Beneficial and detrimental role of adenosine signaling in diseases and therapy

by

Hong Liu\textsuperscript{1,2,3} and Yang Xia\textsuperscript{1,2,4}

\textsuperscript{1}Department of Biochemistry and Molecular Biology, The University of Texas Health Science Center at Houston, Houston, TX, USA; \textsuperscript{2}Graduate School of Biomedical Science, University of Texas Health Science Center at Houston, Houston, TX, USA; \textsuperscript{3}Department of Otolaryngology, \textsuperscript{4}Department of Nephrology, Xiangya Hospital, Central South University, Changsha, Hunan, China

Running Title: Adenosine signaling, disease and therapy

There are no conflicts of interest for any of the authors.

*Correspondence: Yang Xia, Department of Biochemistry and Molecular Biology, University of Texas-Houston Medical School. Email: yang.xia@uth.tmc.edu

Copyright © 2015 by the American Physiological Society.
Abstract

Adenosine is a major signaling nucleoside that orchestrates cellular and tissue adaptation under energy depletion and ischemic/hypoxic conditions by activation of four G-protein coupled receptors (GPCR). The regulation and generation of extracellular adenosine in response to stress is critical in tissue protection. Both mouse and human studies reported that extracellular adenosine signaling plays a beneficial role during acute states. However, prolonged excess extracellular adenosine is detrimental and contributes to the development and progression of various chronic diseases. Recent years, substantial progress has been made to understand the role of adenosine signaling in different conditions and to clarify its significance during the course of disease progression in various organs. These efforts have and will identify potential therapeutic possibilities for protection of tissue injury at acute stage by up-regulation of adenosine signaling or attenuation of chronic disease progression by down-regulation of adenosine signaling. This review is to summarize current progress and the importance of adenosine signaling in different disease stages and its potential therapeutic effects.
1 Introduction

1.1. Metabolism of adenosine

Adenosine is ubiquitously produced in almost all of the cells in our bodies under physiological condition and further produced under hypoxia or energy depletion condition. As a building block and a critical intermediate metabolite of nucleic acids, adenosine is a key signaling molecule that orchestrates the cellular response to hypoxia, energy depletion and tissue damage by activation of its G-protein coupled receptors (GPCR) on multiple cell types. Under normal physiological conditions, both extracellular and intracellular adenosine levels are in the nanomolar range. However, under stress conditions, ATP is released from injured cells such as endothelial cells, neutrophils and glial cells via transmembrane protein channels including pannexins or connexins, and subsequently dephosphorylated to extracellular adenosine by ecto-nucleotidases including CD39, which converts ATP to ADP/AMP and CD73, which converts AMP to adenosine. Under pathological conditions, extracellular adenosine concentrations can reach the millimolar range. Generation of extracellular adenosine through these pathways is the major source of extracellular adenosine production under hypoxia-induced injury. In addition, extracellular adenosine is regulated by adenosine deaminase (ADA), which is responsible for the degradation of extracellular adenosine.
Moreover, extracellular adenosine signaling is terminated by equilibrative nucleoside transporters (ENTs), which are involved in the cellular uptake of adenosine. Once inside the cell, adenosine is metabolized by three enzymes, adenosine kinase (ADK), S-adenosylhomocysteine hydrolase (SAHH) and adenosine deaminase (ADA). ADA catalyzes the irreversible conversion of adenosine to inosine. SAHH converts adenosine to adenosylhomocysteine (AdoHcy). ADK phosphorylates adenosine to AMP, and is critical for regulating intracellular levels of adenosine and maintaining intracellular levels of adenine nucleotides(94). Intracellular adenosine homeostasis is also maintained by bi-directional equilibrative nucleoside transporters (ENTs) on the plasma membrane, through facilitated diffusion of adenosine in the direction of the concentration gradient (Figure 1)(70).

1.2. Adenosine signaling via adenosine receptors

Increases in extracellular adenosine in turn elicit various responses on target cells by engaging cell surface adenosine receptors both in physiological and pathological conditions(44). As GPCRs, adenosine receptors all have a single polypeptide chain which is a structural motif forming seven transmembrane helices. There are four adenosine receptors (ADOR1, ADORA2A, ADORA2B, and ADORA3), and each receptor has a cellular or tissue specific distribution and distinct affinity for adenosine(33). ADORA1, ADORA2A, and
ADORA3 have a high affinity to extracellular adenosine, while ADORA2B has the lowest affinity to extracellular adenosine. Thus, ADORA2B is normally activated under pathological conditions due to excess accumulation of extracellular adenosine. ADORA1 and ADORA3 adenosine receptors are coupled to adenylyl cyclase by the inhibitory G-protein subunit (Gαi) and thereby can lower intracellular levels of the second messenger cyclic adenosine monophosphate (cAMP). In contrast, the ADORA2A and ADORA2B adenosine receptors are commonly coupled to adenylyl cyclase by the stimulatory G-protein subunit (Gαs) and therefore can induce intracellular cAMP levels. Therefore, signaling through adenosine receptors plays important roles in the regulation of intracellular cAMP and thereby regulates multiple cellular functions including vasodilation in endothelial cells, neurotransmitter release from neuronal cells, neutrophil chemotaxis and vascular smooth muscle cell relaxation(63, 64) (Figure 2). In addition, other signaling molecules including phospholipase C (PLC), calcium, nitric oxide (NO), reactive oxygen species (ROS), phosphatidylinositol 3-kinase (PI3K)-AKT, extracellular signal-protein kinase (ERK), and mitogen-activated protein kinases (MAPKs) are implicated functioning downstream of adenosine receptors and subsequently regulating multiple cellular functions. For example, activation of ADORA2A stimulates the PLC pathway and adenylate cyclase pathway(47). ADORA2A signaling is also engaged in modulation of neutrophil function by regulating production of ROS(23, 119). By modulation of NO production via vascular
endothelial cells, adenosine through ADORA2A receptor functions as a potent vasodilator involved in tissue blood flow and cellular homeostasis(55, 80). In addition, shear stress-mediated elevation of adenosine activates ADORA2B, subsequently contributes to endothelial NO synthase phosphorylation via PI3K–AKT, and further generates NO(122). Both pharmacological and genetic studies show that adenosine ADORA2B induces inflammatory cytokine interleukin 6 (IL-6), and contributes to the renal fibrosis(24). The activation of ADORA3 triggers MAPK, and contributes to the critical role of cell growth, survival and differentiation(104). Other studies reported that activation of ADORA3 modulates the proliferation of melanoma cells by regulation of ERK pathway (Figure 2)(86). Thus, activation of adenosine receptors are involved in multiple cellular function via multiple downstream signaling cascade.

1.3. Adenosine signaling in physiological and pathological conditions

Adenosine is involved in numerous critical physiological processes via activation of its adenosine receptors including modulation of nervous system, immune response, vascular function and metabolism(64, 93). Adenosine-mediated biological function is mainly dependent on activation of adenosine receptors, and responses of these cell surface receptors are predominantly determined by adenosine concentrations. Since adenosine levels are generally lower than 1 μM under physiological condition, most function of adenosine signaling is through activation of A1, A2A or A3 adenosine receptors, which have EC50 values between 0.01
μM and 1 μM. In contrast, activation of ADOAR2B requires a higher adenosine concentrations which generally exist under pathophysiological conditions (46). With the development and generation of various adenosine receptors agonists or antagonists and four adenosine receptor knockout mouse models, adenosine signaling has been demonstrated as an essential player under pathophysiological conditions by modulation of inflammation, ischemic tissue injury, fibrosis and tissue remodeling (38, 91, 109).

2. Beneficial role of adenosine signaling during acute states

Recent studies indicate that extracellular adenosine functions as a signaling molecule, which plays an essential role in adaptation to stress especially hypoxia (10, 91, 108). Extracellular adenosine is induced during limited oxygen availability or acute injury, and adenosine is critical for hypoxia adaptation, maintenance of cellular function and protection of hypoxia-induced tissue injury. Under acute hypoxic conditions, adenosine plays various beneficial roles including vasodilatory effect, anti-vascular endothelial leakage and anti-inflammatory response (28, 35, 60, 67, 78).

2.1. Beneficial role in acute heart injury

Beneficial role of adenosine in acute stage was initially found in cardiovascular system showing that adenosine functions as a potent vasodilator increasing blood flow to coronary arteries (106). Later on,
adenosine was implicated to play a generally protective role in the heart by regulation of heart rate, coronary flow, contraction, inflammatory control and tissue remodeling (127). All four adenosine receptors are known to be involved in coronary flow. Generally, previous study identify the expression of ADORA1 in atrial muscle cells and adenosine exerts its cardiac electrophysiologic effects mainly through the activation of ADORA1 that leads to a reduction in contraction rate (12, 73, 103). Adenosine A2A receptor is the major receptor subtype responsible for coronary blood flow regulation in endothelial-dependent and -independent manner (5), previous study reported that adenosine increase coronary flow via vasodilation by promotion of prostacyclin release (7). Additional studies showed that adenosine via ADORA2A contribute to coronary reactive hyperemia by promoting the ROS release (135). Regadenoson (Lexiscan), a specific ADORA2A agonist, was approved by FDA and utilized for diagnosis of myocardial perfusion imaging (89). In addition, the Eltzschig group demonstrated that CD73-mediated adenosine signaling via the ADORA2B is important in cardioprotection by ischemic preconditioning (32). However, Chen and colleagues reported that selective inhibition of adenosine A3 receptor significantly attenuate pressure overload-induced left ventricular hypertrophy and dysfunction (84). These results suggest that selective CD73 agonists and ADORA2B agonists are potential therapeutic possibilities for myocardial ischemia, and specific ADORA3 antagonists
may be a novel strategy to counteract pressure overload-induced left ventricular hypertrophy and
dysfunction(32, 84).

2.2. Beneficial role in acute lung injury

Acute lung injury (ALI) is defined as pulmonary edema and severe hypoxia. Multiple factors including
pneumonia, aspiration or lung contusion, or indirect injury such as sepsis, severe trauma, blood transfusion
cause ALI. Approximately 200,000 patients develop ALI in the US annually. However, due to the lack of
understanding the molecular mechanism involved in the development and progression of ALI, no effective
therapeutic options are available. Several groups reported that adenosine serves beneficial functions on
features of ALI such as enhancing alveolar-capillary barrier function and dampening inflammation, and
substantially protects against ALI resulting from hypoxia or ischemia(31, 112). Follow-up genetic and
pharmacological studies reported that the adenosine mediated beneficial role in ALI is via ADORA2B in a
CD73 dependent manner(30, 101). Therefore, these studies provide potential development of adenosine-

2.3. Beneficial role in acute kidney injury
Acute kidney injury (AKI), characterized as the rapid dysfunction of kidney, is currently the leading cause of mortality and morbidity in hospitalized patients, therefore effective therapeutic strategies are urgently needed. Among multiple factors, renal ischemia is the most common cause of AKI. Previous studies indicated that all four adenosine receptors are expressed in the kidney and are involved in progression of AKI. Particularly, several studies reported adenosine ADORA1 receptor signaling protects the kidney from ischemia-reperfusion injury. Linder’s group showed that adenosine ADORA2A receptor signaling prevents ischemia-induced injury via modulation of inflammatory cells. Additional studies showed that adenosine ADORA2A coupled with epoxeicosatrienoic acids (EETs) plays an important role in regulation of preglomerular microvascular tone, and adenosine-mediated induction of EETs via ADORA2A is required for maintenance of normal renal function under high dietary salt intake, and ADORA2A and EETs are therapeutic targets for salt-sensitive hypertension. In addition, pharmacological and genetic studies demonstrated that adenosine A2B receptor signaling is involved in renal protection during preconditioning. In contrast, activation of the ADORA3 is implicated as detrimental during renal ischemia.

2.4. Beneficial role in brain
As an important signaling molecule, adenosine coupling with its specific receptor functions as an upstream neuromodulator of neurotransmitters involved in the homeostasis and modulation of multiple brain function\(^{(17, 95)}\). For example, previous studies demonstrated that adenosine is present in the extracellular fluid in brain, its level is dramatically induced in the condition of hypoxia or ischemia, and subsequently plays critical effects through activation of its specific receptors. Although all four adenosine receptors are expressed in the mouse forebrain, ADORA1 and ADORA2A have the highest abundance in the brain. Thus, those two adenosine receptors play critical roles in the brain function, while ADORA2B and ADORA3 have relatively modest impact on brain function\(^{(45, 50)}\). It is found that ADORA1 is located presynaptically, postsynaptically and nonsynaptically in brain\(^{(45)}\), and mainly underlies effect of adenosine in neuronal circuits by selectively depressing excitatory synaptic transmission\(^{(27)}\). Both pharmacological evidence and genetic ADORA1 knockout mouse studies demonstrated neuro-protective role of ADORA1 in ischemia/hypoxia models of brain injury\(^{(120)}\). In contrast, ADORA2A are demonstrated to have a widespread distribution in the brain\(^{(45)}\). Chen and other groups suggested that adenosine signaling via ADORA2A is neuroprotective under different pathological conditions\(^{(120)}\) including hypoxia\(^{(52)}\), ischemia\(^{(75, 83)}\), and hypoglycemia\(^{(11)}\). Mechanistic studies demonstrated that adenosine is involved in the brain repair through activation of ADORA2A in glial cells, which promotes the effects cytokines release
including induction of oxygenase 2, NOS activity and NO production and up-regulation of nerve growth factor expression(9). More importantly, adenosine plays a protective effect in the brain through ADORA2A by controlling brain vascular function through endothelial cells. For instance, ADORA2A plays a beneficial role in preventing brain ischemia by induction of cerebral blood flow (CBF) in multiple conditions including energy failure, tissue acidosis, imbalance of ion homeostasis and cytotoxic edema(97, 107). In addition, Winn and colleagues found that the capacity to modulate CBF in response to hypotension was significantly impaired in ADORA2A knockout mice, and treatment with extracellular adenosine transporter inhibitor, dipyridamole, significantly increases circulating adenosine concentrations and subsequently improves CBF in mice, indicating the importance of adenosine ADORA2A in physiologic vascular regulation of CBF(74).

Furthermore, both pharmacological and genetic studies demonstrated that ADORA2A stimulates proliferation of Schwann cells(111). Other studies indicated that adenosine is one of the mediators of cerebral vasodilation by triggering release of ROS via both ADORA2A and ADORA2B in brain(48). Additional studies showed that specific activation of other adenosine receptors contributes to the adenosine-mediated neuroprotective effects as well. For instance, preclinical study showed that A3 specific agonist prevents ischemic brain injury through suppression of apoptosis in wild type mice, but not in the ADORA3-deficient mice(15). Clinical human studies demonstrated that adenosine plays a role of vasodilatation in the cerebral
circulation which can be applied for investigation of cerebrovascular perfusion capacity in patients with carotid occlusive disease (48). Overall, adenosine singling via its specific receptors plays an important role in brain function and modulating adenosine signaling is likely an effective treatment for brain ischemic injury and damage.

2.5. Beneficial role in multiple organ damage at acute states

Adenosine was reported to be beneficial under stress conditions in various organs and tissues through different adenosine receptors (81, 88, 115, 117). Several studies reported that adenosine plays an otoprotective role in the auditory system to counteract intense noise exposure via activation of ADORA1 (57, 58, 126). Cronstein’s group demonstrated that adenosine ADORA2A signaling plays beneficial role in skin by promoting wound healing and angiogenesis (88). Colgan and colleagues used pharmacologic and genetic approaches to show that adenosine signaling via the ADORA2B receptor attenuates tissue injury and inflammation in mucosal organs during intestinal ischemia and colitis (21, 37, 53, 54). Gnad et al. demonstrated that adenosine stimulates brown adipose tissue thermogenesis via ADORA2A, and ADORA2A selective agonist prevents high fat diet-induced obesity in mice (49). Linden’s group reported that elevated adenosine protects against ischemic reperfusion liver injury via ADORA2A signaling (26). In addition, they showed that ADORA2A signaling prevents pulmonary inflammation in a sickle cell disease (SCD) mouse.
model by reducing invariant natural killer cells. Therefore, FDA-approved ADOA2A specific agonist, regadenoson, is currently utilized to conduct a clinical trial in the treatment of patients with SCD(42, 43).

2.6. Summary for beneficial role of adenosine signaling in acute states

Adenosine is induced under stress conditions including hypoxia, ischemia or inflammation. Elevated adenosine subsequently activates four widely expressed adenosine receptors and attenuates tissue injury or promote regeneration of damage tissues.

3. Detrimental role of adenosine signaling in chronic disease states

Although elevated adenosine signaling shows beneficial effects in various organs in response to acute stress or injury, numerous examples indicate that prolonged excessive adenosine signaling is detrimental and contributes to the development and progression of certain chronic disease states.

3.1. Elevated adenosine contributes to sickling and progression of sickle cell disease

Sickle cell disease (SCD) is a devastating genetic hemolytic disorder associated high morbidity and mortality worldwide. Adenosine is well-known to be induced under hypoxic conditions and SCD patients are under a chronic state of hypoxia. By using high throughput metabolomic screening combined with a multidisciplinary approach, Zhang et al. identified the adenosine signaling via erythrocyte ADORA2B
causing induction of 2,3-bisphosphoglycerate, increasing deoxygenation of sickle hemoglobin and subsequently triggering sickling and disease progression in SCD(114, 133). They found that polyethylene glycol-modified adenosine deaminase (PEG-ADA) treatment significantly decreased circulating adenosine levels in the SCD Berkeley mice, and subsequently reduced sickling and improved multi-organ damage, which was reflected by less vascular damage and vascular congestion in liver and lung, attenuated splenomegaly and decreased proteinuria. Similar improvement was also seen in SCD Berkeley mice treated with PSB1115, an ADORA2B-specific antagonist. These findings demonstrated that elevated plasma adenosine via activation of A2B receptor on erythrocyte leads to RBC sickling, hemolysis and multi-tissue damage in sickle cell transgenic mice. Suppression of adenosine A2B signaling by PEG-ADA or an A2B receptor specific antagonist reduced the disease phenotype, thereby revealing potential therapeutic possibilities for SCD(134).

3.2. Differential role of adenosine signaling in priapism and erectile dysfunction

Priapism is defined as persistent penile erection without sexual excitation. The condition stems from a persist relaxation of corpus cavernosal smooth muscle cells, therefore allowing continued engorgement of the corpus cavernosum and persistent unwanted erection. Priapism is a painful pathological condition and it carries a risk of fibrosis which may cause permanent damage to the penis and ultimately erectile dysfunction(90). By
using two independent lines of mutant mice including adenosine deaminase-deficient mice and SCD transgenic mice, Mi et al. reported an unexpected discovery that excess adenosine in the penis, coupled with elevated A2BR signaling, contributes to priapism. Follow-up mechanistic studies demonstrated that adenosine A2B signaling-mediated prolonged penile erection is via cAMP and cGMP activation(87). These findings provide evidence that excess extracellular adenosine contributes to development of priapism via adenosine A2B receptor, and adenosine-mediated therapeutic strategies including PEG-ADA and ADORA2B antagonists are likely novel effective therapeutic treatments for priapism(121-125).

### 3.3. Persistently elevated placental adenosine is pathogenic for preeclampsia

Preeclampsia (PE), a gestation-specific hypertensive syndrome, has a high incidence of mother and infant morbidity and mortality. The placentas which links mothers and fetuses are newly formed organs during pregnancy. Impairment in placental development and function is one of the major factors contributing to the pathogenesis of PE. However, the molecular basis responsible for placental impairment-mediated PE has not been fully understood. Intriguingly, previous studies reported that adenosine levels are significantly elevated in the maternal or fetal circulation of PE patients compared with normal pregnant women and are correlated to disease severity(39, 129). Another earlier study found that elevated adenosine in PE patients is correlated
to Th1/Th2 imbalance(130). In vitro studies indicated that elevated adenosine is related to increased platelet aggregation and P-selectin expression(128). Genetic and pharmacologic studies revealed that chronic elevated adenosine is a previously unrecognized key factor contributing to preeclampsia. Mechanistic studies demonstrated that chronic elevated placental CD73-mediated accumulation of placental adenosine coupled with excess ADORA2B contributes to the features of PE including hypertension, proteinuria, and small for gestational age fetuses(62). Therefore, these studies implicate the novel therapeutic approach by using adenosine-based strategies including PEG-ADA, CD73 inhibitor and ADORA2B antagonist to prevent features of PE and attenuate the morbidity and mortality of PE in humans.

3.4. Sustained elevated adenosine causes chronic lung disease

Chronic lung diseases include pulmonary hypertension and pulmonary fibrosis. Pulmonary hypertension is a common complication of interstitial lung diseases, and pulmonary fibrosis is a component of various interstitial pneumonias(116). These disorders are defined by severity of inflammation, abnormal fibroblast proliferation, and extracellular matrix deposition which cause distortion of pulmonary architecture and pulmonary dysfunction. To elucidate the role of adenosine signaling in the pathophysiology of chronic lung diseases, multiple animal models were used(137). In particular, the Blackburn’s group utilized two independent animal models including ADA knockout mice and bleomycin-induced mice models(6). They
demonstrated that chronic elevation of adenosine results in severe features of chronic lung injury such as airspace enlargement, fibrosis, cardinal signs of chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF)\(^{(6, 20, 71, 102, 113)}\), dysfunction of gas exchange, and the development of hallmarks of pulmonary hypertension\(^{(40, 71)}\). Mechanistically, ADORA2B signaling was found to be correlated with elevation of pulmonary hypertension factors such as IL-6, matrix metalloproteins, and endothelin-1\(^{(71, 113, 136)}\). Furthermore, pharmacologic or genetic suppression of ADORA2B abolished the progression of airspace enlargement\(^{(113)}\), fibrosis\(^{(20)}\) and pulmonary hypertension\(^{(69, 71, 131)}\).

3.5. Excess adenosine plays an important role in chronic kidney disease

Chronic kidney disease (CKD) is a worldwide devastating disease including kidney injury, progression to renal fibrosis and end-stage renal failure. The reactive treatments rarely restore normal kidney function and preventive approaches to limit renal fibrosis are lacking due to poorly understood underlying mechanism for its progression. Zhang et al. found that, in three independent lines of mice, one with a genetic deficiency in adenosine deaminase (ADA), one with angiotensin II-infused mice, and another with surgically manipulated unilateral ureteral obstruction, chronic elevations of renal adenosine level contribute to hallmarks of chronic kidney disease (CKD) including severe kidney injury, fibrosis and hypertension. Moreover, follow-up studies demonstrated that the adenosine-mediated detrimental role in CKD is in an A2B receptor dependent manner,
and that inhibition of adenosine A2B signaling attenuate the progression and features of CKD(24). Taken together, these studies identified the pathophysiological role and determine molecular basis of chronic elevated adenosine signaling in CKD, hypertension and renal fibrosis, and highlighted novel adenosine-based therapeutic possibilities(132).

3.6. Adenosine signaling in multiple chronic conditions.

Adenosine signaling is known in the prevention of acute tissue injury under most pathophysiological conditions. In contrast, excess elevation of adenosine has been indicated in the progression and development of chronic diseases states. In particular, previous study reported that accumulation of extracellular adenosine activates cAMP pathway through ADORA2B, and subsequently induces the apoptosis of arterial smooth muscle cells and contributes to the pathogenesis of atherosclerosis or restenosis(93). Chan and colleagues reported that ADORA2A antagonist significantly prevents the development of dermal fibrosis in the model of excess elevated tissue adenosine, and ADORA2A-deficient mice are protected from bleomycin-induced dermal fibrosis, which implicates the detrimental role of adenosine in the development of skin disorder(13, 41). Other studies demonstrated that adenosine via ADORA2A receptor contributes to the pathogenesis of hepatic fibrosis, which suggests a novel therapeutic strategy in the treatment of hepatic cirrhosis(14). In addition, Chen and colleagues reported that mice lacking ADORA2A display reduced brain damage post
focal ischemia(16) and pharmacologic studies using ADORA2A antagonists showed that ADORA2A blockade confers the protective effect in brain ischemia animal models by regulation of glutamate release, excitotoxicity and generation of oxidents (85, 96), indicating neuroprotective role of ADORA2A blockade. Recently, both human epidemiologic studies and animal results suggest that inactivation of ADORA2A plays a neuroprotective role to prevent neuronal degeneration(18, 68, 105). Thereby blockade of ADORA2A is considered as a leading non-dopaminergic drug for Parkinson's disease (PD) patients, and several ADORA2A antagonists have entered phase II and III clinical trials for advanced PD patients (3, 65, 79, 110).

In addition, several perspective studies reported that with increased consumption of caffeine, a common adenosine antagonist, reduced risk of developing multiple diseases including PD disease(2, 99), Alzheimer's disease(82, 98), chronic liver disorder(56) and diabetes(100, 118).

4. Conclusion

Acutely accumulated extracellular adenosine is considered a beneficial metabolite involved in cellular and tissue adaptation under energy depletion and ischemic/hypoxic conditions. However, prolonged excess extracellular adenosine is detrimental and contributes to development and progression of various chronic diseases. Therefore, it is critical to define the specific roles of adenosine signaling during the course of disease progression in various organs. Understanding the differential roles of adenosine signaling will provide
potential therapeutic possibilities for protection of tissue injury at acute stage by up-regulation of adenosine
signaling or attenuation of chronic disease progression by down-regulation of adenosine signaling.

Sources of Funding

This work was supported by National Institute of Health Grants HL119549 (to MRK, HKK and YX),
DK083559 (to YX), HL113574 (to YX), and American Heart Association Grant 12IRG9150001 (to
YX).

5. References

1. Alencar AK, Pereira SL, Montagnoli TL, Maia RC, Kummerle AE, Landgraf SS, Caruso-Neves C, Ferraz EB,
   Tesch R, Nascimento JH, de Sant'Anna CM, Fraga CA, Barreiro EJ, Sudo RT, and Zapata-Sudo G. Beneficial
effects of a novel agonist of the adenosine A2A receptor on monocrotaline-induced pulmonary hypertension in


   and Chase TN. Adenosine A(2A) receptor antagonist treatment of Parkinson's disease. *Neurology* 61: 293-296,
   2003.


   consequences of adenosine deaminase deficiency in mice are associated with defects in alveogenesis,

7. Blass KE, Forster W, and Zehl U. Coronary vasodilation: interactions between prostacyclin and

8. Bodin P, and Burnstock G. Increased release of ATP from endothelial cells during acute inflammation.

9. Boison D, Chen JF, and Fredholm BB. Adenosine signaling and function in glial cells. *Cell Death Differ* 17:


Figure legends:

Figure 1. **Metabolism of adenosine signaling.** Cells release ATP through Connexins or Pannexins channels under hypoxia and other stress conditions. The accumulation of extracellular ATP is dephosphorylated to adenosine (A) by two ecto-nucleotidases including CD39 and CD73. Adenosine can further be metabolized by adenosine deaminase (ADA) to inosine, or functions as a signaling molecule by activation of its adenosine receptors (AR) on multiple cell types. Once uptake by equilibrative nucleoside transporters (ENTs), adenosine is further metabolized by adenosine kinase.
(ADK) to AMP, adenosine deaminase (ADA) to inosine, or S-adenosylhomocysteine hydrolase (SAHH) to adenosylhomocysteine (AdoHcy).

Figure 2. **Adenosine receptor-mediated signaling pathways.** Extracellular adenosine functions as signaling molecule by engaging cell surface adenosine receptors (ADORA1, ADORA2A, ADORA2B, ADORA3). ADORA1 and ADORA3 adenosine receptors are coupled to adenylyl cyclase (AC) by the inhibitory G-protein subunit (Gαi) and thereby can lower intracellular levels of the second messenger cyclic adenosine monophosphate (cAMP). In contrast, the ADORA2A and ADORA2B adenosine receptors can induce AC by the stimulatory G-protein subunit (Gαs) and therefore can induce intracellular cAMP levels. Activation of both ADORA1 and ADORA2B stimulates PI3K (phosphatidylinositol 3-kinase)/AKT pathway, and activation of both ADORA2A and ADORA2B induces release of ROS, EETs, PGI2. PKA, protein kinase A; PLC, phospholipase C; ROS, reactive oxygen species; EETs, epoxyeicosatrienoic acids; PGI2, prostacyclin; eNOS, endothelial NO synthase; NO, nitric oxide; IL-6, interleukin 6; MAPK, mitogen-activated protein kinases; ERK, extracellular signal-protein kinase.
Physiological and pathological functions
Fig. 2

ADORA1 → Gal → PI3K → PLC → ROS → EETs → PGI2 → NO → AC → cAMP → PKA

ADORA2A → Gal → PI3K → PLC → ROS → eNOS → AC → cAMP → PKA

ADORA2B → Gal → PI3K → ROS → eNOS → AC → cAMP → NO → NO

ADORA3 → Gal → PI3K → MAPK → ERK

AC → cAMP → PKA

EETs PGI2

Prepared for publication by 10.220.33.2 on October 1, 2017.
<table>
<thead>
<tr>
<th>Organs</th>
<th>Ado receptors</th>
<th>Functions</th>
<th>Cell types</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>ADORA1</td>
<td>Slow heart rate</td>
<td>Atrial muscle cells</td>
<td>12,73,103</td>
</tr>
<tr>
<td></td>
<td>ADORA2A</td>
<td>Vasodilation</td>
<td>Endothelial cells</td>
<td>5,7,89</td>
</tr>
<tr>
<td></td>
<td>ADORA2B</td>
<td>Ischemic preconditioning</td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>Lung</td>
<td>ADORA2A</td>
<td>Anti-inflammation</td>
<td>Immune cells</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>ADORA2B</td>
<td>Vascular barrier function</td>
<td>Endothelial cells</td>
<td>29,101,112</td>
</tr>
<tr>
<td></td>
<td>ADORA3</td>
<td>Anti-inflammation</td>
<td>Eosinophils/neutrophils</td>
<td>31</td>
</tr>
<tr>
<td>Kidney</td>
<td>ADORA1</td>
<td>Anti-inflammation/apoptosis</td>
<td>Immune cells</td>
<td>66,72,76,77</td>
</tr>
<tr>
<td></td>
<td>ADORA2A</td>
<td>Anti-inflammation</td>
<td>Immune cells</td>
<td>25,92</td>
</tr>
<tr>
<td></td>
<td>ADORA2B</td>
<td>Vascular barrier function</td>
<td>Endothelial cells</td>
<td>51</td>
</tr>
<tr>
<td>Brain</td>
<td>ADORA1</td>
<td>Inhibit excitatory transmission</td>
<td>Synapse</td>
<td>27,45,120</td>
</tr>
<tr>
<td></td>
<td>ADORA2A</td>
<td>Increase cerebral blood flow</td>
<td>Endothelial/glial cells</td>
<td>9,74</td>
</tr>
<tr>
<td></td>
<td>ADORA3</td>
<td>Anti-apoptosis</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Cochlea</td>
<td>ADORA1</td>
<td>Anti-oxidants</td>
<td>Cochlear hair cell</td>
<td>57,58,126</td>
</tr>
<tr>
<td>Obesity</td>
<td>ADORA2A</td>
<td>Promote thermogenesis</td>
<td>Brown adipose tissue</td>
<td>49</td>
</tr>
<tr>
<td>Liver</td>
<td>ADORA2A</td>
<td>Anti-inflammation</td>
<td>Immune cells</td>
<td>26</td>
</tr>
<tr>
<td>Skin</td>
<td>ADORA2A</td>
<td>Would healing</td>
<td>Endothelial cells/Immune cells</td>
<td>88</td>
</tr>
<tr>
<td>Intestine</td>
<td>ADORA2B</td>
<td>Anti-inflammation</td>
<td>Immune cells</td>
<td>21,37,53,54</td>
</tr>
<tr>
<td>Organs</td>
<td>Functions</td>
<td>References</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------</td>
<td>-------------------------------------------------------------</td>
<td>--------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin disorder</td>
<td>Dermal fibrosis</td>
<td>13,41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatic dysfunction</td>
<td>Hepatic fibrosis and hepatic cirrhosis</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain damage</td>
<td>Glutamate release, excitotoxicity and generation of oxidents</td>
<td>16,85,96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parkinson's disease</td>
<td>Neuronal degeneration</td>
<td>2,3,65,79,99,105</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sickle cell disease</td>
<td>Erythrocyte sickling and multiple organ damage in SCD</td>
<td>114,133,134</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Priapism</td>
<td>Priapism and penis fibrosis</td>
<td>87,90,121-125</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>PE including proteinuria, hypertension small fetus</td>
<td>62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic pulmonary disease</td>
<td>Pulmonary fibrosis, hypertension</td>
<td>6,20,71,102</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>Renal fibrosis and hypertension</td>
<td>24,132</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>