Adenosine Generation and Signaling during Acute Kidney Injury

Jessica D. Bauerle,* Almut Grenz,* Jae-Hwan Kim,*† H. Thomas Lee,‡ and Holger K. Eltzschig*§

*Mucosal Inflammation Program, Department of Anesthesiology, University of Colorado Denver, Aurora, Colorado; †Department of Anesthesiology, Korea University College of Medicine, Seoul, Korea; ‡Department of Anesthesiology, Anesthesiology Research Laboratories, College of Physicians and Surgeons, Columbia University, New York, New York; and §Department of Anesthesiology and Critical Care Medicine, University of Tübingen, Tübingen, Germany

Acute kidney injury (AKI) is defined as a rapid loss of renal function due to kidney damage, resulting in retention of waste products and uremic toxins that are normally excreted by the kidney. Important mechanisms causing AKI include renal ischemia1–3 or sepsis.4,5 AKI associates with dramatic increases in morbidity and mortality; a recent study in hospitalized patients indicates a rise of serum creatinine of only 0.3 mg/dl is associated with a 70% increase in the risk of death.6 Despite continued poor clinical outcomes, there are only few successful preventive or therapeutic strategies available. Given the increase in morbidity and mortality, the lack of therapeutic and preventative measures available, and the incredible cost associated with renal failure, it is not surprising that the search for therapeutic approaches to prevent or treat AKI is an area of intense investigation.

Renal ischemia after attenuated blood supply to the kidneys or the renal tissue is among the leading causes of AKI.5,7,8 Ischemic tissue damage involves several different mechanisms, including renal inflammation, direct tubular damage, and alterations of vascular responses.9 Inflammatory markers during renal ischemia include increased neutrophil infiltrates, elevated levels of adhesion molecules, increased proinflammatory cytokines, and also increased reactive oxygen species.5,10 Other features of renal damage include damage within the tubules themselves. Necrosis, apoptosis, and a loss of the brush border in the proximal tubule are all observed after an ischemic event.9 This can lead to cast formation and obstruction of outflow from the tubules.5,11,12

Recent evidence suggests that adenosine plays a critical role in cellular adaptation to hypoxia.13–22 Adenosine is an endogenous compound that exists in the intra- or extracellular space.23 Several studies implicate extracellular adenosine generation and signaling in adaptation to hypoxia.24,23,25 As such, extracellular adenosine production is enhanced during limited oxygen availability23,26–29 and is critical to maintaining cellular functions during hypoxia,30,23,31 or to dampen hypoxia-driven inflammation of the kidneys. Moreover, experimental therapeutics targeting individual adenosine receptors demonstrate strong prophylactic or therapeutic effects during murine AKI. If these experimental strategies can be translated into a clinical setting, adenosine receptor therapeutics may become an integral part in the prevention or treatment of AKI from renal ischemia.
driven inflammation. On the basis of such findings, recent studies in the kidneys implicate extracellular adenosine generation and signaling as pharmacologic targets for protection from ischemia.

### PROPERTIES AND CLINICAL USE OF ADENOSINE

Adenosine (Figure 1) is a nucleoside, composed of an adenine base and ribose sugar. It is present in every tissue of the body and represents a vital component of the energy-producing machinery. In the extracellular space, adenosine acts as a signaling molecule. The first report regarding adenosine signaling was published in 1929 by Drury and Szent-Györgyi. They made the keen observation that intravenous injection of an extract from cardiac tissues associates with a dramatic slowing of the heart rate in a whole-animal model. The moiety within the cardiac extract responsible for inducing the bradycardia or heart block was an adenine compound, subsequently identified as adenosine.

In the mid-1960s, the first studies on the role of adenosine in renal physiology were published, demonstrating a decrease in urinary flow after adenosine administration. It was not until much later, in the 1980s, when intravenous adenosine was used regularly in the clinic, particularly in the treatment of supraventricular tachycardia, where it still remains the only clinically indicated use of adenosine today. However, numerous other drugs utilize adenosine indirectly. For example, dipyridamole is an equilibrium nucleoside transporter blocker that functions as an adenosine-uptake inhibitor. Dipyridamole treatment leads to elevated levels of extracellular adenosine to facilitate its function through adenosine receptors, causing abrupt coronary vasodilation during cardiac stress echocardiography, or as an inhibitor of platelet aggregation in the prevention of recurrent stroke.

Dipyridamole also decreases albuminuria in diabetic patients by an unknown mechanism. Methylxanthines, such as theophylline and caffeine, are known diuretics and act as adenosine receptor antagonists. Theophylline has also been used in the treatment of asthma; however, because of its small therapeutic window and requirement for frequent monitoring of serum levels, it has been largely replaced by other bronchodilators.

### EXTRACELLULAR ADENOSINE GENERATION

In the extracellular space, adenosine mainly derives from the phosphohydrolisis of precursor molecules, particularly adenosine triphosphate (ATP) and adenosine monophosphate (AMP). In fact, during conditions of limited oxygen availability, inflammation, or acute injury, several cells release extracellular nucleotides, particularly in the form of ATP and adenosine diphosphate (ADP). Intracellular ATP concentrations are relatively high (>5 mM), and during injurious conditions intracellular ATP is released from apoptotic or necrotic cells. There are also several coordinated and regulated nucleotide release mechanisms. For example, ADP is delivered into the extracellular space from activated platelets by granular release or from inflammatory cells after activation. Although extracellular nucleotides such as ATP and ADP serve as signaling molecules themselves, and have been implicated in ischemia-driven inflammation or purinergic chemotaxis, they also serve as the main biologic source for the enzymatic production of adenosine in the extracellular space.

As such, ATP and AMP undergo steps of enzymatic phosphohydrolisis to generate adenosine. The first step of this process is initiated by the enzymatic activity of the ectonucleoside-triphosphate-diphosphohydrolase-1 (also known as ecto-apyrase, CD39; Figure 2). CD39 is present in most cell types throughout the body, including the kidneys. Genetic deletion or pharmacologic inhibition of CD39 leads to a multitude of problems with thrombosis and inflammation in animal models. As such, mice absent CD39 face an increase in extracellular nucleotide signaling (particularly ATP and ADP), causing a dysregulation in hemostatic responses and thromboregulation. At the same time, genetic deletion of CD39 also blocks extracellular adenosine generation.

![Figure 1](image1.png)  
**Figure 1.** Chemical structures of adenosine, 5’-adenosine monophosphate, and 5’-adenosine triphosphate.

![Figure 2](image2.png)  
**Figure 2.** Extracellular ATP is rapidly converted to AMP by the surface enzyme ecto-nucleoside-triphosphate-diphosphohydrolase1 (E-NTPDase1 or CD39) with high expression levels in kidneys.
As such, gene-targeted animals for CD39 experience lower adenosine levels during ischemia, and are prone to a proinflammatory phenotype during ambient hypoxia, or acute lung injury. The final step in generating adenosine in the extracellular space is under the control of the enzyme, ecto-5’-nucleotidase (CD73; Figure 3), that is expressed abundantly along the extracellular membrane of most cells. Genetic deletion of CD73 in mice results in a general deficiency of extracellular adenosine. It is present in especially high concentrations in the kidney, brain, lungs, and intestines. Genetic deletion of CD73 in mice shows the receptor is predominantly located in the renal vasculature, with little to no expression in kidneys based on functional studies, immunohistochemistry, or transgenic adenosine receptor reporter mice. Although all four adenosine receptors are detectable in whole kidney homogenates (Figure 4), some studies claim to identify certain receptor locations. A very elegant study from the laboratory of Juergen Schnermann investigated the location of the A1AR. His group administered infusions containing A1AR agonist and antagonist to isolated glomeruli and attached vasculature. Surprisingly, there is persistent vasoconstriction at the distal afferent arteriole closest to the glomerulus. When these same studies are performed in A1AR−/− mice, no vasoconstriction is observed, indicating that A1AR is likely expressed at this site. A2AAR is predominantly located in the glomerular epithelium and adjacent vasculature. It is also present at low levels within the cortex and medulla. A recent study investigating the location of A2BAR using a A2BAR β-galactosidase reporter mouse (A2BAR−/−/β-gal-knock-in) showed the receptor is predominantly located in the renal vasculature, with little to no expression in the cortex and medulla. All four adenosine receptors are expressed in kidneys based on functional studies, immunohistochemistry, or transgenic adenosine receptor reporter mice.

### Extracellular Adenosine Signaling, Uptake, and Metabolism

Adenosine can signal through four adenosine receptors (ARs), the A1AR, A2AAR, A2BAR, and A3AR. These are G protein-coupled receptors that consist of a seven transmembrane domain and use cyclic AMP (cAMP) as their second messenger. In general, the A1AR and A3AR are coupled to the Gs subunit and thereby cause an inhibition of the cAMP pathway. In contrast, the A2AAR and A2BAR are coupled to the Gi subunit and result in stimulation of cAMP levels. The A1AR, A2AAR, and A3ARs are high-affinity receptors, requiring low concentrations (10 nM to 1 μM) of adenosine for receptor activation. These levels are present under physiologic conditions. In contrast, the A2BAR is a low-affinity receptor, requiring higher concentrations of adenosine (>10 μM) for receptor activation. These levels of adenosine are typically seen only in pathologic disturbances, such as during periods of hypoxia, ischemia, and other cellular distress. Extracellular adenosine has a very short half-life in the extracellular space. This is mainly due to its rapid uptake into the intracellular space by nucleoside transporters, where it is rapidly metabolized into inosine by adenosine deaminase or into AMP by adenosine kinase.

### Renal Adenosine Receptor Expression

Individual adenosine receptors associate with different actions, such as elevation or attenuation of CAMP levels, which makes identifying the precise location of the individual receptors important for delineating exact mechanisms of adenosine in the kidney. However, because of the potential cross-reactivity of antibodies, this has proven difficult. Therefore, information about the different individual adenosine receptor locations on specific structures in the kidneys is relatively limited.
expression observed in the renal epithelia under physiologic conditions. Another study isolated the preglomerular vasculature and measured levels of the four different receptors in the afferent arterioles; A1AR and A2BAR are predominant receptors in the preglomerular vessels, with scant expression of the A2AAR and A3AR. It is important to point out that most of these studies are carried out under baseline conditions, and conditions such as ischemia, hypoxia, or inflammation alter adenosine receptor expression significantly.

ALTERNATIONS OF RENAL BLOOD FLOW BY ADENOSINE

Extracellular adenosine is implicated in the regulation of renal blood flow, particularly during tubuloglomerular feedback (TGF). TGF is a fascinating mechanism that involves a complex interplay of the ability of a single nephron to modulate its GFR based on the amount of solute that is delivered to the area of the thick ascending loop at the macula densa. The predominant action of the juxtaglomerular apparatus is to regulate the diameter of the afferent arteriole to alter the amount of solute being filtered and to coordinate renin secretion. TGF serves as a means to internally check that the kidney adjusts the GFR to a range of work it can handle. Mediated by TGF, elevated solute levels present at the macula densa result in vasoconstriction of the afferent arteriole. Studies with mice deficient in extracellular adenosine generation or adenosine signaling through the A1AR lack TGF, indicating that adenosine is central to this feedback loop. This is particularly surprising as adenosine usually mediates a vasodilatory effect. At present, the role of adenosine-mediated TGF in kidney protection from ischemia or during AKI is not understood mechanistically.

TARGETING ADENOSINE GENERATION IN AKI

Adenosine Generation during AKI

Adenosine is a critical compound for tissue adaptation during periods of limited oxygen availability. It is well established that levels of adenosine dramatically increase in the extracellular space during periods of renal hypoxia and ischemia. For instance, during periods of renal ischemia, levels of adenosine in the kidney rise approximately fivefold. Subsequent studies utilizing genetic or pharmacologic inhibition of the enzymes (CD39, CD73) responsible for extracellular adenosine generation show the rise in extracellular adenosine is attenuated greatly. Adenosine is derived mainly at the extracellular surface from the nucleotides, ATP and ADP.

It is hypothesized that the two key enzymes in extracellular adenosine production are instrumental in the protective pathways crucial to kidney protection during periods of ischemia. The role of CD39 was investigated in the kidney using a hanging weight mouse model to induce renal ischemia and renal ischemic preconditioning (IP). IP was first described in the 1980s by Murray and consists of short bursts of ischemia followed by periods of reperfusion. This is protective when it precedes longer ischemic events. Interestingly, when mice are given an inhibitor of CD39, the protection normally conferred by IP is abolished completely. Similarly, kidney protection is eliminated in CD39−/− mice. When soluble apyrase derived from potato extracts with enzymatic activity similar to that of CD39 (ATP/ADP phosphohydrolysis) is administered, the protection by IP is restored again. These data indicate strong evidence for a protective effect and possible therapeutic role for CD39 in renal ischemia.

The enzyme ecto-5′-nucleotidase (CD73) is the rate-limiting step for production of adenosine at the extracellular space. Therefore, similar studies were conducted investigating a potential protective role for CD73 in renal ischemia and ischemic preconditioning of the kidneys. Again, with pharmacologic inhibition of CD73, the protection by renal IP is abolished. However, when mice with gene deletions of CD73 are given soluble nucleotidase derived from snake venom (converting AMP to adenosine), IP protection is restored again. Moreover, pretreatment with soluble nucleotidase imitated the renal-protective effects of preconditioning. Taken together, these studies identify CD39 and CD73 as endogenous mediators of renal protection from ischemia and implicate pharmacologic strategies to elevate extracellular adenosine levels in the treatment or prevention of ischemic AKI.

Adenosine Signaling during AKI

All four adenosine receptors are implicated in affecting outcome parameters of AKI (Figure 5). As such, several studies indicate a protective role of signaling events through A1AR in kidney protection from ischemia. Initial insight was gained from studies in A1AR−/− mice, which exhibit a more severe degree of kidney injury after exposure to ischemia than their wild-type controls. Additional studies examined the role of the A1AR in acute and delayed protection from renal ischemia. Interestingly, mice given an A1AR agonist 1 day before renal ischemia, and also mice given the agonist acutely before the ischemic event, are both protected, with less necrosis and apoptosis. However, the mechanisms

Figure 5. The A1AR, A2AAR, and A2BAR are implicated in renal protection from ischemia, whereas A3AR signaling appears to be detrimental to kidney function during renal ischemia.
by which the protection is conferred are of different origins. Other studies use a lentiviral-mediated approach to introduce human A1AR in wild-type or A1AR-deficient mice. After renal ischemia, A1AR-deficient mice reconstituted with human A1AR or wild-type mice treated with human A1AR lentivirus demonstrate dramatic improvements in renal function with lower creatinine levels, and also reduced inflammatory infiltrates and increased levels of heat-shock protein-27, as compared with controls.

Other studies implicate A2AAR in renal protection from ischemia, particularly through signaling events involving inflammatory cells. For this purpose, Linden and Okusa generated A2AAR bone-marrow chimeric mice in which A2AAR−/− mice were infused with wild-type bone marrow after irradiation, and vice versa. Renal studies in these models reveal that, after ischemia, mice in which A2AAR is present on bone marrow–derived cells, but not on renal tissue, are protected.2 Adoptive transfer experiments in mice lacking T and B cells (Rag-1−/−) indicate that A2AAR signaling through CD4+ cells play a key role in renal protection from ischemic injury.3 These may be CD4+ T regulatory cells.

A2BAR is also beneficial during periods of renal ischemia. One study examined the role of different adenosine receptors during renal ischemia after ischemic preconditioning. Surprisingly, when mice deficient in each of the four adenosine receptors are compared, only the A2BAR−/− are unprotected by renal IP treatment, indicating A2BAR signaling in renal protection during preconditioning. Inflammatory markers such as NF-κB activation, myeloperoxidase accumulation, and granulocyte infiltration greatly attenuate after IP treatment of wild-type mice before ischemia. In contrast, these markers are significantly elevated in A2BAR−/− mice when IP protection is abolished. Moreover, treatment of wild-type mice with a specific A2BAR agonist (Bay 60-6583) before renal ischemia associates with kidney protection similar to that seen with IP treatment, including dramatic improvements in GFR, creatinine clearance, and renal histology.

In contrast, treatment of A2BAR−/− mice with Bay 60-6583 is not associated with renal protection from ischemia, indicating the specificity of this compound for murine A2BAR in vivo. Studies were also conducted in chimeric mice, with transfer of bone marrow from wild-type mice into A2BAR−/− mice, and bone marrow from A2BAR−/− mice into wild-type mice. Interestingly, A2BAR−/− mice with wild-type bone marrow show protection similar to that of wild-type mice. Such findings suggest the A2BAR confers protection during renal ischemia at the level of the renal tissues rather than myeloid cells.

In contrast to other adenosine receptors, activation of the A3AR is implicated as antiprotective during renal ischemia. When wild-type mice are given an A3AR agonist before renal ischemia, the severity of damage is accelerated greatly. Moreover, renal protection is seen when mice are treated with an A3AR antagonist before ischemia.

**SUMMARY AND FUTURE CHALLENGES**

AKI from renal ischemia continues to be a major cause of morbidity and mortality in critical care and perioperative patients. Efforts to develop new preventive or therapeutic measures to attenuate kidney injury from ischemia are urgently needed. Extracellular adenosine is a central signaling molecule involved in modulation of inflammatory events and mediating adaptation to hypoxia. Adenosine signaling helps alleviate ischemic injury in other organs, and more recently, experimental studies show promising results in AKI as well. Such studies highlight pharmacologic strategies to enhance extracellular adenosine signaling or the targeting of individual adenosine receptors, particularly, the A1, A2A, or A2BAR are effective in preventing or treating AKI from ischemia in murine models. Although the exact mechanism of kidney protection from ischemia by adenosine needs further work, it remains a major challenge to translate these experimental studies from bench to bedside. Specific adenosine receptor agonists are being tested in clinical trials now, and it will be exciting to see if such studies demonstrate a renal protective effect in patients with AKI.

**DISCLOSURES**

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