

# Adenosine A<sub>3</sub> Receptor and Cardioprotection

## Enticing, Enigmatic, Elusive

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Ischemic injury to the myocardium is a leading cause of morbidity and mortality around the globe.<sup>1</sup> It is worth remembering, however, that this insult to the heart emerged only in the last few centuries, an instant in time from an evolutionary perspective. Thus, any cardioprotective responses to ischemic injury undoubtedly evolved for an entirely different purpose. Beyond pure academic interest, however, there is a profound clinical need to identify new means of protecting the myocardium from disease-related stress. Among the strategies in development is a concerted effort to understand and enhance the body's innate mechanisms of cardioprotection.

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### Article p 1713

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Among these cardioprotective mechanisms is one mediated by the molecule adenosine. This purine nucleoside, derived largely as a metabolite of 5'-AMP, coordinates organ metabolism and blood supply and modulates immune responses. Under conditions of cellular/metabolic stress, activation of the adenosinergic system has beneficial effects, and in heart, adenosine has well-established functions to mitigate myocardial damage from ischemia-reperfusion injury.<sup>2</sup> For this reason, there is great interest in adenosinergic signaling as a means of treating ischemic heart disease. However, the biology of adenosinergic signaling in several organ systems has proven complex, manifesting context-dependent pro- and anti-survival actions.

A number of mechanisms have been invoked to explain the cardioprotective actions of adenosine. These include preservation of ATP levels, stimulation of glycolysis, and limitation of oxygen demand. Some of these events are mediated by alterations in protein kinase C, phosphoinositide-3 kinase, and mitogen-activated protein kinase signaling pathways. To cite 1 example, A<sub>1</sub> adenosine receptor antagonism prevents ischemia-induced sensitization of adenylyl cyclase via a protein kinase C-mediated pathway.<sup>3</sup> However, there is considerable disagreement in the literature, possibly resulting from the fact that several commonly used signaling inhibitors,

including inhibitors of protein kinase C, significantly limit agonism of adenosine receptors.<sup>4</sup>

Adenosine signals through 4 receptors called A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, and A<sub>3</sub>. These receptors, which are expressed in a broad range of tissues, couple to G proteins to elicit a range of responses. It is established that A<sub>1</sub> and A<sub>2A</sub> receptor subtypes are expressed in adult cardiomyocytes, but it is less clear whether A<sub>2B</sub> and A<sub>3</sub> receptors are expressed in these cells. Given a literature demonstrating important actions of adenosine to mitigate cardiac injury, including in isolated cardiomyocytes, recent work has focused on dissecting the relative contributions of the 4 receptor isoforms, including potential cross-talk among them.<sup>2</sup>

Most work has focused on A<sub>1</sub> receptors; relatively less is known about A<sub>3</sub>-dependent signaling despite the fact that the A<sub>3</sub> receptor is widely expressed in a broad range of tissues. What is known about A<sub>3</sub> signaling in the heart or other organ systems is puzzling and often contradictory. For example, activation of this receptor has important and countervailing actions in a variety of settings. In the immune system, A<sub>3</sub>-mediated events can be either proinflammatory or antiinflammatory. A<sub>3</sub> signaling can elicit either antitumor or tumorigenic actions in cancer. A<sub>3</sub> receptor signaling has been reported to protect the heart against ischemic<sup>5-9</sup> and doxorubicin-induced damage.<sup>10</sup> However, in other tissues subjected to ischemia such as brain, kidney, lung, and eye, its actions can be either protective or harmful, again depending on the situation. Despite the existence of conflicting reports in the literature, a consensus suggests that the kinetics and magnitude of A<sub>3</sub> receptor activation in the setting of ischemic insult are critical to the dual actions of adenosine.<sup>11</sup>

This is where a recent report by Lu et al<sup>12</sup> enters the picture. These investigators have conducted a study to dissect the relative contributions of A<sub>1</sub> and A<sub>3</sub> receptors to the cardioprotective actions of adenosine in the setting of pressure-overload stress induced by thoracic aortic constriction (TAC). The study was formulated to test the hypothesis that the cardioprotective actions of adenosine are mediated via A<sub>1</sub>- and A<sub>3</sub>-dependent events. Consistent with this hypothesis, Lu and coworkers found that genetic inactivation of the A<sub>1</sub> receptor was associated with heightened mortality in the setting of severe pressure-overload stress. These data, then, suggest that signaling mediated by this receptor (presumably from circulating or, more likely, locally released adenosine) confers a protective action to counter hemodynamic stress. Unexpectedly, however, genetic ablation of the A<sub>3</sub> receptor had the opposite effect; in the absence of A<sub>3</sub> signaling, pathological cardiac remodeling, including hypertrophic growth and fibrotic change, was blunted. These data suggest that the A<sub>3</sub> receptor governs events that are progrowth.

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Analogous experiments in vitro using selective pharmacological antagonists of each receptor subtype similarly pointed to A<sub>3</sub>-mediated signaling as countering the antihypertrophic actions of adenosine.

This report makes a convincing case that A<sub>3</sub> receptor inactivation modestly attenuates TAC-induced left ventricular hypertrophy, which is a significant finding. However, in vitro studies using rat neonatal cardiomyocytes are more puzzling in that pharmacological inhibition of the A<sub>3</sub> receptor blunted phenylephrine-induced protein synthesis but was associated with an amplified phenylephrine-induced increase in rat neonatal cardiomyocyte cross-sectional area.

With respect to the A<sub>1</sub> receptor in cardioprotection, prior reports have been conflicting,<sup>13,14</sup> and aspects of the present study are similarly puzzling. For example, genetic inactivation of the A<sub>1</sub> receptor had no significant effect on TAC-induced pathological cardiac remodeling, and mortality in the setting of moderate pressure stress was not altered. However, mortality was increased in A<sub>1</sub> knockout mice subjected to severe pressure stress, and pharmacological antagonism of the A<sub>1</sub> receptor slightly reversed the effects of adenosine on cell cross-sectional area in vitro. The combined facts that A<sub>3</sub> receptor inactivation only modestly attenuated pressure-overload hypertrophy and A<sub>1</sub> receptor inactivation had no effect suggest that other mechanisms, possibly mediated by other adenosine receptor isoforms, play a role.

An important question for the future pertains to the localization of A<sub>3</sub> receptors involved in cardioprotection. Whereas A<sub>3</sub> receptors are expressed in a near-ubiquitous manner, particularly high levels of expression are found in mast cells, neutrophils, eosinophils, and other inflammatory cells.<sup>11</sup> Detection of A<sub>3</sub> receptors in cardiomyocytes has proved elusive, below the limits of detection of radioligand binding or Northern blot.<sup>15</sup> In fact, transgenic overexpression of A<sub>3</sub> receptors at modest levels (66 fmol/mg protein) triggers maladaptive events that culminate in dilated cardiomyopathy.<sup>15</sup> Future studies are required to determine whether, relevant to the studies presented here, the pertinent molecular events are autonomous to the cardiomyocyte or entail paracrine actions within cardiac tissue.

A larger goal is to dissect the downstream signaling events elicited by A<sub>1</sub> and A<sub>3</sub> receptors in heart beyond their shared activation of G $\alpha$ i and to parse their role(s) in the various cellular elements within the myocardium. A<sub>3</sub> activation triggers phospholipase C stimulation, mobilization of inositol triphosphate and intracellular Ca<sup>2+</sup>, and ATP-sensitive potassium (K<sub>ATP</sub>) channel activation. Interestingly, Lu et al report diminished activation of extracellular signal-regulated kinase, Jun N-terminal kinase, and phosphoinositide-3 kinase pathways in TAC-stressed A<sub>3</sub> knockouts.<sup>12</sup> In future studies, it will be of interest to explore the role of mitochondrial K<sub>ATP</sub> channels and/or the mitochondrial permeability transition pore, both of which have been implicated in ischemic cardioprotection.<sup>16,17</sup>

As with all studies, this interesting report raises new questions for the future. As noted above, adenosine is known to affect many processes, including inflammation, neurohumoral responses, blood flow, and blood pressure. In fact, Lu et al report decreased levels of several inflammatory markers

in TAC-stressed A<sub>3</sub> knockouts.<sup>12</sup> In future studies, it will be of interest to tease apart the potential actions of A<sub>3</sub> signaling in all of these cellular events. For example, does A<sub>3</sub> receptor ablation alter inflammatory cell infiltration, cytokine and vasoactive peptide release, etc? In addition, what was the cause of excess mortality in the A<sub>1</sub> receptor knockouts subjected to severe TAC? How is it that TAC-induced hypertrophic growth or systolic dysfunction in these animals was not different from wild-type animals? Could strain differences play a role, given that the A<sub>3</sub> knockouts<sup>18</sup> were maintained on a C57BL6 background and the A<sub>1</sub><sup>19</sup> and CD73<sup>20</sup> knockouts were bred on a 129 background? Do protein-protein interactions among the various adenosine receptor isoforms and the consequent cross-talk among signaling pathways contribute to these effects? Are the reported changes in markers of oxidative stress in TAC-treated A<sub>3</sub> receptor knockouts mechanistically linked to the blunting of remodeling, or do they occur as a secondary consequence of that blunted remodeling?

Finally, what is the source of the functional adenosine? These investigators studied mice with genetic inactivation of ecto-5'-nucleotidase (CD73), a widely distributed enzyme anchored on the cell surface that produces nucleosides (such as adenosine) from noncyclic nucleoside monophosphates in the extracellular space. In future studies, it will be of considerable interest to define the cellular source of adenosine in the ischemia-injured heart.

The biology of A<sub>3</sub> adenosine receptors is complex and sometimes confusing.<sup>11</sup> Yet, adenosine receptor agonists and antagonists are being developed for oncology, inflammation, and potentially cardiac indications.<sup>21,22</sup> Whereas a plethora of studies have implicated adenosinergic signaling in cardioprotection, the present report by Lu et al suggests that A<sub>3</sub>-mediated signaling may be detrimental in the load-stressed myocardium. As we continue to pursue the lofty goal of cardioprotection, these investigators have done the field a service by reminding us of Hippocrates' aphorism "primum non nocere."

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## Disclosures

None.

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