Adenosine
A Modulator of the Cardiac Response to Stress
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Adenosine has been recognized as a potentially important signaling molecule in the heart for nearly a half century. The original adenosine hypothesis proposed that production of adenosine by cardiac myocytes reflects the metabolic state of the myocardium and serves to regulate vasomotor tone in the coronary resistance vessels, thereby coupling blood flow to the energetic needs of the heart. There are two major pathways for adenosine production in the cardiac myocyte. The transmethylation pathway involves the hydrolysis of S-adenosylhomocysteine (SAH) by SAH hydrolase to l-homocysteine and adenosine. A second pathway, the hydrolysis of AMP by 5'-nucleotidase, predominates during ischemic or hypoxic conditions. According to the adenosine hypothesis, when the energy requirements of the heart increase, the increased rate of ATP hydrolysis would cause cytosolic free ADP levels to rise. In this situation, the myokinase reaction can utilize two molecules of ADP to produce one molecule each of ATP and AMP, the latter being the substrate for 5'-nucleotidase to produce adenosine, which can enter the interstitial space to cause coronary vasodilation. Although this hypothesis for preserving the oxygen supply/demand relationship is attractive, it does not appear to operate during physiological conditions in the normal heart, since adenosine receptor inhibition does not decrease coronary blood flow or impair the increase of coronary flow during exercise.

Implicit in the adenosine hypothesis is the assumption that during increased cardiac work, increases of cytosolic free [ADP] would be required to drive mitochondrial respiration and would serve to increase adenosine production. In fact, over a wide range of workloads, cytosolic free [ADP] remains at a stable low level; only at extreme workloads does ADP rise significantly in the normal heart. Although it has been difficult to demonstrate adenosine effects during normal physiological conditions, adenosine is clearly able to exert important effects during periods of cardiac “stress,” often with a cardioprotective outcome. Thus, myocardial free ADP and interstitial adenosine levels become markedly increased during ischemia and hypoxia and clearly contribute to coronary vasodilation in the ischemic region. Myocardial ADP levels also can be increased in severely hypertrophied or failing hearts. Adenosine produced during a brief period of ischemia, or exogenously administered adenosine, can exert a cardioprotective effect manifested by a reduction of infarct size or decreased myocardial stunning during a subsequent more prolonged period of ischemia (“preconditioning”). Thus, adenosine can exert effects on the ischemic or overloaded heart that are of considerable scientific and potential therapeutic interest.

Adenosine effects are mediated through four distinct receptors: A1, A2A, A2B, and A3, initially defined pharmacologically based on their effect on adenylyl cyclase (inhibition or stimulation) and on their selectivity for agonists and antagonists. All four receptors are members of the G protein–coupled receptor superfamily but have differing primary amino acid sequences and molecular weights. G protein–coupled cell surface receptors are composed of three protein subunits, α, β, and γ (Figure). In the unstimulated state, the α subunit is GDP bound and the G protein is inactive. When stimulated by an activated receptor, the α subunit releases its bound GDP, allowing GTP to bind in its place, causing the G protein complex to dissociate into two activated components, an α subunit and a βγ complex. G proteins are divided into Gs, Gi, Gq, and Go; activation of Gs increases adenylyl cyclase activity and opens Ca2+ channels, while activation of Gi inhibits adenylyl cyclase and decreases cAMP production. Activation of Gq activates phospholipase C-β, Go activates K+ and Ca2+ channels and possibly phospholipase C-β. Activation of Gs-coupled receptors by specific pharmacological agonists, or genetic overexpression of Gs-coupled β1-adrenergic receptors (both in vivo and in vitro), results in cardiac hypertrophy. In addition, activation of Gq-coupled receptors (such as α1-adrenergic receptors) or overexpression of Gq protein in cardiomyocytes resulted in cardiac hypertrophy and failure. G protein–mediated responses are terminated by α subunit–specific GTPase activating proteins termed regulators of G protein signaling (RGS). RGS4 protein was found to counterregulate Gq-mediated hypertrophic signaling in mice. Dramatically increased RGS4 mRNA and protein contents were found in hearts with hypertrophy and failure, suggesting that this protein has the potential to modulate myocardial hypertrophic responses.
Potential signaling pathways for adenosine in modulating cardiac myocyte hypertrophy. Stimulation of Gq-coupled receptors by neurohormonal factors such as NE (norepinephrine), PE (phenylephrine), Ang II (angiotensin II), or ET-1 (endothelin-1) activates a Gq-PLC/PLD (phospholipase C and phospholipase D) signaling pathway, while stimulation of Gs-coupled receptors such as β-adrenergic receptors by Iso (isoproterenol) activates the Gs-cAMP signaling pathway. Activation of Gq and Gs proteins results in activation of Ca²⁺ and cAMP signaling, which increases cardiomycocyte contractility and energy demands and results in hypertrophy. Activation of the Gi-coupled adenosine A₁ receptor inhibits Gs and Gq signaling and protects the myocytes from hypertrophy. PKA indicates protein kinase A; AC, adenylate cyclase; RGS, regulators of G protein signaling; KATP channel, ATP-sensitive potassium channels; CaM-kinase, Ca²⁺/calmodulin-dependent kinase; A₁/A₃, adenosine A₁/A₃ receptors; α₁, α subunit of G protein; and αs/γ, α subunit of Gs or Gq protein.

The cell-specific distribution of adenosine receptors and their corresponding G proteins have the potential to influence multiple signaling pathways involved in the development of cardiac hypertrophy and heart failure. In cardiac myocytes, adenosine A₁ receptor stimulation activates Gi to inhibit adenylyl cyclase. In this way adenosine A₁ receptor stimulation could attenuate effects of β-adrenergic receptor stimulation. Inhibition of Gi signaling with pertussis toxin partially inhibited endothelin-1-stimulated hypertrophy in cultured neonatal rat ventricular myocytes, suggesting that Gi signaling can modulate hypertrophy. Adenosine A₁ receptor overexpression increased myocardial resistance to ischemia, but neither overexpression of adenosine A₁ receptors nor adenosine A₁ receptor gene deletion caused a significant change of heart size, although the response to cardiac overload was not studied. Adenosine inhibits norepinephrine release from presynaptic vesicles, attenuates the renin-angiotensin system, decreases endothelin-1 release, and exerts antiinflammatory effects, actions that would be expected to exert beneficial effects on the overloaded heart. Adenosine A₁ and A₃ receptors contribute to myocardial preconditioning. Alterations of adenosine A₃ gene expression in mice conferred resistance to ischemia, but the response to hypertrophy-inducing stimuli was not studied. Adenosine A₂A receptors are predominantly expressed in the vascular system where they mediate vasodilation. A₂A receptor mRNA found in cardiac myocytes with functional coupling to cAMP has been reported in the rat but not in porcine cardiomyocytes.

It is clear from this brief survey that many adenosine effects have the potential to influence the cardiac response to stress.

In this issue of Circulation Research, Liao and associates report that adenosine can attenuate myocardial hypertrophy both in vivo and in vitro. In cultured rat neonatal cardiomyocytes, 2-chloroadenosine (CAD), a stable analogue of adenosine, inhibited the hypertrophic response to phenylephrine, endothelin-1, angiotensin II, or isoproterenol. This effect was mimicked by the selective adenosine A₁ agonist N-cyclopentyl adenosine (CPA), but not by selective A₂ or A₃ agonists, implying that the antihypertrophic effect was mediated by adenosine A₁ receptors. In in vivo studies of male mice subjected to transverse aortic constriction, continuous infusion of CAD markedly attenuated the hypertrophic response and decreased the development of LV dysfunction. Again, this effect was mimicked by treatment with CPA, indicating that the effect was mediated through adenosine A₁ receptors. It is noteworthy that although the increased LV wall thickness associated with myocardial hypertrophy might be expected to enhance the ability of the heart to pump against an increased load, in fact the attenuated hypertrophy in the animals treated with the adenosine mimetics was associated with improved cardiac function. This finding is in agreement with the concept that the impaired function of the pathologically hypertrophied myocyte outweighs any potential benefit that might accrue from the decreased wall stress resulting from cardiac hypertrophy.

The investigators found that animals treated with the adenosine analogues had decreased plasma concentrations of norepinephrine and renin and suggest that inhibition of sympathetic outflow and activation of the renin-angiotensin system might be responsible for the attenuated hypertrophy. Adenosine is known to attenuate release of norepinephrine from presynaptic vesicles, an action in agreement with the finding of decreased norepinephrine plasma levels in the CAD-treated mice. However, the present data do not allow understanding of whether this is a cause or effect of the adenosine action. Inhibition of neurohormonal activation might exert a protective effect on the overloaded heart, but conversely, an intervention that prevented the development of decompensation would also decrease plasma norepinephrine and renin levels.

A puzzling aspect of this report is that despite the potent antihypertrophic effect of exogenous adenosine receptor agonists, adenosine receptor blockade had no effect on the hypertrophic response to aortic banding. The investigators noted that myocardial 5'-nucleotidase activity (a possible source of adenosine) was significantly increased 4 weeks after aortic banding, a change that could result in increased adenosine production. Consequently, it might be anticipated that endogenous adenosine would act to modulate hypertrophy so that blockade of endogenous adenosine would amplify the hypertrophic response to systolic overload. This was not seen; 8-sulfophenyltheophylline did not aggravate the hypertrophy or increase the incidence of heart failure. This did not result from an inadequate concentration of 8-sulfophenyltheophylline, since the same infusion protocol fully blocked the salutary effects of CAD and CPA. It is possible that endogenous adenosine levels,
although elevated, were insufficient or occurred too late to produce the beneficial effects observed with exogenous adenosine agonists.

Although a role for adenosine in regulation of normal cardiac function during physiological conditions has yet to be clearly defined, it is clear that adenosine (both endogenous and exogenous) can importantly influence the response of the heart to ischemia and hemodynamic overload. It is likely that further exploration of adenosine interactions with the multiple pathways that determine cardiac responses to overload or injury will generate new hypotheses for study and may yield new therapies for cardiac disease.

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References

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