac and renal fibrosis and dysfunction	ZM241385 exacerbated cardiorenal remodelling and dysfunction	181
		CGS21680 (selective)			Sprague-Dawley rats (STZ injection)	↓ Glomerular hyperfiltration ↓ Albuminuria ↓ Immune cells infiltration	$A_{2A}AR$ stimulation did not decrease filtrated fraction during NOS inhibition, demonstrating that $A_{2A}AR$-mediated	182

						↓ Urine excretion of pro-inflammatory cytokines	vasodilatation of efferent arterioles to be NO dependent	
		CGS21680 (selective)			Sprague-Dawley rats (STZ injection)	↓ Proteinuria and glomerular damage ↓ TNF-α[w] secretion and macrophages infiltration	These results that $A_{2A}AR$ stimulation exerts renoprotective effects via an anti-inflammatory mechanism	184
	-	CADO[x] (non selective)			SHR[y]-STZ rats	↓ Hyperglycaemia and glycosuria ↓ Proteinuria ↓ Collagen deposition and oxidative stress in the renal glomeruli ↑ Immunoreactivity against $A_{2A}AR$		186
			DPSPX[z] (non selective)			↑ Renal fibrosis ↓ Immunoreactivity against A_3AR	Treatment with DPSPX did not altered the global metabolic status and renal function. The downregulation of A_3AR may reflect a renoprotective mechanism	
	A_3AR		LJ-6898 (selective)		C57BLKS/J-db/db	↓ Albuminuria and glomerular hypertrophy ↓ Renal fibrosis, inflammation, and oxidative stress ↓ Lipid accumulation ↑ PCG1α[z] ↑ $A_{2A}AR$ expression	These effects were on the same range of losartan PCG1α is key regulator of mitochondrial biogenesis	187

Abbreviations: ADAR1[a]– ADA acting on RNA 1; HFD[b]– High fat diet; AR[c] – Adenosine receptor; CPA [d] – N6-cyclopentyladenosine; STZ[e]– Streptozotocin; FFA[f]– Free fatty acid; DPCPX[g] – 8-Cyclopentyl-1,3-dipropylxanthine; HSu[h] – High-sucrose; ADK[i] – Adenosine kinase; NO[j] – Nitric oxide; NOS[k] – Nitric oxide synthase; NF-κB[l]– Nuclear factor-kappa B; MCP-1[m]– Monocyte chemoattractant protein-1; eNOS[n]– Endothelial nitric oxide synthase; MAPK[o]– Mitogen-activated protein kinases; CCPA[p]– 2-Chloro-N6-cyclopentyladenosine; HSP27 [q]– Heat shock protein 27; CrCl[r]– Clearance of creatinine; HF[s] – Heart failure; GFR [t] – Glomerular filtration rate; VEGF-A[u] – Vascular endothelial growth factor-A; EndMT[v] – Endothelial-to-mesenchymal transition; TNF-α[w]– Tumour necrosis factor- alpha; CADO[x] – 2-chloroadenosine; SHR[y] – Spontaneously hypertensive rat; DPSPX[z] – 1,3-dipropyl-8-sulfophenylxanthine.

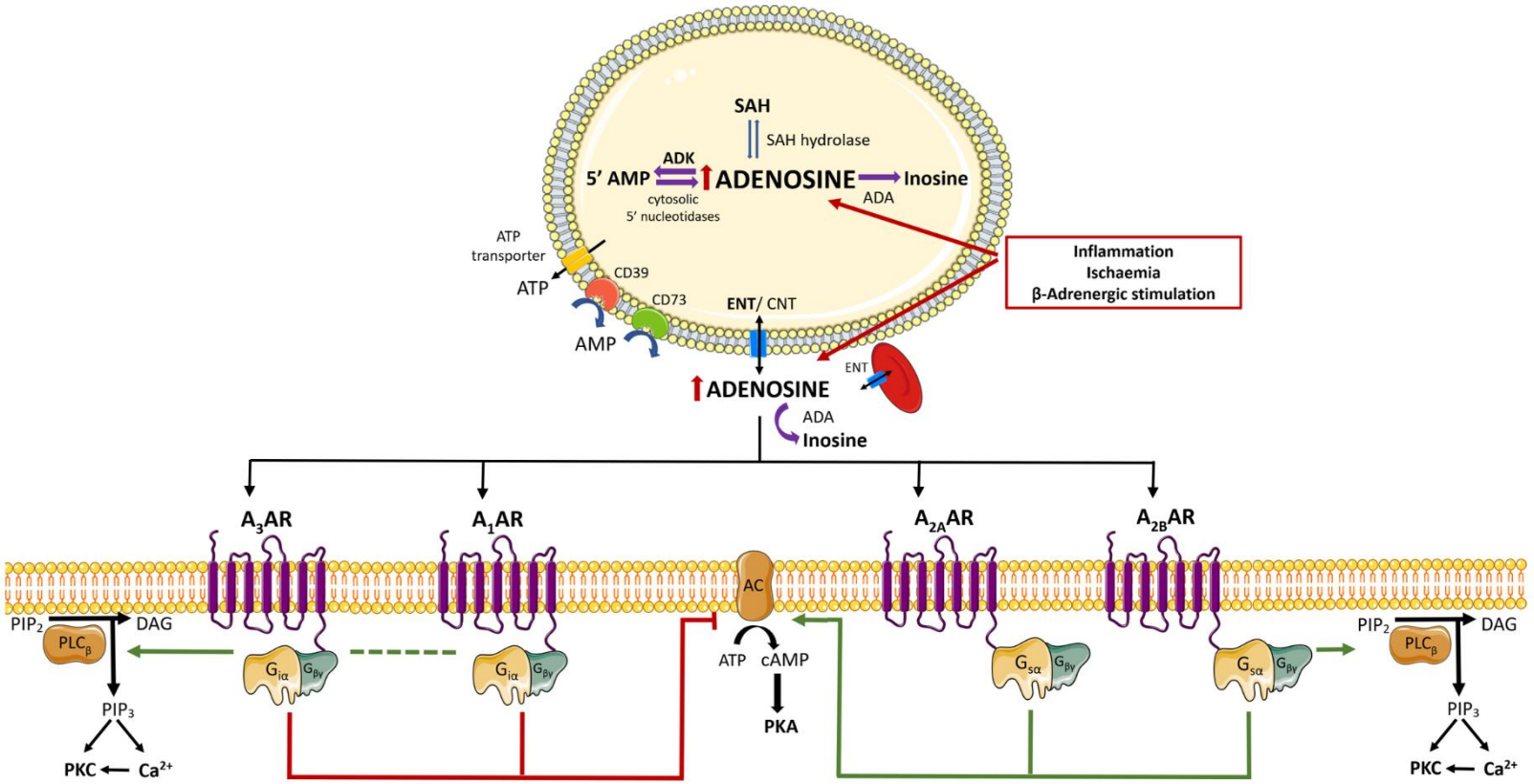
Table III: Pros and cons of targeting adenosine receptors, enzymes and metabolism in molecular pathways underlying heart failure with preserved ejection fraction and cardioprotection.

Adenosine signalling manipulation	Pharmacological approach		Refs
	Activation	Inactivation	
Protective role			
A_1AR[a]	Decreased cardiac hypertrophy and dysfunction		99, 134, 137
	Counteracted β-adrenergic effects on cardiac remodelling through inhibition of cAMP[b] stimulated-PKA[c] activation and inhibition of noradrenaline release from cardiac nerves		133,138
	Attenuated mitochondrial dysfunction by decreased opening rate of mitochondrial pores		99
	Change in energy substrate utilization with increased expression plasma membrane expression of GLUT[d]-1 and -4		99
	Ameliorated SERCA2a[e] function and improved intracellular calcium handling		99
	Myocardial protection under ischemic preconditioning and ischemia		43-45
	Increased capillary density and oxygen diffusion distance		99
	Improved metabolic profile through regulating insulin sensitivity and glucose tolerance		146,151,153
	Attenuated kidney metabolic demand, tubular cell apoptosis and inflammatory reaction, along with a reduction of the glomerular filtration pressure		171, 172
$A_{2A}AR$ $A_{2B}AR$	Endothelial dependent and independent coronary vasodilation		52, 53,54, 55
	Angiogenic properties		56,57
$A_{2A}AR$	Decreased cardiac inflammation, fibrosis, hypertrophy and dysfunction		107
	Enhanced intracellular calcium homeostasis		85
	Enhanced cardiac contractility		46
	Cardioprotection during early reperfusion after MI[f]		58-60
	Improved lipolysis, browning process of white adipose tissue, and insulin signalling; decreased levels of free fatty acids		150
	Decreased renal inflammation, fibrosis and oxidative stress Attenuated glomerular hyperfiltration Improved renal function		169,181-184,186
	Decreased pulmonary vascular remodelling Attenuated right ventricle hypertrophy and dysfunction		194-196
$A_{2B}AR$	Attenuated cardiac fibrosis *in vitro*		112-114

	Reduced infarct size under ischemic preconditioning		209
	Decreased adipogenesis, increases lipolysis and improved insulin signalling		157,158
	Decreased pulmonary artery pressure and proliferation of smooth muscle fibers		33
A_3AR	Stimulated angiogenesis		71
	Exerted cardioprotective effects during reperfusion phase after MI		68-70
ENT[g]	Increased plasma adenosine Significantly improved LV[h] filling and decreased pulmonary congestion Attenuated β-adrenergic dysfunction		135
	Coronary vasodilation		210
CD73		Increased cardiac inflammation, fibrosis, hypertrophy, and dysfunction	83,136
		Increased infarct size under ischemic preconditioning	209
ADK[i]		Attenuated the adenosine mediated anti-hypertrophic effect	141,142
Deleterious role			
A_1AR		Increased natriuresis and diuresis	174,175, 176
A_{2A}AR A_{2B}AR		Rescue the whole-body insulin sensitivity under high-sucrose diet	160
A_{2B}AR		Attenuated the post-MI cardiac remodelling	121,122
		Attenuated glomerulosclerosis and improved renal function	177,180
		Attenuation of pulmonary vascular remodelling and hypertension	185
A_3AR		Attenuated cardiac hypertrophy, fibrosis and oxidative stress and dysfunction	140
		Decreased renal fibrosis, inflammation, oxidative stress, and lipid accumulation Decreased glomerular damage	186
ADK		Epigenetic upregulation of pro-angiogenic genes and consequent endothelial proliferation and ischemia-induced angiogenesis	100
		Protection against the high fat diet induced insulin resistance and metabolic syndrome, in part due to its endothelial anti-inflammatory effects	163
		Decreased renal inflammation and oxidative stress Attenuated glomerular damage	185
ADA[j]		Attenuated the diet-induced obesity and insulin resistance through decreased food intake	147

Abbreviations: AR[a] – Adenosine receptor; cAMP[b] – cyclic 5'-adenosine monophosphate; PKA[c] – protein kinase A; GLUT[d] – Glucose transporter type; SERCA[e] – sarco/endoplasmic reticulum Ca^{2+}– ATPase; MI[f] – Myocardial infarction; ENT[g] – Equilibrative nucleoside transporters; LV[h] – Left ventricle; ADK[i] – Adenosine kinase; ADA[j] – Adenosine deaminase.

Figure 1:

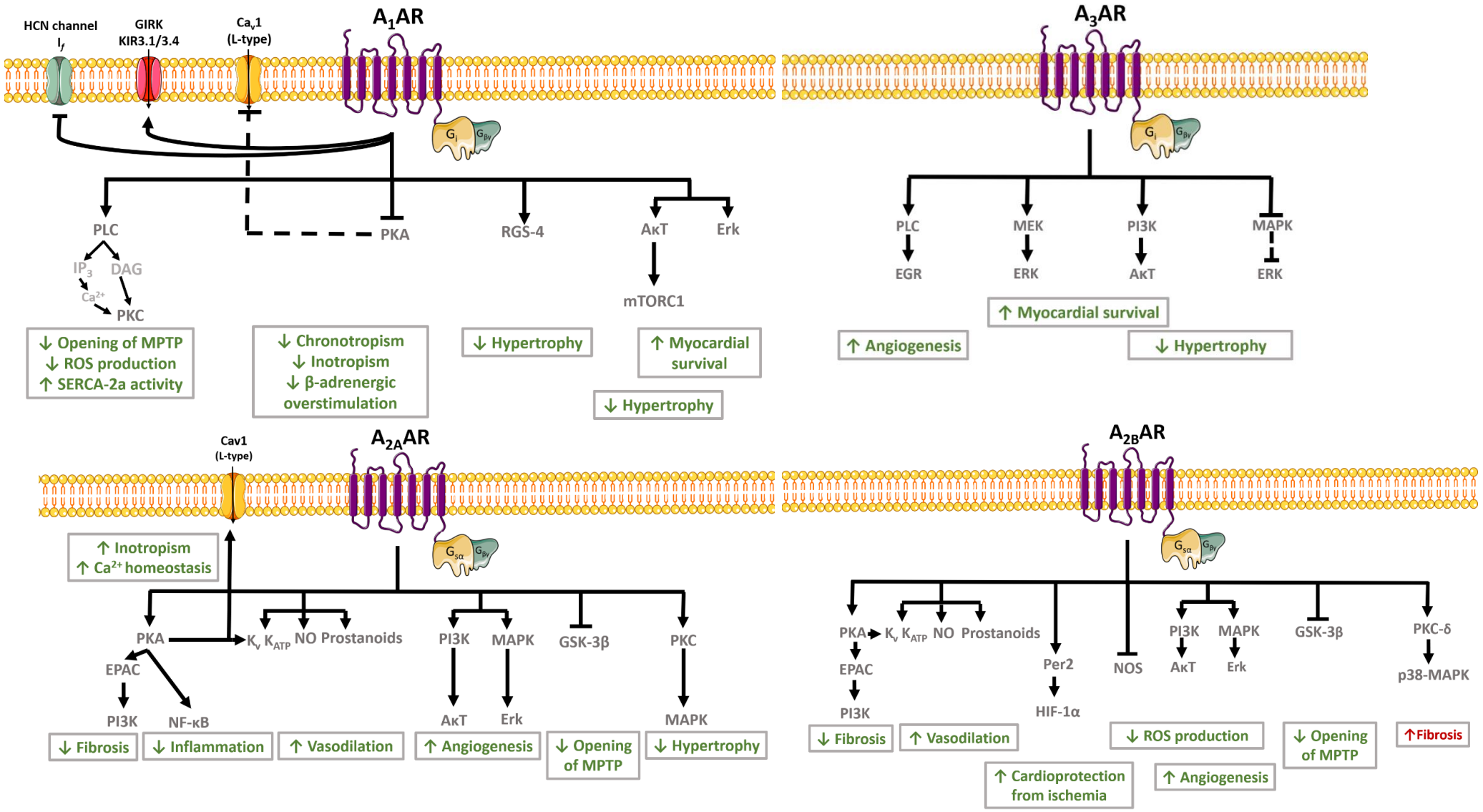


Schematic representation of adenosine biosynthesis and complex signalling pathways induced by adenosine receptors activation. Adenosine is a purine nucleoside continuously generated from the catabolism of adenine nucleotides via a cascade of nucleotidases. Regarding the fate of intracellular adenosine, it may (preferentially) re-enter the purines savage pathway by intracellular phosphorylation via adenosine kinase (ADK) or it is inactivated to inosine by adenosine deaminase (ADA). The main source of adenosine results from the hydrolysis of S-adenosylhomocysteine (SAH) by SAH hydrolase. Under low oxygen availability and/or during high working loads energy, recruitment from ATP hydrolysis increases favouring intracellular adenosine accumulation, which promotes its diffusion to the extracellular medium via equilibrative nucleoside transporters (ENTs) and concentrative nucleoside transporters (CNTs).

Extracellular adenosine may also result from the extracellular breakdown of released adenine nucleotides, namely 5'-adenosine triphosphate (ATP), ADP (5'adenosine diphosphate) and AMP (5'-adenosine monophosphate), by a cascade of ecto-nucleotidases bound to the plasma membrane. CD39 dephosphorylates ATP directly into AMP; the rate limiting enzyme of the ecto-nucleotidase cascade is ecto-5'-nucleotidase/CD73, which dephosphorylates AMP to adenosine and inorganic phosphate. Extracellular adenosine levels are tightly regulated by cellular uptake via ENTs and/or by deamination into inosine by ADA. Adenosine activates four G protein-coupled receptor (GPCRs) known as P1 receptors (adenosine receptors (ARs): A_1AR, A_{2A}AR, A_{2B}AR, A_3AR). In brief, A_1AR and A_3AR are negatively coupled to adenylate cyclase (AC) through binding to G_i and G_o proteins, resulting in decreased intracellular cyclic AMP (cAMP) levels. Both A_{2A}AR and A_{2B}AR are coupled to G_s proteins and stimulate AC leading to increases in cAMP accumulation. Despite the canonical positive and negative coupling to the AC/cAMP system, A_1AR, A_{2B}AR and A_3AR are also entitled to activate phospholipase C-beta (PLC- β) isoform, resulting in increased inositol 1,4,5-trisphosphate (IP3) and intracellular Ca^{2+} mobilization; intracellular Ca^{2+} and diacyglycerol (DAG) production contribute to stimulate Ca^{2+}-dependent protein kinase C (PKC) and/or downstream Ca^{2+}-dependent pathways. Adapted from Borea et al. (2018)[9]. Illustration used elements from Servier Medical Art (http://smart.servier.com).

Abbreviations: AC – Adenosine cyclase; ADA – Adenosine deaminase; ADP – 5'adenosine diphosphate; ADK – Adenosine kinase; AMP – 5'-adenosine monophosphate; AR – Adenosine receptor; ATP – 5'-adenosine triphosphate; cAMP – cyclic AMP; CNT – Concentrative nucleoside transporter; DAG – Diacyglycerol; ENT – Equilibrative nucleoside transporter; GCPR – G protein-coupled receptor; IP3 – inositol 1,4,5-trisphosphate; PKA – protein kinase A; PKC – protein kinase C; PLC- β – Phospholipase C- beta; SAH – S-adenosylhomocysteine.

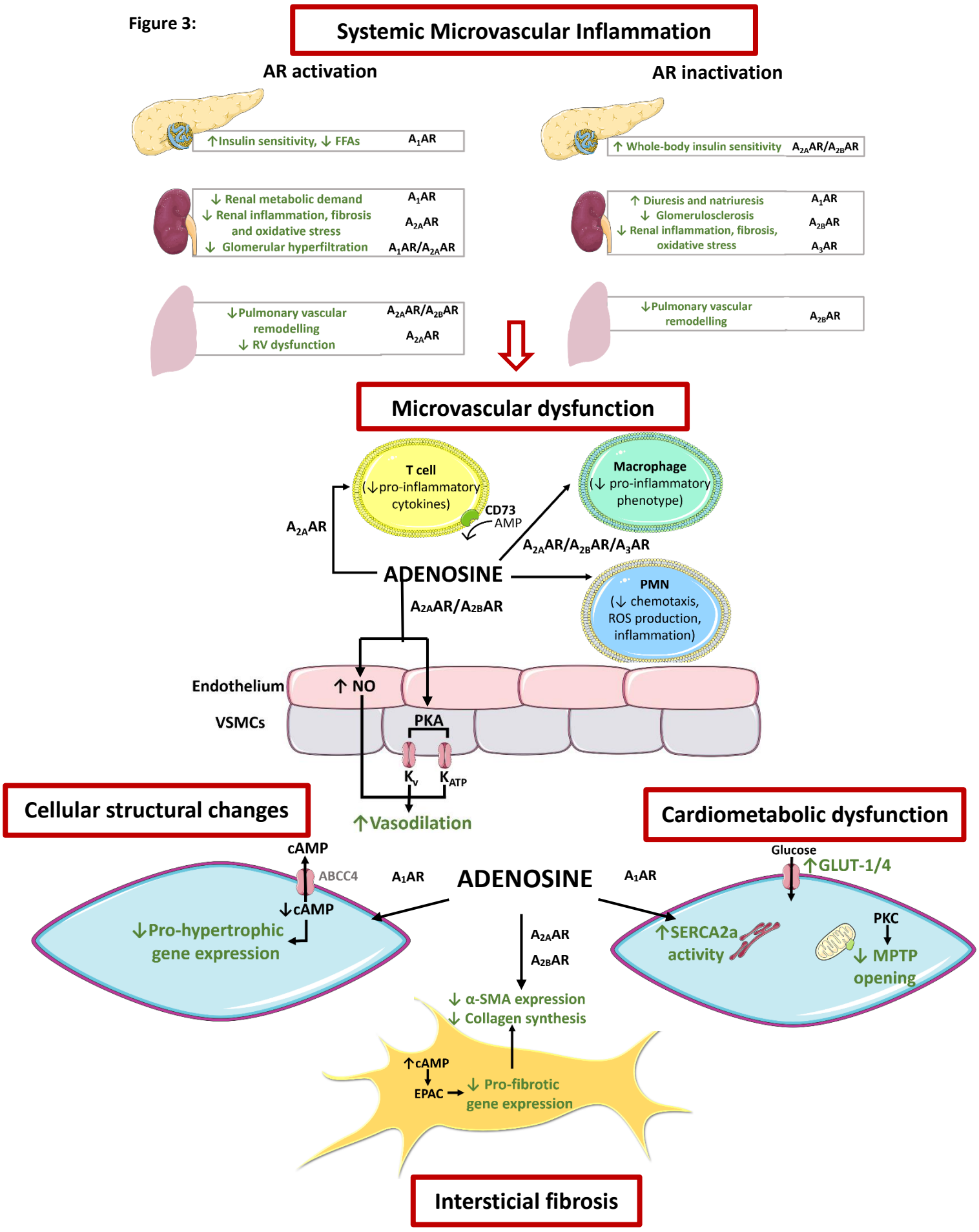
Figure 2:


HCN channel
I_f
GIRK
KIR3.1/3.4
Ca_v1
(L-type)
A_1AR
G_i $G_{\beta\gamma}$
PLC
IP_3 DAG
Ca^{2+}
PKC
PKA
RGS-4
AκT
Erk
mTORC1
↓ Opening of MPTP
↓ ROS production
↑ SERCA-2a activity
↓ Chronotropism
↓ Inotropism
↓ β-adrenergic overstimulation
↓ Hypertrophy
↑ Myocardial survival
↓ Hypertrophy
A_3AR
G_i $G_{\beta\gamma}$
PLC
MEK
PI3K
MAPK
EGR
ERK
AκT
ERK
↑ Myocardial survival
↑ Angiogenesis
↓ Hypertrophy
Cav1
(L-type)
$A_{2A}AR$
$G_{s\alpha}$ $G_{\beta\gamma}$
↑ Inotropism
↑ Ca^{2+} homeostasis
PKA
K_v K_{ATP}
NO
Prostanoids
PI3K
MAPK
GSK-3β
PKC
EPAC
PI3K
NF-κB
AκT
Erk
MAPK
↓ Fibrosis
↓ Inflammation
↑ Vasodilation
↑ Angiogenesis
↓ Opening of MPTP
↓ Hypertrophy
$A_{2B}AR$
$G_{s\alpha}$ $G_{\beta\gamma}$
PKA
K_v K_{ATP}
NO
Prostanoids
Per2
NOS
PI3K
MAPK
GSK-3β
PKC-δ
EPAC
PI3K
HIF-1α
AκT
Erk
p38-MAPK
↓ Fibrosis
↑ Vasodilation
↓ ROS production
↓ Opening of MPTP
↑Fibrosis
↑ Cardioprotection from ischemia
↑ Angiogenesis

Schematic representation of cardiovascular effects of adenosine and related signalling pathways. In the cardiovascular system, adenosine exerts protective effects to control neuronal output and inflammation, while decreasing cardiac metabolism, myocardial contractility, impulse generation and conduction, and the coronary tone, as well as adrenergic responsiveness. These effects depend on the cellular type involved. The A_1AR activation mediates cardioprotective effects by reversing cardiac hypertrophy/remodelling, improving mitochondrial function, enhancing SERCA2a activity and improving Ca^{2+} handling; also, an anti-ischemic effect by decreasing catecholamine release and counteracting β-adrenergic dysfunction is associated with A_1AR activation. Activation of A_{2A}AR activation attenuates inflammation, fibrosis and hypertrophy, in addition to vasodilation and angiogenesis. Despite the A_{2B}AR activation-related vasodilation, angiogenesis, and protection from ischemic preconditioning, conflicting evidence exists regarding its role in ischemia and related cardiac remodelling. The A_3AR activation also exhibits contradictory results; it has cardioprotective effects regarding angiogenesis and in reperfusion setting while may aggravate cardiomyocytes hypertrophy. The arrows and bars indicate the effect mediated by the activation or inactivation of the adenosine receptors, respectively. The dashed bars indicate the effect indirectly blocked by adenosine receptor activation. Illustration used elements from Servier Medical Art (http://smart.servier.com).

Abbreviations: AR – Adenosine receptor; AκT – Protein kinase B; Ca_v1 channel – L-type calcium channel subunit 1; DAG– Diacyglycerol; EGR – Early Growth Response; EPAC – Exchange protein directly activated by cAMP; ERK – Extracellular signal-regulated protein kinase; GIRK and KIR3.1/3.4 – G protein coupled inwardly rectifying K^+ channels; GSK-3 – Glycogen synthase kinase-3; HCN channels – Hyperpolarization-activated cyclic nucleotide-gated channel; HIF-1α – Hypoxia-inducible factor- alpha; IP_3– Inositol 1,4,5-trisphosphate; I_f– "funny" hyperpolarization-activated current; K_{ATP} – ATP-sensitive K^+ channel; K_V – voltage-gated K^+ channels; MAPK – Mitogen activated protein kinase; MEK – Mitogen-activated protein kinase kinase; MPTP – Mitochondrial permeability transition pores; mTORC1 – Rapamycin complex 1; NF-κB – Nuclear factor-κB; NO – Nitric oxide; Per2 – Period 2; PI3K – Phosphoinositol-3 kinase; PKA – Protein kinase A; PKC– Protein kinase C; PLC – Phospholipase C; ROS – Reactive oxygen species; SERCA2a – Sarco/endoplasmic reticulum Ca^{2+}-ATPase 2a.

Figure 3:


Systemic Microvascular Inflammation
AR activation
AR inactivation
↑Insulin sensitivity, ↓ FFAs
A1AR
↑ Whole-body insulin sensitivity
A2AAR/A2BAR
↓ Renal metabolic demand
↓ Renal inflammation, fibrosis and oxidative stress
↓ Glomerular hyperfiltration
A1AR
A2AAR
A1AR/A2AAR
↑ Diuresis and natriuresis
↓ Glomerulosclerosis
↓ Renal inflammation, fibrosis, oxidative stress
A1AR
A2BAR
A3AR
↓Pulmonary vascular remodelling
↓ RV dysfunction
A2AAR/A2BAR
A2AAR
↓Pulmonary vascular remodelling
A2BAR
Microvascular dysfunction
T cell
(↓pro-inflammatory cytokines)
CD73
AMP
A2AAR
Macrophage
(↓ pro-inflammatory phenotype)
A2AAR/A2BAR/A3AR
ADENOSINE
PMN
(↓ chemotaxis, ROS production, inflammation)
A2AAR/A2BAR
Endothelium
↑ NO
VSMCs
PKA
Kv
KATP
↑Vasodilation
Cellular structural changes
cAMP
ABCC4
A1AR
ADENOSINE
A1AR
↓cAMP
↓Pro-hypertrophic gene expression
A2AAR
A2BAR
↓ α-SMA expression
↓ Collagen synthesis
↑cAMP
EPAC
↓ Pro-fibrotic gene expression
Cardiometabolic dysfunction
Glucose
↑GLUT-1/4
↑SERCA2a activity
PKC
↓ MPTP opening
Interstitial fibrosis

Schematic representation of the cardiac pathophysiology of HFpEF and the related role of adenosine. HFpEF is a state of systemic inflammation evidenced by increased levels of circulating pro-inflammatory markers. The most important comorbidities inducing a systemic inflammatory state are diabetes mellitus, metabolic syndrome, pulmonary disease and kidney disease. Microvascular dysfunction is proposed as the cornerstone of the complex molecular pathways that drive clinical manifestations in HFpEF**.** Structural abnormalities in HFpEF consist mainly on cardiomyocyte hypertrophy and interstitial fibrosis and develop as a consequence of pathophysiological myocardial stressful inflammatory and pressure insults. Together with abnormal mitochondrial structure and function, and intracellular calcium overload, these pathological changes ultimately lead to LV diastolic dysfunction, an hallmark of HFpEF. Adenosine was found to counteract cardiac pathophysiological mechanisms implicated in this clinical syndrome, such as cardiac inflammation and microvascular dysfunction, via A_2AR activation, extracellular and cellular structural abnormalities, through A_2AR and A_1AR activation, and energy metabolism and calcium handling, via A_1AR activation. Consequently, adenosine also counteracts cardiac remodelling and related diastolic dysfunction, the pathological hallmark that clinically manifested by a congestive phenotype. Moreover, adenosine receptors play a role in cardiometabolic comorbidities related to this clinical syndrome, specially through A_1AR and A_{2A}AR activation. The arrows and bars indicate the effect mediated by the activation or inactivation of the adenosine receptors, respectively. Adapted from Lam et al. (2018)[75] and Headrick et al. (2013)[30]. Illustration used elements from Servier Medical Art (http://smart.servier.com).

Abbreviations: ABCC4 – ATP-binding cassette sub-family C member 4; AR – Adenosine receptor; cAMP – cyclic adenosine monophosphate; cGMP – cyclic guanosine monophosphate; Epac – exchange factor directly activated by cAMP; FFA – Free fatty acid; GLUT – Glucose transporter; MPTP – mitochondrial permeability transition pores; NO – Nitric oxide; PI3K – Phosphoinositol-3 kinase; PKA – Protein kinase A; PKC – Protein kinase C; PKG – Protein kinase G; SERCA2a – sarco/endoplasmic reticulum Ca^{2+}-ATPase 2a; SMA – Smooth muscle actin; VSMC – Vascular smooth muscle cell.

Bibliography | Bibliografia

1. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur Heart J 2016;37(27):2129-2200. (In eng). DOI: 10.1093/eurheartj/ehw128.
2. Dunlay SM, Roger VL, Redfield MM. Epidemiology of heart failure with preserved ejection fraction. Nat Rev Cardiol 2017;14(10):591-602. (In eng). DOI: 10.1038/nrcardio.2017.65.
3. Paulus WJ, Tschöpe C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. J Am Coll Cardiol 2013;62(4):263-71. (In eng). DOI: 10.1016/j.jacc.2013.02.092.
4. Simmonds SJ, Cuijpers I, Heymans S, Jones EAV. Cellular and Molecular Differences between HFpEF and HFrEF: A Step Ahead in an Improved Pathological Understanding. Cells 2020;9(1) (In eng). DOI: 10.3390/cells9010242.
5. Ilieșiu AM, Hodorogea AS. Treatment of Heart Failure with Preserved Ejection Fraction. Adv Exp Med Biol 2018;1067:67-87. (In eng). DOI: 10.1007/5584_2018_149.
6. Drury AN, Szent-Györgyi A. The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart. J Physiol 1929;68(3):213-37. (In eng). DOI: 10.1113/jphysiol.1929.sp002608.
7. Newby A, Worku Y, Meghji P, Nakazawa M, Skladanowski A. Adenosine: A Retaliatory Metabolite or Not? Physiology 1990;5(2):67-70. DOI: 10.1152/physiologyonline.1990.5.2.67.
8. Borah P, Deka S, Mailavaram RP, Deb PK. P1 Receptor Agonists/Antagonists in Clinical Trials - Potential Drug Candidates of the Future. Curr Pharm Des 2019;25(26):2792-2807. (In eng). DOI: 10.2174/1381612825666190716111245.
9. Borea PA, Gessi S, Merighi S, Vincenzi F, Varani K. Pharmacology of Adenosine Receptors: The State of the Art. Physiol Rev 2018;98(3):1591-1625. (In eng). DOI: 10.1152/physrev.00049.2017.
10. Rubio R, Berne RM, Dobson JG, Jr. Sites of adenosine production in cardiac and skeletal muscle. Am J Physiol 1973;225(4):938-53. (In eng). DOI: 10.1152/ajplegacy.1973.225.4.938.
11. Schrader J, Schütz W, Bardenheuer H. Role of S-adenosylhomocysteine hydrolase in adenosine metabolism in mammalian heart. Biochem J 1981;196(1):65-70. (In eng). DOI: 10.1042/bj1960065.
12. Governo RJ, Deuchars J, Baldwin SA, King AE. Localization of the NBMPR-sensitive equilibrative nucleoside transporter, ENT1, in the rat dorsal root ganglion and lumbar spinal cord. Brain Res 2005;1059(2):129-38. (In eng). DOI: 10.1016/j.brainres.2005.08.017.
13. Dip RG. Adenosine receptor modulation: potential implications in veterinary medicine. Vet J 2009;179(1):38-49. (In eng). DOI: 10.1016/j.tvjl.2007.08.005.
14. Eltzschig HK, Abdulla P, Hoffman E, et al. HIF-1-dependent repression of equilibrative nucleoside transporter (ENT) in hypoxia. J Exp Med 2005;202(11):1493-1505. (In eng). DOI: 10.1084/jem.20050177.
15. Morote-Garcia JC, Rosenberger P, Nivillac NM, Coe IR, Eltzschig HK. Hypoxia-inducible factor-dependent repression of equilibrative nucleoside transporter 2 attenuates mucosal inflammation during intestinal hypoxia. Gastroenterology 2009;136(2):607-18. (In eng). DOI: 10.1053/j.gastro.2008.10.037.

16. Ciacciarelli M, Zerbinati C, Violi F, Iuliano L. Dipyridamole: a drug with unrecognized antioxidant activity. Curr Top Med Chem 2015;15(9):822-9. (In eng). DOI: 10.2174/1568026615666150220111942.
17. Pastor-Anglada M, Pérez-Torras S. Emerging Roles of Nucleoside Transporters. Front Pharmacol 2018;9:606. (In eng). DOI: 10.3389/fphar.2018.00606.
18. Lazarowski ER, Sesma JI, Seminario-Vidal L, Kreda SM. Molecular mechanisms of purine and pyrimidine nucleotide release. Adv Pharmacol 2011;61:221-61. (In eng). DOI: 10.1016/b978-0-12-385526-8.00008-4.
19. Yegutkin GG. Nucleotide- and nucleoside-converting ectoenzymes: Important modulators of purinergic signalling cascade. Biochim Biophys Acta 2008;1783(5):673-94. (In eng). DOI: 10.1016/j.bbamcr.2008.01.024.
20. Vieira C, Magalhães-Cardoso MT, Ferreirinha F, et al. Feed-forward inhibition of CD73 and upregulation of adenosine deaminase contribute to the loss of adenosine neuromodulation in postinflammatory ileitis. Mediators Inflamm 2014;2014:254640. (In eng). DOI: 10.1155/2014/254640.
21. Bono MR, Fernández D, Flores-Santibáñez F, Rosemblatt M, Sauma D. CD73 and CD39 ectonucleotidases in T cell differentiation: Beyond immunosuppression. FEBS Lett 2015;589(22):3454-60. (In eng). DOI: 10.1016/j.febslet.2015.07.027.
22. Grenz A, Homann D, Eltzschig HK. Extracellular adenosine: a safety signal that dampens hypoxia-induced inflammation during ischemia. Antioxid Redox Signal 2011;15(8):2221-2234. (In eng). DOI: 10.1089/ars.2010.3665.
23. Idzko M, Ferrari D, Eltzschig HK. Nucleotide signalling during inflammation. Nature 2014;509(7500):310-7. (In eng). DOI: 10.1038/nature13085.
24. Sumi Y, Woehrle T, Chen Y, Yao Y, Li A, Junger WG. Adrenergic receptor activation involves ATP release and feedback through purinergic receptors. Am J Physiol Cell Physiol 2010;299(5):C1118-26. (In eng). DOI: 10.1152/ajpcell.00122.2010.
25. Meyskens FL, Williams HE. Adenosine metabolism in human erythrocytes. Biochim Biophys Acta 1971;240(2):170-9. (In eng). DOI: 10.1016/0005-2787(71)90654-x.
26. Funaya H, Kitakaze M, Node K, Minamino T, Komamura K, Hori M. Plasma adenosine levels increase in patients with chronic heart failure. Circulation 1997;95(6):1363-5. (In eng). DOI: 10.1161/01.cir.95.6.1363.
27. Asakura M, Asanuma H, Kim J, et al. Impact of adenosine receptor signaling and metabolism on pathophysiology in patients with chronic heart failure. Hypertens Res 2007;30(9):781-7. (In eng). DOI: 10.1291/hypres.30.781.
28. Correia-de-Sá M, Magalhães-Cardoso MT, Lobo L, et al. Biomarker sensitivity of plasma adenosine levels to predict heart failure in dogs. Autonomic Neuroscience: Basic and Clinical 2015;192:75. DOI: 10.1016/j.autneu.2015.07.071.
29. Fujita M, Asakura M, Sanada S, et al. Activation of ecto-5'-nucleotidase in the blood and hearts of patients with chronic heart failure. J Card Fail 2008;14(5):426-30. (In eng). DOI: 10.1016/j.cardfail.2008.01.011.
30. Headrick JP, Ashton KJ, Rose'meyer RB, Peart JN. Cardiovascular adenosine receptors: expression, actions and interactions. Pharmacol Ther 2013;140(1):92-111. DOI: 10.1016/j.pharmthera.2013.06.002.
31. Zhou P, Pu WT. Recounting Cardiac Cellular Composition. Circulation Research 2016;118(3):368-370. DOI: doi:10.1161/CIRCRESAHA.116.308139.
32. Sun Y, Huang P. Adenosine A2B Receptor: From Cell Biology to Human Diseases. Frontiers in Chemistry 2016;4(37) (Review) (In English). DOI: 10.3389/fchem.2016.00037.
33. Bessa-Gonçalves M, Bragança B, Martins-Dias E, Correia-de-Sá P, Fontes-Sousa AP. Is the adenosine A(2B) 'biased' receptor a valuable target for the treatment of pulmonary arterial hypertension? Drug Discov Today 2018;23(6):1285-1292. (In eng). DOI: 10.1016/j.drudis.2018.05.005.

34. Belardinelli L, Shryock JC, Song Y, Wang D, Srinivas M. Ionic basis of the electrophysiological actions of adenosine on cardiomyocytes. Faseb j 1995;9(5):359-65. (In eng). DOI: 10.1096/fasebj.9.5.7896004.
35. Belardinelli L, Isenberg G. Isolated atrial myocytes: adenosine and acetylcholine increase potassium conductance. Am J Physiol 1983;244(5):H734-7. (In eng). DOI: 10.1152/ajpheart.1983.244.5.H734.
36. Bragança B, Oliveira-Monteiro N, Ferreirinha F, et al. Ion Fluxes through KCa2 (SK) and Cav1 (L-type) Channels Contribute to Chronoselectivity of Adenosine A1 Receptor-Mediated Actions in Spontaneously Beating Rat Atria. Frontiers in pharmacology 2016;7:45-45. (In eng). DOI: 10.3389/fphar.2016.00045.
37. Lim SH, Anantharaman V, Teo WS, Chan YH. Slow infusion of calcium channel blockers compared with intravenous adenosine in the emergency treatment of supraventricular tachycardia. Resuscitation 2009;80(5):523-8. (In eng). DOI: 10.1016/j.resuscitation.2009.01.017.
38. Savelieva I, Camm J. Anti-arrhythmic drug therapy for atrial fibrillation: current anti-arrhythmic drugs, investigational agents, and innovative approaches. EP Europace 2008;10(6):647-665. DOI: 10.1093/europace/eun130.
39. Dobson JG, Jr. Mechanism of adenosine inhibition of catecholamine-induced responses in heart. Circ Res 1983;52(2):151-60. (In eng). DOI: 10.1161/01.res.52.2.151.
40. Romano FD, Dobson JG, Jr. Adenosine modulates beta-adrenergic signal transduction in guinea-pig heart ventricular membranes. J Mol Cell Cardiol 1990;22(12):1359-70. (In eng). DOI: 10.1016/0022-2828(90)90981-7.
41. Xiang F, Huang Y-s, Zhang D-x, Chu Z-g, Zhang J-p, Zhang Q. Adenosine A1 receptor activation reduces opening of mitochondrial permeability transition pores in hypoxic cardiomyocytes. Clinical and Experimental Pharmacology and Physiology 2010;37(3):343-349. DOI: https://doi.org/10.1111/j.1440-1681.2009.05300.x.
42. Williams-Pritchard G, Knight M, Hoe LS, Headrick JP, Peart JN. Essential role of EGFR in cardioprotection and signaling responses to A1 adenosine receptors and ischemic preconditioning. American Journal of Physiology-Heart and Circulatory Physiology 2011;300(6):H2161-H2168. DOI: 10.1152/ajpheart.00639.2010.
43. Greene SJ, Sabbah HN, Butler J, et al. Partial adenosine A1 receptor agonism: a potential new therapeutic strategy for heart failure. Heart Fail Rev 2016;21(1):95-102. (In eng). DOI: 10.1007/s10741-015-9522-7.
44. Reichelt ME, Willems L, Molina JG, et al. Genetic deletion of the A1 adenosine receptor limits myocardial ischemic tolerance. Circ Res 2005;96(3):363-7. (In eng). DOI: 10.1161/01.Res.0000156075.00127.C3.
45. Morrison RR, Teng B, Oldenburg PJ, Katwa LC, Schnermann JB, Mustafa SJ. Effects of targeted deletion of A1 adenosine receptors on postischemic cardiac function and expression of adenosine receptor subtypes. Am J Physiol Heart Circ Physiol 2006;291(4):H1875-82. (In eng). DOI: 10.1152/ajpheart.00158.2005.
46. Dobson JG, Jr., Fenton RA. Adenosine A2 receptor function in rat ventricular myocytes1. Cardiovascular Research 1997;34(2):337-347. DOI: 10.1016/S0008-6363(97)00023-0.
47. Dobson JG, Jr., Shea LG, Fenton RA. Adenosine A2A and beta-adrenergic calcium transient and contractile responses in rat ventricular myocytes. Am J Physiol Heart Circ Physiol 2008;295(6):H2364-72. (In eng). DOI: 10.1152/ajpheart.00927.2008.
48. Fenton RA, Dobson JG, Jr. Adenosine A1 and A2A receptor effects on G-protein cycling in beta-adrenergic stimulated ventricular membranes. J Cell Physiol 2007;213(3):785-92. (In eng). DOI: 10.1002/jcp.21149.
49. Shryock JC, Snowdy S, Baraldi PG, et al. A2A-adenosine receptor reserve for coronary vasodilation. Circulation 1998;98(7):711-8. (In eng). DOI: 10.1161/01.cir.98.7.711.

50. Iwamoto T, Umemura S, Toya Y, et al. Identification of adenosine A2 receptor-cAMP system in human aortic endothelial cells. Biochem Biophys Res Commun 1994;199(2):905-10. (In eng). DOI: 10.1006/bbrc.1994.1314.
51. Kusano Y, Echeverry G, Miekisiak G, et al. Role of adenosine A2 receptors in regulation of cerebral blood flow during induced hypotension. J Cereb Blood Flow Metab 2010;30(4):808-15. (In eng). DOI: 10.1038/jcbfm.2009.244.
52. Berwick ZC, Payne GA, Lynch B, Dick GM, Sturek M, Tune JD. Contribution of adenosine A(2A) and A(2B) receptors to ischemic coronary dilation: role of K(V) and K(ATP) channels. Microcirculation 2010;17(8):600-607. (In eng). DOI: 10.1111/j.1549-8719.2010.00054.x.
53. Li JM, Fenton RA, Cutler BS, Dobson JG, Jr. Adenosine enhances nitric oxide production by vascular endothelial cells. Am J Physiol 1995;269(2 Pt 1):C519-23. (In eng). DOI: 10.1152/ajpcell.1995.269.2.C519.
54. Li J, Fenton RA, Wheeler HB, et al. Adenosine A2a receptors increase arterial endothelial cell nitric oxide. J Surg Res 1998;80(2):357-64. (In eng). DOI: 10.1006/jsre.1998.5439.
55. Faria M, Magalhães-Cardoso T, Lafuente-de-Carvalho JM, Correia-de-Sá P. Corpus cavernosum from men with vasculogenic impotence is partially resistant to adenosine relaxation due to endothelial A(2B) receptor dysfunction. J Pharmacol Exp Ther 2006;319(1):405-13. (In eng). DOI: 10.1124/jpet.106.107821.
56. Ahmad A, Schaack JB, White CW, Ahmad S. Adenosine A2A receptor-dependent proliferation of pulmonary endothelial cells is mediated through calcium mobilization, PI3-kinase and ERK1/2 pathways. Biochem Biophys Res Commun 2013;434(3):566-71. (In eng). DOI: 10.1016/j.bbrc.2013.03.115.
57. Du X, Ou X, Song T, et al. Adenosine A2B receptor stimulates angiogenesis by inducing VEGF and eNOS in human microvascular endothelial cells. Exp Biol Med (Maywood) 2015;240(11):1472-1479. (In eng). DOI: 10.1177/1535370215584939.
58. Jordan JE, Zhao ZQ, Sato H, Taft S, Vinten-Johansen J. Adenosine A2 receptor activation attenuates reperfusion injury by inhibiting neutrophil accumulation, superoxide generation and coronary endothelial adherence. J Pharmacol Exp Ther 1997;280(1):301-9. (In eng).
59. Rork TH, Wallace KL, Kennedy DP, Marshall MA, Lankford AR, Linden J. Adenosine A2A receptor activation reduces infarct size in the isolated, perfused mouse heart by inhibiting resident cardiac mast cell degranulation. Am J Physiol Heart Circ Physiol 2008;295(5):H1825-33. (In eng). DOI: 10.1152/ajpheart.495.2008.
60. Yang Z, Day YJ, Toufektsian MC, et al. Myocardial infarct-sparing effect of adenosine A2A receptor activation is due to its action on CD4+ T lymphocytes. Circulation 2006;114(19):2056-64. (In eng). DOI: 10.1161/circulationaha.106.649244.
61. Cury RC, Kitt TM, Feaheny K, Akin J, George RT. Regadenoson-stress myocardial CT perfusion and single-photon emission CT: rationale, design, and acquisition methods of a prospective, multicenter, multivendor comparison. J Cardiovasc Comput Tomogr 2014;8(1):2-12. (In eng). DOI: 10.1016/j.jcct.2013.09.004.
62. Gile J, Eckle T. ADORA2b Signaling in Cardioprotection. J Nat Sci 2016;2(10):e222. (In eng) (https://pubmed.ncbi.nlm.nih.gov/27747290).
63. Eckle T, Hartmann K, Bonney S, et al. Adora2b-elicited Per2 stabilization promotes a HIF-dependent metabolic switch crucial for myocardial adaptation to ischemia. Nat Med 2012;18(5):774-82. (In eng). DOI: 10.1038/nm.2728.
64. Xi J, McIntosh R, Shen X, et al. Adenosine A2A and A2B receptors work in concert to induce a strong protection against reperfusion injury in rat hearts. J Mol Cell Cardiol 2009;47(5):684-90. (In eng). DOI: 10.1016/j.yjmcc.2009.08.009.
65. Yang X, Xin W, Yang XM, et al. A2B adenosine receptors inhibit superoxide production from mitochondrial complex I in rabbit cardiomyocytes via a mechanism sensitive to

Pertussis toxin. Br J Pharmacol 2011;163(5):995-1006. (In eng). DOI: 10.1111/j.1476-5381.2011.01288.x.
66. Kuno A, Critz SD, Cui L, et al. Protein kinase C protects preconditioned rabbit hearts by increasing sensitivity of adenosine A2b-dependent signaling during early reperfusion. Journal of Molecular and Cellular Cardiology 2007;43(3):262-271. DOI: https://doi.org/10.1016/j.yjmcc.2007.05.016.
67. Seo SW, Koeppen M, Bonney S, et al. Differential Tissue-Specific Function of Adora2b in Cardioprotection. J Immunol 2015;195(4):1732-43. (In eng). DOI: 10.4049/jimmunol.1402288.
68. Ge ZD, van der Hoeven D, Maas JE, Wan TC, Auchampach JA. A(3) adenosine receptor activation during reperfusion reduces infarct size through actions on bone marrow-derived cells. J Mol Cell Cardiol 2010;49(2):280-6. (In eng). DOI: 10.1016/j.yjmcc.2010.01.018.
69. Tian Y, Marshall M, French BA, Linden J, Yang Z. The infarct-sparing effect of IB-MECA against myocardial ischemia/reperfusion injury in mice is mediated by sequential activation of adenosine A3 and A 2A receptors. Basic Res Cardiol 2015;110(2):16. (In eng). DOI: 10.1007/s00395-015-0473-x.
70. Hussain A, Gharanei AM, Nagra AS, Maddock HL. Caspase inhibition via A3 adenosine receptors: a new cardioprotective mechanism against myocardial infarction. Cardiovasc Drugs Ther 2014;28(1):19-32. (In eng). DOI: 10.1007/s10557-013-6500-y.
71. Hinze AV, Mayer P, Harst A, von Kügelgen 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(5):639-45. (In eng). DOI: 10.1016/j.yjmcc.2012.08.003.
72. Kalogeropoulos A, Georgiopoulou V, Psaty BM, et al. Inflammatory markers and incident heart failure risk in older adults: the Health ABC (Health, Aging, and Body Composition) study. Journal of the American College of Cardiology 2010;55(19):2129-2137. (In eng). DOI: 10.1016/j.jacc.2009.12.045.
73. Rozenbaum Z, Topilsky Y, Khoury S, Pereg D, Laufer-Perl M. Association of body mass index and diastolic function in metabolically healthy obese with preserved ejection fraction. Int J Cardiol 2019;277:147-152. (In eng). DOI: 10.1016/j.ijcard.2018.08.008.
74. Westermann D, Lindner D, Kasner M, et al. Cardiac inflammation contributes to changes in the extracellular matrix in patients with heart failure and normal ejection fraction. Circ Heart Fail 2011;4(1):44-52. (In eng). DOI: 10.1161/circheartfailure.109.931451.
75. Lam CSP, Voors AA, de Boer RA, Solomon SD, van Veldhuisen DJ. Heart failure with preserved ejection fraction: from mechanisms to therapies. Eur Heart J 2018;39(30):2780-2792. (In eng). DOI: 10.1093/eurheartj/ehy301.
76. Antonioli L, Csóka B, Fornai M, et al. Adenosine and inflammation: what's new on the horizon? Drug Discov Today 2014;19(8):1051-68. (In eng). DOI: 10.1016/j.drudis.2014.02.010.
77. Antonioli L, Fornai M, Blandizzi C, Pacher P, Haskó G. Adenosine signaling and the immune system: When a lot could be too much. Immunol Lett 2019;205:9-15. (In eng). DOI: 10.1016/j.imlet.2018.04.006.
78. Sands WA, Martin AF, Strong EW, Palmer TM. Specific inhibition of nuclear factor-kappaB-dependent inflammatory responses by cell type-specific mechanisms upon A2A adenosine receptor gene transfer. Mol Pharmacol 2004;66(5):1147-59. (In eng). DOI: 10.1124/mol.104.001107.
79. Grinberg S, Hasko G, Wu D, Leibovich SJ. Suppression of PLCbeta2 by endotoxin plays a role in the adenosine A(2A) receptor-mediated switch of macrophages from an inflammatory to an angiogenic phenotype. Am J Pathol 2009;175(6):2439-2453. (In eng). DOI: 10.2353/ajpath.2009.090290.

80. Silverman MH, Strand V, Markovits D, et al. Clinical evidence for utilization of the A3 adenosine receptor as a target to treat rheumatoid arthritis: data from a phase II clinical trial. J Rheumatol 2008;35(1):41-8. (In eng).
81. Antonioli L, Novitskiy SV, Sachsenmeier KF, Fornai M, Blandizzi C, Haskó G. Switching off CD73: a way to boost the activity of conventional and targeted antineoplastic therapies. Drug Discov Today 2017;22(11):1686-1696. (In eng). DOI: 10.1016/j.drudis.2017.06.005.
82. Chan ES, Cronstein BN. Methotrexate--how does it really work? Nat Rev Rheumatol 2010;6(3):175-8. (In eng). DOI: 10.1038/nrrheum.2010.5.
83. Quast C, Alter C, Ding Z, Borg N, Schrader J. Adenosine Formed by CD73 on T Cells Inhibits Cardiac Inflammation and Fibrosis and Preserves Contractile Function in Transverse Aortic Constriction-Induced Heart Failure. Circ Heart Fail 2017;10(4) (In eng). DOI: 10.1161/circheartfailure.116.003346.
84. Eltzschig HK, Thompson LF, Karhausen J, et al. Endogenous adenosine produced during hypoxia attenuates neutrophil accumulation: coordination by extracellular nucleotide metabolism. Blood 2004;104(13):3986-92. (In eng). DOI: 10.1182/blood-2004-06-2066.
85. Hamad EA, Zhu W, Chan TO, et al. Cardioprotection of controlled and cardiac-specific over-expression of A(2A)-adenosine receptor in the pressure overload. PLoS One 2012;7(7):e39919. (In eng). DOI: 10.1371/journal.pone.0039919.
86. Oliveira L, Correia A, Cristina Costa A, et al. Deficits in endogenous adenosine formation by ecto-5'-nucleotidase/CD73 impair neuromuscular transmission and immune competence in experimental autoimmune myasthenia gravis. Mediators Inflamm 2015;2015:460610. (In eng). DOI: 10.1155/2015/460610.
87. D'Amario D, Migliaro S, Borovac JA, et al. Microvascular Dysfunction in Heart Failure With Preserved Ejection Fraction. Front Physiol 2019;10:1347-1347. (In eng). DOI: 10.3389/fphys.2019.01347.
88. Tschöpe C, Bock CT, Kasner M, et al. High prevalence of cardiac parvovirus B19 infection in patients with isolated left ventricular diastolic dysfunction. Circulation 2005;111(7):879-86. (In eng). DOI: 10.1161/01.Cir.0000155615.68924.B3.
89. Griendling KK, Sorescu D, Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. Circ Res 2000;86(5):494-501. (In eng). DOI: 10.1161/01.res.86.5.494.
90. Landmesser U, Cai H, Dikalov S, et al. Role of p47(phox) in vascular oxidative stress and hypertension caused by angiotensin II. Hypertension 2002;40(4):511-5. (In eng). DOI: 10.1161/01.hyp.0000032100.23772.98.
91. Thakur S, Du J, Hourani S, Ledent C, Li JM. Inactivation of adenosine A2A receptor attenuates basal and angiotensin II-induced ROS production by Nox2 in endothelial cells. J Biol Chem 2010;285(51):40104-13. (In eng). DOI: 10.1074/jbc.M110.184606.
92. Pugliese AM, Traini C, Cipriani S, et al. The adenosine A2A receptor antagonist ZM241385 enhances neuronal survival after oxygen-glucose deprivation in rat CA1 hippocampal slices. British journal of pharmacology 2009;157(5):818-830. (In eng). DOI: 10.1111/j.1476-5381.2009.00218.x.
93. Ribé D, Sawbridge D, Thakur S, et al. Adenosine A2A receptor signaling regulation of cardiac NADPH oxidase activity. Free Radic Biol Med 2008;44(7):1433-1442. (In eng). DOI: 10.1016/j.freeradbiomed.2007.12.035.
94. Nadeem A, Ponnoth DS, Ansari HR, et al. A2A adenosine receptor deficiency leads to impaired tracheal relaxation via NADPH oxidase pathway in allergic mice. J Pharmacol Exp Ther 2009;330(1):99-108. (In eng). DOI: 10.1124/jpet.109.151613.
95. Chiang PH, Wu SN, Tsai EM, et al. Adenosine modulation of neurotransmission in penile erection. Br J Clin Pharmacol 1994;38(4):357-62. (In eng). DOI: 10.1111/j.1365-2125.1994.tb04366.x.

96. Thompson IM, Tangen CM, Goodman PJ, Probstfield JL, Moinpour CM, Coltman CA. Erectile dysfunction and subsequent cardiovascular disease. Jama 2005;294(23):2996-3002. (In eng). DOI: 10.1001/jama.294.23.2996.
97. Mierzejewska P, Zabielska MA, Kutryb-Zajac B, et al. Impaired L-arginine metabolism marks endothelial dysfunction in CD73-deficient mice. Mol Cell Biochem 2019;458(1-2):133-142. (In eng). DOI: 10.1007/s11010-019-03537-4.
98. Mohammed SF, Hussain S, Mirzoyev SA, Edwards WD, Maleszewski JJ, Redfield MM. Coronary microvascular rarefaction and myocardial fibrosis in heart failure with preserved ejection fraction. Circulation 2015;131(6):550-9. (In eng). DOI: 10.1161/circulationaha.114.009625.
99. Sabbah HN, Gupta RC, Kohli S, et al. Chronic Therapy With a Partial Adenosine A1-Receptor Agonist Improves Left Ventricular Function and Remodeling in Dogs With Advanced Heart Failure. Circulation: Heart Failure 2013;6(3):563-571. DOI: doi:10.1161/CIRCHEARTFAILURE.112.000208.
100. Xu Y, Wang Y, Yan S, et al. Intracellular adenosine regulates epigenetic programming in endothelial cells to promote angiogenesis. EMBO Mol Med 2017;9(9):1263-1278. (In eng). DOI: 10.15252/emmm.201607066.
101. Wernly B, Erlinge D, Pernow J, Zhou Z. Ticagrelor: a cardiometabolic drug targeting erythrocyte-mediated purinergic signaling? Am J Physiol Heart Circ Physiol 2021;320(1):H90-h94. (In eng). DOI: 10.1152/ajpheart.00570.2020.
102. Koupenova M, Johnston-Cox H, Ravid K. Regulation of atherosclerosis and associated risk factors by adenosine and adenosine receptors. Curr Atheroscler Rep 2012;14(5):460-8. (In eng). DOI: 10.1007/s11883-012-0263-y.
103. Bhatt DL, Steg PG, Mehta SR, et al. Ticagrelor in patients with diabetes and stable coronary artery disease with a history of previous percutaneous coronary intervention (THEMIS-PCI): a phase 3, placebo-controlled, randomised trial. Lancet 2019;394(10204):1169-1180. (In eng). DOI: 10.1016/s0140-6736(19)31887-2.
104. Souders CA, Bowers SL, Baudino TA. Cardiac fibroblast: the renaissance cell. Circ Res 2009;105(12):1164-76. (In eng). DOI: 10.1161/circresaha.109.209809.
105. Sassi Y, Ahles A, Truong DJ, et al. Cardiac myocyte-secreted cAMP exerts paracrine action via adenosine receptor activation. J Clin Invest 2014;124(12):5385-97. (In eng). DOI: 10.1172/jci74349.
106. Vecchio EA, White PJ, May LT. Targeting Adenosine Receptors for the Treatment of Cardiac Fibrosis. Frontiers in Pharmacology 2017;8:243. (10.3389/fphar.2017.00243) (https://www.frontiersin.org/article/10.3389/fphar.2017.00243).
107. Zhou YP, Ruan CC, Kong LR, Gao PJ. Adenosine A(2A) receptor activation prevents DOCA-salt induced hypertensive cardiac remodeling via iBAT. Biochem Biophys Res Commun 2020 (In eng). DOI: 10.1016/j.bbrc.2020.02.035.
108. Herman-de-Sousa C, Pinheiro AR, Paramos-de-Carvalho D, et al. Opposing Effects of Adenosine and Inosine in Human Subcutaneous Fibroblasts May Be Regulated by Third Party ADA Cell Providers. Cells 2020;9(3) (In eng). DOI: 10.3390/cells9030651.
109. Chan ES, Montesinos MC, Fernandez P, et al. Adenosine A(2A) receptors play a role in the pathogenesis of hepatic cirrhosis. Br J Pharmacol 2006;148(8):1144-55. (In eng). DOI: 10.1038/sj.bjp.0706812.
110. Epperson SA, Brunton LL, Ramirez-Sanchez I, Villarreal F. Adenosine receptors and second messenger signaling pathways in rat cardiac fibroblasts. Am J Physiol Cell Physiol 2009;296(5):C1171-7. (In eng). DOI: 10.1152/ajpcell.00290.2008.
111. Chen Y, Epperson S, Makhsudova L, et al. Functional effects of enhancing or silencing adenosine A2b receptors in cardiac fibroblasts. Am J Physiol Heart Circ Physiol 2004;287(6):H2478-86. (In eng). DOI: 10.1152/ajpheart.00217.2004.
112. Delaunay M, Osman H, Kaiser S, Diviani D. The Role of Cyclic AMP Signaling in Cardiac Fibrosis. Cells 2019;9(1) (In eng). DOI: 10.3390/cells9010069.

113. Phosri S, Bunrukchai K, Parichatikanond W, Sato VH, Mangmool S. Epac is required for exogenous and endogenous stimulation of adenosine A(2B) receptor for inhibition of angiotensin II-induced collagen synthesis and myofibroblast differentiation. Purinergic Signal 2018;14(2):141-156. (In eng). DOI: 10.1007/s11302-017-9600-5.
114. Phosri S, Arieyawong A, Bunrukchai K, et al. Stimulation of Adenosine A(2B) Receptor Inhibits Endothelin-1-Induced Cardiac Fibroblast Proliferation and α-Smooth Muscle Actin Synthesis Through the cAMP/Epac/PI3K/Akt-Signaling Pathway. Frontiers in pharmacology 2017;8:428-428. (In eng). DOI: 10.3389/fphar.2017.00428.
115. Phosri S, Arieyawong A, Bunrukchai K, et al. Stimulation of Adenosine A2B Receptor Inhibits Endothelin-1-Induced Cardiac Fibroblast Proliferation and α-Smooth Muscle Actin Synthesis Through the cAMP/Epac/PI3K/Akt-Signaling Pathway. Frontiers in Pharmacology 2017;8(428) (Original Research) (In English). DOI: 10.3389/fphar.2017.00428.
116. Surinkaew S, Aflaki M, Takawale A, et al. Exchange protein activated by cyclic-adenosine monophosphate (Epac) regulates atrial fibroblast function and controls cardiac remodelling. Cardiovasc Res 2019;115(1):94-106. (In eng). DOI: 10.1093/cvr/cvy173.
117. Eckle T, Krahn T, Grenz A, et al. Cardioprotection by Ecto-5′-Nucleotidase (CD73) and A_{2B} Adenosine Receptors. Circulation 2007;115(12):1581-1590. DOI: doi:10.1161/CIRCULATIONAHA.106.669697.
118. Ni Y, Liang D, Tian Y, Kron IL, French BA, Yang Z. Infarct-Sparing Effect of Adenosine A2B Receptor Agonist Is Primarily Due to Its Action on Splenic Leukocytes Via a PI3K/Akt/IL-10 Pathway. J Surg Res 2018;232:442-449. (In eng). DOI: 10.1016/j.jss.2018.06.042.
119. Tian Y, Piras BA, Kron IL, French BA, Yang Z. Adenosine 2B Receptor Activation Reduces Myocardial Reperfusion Injury by Promoting Anti-Inflammatory Macrophages Differentiation via PI3K/Akt Pathway. Oxidative Medicine and Cellular Longevity 2015;2015:585297. DOI: 10.1155/2015/585297.
120. Sun Y, Huang P. Adenosine A2B Receptor: From Cell Biology to Human Diseases. Front Chem 2016;4:37. (In eng). DOI: 10.3389/fchem.2016.00037.
121. Zhang H, Zhong H, Everett THt, et al. Blockade of A2B adenosine receptor reduces left ventricular dysfunction and ventricular arrhythmias 1 week after myocardial infarction in the rat model. Heart Rhythm 2014;11(1):101-9. (In eng). DOI: 10.1016/j.hrthm.2013.10.023.
122. Toldo S, Zhong H, Mezzaroma E, et al. GS-6201, a selective blocker of the A2B adenosine receptor, attenuates cardiac remodeling after acute myocardial infarction in the mouse. J Pharmacol Exp Ther 2012;343(3):587-95. (In eng). DOI: 10.1124/jpet.111.191288.
123. Wakeno M, Minamino T, Seguchi O, et al. Long-Term Stimulation of Adenosine A2b Receptors Begun After Myocardial Infarction Prevents Cardiac Remodeling in Rats. Circulation 2006;114(18):1923-1932. DOI: doi:10.1161/CIRCULATIONAHA.106.630087.
124. Feng W, Song Y, Chen C, Lu ZZ, Zhang Y. Stimulation of adenosine A(2B) receptors induces interleukin-6 secretion in cardiac fibroblasts via the PKC-delta-P38 signalling pathway. British journal of pharmacology 2010;159(8):1598-1607. (In eng). DOI: 10.1111/j.1476-5381.2009.00558.x.
125. Alter C, Ding Z, Flögel U, Scheller J, Schrader J. A(2b)R-dependent signaling alters immune cell composition and enhances IL-6 formation in the ischemic heart. Am J Physiol Heart Circ Physiol 2019;317(1):H190-h200. (In eng). DOI: 10.1152/ajpheart.00029.2019.
126. Hinz S, Navarro G, Borroto-Escuela D, et al. Adenosine A(2A) receptor ligand recognition and signaling is blocked by A(2B) receptors. Oncotarget 2018;9(17):13593-13611. (In eng). DOI: 10.18632/oncotarget.24423.
127. Ryzhov S, Goldstein AE, Matafonov A, Zeng D, Biaggioni I, Feoktistov I. Adenosine-activated mast cells induce IgE synthesis by B lymphocytes: an A2B-mediated process

involving Th2 cytokines IL-4 and IL-13 with implications for asthma. J Immunol 2004;172(12):7726-33. (In eng). DOI: 10.4049/jimmunol.172.12.7726.
128. Varani K, Caramori G, Vincenzi F, et al. Oxidative/nitrosative stress selectively altered A(2B) adenosine receptors in chronic obstructive pulmonary disease. Faseb j 2010;24(4):1192-204. (In eng). DOI: 10.1096/fj.09-139485.
129. Nakamura M, Sadoshima J. Mechanisms of physiological and pathological cardiac hypertrophy. Nat Rev Cardiol 2018;15(7):387-407. (In eng). DOI: 10.1038/s41569-018-0007-y.
130. Stowe DF, O'Brien WC, Chang D, Knop CS, Kampine JP. Reversal of endothelin-induced vasoconstriction by endothelium-dependent and -independent vasodilators in isolated hearts and vascular rings. J Cardiovasc Pharmacol 1997;29(6):747-54. (In eng). DOI: 10.1097/00005344-199706000-00007.
131. Taddei S, Arzilli F, Arrighi P, Salvetti A. Dipyridamole decreases circulating renin-angiotensin system activity in hypertensive patients. Am J Hypertens 1992;5(1):29-31. (In eng). DOI: 10.1093/ajh/5.1.29.
132. Wagner DR, McTiernan C, Sanders VJ, Feldman AM. Adenosine inhibits lipopolysaccharide-induced secretion of tumor necrosis factor-alpha in the failing human heart. Circulation 1998;97(6):521-4. (In eng). DOI: 10.1161/01.cir.97.6.521.
133. Burgdorf C, Richardt D, Kurz T, et al. Adenosine inhibits norepinephrine release in the postischemic rat heart: the mechanism of neuronal stunning. Cardiovasc Res 2001;49(4):713-20. (In eng). DOI: 10.1016/s0008-6363(00)00309-6.
134. Liao Y, Takashima S, Asano Y, et al. Activation of adenosine A1 receptor attenuates cardiac hypertrophy and prevents heart failure in murine left ventricular pressure-overload model. Circ Res 2003;93(8):759-66. (In eng). DOI: 10.1161/01.Res.0000094744.88220.62.
135. Chung ES, Perlini S, Aurigemma GP, Fenton RA, Dobson JG, Jr., Meyer TE. Effects of chronic adenosine uptake blockade on adrenergic responsiveness and left ventricular chamber function in pressure overload hypertrophy in the rat. J Hypertens 1998;16(12 Pt 1):1813-22. (In eng). DOI: 10.1097/00004872-199816120-00015.
136. Xu X, Fassett J, Hu X, et al. Ecto-5'-nucleotidase deficiency exacerbates pressure-overload-induced left ventricular hypertrophy and dysfunction. Hypertension 2008;51(6):1557-64. (In eng). DOI: 10.1161/hypertensionaha.108.110833.
137. Puhl SL, Kazakov A, Müller A, et al. Adenosine A1 receptor activation attenuates cardiac hypertrophy and fibrosis in response to α1 -adrenoceptor stimulation in vivo. Br J Pharmacol 2016;173(1):88-102. (In eng). DOI: 10.1111/bph.13339.
138. Meyer TE, Chung ES, Perlini S, et al. Antiadrenergic effects of adenosine in pressure overload hypertrophy. Hypertension 2001;37(3):862-8. (In eng). DOI: 10.1161/01.hyp.37.3.862.
139. Prijic S, Buchhorn R. Mechanisms of Beta-Blockers Action in Patients with Heart Failure. Rev Recent Clin Trials 2014;9(2):58-60. (In eng). DOI: 10.2174/1574887109666140908125402.
140. Lu Z, Fassett J, Xu X, et al. Adenosine A3 receptor deficiency exerts unanticipated protective effects on the pressure-overloaded left ventricle. Circulation 2008;118(17):1713-21. (In eng). DOI: 10.1161/circulationaha.108.788307.
141. Fassett JT, Hu X, Xu X, et al. Adenosine kinase regulation of cardiomyocyte hypertrophy. Am J Physiol Heart Circ Physiol 2011;300(5):H1722-32. (In eng). DOI: 10.1152/ajpheart.00684.2010.
142. Fassett J, Xu X, Kwak D, et al. Adenosine kinase attenuates cardiomyocyte microtubule stabilization and protects against pressure overload-induced hypertrophy and LV dysfunction. J Mol Cell Cardiol 2019;130:49-58. (In eng). DOI: 10.1016/j.yjmcc.2019.03.015.

143. Birkenfeld AL, Jordan J, Dworak M, Merkel T, Burnstock G. Myocardial metabolism in heart failure: Purinergic signalling and other metabolic concepts. Pharmacol Ther 2019;194:132-144. (In eng). DOI: 10.1016/j.pharmthera.2018.08.015.
144. Xiang F, Huang YS, Zhang DX, Chu ZG, Zhang JP, Zhang Q. Adenosine A1 receptor activation reduces opening of mitochondrial permeability transition pores in hypoxic cardiomyocytes. Clin Exp Pharmacol Physiol 2010;37(3):343-9. (In eng). DOI: 10.1111/j.1440-1681.2009.05300.x.
145. Yang X, Xin W, Yang X-M, et al. A2B adenosine receptors inhibit superoxide production from mitochondrial complex I in rabbit cardiomyocytes via a mechanism sensitive to Pertussis toxin. British Journal of Pharmacology 2011;163(5):995-1006. DOI: https://doi.org/10.1111/j.1476-5381.2011.01288.x.
146. Staehr PM, Dhalla AK, Zack J, et al. Reduction of free fatty acids, safety, and pharmacokinetics of oral GS-9667, an A(1) adenosine receptor partial agonist. J Clin Pharmacol 2013;53(4):385-92. (In eng). DOI: 10.1002/jcph.9.
147. Cui XB, Fei J, Chen S, Edwards GL, Chen SY. ADAR1 deficiency protects against high-fat diet-induced obesity and insulin resistance in mice. Am J Physiol Endocrinol Metab 2021;320(1):E131-e138. (In eng). DOI: 10.1152/ajpendo.00175.2020.
148. Campbell RT, Jhund PS, Castagno D, Hawkins NM, Petrie MC, McMurray JJ. What have we learned about patients with heart failure and preserved ejection fraction from DIG-PEF, CHARM-preserved, and I-PRESERVE? J Am Coll Cardiol 2012;60(23):2349-56. (In eng). DOI: 10.1016/j.jacc.2012.04.064.
149. Lindman BR, Dávila-Román VG, Mann DL, et al. Cardiovascular phenotype in HFpEF patients with or without diabetes: a RELAX trial ancillary study. J Am Coll Cardiol 2014;64(6):541-9. (In eng). DOI: 10.1016/j.jacc.2014.05.030.
150. de Oliveira M, Mathias LS, de Sibio MT, et al. Pitfalls and challenges of the purinergic signaling cascade in obesity. Biochem Pharmacol 2020;182:114214. (In eng). DOI: 10.1016/j.bcp.2020.114214.
151. Johansson SM, Lindgren E, Yang J-N, Herling AW, Fredholm BB. Adenosine A1 receptors regulate lipolysis and lipogenesis in mouse adipose tissue — Interactions with insulin. European Journal of Pharmacology 2008;597(1):92-101. DOI: https://doi.org/10.1016/j.ejphar.2008.08.022.
152. Dhalla AK, Wong MY, Voshol PJ, Belardinelli L, Reaven GM. A1 adenosine receptor partial agonist lowers plasma FFA and improves insulin resistance induced by high-fat diet in rodents. American Journal of Physiology-Endocrinology and Metabolism 2007;292(5):E1358-E1363. DOI: 10.1152/ajpendo.00573.2006.
153. Cheng JT, Chi TC, Liu IM. Activation of adenosine A1 receptors by drugs to lower plasma glucose in streptozotocin-induced diabetic rats. Auton Neurosci 2000;83(3):127-33. (In eng). DOI: 10.1016/s0165-1838(00)00106-5.
154. Jacobson KA, Tosh DK, Jain S, Gao Z-G. Historical and Current Adenosine Receptor Agonists in Preclinical and Clinical Development. Front Cell Neurosci 2019;13:124-124. (In eng). DOI: 10.3389/fncel.2019.00124.
155. Liang H-X, Belardinelli L, Ozeck MJ, Shryock JC. Tonic activity of the rat adipocyte A1-adenosine receptor. British journal of pharmacology 2002;135(6):1457-1466. (In eng). DOI: 10.1038/sj.bjp.0704586.
156. Wu L, Belardinelli L, Zablocki JA, Palle V, Shryock JC. A partial agonist of the A1-adenosine receptor selectively slows AV conduction in guinea pig hearts. American Journal of Physiology-Heart and Circulatory Physiology 2001;280(1):H334-H343. DOI: 10.1152/ajpheart.2001.280.1.H334.
157. Gharibi B, Abraham AA, Ham J, Evans BAJ. Contrasting effects of A1 and A2b adenosine receptors on adipogenesis. International Journal of Obesity 2012;36(3):397-406. DOI: 10.1038/ijo.2011.129.

158. Peleli M, Hezel M, Zollbrecht C, et al. In adenosine A2B knockouts acute treatment with inorganic nitrate improves glucose disposal, oxidative stress, and AMPK signaling in the liver. Front Physiol 2015;6(222) (Original Research) (In English). DOI: 10.3389/fphys.2015.00222.
159. van Dam RM, Willett WC, Manson JE, Hu FB. Coffee, caffeine, and risk of type 2 diabetes: a prospective cohort study in younger and middle-aged U.S. women. Diabetes Care 2006;29(2):398-403. (In eng). DOI: 10.2337/diacare.29.02.06.dc05-1512.
160. Sacramento JF, Martins FO, Rodrigues T, et al. A2 Adenosine Receptors Mediate Whole-Body Insulin Sensitivity in a Prediabetes Animal Model: Primary Effects on Skeletal Muscle. Frontiers in Endocrinology 2020;11(262) (Original Research) (In English). DOI: 10.3389/fendo.2020.00262.
161. Boison D. Adenosine kinase: exploitation for therapeutic gain. Pharmacol Rev 2013;65(3):906-43. (In eng). DOI: 10.1124/pr.112.006361.
162. Navarro G, Abdolazimi Y, Zhao Z, et al. Genetic Disruption of Adenosine Kinase in Mouse Pancreatic β-Cells Protects Against High-Fat Diet-Induced Glucose Intolerance. Diabetes 2017;66(7):1928-1938. (In eng). DOI: 10.2337/db16-0816.
163. Xu J, Yang Q, Zhang X, et al. Endothelial adenosine kinase deficiency ameliorates diet-induced insulin resistance. J Endocrinol 2019;242(2):159-172. (In eng). DOI: 10.1530/joe-19-0126.
164. Xu Y, Wang Y, Yan S, et al. Regulation of endothelial intracellular adenosine via adenosine kinase epigenetically modulates vascular inflammation. Nat Commun 2017;8(1):943. (In eng). DOI: 10.1038/s41467-017-00986-7.
165. Fang JC. Heart Failure With Preserved Ejection Fraction: A Kidney Disorder? Circulation 2016;134(6):435-7. (In eng). DOI: 10.1161/circulationaha.116.022249.
166. Löfman I, Szummer K, Dahlström U, Jernberg T, Lund LH. Associations with and prognostic impact of chronic kidney disease in heart failure with preserved, mid-range, and reduced ejection fraction. Eur J Heart Fail 2017;19(12):1606-1614. (In eng). DOI: 10.1002/ejhf.821.
167. Patinha D, Afonso J, Sousa T, Morato M, Albino-Teixeira A. Activation of adenosine receptors improves renal antioxidant status in diabetic Wistar but not SHR rats. Ups J Med Sci 2014;119(1):10-8. (In eng). DOI: 10.3109/03009734.2013.851748.
168. Katz DH, Burns JA, Aguilar FG, Beussink L, Shah SJ. Albuminuria is independently associated with cardiac remodeling, abnormal right and left ventricular function, and worse outcomes in heart failure with preserved ejection fraction. JACC Heart Fail 2014;2(6):586-96. (In eng). DOI: 10.1016/j.jchf.2014.05.016.
169. Pandey S, Aggarwal D, Gupta K, et al. “Adenosine an old player with new possibilities in kidney diseases”: Preclinical evidences and clinical perspectives. Life Sciences 2021;265:118834. DOI: https://doi.org/10.1016/j.lfs.2020.118834.
170. Kidokoro K, Cherney DZI, Bozovic A, et al. Evaluation of Glomerular Hemodynamic Function by Empagliflozin in Diabetic Mice Using In Vivo Imaging. Circulation 2019;140(4):303-315. (In eng). DOI: 10.1161/circulationaha.118.037418.
171. Lee HT, Gallos G, Nasr SH, Emala CW. A₁ Adenosine Receptor Activation Inhibits Inflammation, Necrosis, and Apoptosis after Renal Ischemia-Reperfusion Injury in Mice. Journal of the American Society of Nephrology 2004;15(1):102-111. DOI: 10.1097/01.Asn.0000102474.68613.Ae.
172. Xiong B, Li M, Xiang S, Han L. A1AR-mediated renal protection against ischemia/reperfusion injury is dependent on HSP27 induction. International Urology and Nephrology 2018;50(7):1355-1363. DOI: 10.1007/s11255-018-1797-x.
173. Gottlieb SS, Brater DC, Thomas I, et al. BG9719 (CVT-124), an A1 adenosine receptor antagonist, protects against the decline in renal function observed with diuretic therapy. Circulation 2002;105(11):1348-53. (In eng). DOI: 10.1161/hc1102.105264.

174. Dittrich HC, Gupta DK, Hack TC, Dowling T, Callahan J, Thomson S. The effect of KW-3902, an adenosine A1 receptor antagonist, on renal function and renal plasma flow in ambulatory patients with heart failure and renal impairment. J Card Fail 2007;13(8):609-17. (In eng). DOI: 10.1016/j.cardfail.2007.08.006.
175. Givertz MM, Massie BM, Fields TK, Pearson LL, Dittrich HC. The effects of KW-3902, an adenosine A1-receptor antagonist,on diuresis and renal function in patients with acute decompensated heart failure and renal impairment or diuretic resistance. J Am Coll Cardiol 2007;50(16):1551-60. (In eng). DOI: 10.1016/j.jacc.2007.07.019.
176. Voors AA, Dittrich HC, Massie BM, et al. Effects of the adenosine A1 receptor antagonist rolofylline on renal function in patients with acute heart failure and renal dysfunction: results from PROTECT (Placebo-Controlled Randomized Study of the Selective Adenosine A1 Receptor Antagonist Rolofylline for Patients Hospitalized with Acute Decompensated Heart Failure and Volume Overload to Assess Treatment Effect on Congestion and Renal Function). J Am Coll Cardiol 2011;57(19):1899-907. (In eng). DOI: 10.1016/j.jacc.2010.11.057.
177. Tofovic SP, Salah EM, Smits GJ, et al. Dual A1/A2B Receptor Blockade Improves Cardiac and Renal Outcomes in a Rat Model of Heart Failure with Preserved Ejection Fraction. J Pharmacol Exp Ther 2016;356(2):333-40. (In eng). DOI: 10.1124/jpet.115.228841.
178. Nakagawa T. Uncoupling of the VEGF-endothelial nitric oxide axis in diabetic nephropathy: an explanation for the paradoxical effects of VEGF in renal disease. Am J Physiol Renal Physiol 2007;292(6):F1665-72. (In eng). DOI: 10.1152/ajprenal.00495.2006.
179. Wilkinson PF, Farrell FX, Morel D, Law W, Murphy S. Adenosine Signaling Increases Proinflammatory and Profibrotic Mediators through Activation of a Functional Adenosine 2B Receptor in Renal Fibroblasts. Ann Clin Lab Sci 2016;46(4):339-45. (In eng).
180. Patel L, Thaker A. The effects of adenosine A2B receptor inhibition on VEGF and nitric oxide axis-mediated renal function in diabetic nephropathy. Ren Fail 2014;36(6):916-24. (In eng). DOI: 10.3109/0886022x.2014.900404.
181. Chen X, Ge W, Dong T, et al. Spironolactone inhibits endothelial-mesenchymal transition via the adenosine A2A receptor to reduce cardiorenal fibrosis in rats. Life Sci 2019;224:177-186. (In eng). DOI: 10.1016/j.lfs.2019.01.017.
182. Persson P, Hansell P, Palm F. Reduced adenosine A_{2a} receptor–mediated efferent arteriolar vasodilation contributes to diabetes-induced glomerular hyperfiltration. Kidney International 2015;87(1):109-115. DOI: 10.1038/ki.2014.219.
183. Awad AS, Huang L, Ye H, et al. Adenosine A2A receptor activation attenuates inflammation and injury in diabetic nephropathy. Am J Physiol Renal Physiol 2006;290(4):F828-37. (In eng). DOI: 10.1152/ajprenal.00310.2005.
184. Persson P, Friederich-Persson M, Fasching A, Hansell P, Inagi R, Palm F. Adenosine A2 a receptor stimulation prevents proteinuria in diabetic rats by promoting an anti-inflammatory phenotype without affecting oxidative stress. Acta Physiol (Oxf) 2015;214(3):311-8. (In eng). DOI: 10.1111/apha.12511.
185. Pye C, Elsherbiny NM, Ibrahim AS, et al. Adenosine kinase inhibition protects the kidney against streptozotocin-induced diabetes through anti-inflammatory and anti-oxidant mechanisms. Pharmacol Res 2014;85:45-54. (In eng). DOI: 10.1016/j.phrs.2014.05.004.
186. Patinha D, Abreu C, Carvalho C, et al. Adenosine A(2A) and A(3) Receptors as Targets for the Treatment of Hypertensive-Diabetic Nephropathy. Biomedicines 2020;8(11) (In eng). DOI: 10.3390/biomedicines8110529.
187. Dorotea D, Cho A, Lee G, et al. Orally active, species-independent novel A(3) adenosine receptor antagonist protects against kidney injury in db/db mice. Exp Mol Med 2018;50(4):1-14. (In eng). DOI: 10.1038/s12276-018-0053-x.

188. Levine AR, Simon MA, Gladwin MT. Pulmonary vascular disease in the setting of heart failure with preserved ejection fraction. Trends Cardiovasc Med 2019;29(4):207-217. (In eng). DOI: 10.1016/j.tcm.2018.08.005.
189. Sabbah HN, Gupta RC, Kohli S, et al. Chronic therapy with a partial adenosine A1-receptor agonist improves left ventricular function and remodeling in dogs with advanced heart failure. Circ Heart Fail 2013;6(3):563-71. (In eng). DOI: 10.1161/circheartfailure.112.000208.
190. Saadjian AY, Paganelli F, Gaubert ML, Levy S, Guieu RP. Adenosine plasma concentration in pulmonary hypertension. Cardiovasc Res 1999;43(1):228-36. (In eng). DOI: 10.1016/s0008-6363(99)00059-0.
191. Cheng DY, DeWitt BJ, Suzuki F, Neely CF, Kadowitz PJ. Adenosine A1 and A2 receptors mediate tone-dependent responses in feline pulmonary vascular bed. Am J Physiol 1996;270(1 Pt 2):H200-7. (In eng). DOI: 10.1152/ajpheart.1996.270.1.H200.
192. Xu MH, Gong YS, Su MS, et al. Absence of the adenosine A2A receptor confers pulmonary arterial hypertension and increased pulmonary vascular remodeling in mice. J Vasc Res 2011;48(2):171-83. (In eng). DOI: 10.1159/000316935.
193. Shang P, He ZY, Chen JF, et al. Absence of the Adenosine A2A Receptor Confers Pulmonary Arterial Hypertension Through RhoA/ROCK Signaling Pathway in Mice. J Cardiovasc Pharmacol 2015;66(6):569-75. (In eng). DOI: 10.1097/fjc.0000000000000305.
194. Alencar AK, Pereira SL, Montagnoli TL, 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(5):953-62. (In eng). DOI: 10.1111/bph.12193.
195. Alencar AK, Pereira SL, da Silva FE, et al. N-acylhydrazone derivative ameliorates monocrotaline-induced pulmonary hypertension through the modulation of adenosine AA2R activity. Int J Cardiol 2014;173(2):154-62. (In eng). DOI: 10.1016/j.ijcard.2014.02.022.
196. Alencar AK, Carvalho FI, Silva AM, et al. Synergistic interaction between a PDE5 inhibitor (sildenafil) and a new adenosine A2A receptor agonist (LASSBio-1359) improves pulmonary hypertension in rats. PLoS One 2018;13(4):e0195047. (In eng). DOI: 10.1371/journal.pone.0195047.
197. Moles VM, Cascino T, Saleh A, et al. Safety of regadenoson stress testing in patients with pulmonary hypertension. J Nucl Cardiol 2018;25(3):820-827. (In eng). DOI: 10.1007/s12350-016-0734-6.
198. Bragança B, Rodrigues, T., Gonçalves, M., Ferreirinha, F., Correia-de-Sá, P., and Fontes-Sousa, A.P. Unveiling the potential benefit of adenosine A_{2B} receptor blockage to counteract right cardiac overload in rat pulmonary arterial hypertension. European Heart Journal2015.
199. Karmouty-Quintana H, Weng T, Garcia-Morales LJ, et al. Adenosine A2B receptor and hyaluronan modulate pulmonary hypertension associated with chronic obstructive pulmonary disease. Am J Respir Cell Mol Biol 2013;49(6):1038-47. (In eng). DOI: 10.1165/rcmb.2013-0089OC.
200. Ranchoux B, Nadeau V, Bourgeois A, et al. Metabolic Syndrome Exacerbates Pulmonary Hypertension due to Left Heart Disease. Circ Res 2019;125(4):449-466. (In eng). DOI: 10.1161/circresaha.118.314555.
201. Albrecht-Küpper BE, Leineweber K, Nell PG. Partial adenosine A1 receptor agonists for cardiovascular therapies. Purinergic signalling 2012;8(Suppl 1):91-99. (In eng). DOI: 10.1007/s11302-011-9274-3.
202. Shah SJ, Voors AA, McMurray JJV, et al. Effect of Neladenoson Bialanate on Exercise Capacity Among Patients With Heart Failure With Preserved Ejection Fraction: A Randomized Clinical Trial. JAMA 2019;321(21):2101-2112. DOI: 10.1001/jama.2019.6717.

203. Chandrasekera PC, McIntosh VJ, Cao FX, Lasley RD. Differential effects of adenosine A2a and A2b receptors on cardiac contractility. American Journal of Physiology-Heart and Circulatory Physiology 2010;299(6):H2082-H2089. DOI: 10.1152/ajpheart.00511.2010.
204. Tang XL, Wang HX, Cho CH, Wong TM. Reduced responsiveness of [Ca2+]i to adenosine A1- and A2-receptor stimulation in the isoproterenol-stimulated ventricular myocytes of spontaneously hypertensive rats. J Cardiovasc Pharmacol 1998;31(4):493-8. (In eng). DOI: 10.1097/00005344-199804000-00004.
205. Voors AA, Shah SJ, Bax JJ, et al. Rationale and design of the phase 2b clinical trials to study the effects of the partial adenosine A1-receptor agonist neladenoson bialanate in patients with chronic heart failure with reduced (PANTHEON) and preserved (PANACHE) ejection fraction. European Journal of Heart Failure 2018;20(11):1601-1610. DOI: https://doi.org/10.1002/ejhf.1295.
206. Voors AA, Düngen H-D, Senni M, et al. Safety and Tolerability of Neladenoson Bialanate, a Novel Oral Partial Adenosine A1 Receptor Agonist, in Patients With Chronic Heart Failure. The Journal of Clinical Pharmacology 2017;57(4):440-451. DOI: https://doi.org/10.1002/jcph.828.
207. El-Tayeb A, Iqbal J, Behrenswerth A, et al. Nucleoside-5'-monophosphates as prodrugs of adenosine A2A receptor agonists activated by ecto-5'-nucleotidase. J Med Chem 2009;52(23):7669-77. (In eng). DOI: 10.1021/jm900538v.
208. Flögel U, Burghoff S, van Lent PL, et al. Selective activation of adenosine A2A receptors on immune cells by a CD73-dependent prodrug suppresses joint inflammation in experimental rheumatoid arthritis. Sci Transl Med 2012;4(146):146ra108. (In eng). DOI: 10.1126/scitranslmed.3003717.
209. Eckle T, Krahn T, Grenz A, et al. Cardioprotection by ecto-5'-nucleotidase (CD73) and A2B adenosine receptors. Circulation 2007;115(12):1581-90. (In eng). DOI: 10.1161/circulationaha.106.669697.
210. Drury AN, Szent-Györgyi A. The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart. J Physiol 1929;68(3):213-237. (In eng). DOI: 10.1113/jphysiol.1929.sp002608.
211. Conceição G, Heinonen I, Lourenço AP, Duncker DJ, Falcão-Pires I. Animal models of heart failure with preserved ejection fraction. Neth Heart J 2016;24(4):275-86. (In eng). DOI: 10.1007/s12471-016-0815-9.