Protective Effects of Adenosine
In Myocardial Ischemia

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Adenosine functions in a multiplicity of physiological and pathophysiological ways and serves as a negative feedback regulator in the cardiovascular system as well as in some cell types. Many of the actions of adenosine are homeostatic and protective in nature, and the nucleoside has been termed a retaliatory metabolite because of these properties. The present review is limited to the involvement of endogenous and exogenous adenosine in myocardial ischemia and the means whereby adenosine can protect the heart from the deleterious effects of an inadequate blood flow and oxygen supply.

Adenosine and Myocardial Reperfusion Injury
Myocardial Stunning

Recently, considerable attention has been focused on the pathophysiology of myocardial ischemia with subsequent reperfusion and on methods of reducing reperfusion injury and the associated reversible posts ischemic dysfunction of the ventricles. The prolonged dysfunction of the ventricles after an episode of ischemia has been termed myocardial stunning, as contrasted to irreversible injury characteristic of myocardial infarction. Furthermore, there has been an enormous interest in the pharmacological modification of this phenomenon as a result of the technical advances in the areas of thrombotytic and angioplastic recanalization of stenotic or occluded coronary arteries, cardioplegic arrest during cardiac surgery, and organ preservation techniques for cardiac transplantation.

Stunned myocardium is characterized by metabolic and functional abnormalities that occur after a period of ischemia that may persist for hours to days. There are measurable defects in myocardial cell volume and ion content, loss of intracellular nucleotides, and contractile dysfunction. There are many possible mechanisms of myocardial stunning.

Inability of the cell to produce sufficient energy. Adenosine triphosphate (ATP) levels are known to be depressed for hours to days after an ischemic episode. During ischemia, mitochondrial function (as measured by the ratio of state 3 to state 4 respiration) remains intact while ATP levels fall significantly. Reduced substrate in the form of adenosine monophosphate (AMP) or diphosphate (ADP) may play a role in myocardial stunning. Furthermore, with an interruption of blood flow to the myocardium, there is a fall in tissue P02, mitochondrial electron flow ceases, and there is a rapid mitochondrial ATPase-mediated hydrolysis of ATP. This hydrolysis of ATP is limited by the decrease in pH that develops during ischemia.

Inability to use energy. Creatine kinase activity and free ADP are reduced in stunned myocardium and may contribute to functional abnormalities of the myofibrils.

Inadequate myocardial perfusion. Factors that may contribute to an insufficient blood flow include endothelial and myocardial cell swelling, microvascular thrombosis, leukocyte plugging, and vascular smooth muscle dysfunction.

Generation of oxygen free radicals from activated neutrophils and from the endothelium. Reversible ischemia may result in the formation of oxygen free radicals, including the superoxide anion (O2-), the hydroxyl radical (OH·), and hydrogen peroxide (H2O2). These oxygen free radicals may contribute significantly to depressed myocardial function with reperfusion of ischemic myocardium. Free radicals are highly reactive and capable of causing membrane lipid peroxidation, protein (enzyme) denaturation, and sarcolemmal and sarcoplasmic reticulum dysfunction. Beneficial effects of antioxidants on stunned myocardium support a role for free oxygen radicals in the damage observed in reperfused myocardium.

Other hypotheses proposed for ventricular dysfunction in stunned myocardium include sarcoplasmic reticulum dysfunction with reduced availability of calcium to the contractile apparatus, reduced calcium sensitivity of the contractile apparatus despite elevated intracellular calcium concentrations, and calcium overload, which may be transient and may contribute to damage of intracellular organelles. An extensive review of this topic recently appeared in this journal and the reader is referred to it for more detailed information.

Summary. Myocardial dysfunction after an ischemic episode is multifactorial in etiology. Factors reported to be associated with myocardial stunning are high-energy phosphate production and utilization, inadequate myocardial perfusion, free radical injury, and alterations in calcium metabolism.

Regional Reperfusion Injury

The use of intra-arterial adenosine infusion to hasten recovery of regionally stunned myocardium was initially
Infarct Size Reduction

A series of articles has recently been published by investigators at Vanderbilt University, Nashville, Tenn., on the ability of adenosine to limit infarct size in a canine model of LAD occlusion with reperfusion.62–65 Olafsson et al62 administered an intracoronary infusion of adenosine at a rate of 3.75 mg/min for the first hour of reperfusion after a 90-minute LAD occlusion. Infarct size, measured by vital staining with triphenyltetrazolium at 24 hours, was reduced in the adenosine-treated group, both when expressed as percent infarct of the area at risk (10% versus 41%) and as a percent of the left ventricle (4% versus 18%). Regional ventricular function was assessed by contrast ventriculography. Both regional and global indexes of ventricular function were substantially improved over control in the adenosine-treated group.62 Furthermore, light and electron microscopy revealed that adenosine treatment reduced the degree of neutrophil infiltration and capillary plugging and enhanced endothelial preservation. These findings were confirmed in a study of similar design, but the duration of LAD occlusion was 120 minutes.63 However, when the length of LAD occlusion was extended to 180 minutes, cardioprotective effects of adenosine were lost.64 In a more recent study with this same model (90-minute LAD occlusion, 72-hour reperfusion), adenosine (0.15 mg/kg/min) was infused intravenously during the first hour of reperfusion. There were no significant effects on heart rate or blood pressure, and infarct size (as percentage of area at risk) was 35% in controls versus 17% in the adenosine-treated group. Better regional ventricular function, reduced capillary plugging, and preservation of endothelial cell structure were all seen in the adenosine-treated group.65

Studies on adenosine and infarct size reduction are not all in agreement. Homeister et al66 reported that in a canine model with 90 minutes of left circumflex coronary artery occlusion and 6 hours of reperfusion, intracoronary adenosine infusions that yielded calculated blood concentrations of 200–600 μmol/l had no effect on infarct size unless the adenosine was administered in combination with lidocaine (2.0 mg/kg i.v.). It is also noteworthy that in the previously cited studies,62–65 lidocaine was administered at the time of occlusion and at the onset of reperfusion.

Retrograde coronary venous infusion of adenosine has also been reported to reduce infarct size.67 In this study, adenosine (20 μg/kg/min) was infused retrogradely via the great cardiac vein for 30 minutes after a 60-minute occlusion of the LAD coronary artery in pigs. Lidocaine was not given in this study. Retrograde adenosine infusion resulted in an infarct size of 27% of the area at risk, whereas right atrial infusion and vehicle controls resulted in infarct sizes of 62% and 56% of the area at risk, respectively.

Summary. Intracoronary infusion, retrograde coronary venous infusion, or intravenous infusion of adenosine can significantly reduce infarct size if the length of occlusion is less than 3 hours. Lidocaine may confer additional cardioprotection when used in combination with adenosine.
Global Ischemic Injury

Open heart surgery requires a period of aortic cross-clamping during cardiopulmonary bypass. The degree of myocardial injury during these episodes of global ischemia is markedly reduced with standard cardioplegic techniques of hypothermia and potassium-induced arrest. However, these methods are not wholly efficacious because metabolic and functional injury secondary to ischemia and reperfusion have been documented both in experimental and clinical models.68-73 Whereas there may be many mechanisms involved in reperfusion injury,3,11,12,14-40 a defect in metabolism plays a significant role.

Benson et al74 first suggested that recovery of ATP during reperfusion is limited by a lack of nucleotide precursors that are degraded and washed out with ischemia and subsequent reperfusion.75 This concept led to an ever-growing interest in substrate enhancement of postischemic myocardium. Research efforts have since focused on a multitude of substrates, the discussion of which is beyond the scope of this review but can be found elsewhere.76

A number of investigators have provided evidence for the use of adenosine as a substrate for ATP resynthesis during global ischemia and reperfusion. Reibel and Rovetto77,78 conducted studies in which isolated rat hearts were exposed to low-flow ischemia (60% reduction in coronary flow) until ventricular failure occurred and then for an additional 30 minutes. The hearts were then reperfused for 30 minutes at normal flow rates. Adenosine (50 μmol/l) was added to the perfusion fluid at the time of onset of ischemia and maintained throughout reperfusion. This procedure had no effect on tissue high-energy phosphate concentrations. However, a close relation between ATP concentrations and ventricular power in postischemic hearts was noted.77 but this does not indicate a cause-and-effect relation. In a second study, these authors79 extended the period of reperfusion with 50 μmol/l radiolabeled adenosine from 30 to 300 minutes. After 5 hours of reperfusion with adenosine, tissue ATP levels recovered from a level of 50% of control values at the onset of reperfusion to ~100% by 300 minutes of reperfusion, whereas ATP levels in the group reperfused without adenosine failed to recover over 300 minutes of reperfusion.

Our laboratory observed the direct effects of adenosine on adenine nucleotide concentrations and ventricular function in an isolated perfused rat heart preparation in which heart rate, ventricular preload and afterload, and coronary flow rate were all held constant.79 These hearts were perfused with or without 100 μmol/l adenosine throughout the protocol of 30 minutes of equilibration, 10 minutes of normothermic ischemia, and 60 minutes of reperfusion. Adenosine significantly increased ATP levels at the end of the ischemic period and during the reperfusion phase. Ventricular function was increased during the reperfusion period in the adenosine-treated group; however, these data do not establish a cause-and-effect relation.

Summary. ATP depletion can be retarded by adenosine, and this effect is associated with improved postischemic ventricular function, thus supporting the use of adenosine as a cardioprotective agent in global ischemia. Whether this effect is mediated via a salvage mechanism or through other receptor-mediated mechanisms has yet to be ascertained.

Cardioplegia

In an initial study addressing the cardioplegic potential of adenosine, Foker et al80 used a canine model of global normothermic ischemia for 20 minutes while the animals were supported on cardiopulmonary bypass. In addition to adenosine alone (20 mg/kg i.a. bolus injection), the adenosine deaminase inhibitor EHNA (erythro-9-[2-hydroxy-3-nonyl]adenine hydrochloride) was also used alone (10 mg/kg) or in combination with adenosine during 20 minutes of ischemia and 30 minutes of reperfusion. Adenosine or EHNA alone had no effect on reperfusion ATP concentrations. However, EHNA plus adenosine restored ATP to 88% of the preischemic ATP levels. Silverman et al81 used hypothermic, potassium-arrested dog hearts in situ and a higher dose of adenosine for aortic root infusion during reperfusion (40 mg/kg). They found that after 60 minutes of ischemia, adenosine alone or in combination with EHNA (10 mg/kg) maintained ATP at a level no different from preischemic levels over a 60-minute reperfusion period. Hence, adenosine prevented the fall in ATP normally seen during reperfusion. However, this study did not evaluate ventricular function.

In a study in which heart rate, preload, afterload, and coronary blood flow were held constant in an in vivo canine model subjected to 1 hour of global normothermic ischemia,82 our laboratory compared standard hyperkalemic cardioplegia with purine-enriched asanguinous cardioplegia (adenosine 100 μmol/l, hypoxanthine 100 μmol/l, and ribose 2 mmol/l)83 or blood cardioplegia.84 In the purine-enriched cardioplegia group, ATP degradation during ischemia was significantly reduced, and postischemic recovery of ventricular function was significantly improved when compared with standard asanguinous or blood cardioplegia.

Reports of the optimal dose of adenosine in cardioplegic solutions of the isolated heart model vary. Hohlfeld et al84 evaluated adenosine infusions over a range of concentrations from 0 to 120 μmol/l and found that 15–30 μmol/l concentrations resulted in the greatest increment in baseline ATP levels (53%). However, the addition of 15 μmol/l adenosine to the perfusion fluid did not significantly improve contractile recovery after 20 minutes of normothermic ischemia and 45 minutes of reperfusion, suggesting that ATP and contractile recovery may not be related. Higher doses of adenosine, from 100 to 400 μmol/l, demonstrate beneficial dose-dependent effects on both adenine nucleotide preservation and postischemic ventricular function with an optimal concentration at ~200 μmol/l.85-87 Several studies in both isolated crystalloid-perfused rat hearts and blood-perfused baboon hearts (in vivo) have demonstrated enhanced cardioprotection with adenosine with doses of 1–10 mmol/l.88-91

The use of adenosine as the sole cardioplegic agent90 or in combination with potassium90 is effective when used in high concentrations (1–10 mmol/l) by virtue of the ability of the nucleoside to induce cardiac arrest or to hasten potassium-induced arrest. These effects are caused by an adenosine-induced activation of the outward potassium current and subsequent membrane
Furthermore, adenosine-supplemented cardioplegia is also effective at clinically relevant hypothermic temperatures.\textsuperscript{88,89,93,94}

**Summary.** These studies suggest that adenosine, either alone or in combination with potassium cardioplegia, provides additional cardioprotection during normothermic or hypothermic global ischemia. Adenosine has been shown to reduce ATP degradation during ischemia, to increase ATP resynthesis during reperfusion, and to enhance postischemic ventricular function in crystalloid-perfused and blood-perfused hearts.

**Cardiac Transplantation**

Hyperkalemic arrest and/or hypothermic storage are currently the accepted methods for in vivo preservation of cardiac homografts and provide adequate protection for up to 4 hours.\textsuperscript{95,96} Prolonged storage has been reported to result in tissue edema, a rise in coronary vascular resistance after transplantation, and vascular endothelial injury attributed to hyperkalemic storage solutions.\textsuperscript{97–99} The ability to extend storage time and preserve transplant organ function has been the subject of many studies.

Recently, the use of adenosine in cardiac transplant preservation has gained attention. Petsikas et al.\textsuperscript{100,101} reported that the addition of adenosine (20 \textmu mol/l) or AMP (0.1 mmol/l) to the Krebs-Henseleit perfusion fluid was successful in markedly preserving ventricular function in canine hearts that were stored for 24 hours in a continuous hypothermic perfusion system. Similar results have been reported by Ledingham et al.\textsuperscript{94}

We have also found that adenosine increases the tolerance to ischemia in an isolated normothermic rat heart model.\textsuperscript{79} The preischemic perfusion of 100 \textmu mol/l adenosine for 30 minutes before the onset of total ischemia extended the time to onset of ischemic contracture by 50% and was associated with a reduced rate of ATP degradation.\textsuperscript{79}

**Summary.** Adenosine prolongs the ischemic interval to irreversible contracture and preserves ventricular function after a storage period of up to 24 hours of hypothermic perfusion. Although there are no published studies evaluating adenosine effects on the function of the transplanted heart, further investigation is warranted to evaluate its use in transplant preservation.

**Preconditioning**

Murry et al.\textsuperscript{102} observed that myocardium that is first exposed to multiple brief periods of ischemia is more tolerant to a subsequent episode of prolonged ischemia. They termed this phenomenon ischemic preconditioning. Their data indicate that infarct size in response to a 40-minute coronary occlusion was reduced by 75% (29–7% of area at risk) when the area supplied by the occluded artery had been previously subjected to four successive 5-minute periods of ischemia, each separated by 5 minutes of reperfusion. Preconditioning dramatically increased the tolerance of the myocardium at risk to an otherwise lethal ischemic event. The increased tolerance to ischemia is associated with a reduced rate of energy utilization during ischemia and a slowing of glycolysis and glycogenolysis.\textsuperscript{103} This effect of preconditioning cannot be explained on the basis of metabolic depression derived from postischemic contractile dysfunction.\textsuperscript{104}

Preconditioning has been reported in dogs,\textsuperscript{102} rabbits,\textsuperscript{105} and pigs.\textsuperscript{106} Also, it has been suggested that a preconditioning effect could be conferred in humans by sequential 90-second occlusions in patients undergoing elective coronary angioplasty.\textsuperscript{107} The mechanism responsible for preconditioning is not completely known; other mechanisms in addition to the observed reduction of energy utilization may be involved.\textsuperscript{103} Recently, adenosine has been implicated in preconditioning,\textsuperscript{108} and the beneficial effects of preconditioning could be prevented by pretreatment with the adenosine receptor antagonist 8-sulfophenyltheophylline. This observation suggests that endogenous adenosine is involved in the preconditioning effect.\textsuperscript{108} Furthermore, intravenous or intracoronary adenosine or the selective \(\alpha_1\)-receptor agonist PIA (N\(^6\)-phenylisopropyl adenosine) instead of the ischemic preconditioning protocol conferred a similar protective effect against the subsequent prolonged ischemic episode.\textsuperscript{109,110} These observations suggest that adenosine \(\alpha_1\)-receptor activation may be involved in the protective changes seen in preconditioning.

**Summary.** Intravenous or intracoronary infusion of adenosine or the \(\alpha_1\)-receptor agonist PIA confers a protective effect on the myocardium similar to a preconditioning protocol, and the preconditioning effect can be prevented by adenosine receptor antagonists.

**Cardioprotective Actions by Adenosine**

**Formation and Metabolism**

It is clear that protection of the heart from episodes of ischemia or hypoxia can be achieved with both endogenous and exogenous adenosine. Endogenous adenosine is primarily formed from the dephosphorylation of AMP that may occur intracellularly and extracellularly; 5'-nucleotidase (both membrane bound and cytosolic) dephosphorylates AMP to adenosine.\textsuperscript{111} AMP is derived from cytosolic ATP and ADP and cAMP, or from extracellular sources of nucleotides such as blood elements,\textsuperscript{112} endothelium,\textsuperscript{113,114} and adrenergic nerves.\textsuperscript{115} However, the major source of coronary adenosine is the cardiomyocyte\textsuperscript{116} by the action of cytosolic 5'-nucleotidase.

Adenosine can also arise from the hydrolysis of S-adenosylhomocysteine (SAH) to adenosine and homocysteine, a reaction catalyzed by SAH hydrolase. The equilibrium constant favors the formation of SAH, and a large percentage of the intracellular adenosine is bound to SAH and cannot be degraded by adenosine deaminase. Furthermore, adenosine release from SAH hydrolysis does not increase during hypoxia-induced increases in cardiac adenosine formation and release.\textsuperscript{117}

Endogenous or exogenous adenosine is removed by 1) phosphorylation by adenosine kinase to AMP, 2) degradation to inosine by adenosine deaminase, or 3) washout in the circulation. Because the \(K_m\) for adenosine kinase is 100-fold lower than that for adenosine deaminase,\textsuperscript{118} the preferential pathway for adenosine metabolism is for salvage by phosphorylation to AMP. The purine salvage pathway is critical for the regeneration of cardiomyocyte adenosine nucleotide pools after an ischemic or hypoxic episode because the pathway for
de novo nucleotide synthesis accounts for only 0.4% of the total nucleotide pool per hour.47 Purine salvage can also be accomplished by the degradation of adenosine to inosine, which is then degraded to hypoxanthine, which can be phosphorylated to IMP and then aminated to AMP. Furthermore, adenosine can be converted to adenine, which can be ribosylated to form AMP. Both of these secondary salvage pathways (for hypoxanthine and adenine) are limited because they require phosphoribosylpyrophosphate, which is present in low concentrations in the myocardium.119,120 However, adenine121 and hypoxanthine122 have been shown to be effective in preserving postischemic ATP and myocardial function.

Therefore, the purine salvage pathways represent the primary mechanism whereby endogenous or exogenous adenosine (and its metabolites) contribute to the preservation of the adenine nucleotide pool during periods of oxygen deprivation and reperfusion.78,123–127

**Transport and Deaminase Inhibitors**

Adenosine and other nucleosides traverse the cell membrane via a nucleoside carrier system that represents a reversible process of simple and facilitated diffusion.128 Several agents are known to inhibit the nucleoside transporter and therefore might theoretically keep nucleosides sequestered in the cell to promote the maintenance of adenine nucleotide levels during ischemia and reperfusion. These agents include dipryidamole, mioflazine, and NBMPR (nitrobenzylmercaptopurine riboside).

Dipryidamole has been shown to inhibit nucleoside (adenosine) transport,129,130 and several studies have demonstrated its ability to reduce infarct size,131–133 although not all studies are in agreement.134

Mioflazine is a chemical analogue of lidoflazine and also inhibits the nucleoside transporter.135 Mioflazine has been shown to reduce the release of adenosine136 and preserve high-energy phosphates and ventricular function after an ischemic insult.137 Both dipryidamole and mioflazine increase coronary blood flow131,138 as well as coronary collateral blood flow, which may aid in the reduction of infarct size.131 In addition to inhibiting the nucleoside transporter, mioflazine also is a calcium channel antagonist that may contribute to its cardioprotective properties. In a recent clinical study, Rosseau et al.139 reported that patients treated with dipryidamole after thrombolysis had better indexes of ventricular relaxation and contraction. These preliminary data suggest that adenosine transport inhibition might improve ventricular function after thrombolysis-induced reperfusion. However, these findings await further study and confirmation.

Dipryidamole is also used to assess coronary perfusion in conjunction with myocardial 201Tl scanning in patients as a noninvasive test to assess coronary artery disease. Dipryidamole is believed to dilate coronary vessels indirectly, with its mechanism of action involving local increases in adenosine concentration by adenosine transport inhibition. Dipryidamole140 and, to a lesser extent, adenosine infusions141 have been shown to decrease the subendocardial-to-subepicardial flow ratio, and in the case of dipryidamole,140 endocardial flow has been shown to decrease, implicating a true steal phenomenon. This suggests that dipryidamole may induce subendocardial ischemia in the presence of significant coronary stenosis. Transient myocardial ischemia with regional contractile dysfunction, chest pain, and ischemic electrocardiographic changes has been reported in patients with isolated stenoses of the LAD coronary artery in response to dipryidamole.142

It is also interesting to note that coronary ischemia does not result in maximal vasodilation. It has been suggested that local or reflex α-adrenergic tone may limit ischemia-induced vasodilation.143,144 Vasodilators such as adenosine have been shown to provide additional vasodilation when infused during myocardial ischemia.141

The nucleoside transport blocker NBMPR has also been shown to increase cellular adenosine during ischemia and to enhance ATP repletion during reperfusion.145 This drug, when combined with the adenosine deaminase inhibitor EHNA, markedly improves postischemic ventricular function.146

Inhibitors of adenosine deaminase such as EHNA or 2-deoxycoformycin increase levels of myocardial adenosine and reduce its breakdown to inosine and hypoxanthine.147 Adenosine deaminase inhibitors in the setting of myocardial ischemia and reperfusion increase tissue and interstitial adenosine levels, promote ATP restoration, and improve postischemic ventricular function.86,148–152 These effects may be mediated by enhanced purine salvage and by reduced free radical–induced injury.145,146 Because uric acid also acts as a free radical scavenger, limiting its formation with the use of adenosine deaminase inhibitors could also paradoxically enhance free radical–induced injury. The overall effects of adenosine deaminase inhibition on free radical–induced injury have not been studied.

**Receptors**

Adenosine receptors are classified as A₁, those that inhibit adenyl cyclase, and A₂, those that stimulate this enzyme system.153 These receptors have been characterized on the basis of radioligand binding studies and the specific pharmacological responses to adenosine and its analogues. In the heart, A₁-receptors are found on cardiomyocytes and vascular smooth muscle, whereas A₂-receptors are found on endothelium and vascular smooth muscle. Cardiac cell A₁-receptors mediate the negative chronotropic,154 dromotropic,154 and inotropic155 responses. A₁-receptors in the heart also appear to mediate the beneficial effects of adenosine in prolonging the time to ischemic contracture156 and preconditioning,109,110 which may involve activation of G proteins157 and glycolytic flux.

Adenosine A₂-receptors are apparently located on the coronary vessels, because intravascular A₂-agonists are more potent coronary vasodilators than A₁-agonists.158 Thus, it has been postulated that exogenous adenosine stimulates A₂-receptors, which induce vascular smooth muscle relaxation, although the mechanism for this response has not yet been elucidated. Endothelial receptor stimulation by exogenous adenosine in guinea pig aorta contributes approximately 30% of the vasodilation. The remaining 70% results from direct stimulation of receptors on vascular smooth muscle and is observed after removal of the endothelium.159
Therefore, adenosine and its analogues aid in protection of the heart from injurious effects of ischemia and hypoxia by activation of the adenosine receptors.

**Coronary Blood Flow**

The earliest response to an inadequate blood supply to the myocardium appears to be dilatation of the coronary resistance vessels. Considerable evidence supports the concept that ischemia or hypoxia-induced vasodilatation is mediated by the release of adenosine from the myocardial cells. Furthermore, the degree of vasodilatation and the release of adenosine from the heart are directly proportional to the degree of oxygen deprivation. Adenosine represents the first line of defense in protection of the myocardium against a decrease in the oxygen supply/demand ratio by reducing coronary resistance and maximizing coronary blood flow.

**Atrioventricular Conduction and Excitation**

The difference in the sensitivity of the atrioventricular (AV) node and the coronary vascular smooth muscle to endogenous adenosine produced by hypoxia is that when the coronary resistance vessels become maximally dilated, a greater degree of oxygen deprivation elicits a greater production of adenosine. The resulting higher myocardial adenosine concentration produces AV conduction delay or block, which represents another mechanism to rectify the oxygen supply/demand imbalance. This dromotropic effect, which has been studied extensively in the isolated perfused guinea pig heart, is also mediated by adenosine. A moderate decrease in the global oxygen supply of the isolated perfused guinea pig heart elicits a conduction delay between the atrium and the bundle of His, and a greater reduction in oxygen supply results in AV block. The degree of impairment of AV conduction is roughly proportional to the adenosine formed in the hypoxic or ischemic myocardium.

Conduction delay or block is a result of activation of the A<sub>1</sub>-adenosine receptor and is attenuated or abolished by administrations of adenosine receptor antagonists or adenosine deaminase and potentiated by adenosine transport blockers, which increase interstitial fluid adenosine levels by blocking uptake of the nucleoside. Adenosine has been shown to act similarly on the human AV node; in fact, it is used clinically to transiently block AV conduction as a means of terminating supraventricular tachycardia in which the AV node is part of the reentrant pathway. By producing AV block, the heart rate is decreased, which reduces the oxygen needs of the heart, thereby aiding in protection of the myocardium from the detrimental effects of oxygen deprivation.

With high concentrations of myocardial adenosine, which can occur with severe reductions in oxygen delivery, the sinoatrial (SA) node becomes depressed via activation of A<sub>1</sub>-receptors. The resulting bradycardia reduces the oxygen requirements of the heart further, thus affording additional protection against ischemic damage to the myocardium. This effect of adenosine on the SA node observed in guinea pig hearts has also been demonstrated in humans and can be abolished by A<sub>1</sub>-receptor antagonists and potentiated by adenosine uptake blockers. Further, other pacemaker cells (e.g., His bundle, Purkinje fiber) are also depressed by adenosine.

**Inotropy**

In the atria, adenosine has a direct negative inotropic effect. It shortens or abolishes the action potential and causes hyperpolarization of the membrane by enhancing potassium efflux by activation of the potassium channels. However, in ventricular muscle, a direct effect is lacking and a negative inotropic response is observed only if the tissue is first stimulated by catecholamines and the adenyl cyclase system is activated. As with the chronotropic and dromotropic effects on the heart, adenosine activates the membrane A<sub>1</sub>-receptors, and the elicited responses are attenuated by adenosine deaminase and adenosine antagonists and potentiated by adenosine transport blockers and adenosine deaminase inhibitors. Studies with perfused hearts and membranes prepared from ventricular myocardium indicate that adenosine attenuates the β-adrenergic–enhanced adenylyl cyclase activity but not the basal activity of this enzyme. Furthermore, the A<sub>1</sub>-adenosine agonist phenylisopropyladenosine attenuates the β-adrenergic–induced increase in adenylyl cyclase by its action on signal transduction of the β-receptor. Adenosine also inhibits the release of norepinephrine from stimulated sympathetic nerve fibers in the heart; short periods of myocardial ischemia release sufficient adenosine to significantly reduce the neural release of norepinephrine. Myocardial ischemia results in the release of endogenous catecholamines, which stimulate myocardial metabolism and increase oxygen needs. Ischemia also results in the production and release of adenosine.

Therefore, in ischemia, endogenous adenosine can function in a protective manner by decreasing the release of the metabolic stimulant norepinephrine and by attenuating the stimulating effect of the norepinephrine that is released.

**Glycolysis**

The metabolic response of the heart to hypoxia or ischemia involves an initial increase in glucose uptake and utilization, which helps in the preservation of myocardial ATP by glycolysis. The rate-limiting step in the glycolytic pathway is the enzyme phosphofructokinase, which is stimulated by ADP, AMP, and inorganic phosphate, all of which are increased when oxygen availability is limited. Adenosine production is similarly increased in response to adenosine nucleotide degradation, and a possible role for adenosine in mediating glucose transport has been proposed. Raberger et al demonstrated that intracoronary adenosine infusion in dogs increased myocardial glucose uptake without eliciting a concomitant change in oxygen utilization, although coronary blood flow was increased. Because coronary flow was not controlled and the increased glucose uptake seen during the infusion of adenosine may have been secondary to increased substrate delivery, Turheim et al used the potassium-arrested isolated cat heart and compared constant pressure and constant flow perfusion. Adenosine infusion during constant pressure perfusion (and increased coronary flow) resulted in an increase in glucose uptake.
and utilization, whereas this effect failed to occur when coronary flow was held constant. These results conflict with reports that adenosine increases glucose uptake in isolated hearts with both constant pressure and constant flow perfusion. Furthermore, Jesmok et al demonstrated that adenosine increased glucose uptake in an isolated supported dog heart perfused at constant flow and that the effect was greater with increases in MVO₂. This apparent relation of glucose uptake to MVO₂ could explain the negative effects reported by Turnheim et al in the potassium-arrested heart. More recently, Law and Raymond observed that adenosine increased insulin-dependent glucose uptake in vivo in a euglycemic clamped dog heart and that the effect was not related to a change in MVO₂ or coronary blood flow.

In a study in which glycolytic flux was measured, Wyatt et al used an isolated rat heart model perfused at constant flow and demonstrated that adenosine produced a dose-dependent increase in glycolytic flux over a range of 25–100 μmol/l and that this effect was mediated by A₁-adenosine receptor stimulation. Furthermore, the increase in glycolytic flux in response to hypoxia or exogenous adenosine could be abrogated with the adenosine receptor antagonist 8-sulfophenyltheophylline. Therefore, these studies generally support the hypothesis that adenosine production during hypoxia or ischemia may increase energy production through enhanced glucose transport. What effect this has on cell viability has yet to be defined.

Microvascular Injury

The vascular injury and resulting myocardial damage that occur in ischemia and reperfusion consist of a complex series of related events in which sequence and interaction are not fully understood.

With hypoxia or ischemia, myocardial perfusion can be reduced by a vasoconstrictor substance released by the endothelium or by impairment of endothelium-mediated vessel relaxation by endothelium-derived relaxing factor (EDRF) caused by the action of superoxide anions released from activated neutrophils. In crystalloid-perfused rat hearts, ischemia or hypoxia depressed endothelium-mediated dilation on reperfusion only if the reperfusion solution contained oxygen. Furthermore, perfusion with buffer equilibrated with 95% N₂–5% CO₂ or containing superoxide dismutase prevented endothelial dysfunction. This observation plus the finding of elevated levels of superoxide anions in the cardiac effluent on reperfusion with oxygenated perfusion fluid indicates a cardiac source of the oxygen-derived free radicals. An endothelium-independent injury is observed in the microvasculature as indicated by the blunted dilator response to papaverine during reperfusion after 2 hours of coronary occlusion. Endogenous adenosine from the myocytes released during ischemia probably prevents more extensive damage (than might occur if adenosine were not released) by inducing some degree of relaxation of the vascular smooth muscle and protection of the endothelium and the vascular smooth muscle against the toxic effects of oxygen-derived free radicals. However, toxic effects of the ischemia and reperfusion override any beneficial effects of endogenously produced adenosine.

Hence, higher concentrations of administered adenosine are required to combat the detrimental microvascular and parenchymal effects of ischemia.

A second mechanism whereby ischemia can produce microvascular damage and the related no-reflow phenomenon is by the plugging of capillaries with neutrophils. Engler and colleagues have clearly demonstrated that the no-reflow response after periods of ischemia can be markedly attenuated or abolished if the leukocytes are removed from the blood. These observations are consonant with those of Cronstein et al, who demonstrated that adenosine, acting via an A₁-adenosine receptor on the neutrophil, facilitates the chemotaxis of activated neutrophils. This enhanced chemotactic effect produced by adenosine could contribute to the obstruction of the capillaries by increasing the number of neutrophils in the ischemic region. To compound this injurious effect, activated neutrophils also release superoxide anions that cause considerable local vascular and tissue damage. In contrast to the A₁-mediated effect of adenosine on neutrophils, A₂-adenosine receptor activation prevents adherence of the neutrophils to the vascular endothelium and also prevents the release of superoxide anions from the activated neutrophils. The neutrophil A₂-receptor stimulation occurs at much lower concentrations of adenosine than does the A₁-receptor stimulation. Hence, the endogenous adenosine concentration may be sufficient to promote neutrophil chemotaxis but not sufficient to inhibit superoxide anion release. However, the addition of pharmacological concentrations of adenosine during ischemia and/or reperfusion can prevent free radical release and thereby counter the neutrophil-mediated injury of the endothelium and myocardium.

Another factor that contributes to the microvascular damage and reduced tissue perfusion is the adherence of platelets to injured endothelial cells and aggregation of the platelets at these sites. Platelets release ADP, which enhances aggregation, and thromboxane A₂, which causes thrombus formation and local vasoconstriction. Release of 5-hydroxytryptamine from the platelets also contributes to the vasoconstriction. Adenosine formed in the ischemic myocardium (as well as some contributed by release from platelets) opposes platelet aggregation and prevents microthrombosis. This effect of adenosine represents another protective mechanism for the microcirculation and parenchymal tissue. However, exogenous adenosine provides additional protection because of the washout of endogenous adenosine during reperfusion.

Angiogenesis

Prolonged ischemia leads to irreversible myocardial damage, necrosis, and subsequent scarring. However, chronic myocardial ischemia as occurs with coronary artery stenosis leads to the development of collateral circulation. This type of growth of new blood vessels has been demonstrated in heart and skeletal muscle and results from a reduction in the oxygen supply/demand ratio. Adair et al have shown that adenosine is involved in this angiogenic effect of reduced oxygen supply. In support of this concept, it has been demonstrated that low oxygen concentrations (2%) increased proliferation of endothelial cells in culture and that culture media...
from hypoxic cells stimulated proliferation of cells grown in 20% oxygen. Addition of an adenosine receptor antagonist to the hypoxic conditioned media prevented this increase in endothelial cell proliferation. Furthermore, dipyridamole, an adenosine transport blocker that produces elevation of tissue adenosine levels, increased the formation of new capillaries in the rat heart.19,7 These observations suggest that adenosine serves as a protective agent against prolonged periods of suboptimal blood flow by stimulating the development of new blood vessels. To what extent these observations can be extrapolated to humans remains to be explored. Nevertheless, adenosine may play a role in the development of the coronary collateral circulation frequently seen with coronary artery stenosis.

Summary

Adenosine is released from the myocardium in response to a decrease in the oxygen supply/demand ratio, as is seen in myocardial ischemia; its protective role is manifested by coronary and collateral vessel vasodilation that increase oxygen supply and by multiple effects that act in concert to decrease myocardial oxygen demand (i.e., negative inotropism, chronotropic, and dromotropic). During periods of oxygen deprivation, adenosine enhances energy production via increased glycolytic flux and can act as a substrate for purine salvage to restore cellular energy charge during reperfusion.

Adenosine limits the degree of vascular injury during ischemia and reperfusion by inhibition of oxygen radical release from activated neutrophils, thereby preventing endothelial cell damage, and by inhibition of platelet aggregation. These effects help to preserve endothelial cell function and microvascular perfusion. Long-term exposure to adenosine may also induce coronary angiogenesis.

Conclusions

Endogenous adenosine plays a multifaceted role in protection of the ischemic myocardium. The pharmacological use of adenosine, its analogues, or its transport and metabolic inhibitors may extend its clinical application beyond its approved use as an agent for the termination of supraventricular tachyarrhythmias and provide significant new advances in myocardial protection in regional reperfusion, cardioplegic solutions, and preservation solutions for heart transplantation.

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