Adenosine Receptors and Reperfusion Injury of the Heart

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Abstract Adenosine, a catabolite of ATP, exerts numerous effects in the heart, including modulation of the cardiac response to stress, such as that which occurs during myocardial ischemia and reperfusion. Over the past 20 years, substantial evidence has accumulated that adenosine, administered either prior to ischemia or during reperfusion, reduces both reversible and irreversible myocardial injury. The latter effect results in a reduction of both necrosis or myocardial infarction (MI) and apoptosis. These effects appear to be mediated via the activation of one or more G-protein-coupled receptors (GPCRs), referred to as A_1, A_{2A}, A_{2B} and A_3 adenosine receptor (AR) subtypes. Experimental studies in different species and models suggest that activation of the A_1 or A_3ARs prior to ischemia is cardioprotective. Further experimental studies reveal that the administration of A_{2A}AR agonists during reperfusion can also reduce MI, and recent reports suggest that A_{2B}ARs may also play an important role in modulating myocardial reperfusion injury. Despite convincing

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experimental evidence for AR-mediated cardioprotection, there have been only a limited number of clinical trials examining the beneficial effects of adenosine or adenosine-based therapeutics in humans, and the results of these studies have been equivocal. This review summarizes our current knowledge of AR-mediated cardioprotection, and the roles of the four known ARs in experimental models of ischemia-reperfusion. The chapter concludes with an examination of the clinical trials to date assessing the safety and efficacy of adenosine as a cardioprotective agent during coronary thrombolysis in humans.

Keywords Adenosine receptor subtypes · Cardioprotection · Ischemia · Myocardial infarction · Reperfusion · Signaling

Abbreviations

AR	Adenosine receptor
CCPA	2 Chloro-N^6-cyclopentyladenosine
CHF	Congestive heart failure
CSC	8-(13-Chlorostyryl) caffeine
FMLP	Formyl–Met–Leu–Phe
GPCR	G-protein-coupled receptor
I/R	Ischemia-reperfusion
iNOS	Inducible nitric oxide synthase
IPC	Ischemic preconditioning
KO	Knockout
MI	Myocardial infarction
mito K_{ATP}	Mitochondrial ATP-sensitive K^+ channels
MPTP	Mitochondrial permeability transition pore
NECA	5′-*N*-Ethyl-carboxamidoadenosine
p38-MAPK	p38 Mitogen-activated protein kinase
PC	Preconditioning
PIA	N^6-1-(Phenyl-2*R*-isopropyl)adenosine
PKC	Protein kinase C
PTCA	Percutaneous transluminal coronary angioplasty
ROS	Reactive O_2 species
SPECT	Single-photon emission computed tomography
STEMI	ST-segment elevation myocardial infarction
SR	Sarcoplasmic reticulum

1 Introduction

Since extent of myocardial cell death is the primary determinant of outcome from planned or unplanned cardiac ischemia, protective strategies to limit this damage during ischemia-reperfusion (I/R) are highly sought after. It is now clear that a num-

ber of GPCR families can activate cytoprotective responses. These receptors, including the adenosine, opioid and bradykinin families, may act not only as acute "retaliatory" systems mediating immediate responses to injurious stimuli, but function as sensors of low-level stress to initiate a signaling cascade culminating in the expression of more prolonged protected phenotypes. These adaptive or hormesis responses predate mammals, and offer potential as targets for therapeutic cardioprotection.

The AR family, composed of A_1, A_{2A}, A_{2B}, and A_3 subtypes, has been implicated in both acute protection and adaptive preconditioning (PC) responses. Not only does preischemic activation of ARs generate potent protection, but significant evidence indicates that this receptor class also mediates powerful cardioprotection when targeted during the reperfusion phase. This brief review focuses on temporal properties of AR-mediated cardioprotection (prior to, during, after ischemia), their contributions to PC responses, and their relevance to the protection of human myocardium.

2 Cardioprotection with Tonic A_1AR Agonism: A_1AR Overexpression

Given early evidence of cardioprotection in response to adenosine and (subsequently) selective A_1AR agonism, the A_1AR subtype seemed an obvious target for manipulating myocardial ischemic tolerance. To test the hypothesis that A_1AR density (rather than endogenous [ligand]) limits the resistance of the heart to I/R, Matherne and colleagues developed a cardiac-specific A_1AR overexpression model. The model employed a construct containing the rat A_1AR gene under the control of a mutated α-myosin heavy chain promoter (Matherne et al. 1997), with extent of A_1AR expression varying across the lines generated (with up to 100-fold overexpression of coupled A_1ARs). The resulting phenotype was characterized by modest bradycardia, conduction disturbances, and a small increase in heart mass in some lines (Matherne et al. 1997; Gauthier et al. 1998; Kirchhof et al. 2003). Initial studies of I/R revealed profound reductions in cell death and contractile dysfunction compared with wild-type hearts (Matherne et al. 1997; Headrick et al. 1998; Morrison et al. 2000). Tolerance to hypoxic challenge (Cerniway et al. 2002), and long-term cold storage of hearts (Crawford et al. 2005) were also improved. Cardioprotection was evident in isolated tissue preparations (Matherne et al. 1997; Headrick et al. 1998) and in vivo (Yang et al. 2002). These outcomes were consistent with protective effects of artificially enhanced A_1 (and A_3) expression in isolated myocytes (Dougherty et al. 1998). Differing components of cardiac protection were apparent, with reduced necrosis and infarction (Matherne et al. 1997; Morrison et al. 2000; Yang et al. 2002), inhibition of apoptosis (Regan et al. 2003; Crawford et al. 2005), enhancement of bioenergetic state during ischemia (Headrick et al. 1998), and selective modulation of contractile injury: A_1AR overexpression consistently reduces diastolic (and not systolic) dysfunction during I/R (Matherne et al. 1997; Reichelt et al. 2007). The latter suggests that A_1ARs selectively target processes underlying diastolic contracture (e.g., Ca^{2+} handling, myofibrillar function).

While the signaling basis of cardioprotection with A_1AR overexpression remains to be established, analysis to date implicates players common to protective signaling in wild-type tissue, including mitochondrial ATP-sensitive K^+ (mito K_{ATP}) channels and inducible nitric oxide synthase (iNOS) (Headrick et al. 2000; Nayeem et al. 2003). Curiously, mito K_{ATP} channels (or 5-hydroxydecanoate-sensitive targets) were not implicated in protection against hypoxia (Cerniway et al. 2002). Other work supports a role for p38 mitogen-activated protein kinase (p38-MAPK)-dependent signaling, though this remains to be more fully tested (Jones et al. 1999). Sarcoplasmic reticulum (SR) Ca^{2+} handling is impaired (Zucchi et al. 2002), which could contribute to specific aspects of associated cardioprotection. Another interesting outcome with A_1AR overexpression is restoration of ischemic resistance in aged hearts: aging may limit the capacity of hearts to withstand damage during I/R (Willems et al. 2005), and this effect was reversed by A_1AR overexpression in mice (Headrick et al. 2003b), in parallel with restoration of adenosine responsiveness.

In terms of PC responses, overexpression of A_1ARs mimics the benefit with this stimulus, actually surpassing the degree of protection with ischemic PC (IPC) (Morrison et al. 2000). Protection with A_1AR overexpression is also nonadditive with IPC, suggesting a commonality of signaling/end-effectors and/or maximally effective protection with A_1AR overexpression. However, the latter is inconsistent with reports that acute application of adenosine (Peart et al. 2002) or A_1AR agonist (Nayeem et al. 2003) can augment the protection with A_1AR overexpression.

Overexpression of A_1ARs in cardiac cells did confirm the hypothesis that normal levels of A_1AR expression in wild-type hearts do appear to limit the extent of cardioprotection possible, and thus the heart's intrinsic resistance to I/R (Matherne et al. 1997). Nonetheless, pharmacologically activating A_1ARs does provide benefit in wild-type hearts (see Sect. 2.1.1 below), demonstrating that normally expressed A_1ARs can be targeted to achieve further cardioprotection. This may reflect additional effects of transient AR agonism (and induction of a short-lived PC state), as opposed to the longer-lived effects of tonic A_1AR activity in transgenic hearts.

3 Cardioprotection via Preischemic AR Activation: A Role in PC Responses

Since its discovery by Murry and colleagues (Murry et al. 1986), the molecular basis of IPC has been the subject of intense investigation. An ultimate goal is translation to the clinical setting, enabling activation of similar protection in cardiac patients. Through a simplified scheme, we can examine the roles of ARs in PC responses from the viewpoint of the initial "trigger" phase and the subsequent "mediation" phase.

The initial and rather crude ischemic trigger of PC is now known to involve the release and actions of several GPCR ligands (including opioids, bradykinin, and adenosine). A "threshold" model for triggering PC has evolved, in which summation

of multiple GPCR stimuli is required to activate delayed protection (Goto et al. 1995; Baba et al. 2005). The response may involve not only summation of GPCR triggers but also downstream kinase signaling (Vahlhaus et al. 1998). The kinase cascades involved in PC have been elaborated over recent years, and are currently thought to converge on modulation of mitochondrial effectors, including K_{ATP} channels and the mitochondrial permeability transition pore (MPTP) (Murphy 2004; Hausenloy and Yellon 2007; Liem et al. 2008). Nonetheless, there remains considerable disagreement regarding the roles of different signaling components, and putative end-effectors, in AR-mediated protection and PC. As the focus of this review is on AR involvement in cardioprotection, and since the signaling basis of PC responses has been very well addressed in recent reviews (Murphy 2004; Downey et al. 2007; Hausenloy and Yellon 2007), interested readers are directed to these for further details.

3.1 Adenosine as a Preischemic Trigger of PC

It should be clarified that true PC describes a delayed protective state persisting in the absence of the initial stimulus. Many studies refer to "preconditioning" effects when assessing preischemic receptor or pathway activation. However, application of receptor agonists up to induction of ischemia (with no intervening washout) will modify the same targets during ischemia and possibly early reperfusion. This is an inherent limitation to in vivo studies, since exogenously applied AR agonists (or antagonists) may be slowly removed and thus exert potentially long-lasting effects beyond the desired "window." Thus, while discussion of the effects of preischemic AR activation (or antagonism) can be informative in terms of roles of ARs in PC responses, these experimental scenarios do not simulate PC per se.

In seeking a released factor capable of transducing protection with PC, adenosine seemed a likely candidate: adenosine release increases rapidly in response to different conditions of stress (Headrick et al. 2003a); the interstitial concentrations achieved are sufficient to activate one or more AR subtypes (Van Wylen 1994; Lasley et al. 1995a; Headrick 1996; Harrison et al. 1998); rapid transport and catabolism ensures a brief extracellular half-life and localized signaling; and exogenous AR agonists appear to induce similar protective states.

3.1.1 AR-Triggered Pharmacological PC

In early work Liu et al. showed that preischemic treatment with adenosine or N^6-1-(phenyl-2*R*-isopropyl) adenosine (PIA) mimicked the protective effects of PC in rabbit myocardium (Liu et al. 1991). Subsequent studies confirmed protection via preischemic A_1AR agonism in different models and species (Lasley and Mentzer 1992; Thornton et al. 1992; Liu and Downey 1992; Tsuchida et al. 1993; Strickler et al. 1996; Carr et al. 1997; Liang and Jacobson 1998; de Jonge and de Jong

1999; de Jonge et al. 2002; Germack et al. 2004; Germack and Dickenson 2005). Toombs and colleagues not only showed that preischemic adenosine limited infarct size (Toombs et al. 1992), but further showed that activation of 8-ρ-sulfophenyltheophylline-sensitive ARs (likely A_1 and/or A_2ARs) during the ischemic period itself was required for protection.

Preischemic activation of the A_3AR subtype can also generate cardiac protection. Strickler et al. (1996) presented some of the first evidence that A_3AR activation prior to ischemia could confer protection against ischemia-like insult in myocytes (of avian origin), while Tracey and colleagues acquired evidence for A_3AR-triggered protection in rabbit hearts (Tracey et al. 1997). Other groups confirmed A_3AR-mediated protection in multiple models (Strickler et al. 1996; Carr et al. 1997; Liang and Jacobson 1998; de Jonge et al. 2002; Maddock et al. 2002; Germack et al. 2004; Germack and Dickenson 2005; Wan et al. 2008). Indeed, Liang and Jacobson (1998) found that the A_3AR induced a more sustained state of protection than the A_1AR when activated prior to ischemia.

In contrast to PC-like effects of A_1AR or A_3AR agonism, preischemic activation of A_{2A}ARs or A_{2B}ARs is generally ineffective in limiting myocardial injury during subsequent I/R (Thornton et al. 1992; Lasley and Mentzer 1992; Maddock et al. 2002; Germack and Dickenson 2005). Studies with the natural agonist adenosine yield mixed results, likely due to rapid uptake and catabolism of extracellular adenosine, complications of potent hemodynamic actions of the endogenous agonist, and the impact of mixed AR activation on different cell types.

3.1.2 ARs as Intrinsic Triggers of IPC

Studies demonstrating PC-like responses to preischemic AR activation provided support for AR involvement in IPC. To more directly test for a role of AR activation in triggering nonpharmacological forms of PC, AR antagonists or adenosine deaminase have been added, often in both trigger and mediation phases, to limit any contributions from ARs. A number of these studies independently provided no evidence for essential roles for ARs in PC (Liu and Downey 1992; Lasley et al. 1993; Hendrikx et al. 1993; Bugge and Ytrehus 1995; Lasley et al. 1995b), leading to premature elimination of this class of GPCRs as contributing to PC (Cave et al. 1993; Li and Kloner 1993). In the context of protective thresholds and contributions of multiple stimuli, a more accurate conclusion may be that the roles of ARs in triggering/mediating PC are redundant, with other concomitant stimuli (e.g., endogenous opioids and bradykinin) being able to compensate and surpass the signaling threshold required for protection.

On the other hand, considerable evidence supporting essential AR involvement in PC has been reported. Studies employing different AR antagonists or adenosine deaminase supported roles in rabbit (Liu et al. 1991; Tsuchida et al. 1992; Thornton et al. 1993; Urabe et al. 1993; Weinbrenner et al. 1997) rat (Headrick 1996; de Jonge and de Jong 1999; de Jonge et al. 2001; Tani et al. 1998), dog (Auchampach and Gross 1993; Hoshida et al. 1994), and pig (Schulz et al. 1995;

Vogt et al. 1996; Louttit et al. 1999). Early studies of PC responses in human myocardium also supported involvement of endogenous adenosine, likely via A_1ARs (Walker et al. 1995; Tomai et al. 1996).

Reasons for differing outcomes with AR blockade in varied models of PC are not clear. Evidence has been presented for substantial species differences in adenosine handling and receptor activation (Headrick 1996), which might dictate differing roles for adenosine and certainly contribute to differing abilities of competitive AR antagonists to limit these responses. Moreover, the affinity and selectivity of AR ligands varies across species, and in the event of poor solubility, bioavailability may limit the effects of a ligand. Furthermore, the relative contributions of adenosine and ARs in triggering PC may be species dependent, with a greater and essential contribution in rodent myocardium. Nonetheless, evidence for essential AR involvement has been reported in large animal models (Auchampach and Gross 1993; Hoshida et al. 1994; Schulz et al. 1995; Vogt et al. 1996; Louttit et al. 1999) and in human tissue (Walker et al. 1995; Tomai et al. 1996; Ikonomidis et al. 1997). Responses may be model specific, in part, since some aspects of I/R injury are dependent upon blood components and activation of pathways for inflammation, while others are intrinsic to the myocardial cells themselves (and these cell-dependent responses may also vary across species). Thus, injury and counteracting protective processes may differ between ex vivo or blood-free models and the in situ myocardium. Finally, differences reported with the use of AR antagonists in PC studies may be related to the nature and duration of the PC stimulus (see below), which may influence the contribution of ARs to protection.

In terms of the identity of the ARs implicated in triggering PC, initial work supported the involvement of A_1ARs (Liu et al. 1991; Tsuchida et al. 1992; Auchampach and Gross 1993). However, subsequent studies (Armstrong and Ganote 1994, 1995; Liu et al. 1994; Wang et al. 1997) demonstrated that partially selective A_3AR antagonism also impaired the protective efficacy of PC. Liang and colleagues documented A_1AR and A_3AR involvement in PC responses in chick cardiomyocytes (Strickler et al. 1996; Liang and Jacobson 1998), while Wang et al. (1997) reported additive contributions from A_1AR and A_3ARs to optimize PC in rabbit myocytes. Although other studies initially supported A_3AR involvement in IPC in intact rabbit myocardium (Tracey et al. 1997), this group subsequently presented evidence of a quantitatively more critical role for A_1AR vs. A_3AR (Hill et al. 1998). More recent studies confirm that endogenous adenosine contributes to IPC via A_1AR and/or A_3AR activation, though the contribution of ARs may be dependent upon the nature and duration of the PC stimulus, being less important with shorter periods of triggering ischemia (Liem et al. 2001, 2008). This is consistent with earlier observations of Schulz et al. in pigs (1995).

Ultimately, preservation of AR-dependent protection in human myocardial tissue is of key importance. Walker and colleagues provided some of the first support for mediation of PC by ARs in human myocardium (Walker et al. 1995). Cleveland et al. (1996, 1997) subsequently confirmed AR-mediated PC responses in human myocardial tissue. Carr et al. (1997) further established that A_1ARs and A_3ARs trigger PC in human atrial muscle, while Ikonomidis et al. (1997) demonstrated AR

dependence of PC in human pediatric myocytes. Thus, AR-mediation of PC is relevant to human myocardium. Indeed, an early study by Tomai et al. (1996) supported A_1AR-dependent PC in patients undergoing coronary angioplasty. Furthermore, the importance of ARs in determining resistance to myocardial ischemia is supported by associations between AR polymorphisms, specifically for A_1 and A_3ARs, and infarct size in patients with ischemic cardiomyopathy (Tang et al. 2007).

3.1.3 Evidence from Gene-Modified Models

Essential contributions of ARs to PC are borne out by recent gene manipulation studies. Analysis of A_3AR gene knockout (KO) in mice revealed no impact on induction of IPC (Guo et al. 2001), apparently negating an essential role for this AR subtype. However, A_1AR KO eliminates protection with both IPC (Lankford et al. 2006) and remote PC triggered by cerebral ischemia (Schulte et al. 2004). Moreover, ecto-5′-nucleotidase deletion also eliminates protection with IPC, supporting an essential role for endogenous adenosine generated at the cell surface (Eckle et al. 2007). This latter study also confirmed an essential role for ARs in IPC, although their data differed in implicating only the A_{2B}AR. The basis of this discrepancy is not clear, but may, in part, be model related (in vivo vs. in vitro). This latter observation is, however, consistent with recent data from the laboratory of Downey and colleagues, who reported evidence for protein kinase C (PKC) dependent sensitization of A_{2B}ARs during the trigger or ischemic phases and their role in protection during the subsequent reperfusion phase (Kuno et al. 2007).

Of course, a limitation inherent to gene deletion (or overexpression) is an inability to distinguish events temporally. Since gene deletion eliminates the actions of targeted ARs at all time points, it is unclear from such work when the receptors are involved. For example, A_1ARs or A_3ARs may trigger protection with IPC prior to or during ischemia, while recent evidence implicates a role for A_{2B}AR in mediating the protection with PC during the reperfusion phase (Kuno et al. 2007). This A_{2B}AR-mediated protection during reperfusion could depend to some extent upon A_1AR and/or A_3AR activation of PKC prior to or during ischemia. Such complex responses are not amenable to interrogation by gene manipulation.

3.2 AR Activity During Ischemia

Cardioprotective effects of PC and preischemic GPCR activation were initially thought to manifest primarily during ischemia itself (Cohen et al. 2000). Preischemic AR agonism (or A_1AR overexpression) modifies substrate and energy metabolism, H^+ and Ca^{2+} accumulation, and contracture development during the ischemic episode (Lasley et al. 1990; Fralix et al. 1993; Lasley and Mentzer 1993; Headrick 1996). Similarly, there is evidence of specific protective actions of adenosine and A_1ARs during ischemia versus reperfusion (Peart and Headrick 2000;

Peart et al. 2003). IPC also modifies ischemic events relevant to tissue protection (de Jonge and de Jong 1999), reducing purine moiety accumulation and washout (Van Wylen 1994; Lasley et al. 1995a; Harrison et al. 1998; de Jonge et al. 2002) and ionic perturbations (Fralix et al. 1993). Such observations are consistent with the idea that modulation of injury during ischemia itself contributes to overall protection and improved postischemic outcome. This is supported by early work of Thornton et al. (1993), who showed that protection with IPC is mediated, at least in part, via intrinsic activation of A_1ARs during the subsequent ischemic insult. Studies such as that of Stambaugh et al. (1997) also show that AR activation throughout the period of ischemia/hypoxia is beneficial.

While a majority of studies across differing species support beneficial actions of either exogenously or intrinsically activated ARs during myocardial ischemia, there are a small number of reports of improved outcomes with AR antagonists applied prior to ischemia in vivo (and thus reflecting possible blockade of ARs prior to, during, or following ischemia). Neely et al. (1996) initially documented infarct limitation with three different A_1AR antagonists, DPCPX (1,3 dipropyl-8-cyclopentylxanthine), XAC (xanthine amine congener) and bamiphylline, in a feline regional myocardial infarct model. To rule out that the possibility that these A_1AR antagonists were producing their effects via a nonspecific intracellular action (i.e., inhibition of intracellular enzymes, e.g., phosphodiesterases), Forman and colleagues (2000) reported that another (albeit poorly selective) A_1AR antagonist, DPSPX (1,3-dipropyl-8-*p*-sulfophenylxanthine), which is negatively charged and thus does not accumulate in intracellular spaces because of its high water solubility, also reduced infarct size in dogs. Because DPSPX significantly reduced FMLP (formyl–Met–Leu–Phe)-induced chemoattraction of human neutrophils, the authors of this study suggested that this A_1AR antagonist produced sustained myocardial protection in dogs by reducing inflammation. However, DPSPX is also known to interact with the A_{2B}AR (Feoktistov and Biaggioni 1997), and at the doses applied in this study, to block A_2-dependent coronary dilation (Forman et al. 2000). A later detailed study by Auchampach et al. (2004) described the effect of three different A_1AR antagonists, DPCPX, BG 9928 (1,3-dipropyl-8-[1-(4-propionate)-bicyclo-[2,2,2]octyl)]xanthine) and BG 9719 (1,3-dipropyl-8-[2-(5,6-epoxynorbornyl) xanthine), of varying specificities in a regional myocardial infarct model in vivo in dogs. A_1AR antagonists could limit infarct size in dog hearts, though only with those agents (DPCPX and BG 9928) that also antagonized A_{2A}AR-mediated coronary dilation and possessed appropriate affinities for A_{2B}ARs, raising the possibility of actions at multiple AR subtypes. An alternative explanation by the authors of this study was that differences in the pharmacokinetic and pharmacodynamic properties of BG 9719 may have limited the in vivo potency of this A_1AR antagonist in these studies. They additionally showed that the A_1AR antagonists DPCPX and BG 9928 were equally protective when applied just prior to reperfusion or throughout ischemia-reperfusion, suggesting a primarily postischemic mode of action.

The basis of these mixed observations remains to be determined, though they do raise the possibility of opposing effects of ARs through cell-specific responses. For example, A_1AR activity may augment chemotaxis and neutrophil-dependent injury,

whereas the same receptor limits injury in cardiomyocytes. A number of studies confirm a lack of any infarct-sparing effects of nonselective or subtype-specific AR antagonists in vivo in multiple species (Toombs et al. 1992; Tsuchida et al. 1992; Auchampach and Gross 1993; Thornton et al. 1993; Zhao et al. 1993; Hoshida et al. 1994; Baba et al. 2005; Kin et al. 2005; Lasley et al. 2007). However, with the exception of the study by Zhao et al. (1993), the antagonists used in these studies were administered as single doses and not as continuous infusions or multiple doses to achieve a steady state plasma concentration of the AR antagonist, as was done by Neely et al., Forman et al., and Auchampach et al.. Moreover, problems with the selectivity of AR antagonists for specific AR subtypes, particularly during in vivo studies, limit their interpretation with respect to the definitive roles of the four AR subtypes in the setting of acute myocardial ischemia-reperfusion injury.

4 Reperfusion Injury and ARs in Experimental Studies

Although reperfusion is necessary to salvage ischemic myocardium, the process of restoring blood flow also contributes to the total injury observed in ischemic-reperfused myocardium. Reperfusion injury is caused by intracellular calcium overload and oxidative stress induced by the formation of reactive O_2 species (ROS) in the presence of decreased cellular redox state. Reperfusion injury in intact animals and in humans following myocardial ischemia durations of >15 min produces irreversible injury that is also associated with a general inflammatory process including the release of numerous cytokines, adhesion and infiltration of neutrophils across the damaged coronary endothelium, platelet aggregation, and activation of the complement cascade (Ambrosio and Tritto 1999; Park and Lucchesi 1999; Verma et al. 2002).

Similar to the beneficial protective effects of AR agonists discussed in the first sections of this chapter, there is now convincing evidence that the activation of ARs during reperfusion is cardioprotective in animal models. However, in contrast to reports nearly 20 years old documenting the cardioprotective effects of adenosine treatment prior to ischemia, initial studies on the effects of treatment with adenosine after reperfusion were much more controversial. Two initial reports in canine models indicated that intracoronary and intravenous adenosine infusions for the first 1–2.5 h of reperfusion after 90 min coronary occlusions significantly reduced infarct size after 24 and 72 h reperfusion, respectively (Olafsson et al. 1987; Pitarys et al. 1991). In both of these studies, the ischemic myocardium from animals treated with adenosine exhibited significantly less neutrophil accumulation and erythrocyte plugging of capillaries. These observations are consistent with adenosine's ability to inhibit both neutrophil adherence to endothelium (Cronstein et al. 1992) and platelet aggregation (Söderbäck et al. 1991). Several subsequent reports were, however, unable to reproduce these positive findings (Homeister et al. 1990; Goto et al. 1991; Vander Heide and Reimer 1996). Negative results with adenosine treatment following reperfusion may be due to the use of inadequate doses, which must be high enough to overcome its rapid uptake and metabolism by red blood cells and endothelial cells.

However, high concentrations of adenosine can be associated with severe hypotension, reflex tachycardia, and coronary steal. These side effects will likely limit the use of adenosine as a cardioprotective agent in humans.

4.1 Effects of the A_{2A}AR During Reperfusion

Despite the contradictory reports regarding the beneficial effects of adenosine as a reperfusion treatment, there have been an increasing number of reports that reperfusion treatments with infusions of certain AR agonists are cardioprotective. Such studies support the hypothesis that the cardioprotective effects of adenosine are mediated primarily via activation of one or more AR subtypes. The majority of such studies indicate that the infusion of adenosine A_{2A}AR agonists during reperfusion reduces myocardial infarct size. It appears that the first such study was conducted by Norton et al. (1992), who reported that the A_{2A}AR agonist CGS21680 (4-[2-[[6-Amino-9-(*N*-ethyl-*b*-D-ribofuranuronamidosyl)-9*H*-purin-2-yl]amino]ethyl]benzenepropanoic acid), infused during reperfusion in vivo, significantly reduced myocardial infarct size measured after 48 h of reperfusion in rabbits in the absence of hypotension. Subsequent studies have reproduced similar infarct size-reducing effects of reperfusion A_{2A}AR stimulation in dogs, pigs, rats, and mice (Schlack et al. 1993; Zhao et al. 1996; Jordan et al. 1997; Budde et al. 2000; Lasley et al. 2001; Boucher et al. 2005; Yang et al. 2005, 2006).

Although there is a significant expression of A_{2A}ARs on vascular cells (vascular smooth muscle and endothelial cells), and activation of this receptor is associated with coronary vasodilatation, the beneficial effects of reperfusion A_{2A}AR agonists are independent of increased coronary blood flow and can be achieved without systemic hypotension. The prevailing current hypothesis for the beneficial A_{2A}AR effects during reperfusion are related to its anti-inflammatory properties, such as inhibition of neutrophil production of ROS and adherence to endothelium (Visser et al. 2000; Sullivan et al. 2001). Recent studies in mice further suggest that this A_{2A}AR-mediated reperfusion protection is due to effects on bone marrow-derived cells, more specifically to $CD4^+$ T-helper lymphocytes (Toufektsian et al. 2006).

However, two additional studies conducted in intact animal models of myocardial stunning indicate that reperfusion treatment with A_{2A}AR agonists can exert beneficial effects in the absence of severe inflammation and myocardial necrosis. In porcine regionally stunned myocardium, an intracoronary infusion of the A_{2A}AR agonist CGS21680, initiated after 2 h reperfusion following 15 min coronary occlusion, significantly increased regional preload-recruitable stroke work and stroke work area, both of which are load-insensitive parameters of cardiac contractility. This effect, which appeared to be independent of increased coronary blood flow, occurred in stunned (i.e., no infarction was detected), but not normal, myocardium (Lasley et al. 2001). The fact that the A_{2A}AR agonist exerted its beneficial effects 2 h after reperfusion suggests that the improvement in regional contractility is likely to have been independent of a reduction in myocardial reperfusion injury, but rather

may have been a true positive inotropic effect. Using another myocardial stunning model in dogs, Glover et al. (2007) observed that the A_{2A}AR agonist ATL-146e, given just prior and during reperfusion following multiple brief (5 min) coronary occlusions, improved reperfusion wall thickening in the absence of any increase in coronary blood flow. Infusion of ATL-146e had no effect on regional function in normally perfused myocardium. Whether these beneficial effects of reperfusion A_{2A}AR stimulation in the absence of necrosis are due to a direct effect on the myocardium remains to be determined.

Although the evidence implicating the anti-inflammatory effects of postischemic A_{2A}AR activation in the setting of myocardial infarction is compelling, the above two studies in stunned myocardium indicate that A_{2A}AR activation may also protect the reperfused heart via mechanisms independent of neutrophils and inflammatory processes, as well as increased coronary blood flow. There are several reports that A_{2A}ARs are expressed in porcine, human, and rat ventricular myocytes (Marala and Mustafa 1998; Kilpatrick et al. 2002), which raises the possibility that the beneficial effect of A_{2A}AR agonists during reperfusion may also be due to direct effects on the cardiac myocyte. There have been numerous studies over the past 15 years investigating the effects of A_{2A}AR agonists on cardiac myocyte physiology, but these reports have yielded conflicting findings (Shryock et al. 1993; Stein et al. 1994; Xu et al. 1996, 2005; Boknik et al. 1997; Woodiwiss et al. 1999; Hleihel et al. 2006; Hove-Madsen et al. 2006). The majority of these reports indicate that A_{2A}AR activation alone exerts little, if any, direct effects on normal cardiac ventricular myocytes. However, it is possible that during myocardial ischemia, when endogenous adenosine levels increase and multiple AR subtypes are activated, cardiomyocyte A_{2A}AR may modulate the cardioprotective effects of adenosine.

There remain several interesting and incomplete aspects to our understanding of the cardioprotective effects of reperfusion AR agonist treatment. Although A_{2A}AR agonists administered during reperfusion have been shown to be cardioprotective in intact animals, the administration of A_{2A}AR antagonists does not exacerbate myocardial injury or infarct size in normal animals (Kin et al. 2005; Reid et al. 2005; Lasley et al. 2007). However, there is evidence that the A_{2A}AR does participate in the cardioprotective effect of ischemic postconditioning. Ischemic postconditioning is the phenomenon by which brief interruptions in coronary flow during the initial minutes of reperfusion following a prolonged occlusion reduce myocardial infarct size. This phenomenon is thus somewhat analogous to ischemic preconditioning, which was described earlier. The AR antagonist ZM241385 (4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-*a*][1,3,5]triazin-5-ylamino]ethyl)phenol), which exhibits some selectivity for the A_{2A}AR subtype, has been shown to block ischemic postconditioning in vivo in rat hearts and in isolated perfused mouse hearts (Kin et al. 2005). A more recent report indicated that ischemic postconditioning could not be induced in mouse hearts from A_{2A}AR KO mice (Morrison et al. 2007). These findings indicate that stimulation of A_{2A}ARs plays a pivotal role in reducing myocardial reperfusion injury. Observations in isolated buffer perfused hearts in these latter two reports further support the hypothesis that this protective effect is mediated, at least in part, by the cardiomyocyte A_{2A}AR.

As described above, there are now numerous reports indicating that the infusion of A_{2A}AR agonists during reperfusion is cardioprotective. Although the administration of A_{2A}AR agonists prior to ischemia does not reduce myocardial ischemia-reperfusion injury, there is increasing evidence that A_{2A}ARs may modulate the protective effects of A_1AR stimulation. Reid et al. (2005) and Lasley et al. (2007) reported that the A_{2A}AR antagonist ZM241385 blocked the infarct reducing effects of preischemic treatments with three different AR agonists—AMP579 (1*S*-[1*a*, 2*b*, 3*b*, 4*a*(*S**)]-4-[7-[[2-(3-chloro-2-thienyl)-1-methylpropyl]amino]-3*H*-imidazo[4,5-*b*]pyridyl-3-yl]cyclopentane carboxamide), 2 chloro-N^6-cyclopentyladenosine (CCPA), 5′-*N*-ethyl-carboxamidoadenosine (NECA)—in two different studies. The A_{2A}AR antagonist did not alter the A_1AR-induced bradycardia with these agonists, indicating that the A_1AR was not blocked; however, the ability of ZM241385 to block the protection by these AR agonists was comparable to that achieved with the A_1AR antagonist DPCPX. Preliminary observations in one of these studies suggested that the A_{2A}AR antagonist partially blunted the effects of AMP579 on preischemic mitogen-activated protein kinase (MAPK) signaling (Reid et al. 2005). These findings regarding the effects of A_{2A}AR antagonists on A_1AR cardioprotection are supported by an increasing number of reports of interactions between AR subtypes, including the formation of heterodimers (Karcz-Kubicha et al. 2003; O'Kane and Stone 1998; Lopes et al. 1999, 2002; Nakata et al. 2005).

There is also evidence that the beneficial effects of reperfusion AR agonist treatments may involve interactions among AR subtypes. In the isolated perfused rabbit heart, a reperfusion infusion (500 nM) of the AR agonist AMP579, which has a high affinity for both A_1 and A_{2A}ARs (Smits et al. 1998), reduced infarct size—an effect that was blocked by 8-(13-chlorostyryl) caffeine (CSC), which exhibits some selectivity for A_{2A}ARs, but not by the A_1AR antagonist DPCPX (Xu et al. 2001). The beneficial effect of AMP579 was mimicked by the nonselective agonist NECA at a dose (100 nM) activating both A_1 and A_{2A}ARs, but not by the A_{2A}AR agonist CGS21680 (50 nM). Kis et al. (2003) reported similar findings in the intact rabbit, where an infusion of AMP579 during reperfusion reduced infarct size, and this effect was blocked by the A_{2A}AR antagonist ZM241385 but not mimicked by the same dose of the A_{2A}AR agonist CGS21680. It is not clear why these studies did not observe protection with the A_{2A}AR agonist alone, when numerous other studies have reported such protection; however, these findings support a role for the A_{2A}AR in reduction of myocardial injury. Since ZM241385 has some affinity for A_{2B}ARs, it is also possible that the effects of this agent could be due to antagonism of this receptor subtype (Hasan et al. 2000).

4.2 Effects of A_1 and A_3ARs During Reperfusion

To date, the primary emphasis on AR reduction of reperfusion injury has focused on the role of the A_{2A}AR. However, given that there are four AR subtypes, all of

which appear to be expressed in the heart, it is possible that one or more of these other AR subtypes may modulate reperfusion injury. The one exception to this hypothesis is the A_1AR. Although, as described in the first section of this chapter, there is significant evidence that A_1AR agonists administered prior to ischemia are protective, it is clear that A_1AR agonists administered during reperfusion are not protective (Thornton et al. 1992; Baxter et al. 2000). There is evidence that A_3AR activation during reperfusion may be cardioprotective, as studies in isolated hearts and intact animals indicate that the A_3AR agonists IBMECA (1-deoxy-1-[6-[[(3-iodophenyl)methyl]amino]-9*H*-purin-9-yl]-*N*-methyl-*b*-D-ribofuranuronamide) and Cl-IBMECA (1-[2-chloro-6-[[(3-iodophenyl)methyl]amino]-9*H*-purin-9-yl]-1-deoxy-*N*-methyl-*b*-D-ribofuranuronamide), administered during reperfusion, reduce myocardial infarct size (Maddock et al. 2002; Auchampach et al. 2003; Park et al. 2006). In two of these studies, the effects of the A_3AR agonists were blocked by A_3AR antagonists (Maddock et al. 2002; Park et al. 2006). Interestingly, in the former study (Maddock et al. 2002) the reperfusion A_3AR agonist protection was also blocked by the A_{2A}AR antagonist CSC. Finally, Kin et al. (2005) observed that postconditioning could be blocked by an A_3AR antagonist. Thus, in contrast to the A_1AR, activation of the A_3AR either prior to ischemia or during reperfusion appears to be cardioprotective.

4.3 Emerging Roles for the A_{2B}AR During Reperfusion

With respect to the fourth AR subtype, only now are a limited number of studies supporting a role for the A_{2B}AR in modulating myocardial reperfusion injury appearing. Investigations of this receptor in the heart have been hindered by the fact that there are no radioligand binding studies defining A_{2B}AR receptor density or affinity in mammalian myocardium or cardiomyocytes. The role of this receptor has also been hindered by the lack of studies with well-characterized, selective A_{2B}AR agonists and antagonists. To date there are four pharmacological studies providing some evidence for the involvement of A_{2B}ARs, although the results are conflicting. Auchampach et al. (2004) reported that reperfusion treatments with DPCPX and BG 9928, but not BG 9719, all of which are selective A_1AR antagonists, reduced infarct size in dogs by ~40%. These effects were compared to radioligand binding studies performed with recombinant canine ARs expressed in HEK cells, and blockade of canine A_1 (heart rate) and A_{2A}AR (coronary conductance) effects. Based on these observations, the authors concluded that DPCPX and BG 9928 may exert their infarct-reducing effects by blocking A_{2B}ARs; however, they could not discount the possibility that DPCPX and BG 9928 reduced infarct size by blocking A_1ARs.

Three additional studies in rabbit heart models of ischemia/reperfusion concluded that A_{2B}AR activation, rather than inhibition, contributes to reperfusion cardioprotection (Solenkova et al. 2006; Phillip et al. 2006; Kuno et al. 2007). In the first of these studies, the infarct-reducing effect of IPC was blocked by the A_{2B}AR antagonist, MRS1754 (*N*-(4-cyanophenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,

3-dipropyl-1*H*-purin-8-yl)phenoxy]-acetamide), but not an A_{2A}AR antagonist, CSC, administered at the onset of reperfusion. Subsequently, Phillip et al. (2006) reported that the cardioprotective effect of NECA administration at reperfusion (i.e., pharmacological postconditioning) in intact rabbits was blocked by MRS1754. Interestingly, a previous report from this same laboratory concluded that the reperfusion protection induced by NECA was due to A_{2A}AR activation (Xu et al. 2001). More recently, Kuno et al. (2007) demonstrated that a novel A_{2B}AR agonist, BAY 60–6583, administered during reperfusion, is protective. Given the apparent expression of multiple AR subtypes in the heart and their possible interactions, as well as the lack of selectivity for many of the commonly used AR agonists and antagonists, studies in AR KO mice will likely be needed to address the question of the A_{2B}AR, as well as the definitive roles of other AR subtypes. Interestingly, the results of a recent study by Eckle et al. (2007) indicated that in vivo IPC was ablated in A_{2B}AR KO mice, but not in mice lacking A_1, A_{2A} or A_3 receptors.

5 Reperfusion Injury and ARs in Human Myocardium

Despite all of the experimental evidence to date indicating the cardioprotective effects of adenosine and AR agonists, there have been very few studies examining the beneficial effects of these agents in humans in the setting of myocardial ischemia-reperfusion and thrombolysis. The initial such report was the acute myocardial infarction study of adenosine (AMISTAD) trial conducted between December 1994 and July 1997, the results of which were published in 1999 (Mahaffey et al. 1999). This was an open-label, placebo-controlled, randomized study to determine the safety and efficacy of adenosine as an adjunct to thrombolytic therapy in the treatment of acute myocardial infarction (MI). The effect of an intravenous infusion of adenosine ($70\,\mu g\,kg^{-1}\,min^{-1}$) for 3 h was compared to a placebo infusion in patients treated with thrombolysis within 6 h of the onset of an MI. After modification for slow enrollment, 197 patients were included, with the primary end-point being myocardial infarct size, as determined by Tc-99m sestamibi single-photon emission computed tomography (SPECT) imaging 5–7 days after enrollment. The results indicated that there was a 33% relative reduction in infarct size in patients that received adenosine ($p = 0.03$). Patients with an anterior MI exhibited a 67% relative reduction in infarct size, whereas there was no beneficial effect in patients with a nonanterior MI. Patients receiving adenosine, particularly those with nonanterior MI, experienced more bradycardia, heart block, hypotension and ventricular arrhythmias (Mahaffey et al. 1999).

There is a significant amount of preclinical data on the efficacy of AR agonists in reducing myocardial reperfusion injury, and these studies are clearly more consistently positive than the often contradictory findings with adenosine. Despite this wealth of information, today there remains only one documented clinical trial examining the effects of an AR agonist in the setting of clinical myocardial ischemia-reperfusion injury, the ADMIRE (AMP579 Delivery for Myocardial Infarction

REduction) study. This was a double-blind, multicenter, placebo-controlled trial of 311 patients undergoing primary percutaneous transluminal coronary angioplasty (PTCA) after acute ST-segment elevation MI (Kopecky et al. 2003). Patients were randomly assigned to placebo or to one of three different doses of AMP579 (15, 30 or 60 $\mu g\ kg^{-1}$) continuously infused over 6 h. This AR agonist, which has a high affinity for both A_1 and A_{2A}ARs, has been shown to reduce experimental myocardial ischemia-reperfusion in multiple species when administered both prior to ischemia or during reperfusion (Merkel et al. 1998; McVey et al. 1999; Meng et al. 2000; Xu et al. 2001; Kis et al. 2003; Kristo et al. 2004). The primary end-point was final myocardial infarct size measured by technetium Tc-99m sestamibi scanning at 120–216 h after PTCA. Secondary end-points included myocardial salvage and salvage index at the same time interval (in a subset of patients), left ventricular ejection fraction, duration of hospitalization, heart failure at 4–6 weeks, and cardiac events at four weeks and six months. Results indicated that there was no difference in final infarct size or in any of the secondary end-points. There was a trend towards increased myocardial salvage in patients with anterior MI. The authors of this study concluded that, based on the pharmacokinetic data, the maximal dose used in this trial was comparable to the lowest dose proven effective in animal studies.

The promising results of AMISTAD I led to a second trial (AMISTAD II) to determine the effects of adenosine infusion on clinical outcomes and infarct size in ST-segment elevation myocardial infarction (STEMI) patients undergoing reperfusion therapy (Ross et al. 2005). A total of 2,118 patients receiving thrombolysis or primary angioplasty were randomized to a 3 h infusion of either adenosine (50 or 70 $\mu g\ kg^{-1}\ min^{-1}$) or placebo. The primary end-point was new congestive heart failure (CHF) beginning >24 h after randomization, or the first rehospitalization for CHF, or death from any cause within six months. Infarct size was measured in a subset of 243 patients by Tc-99m sestamibi tomography. There was no effect of either adenosine dose on primary end-points, although patients receiving the higher dose (70 $\mu g\ kg^{-1}\ min^{-1}$) exhibited a median infarct size (11%) that was significantly lower ($p = 0.023$) than that of the placebo group (median infarct size 23%). It was concluded that a larger clinical trial was warranted to determine whether the decreased infarct size observed with adenosine was associated with enhanced long-term outcome. A post hoc subanalysis of these data indicated that patients receiving the adenosine infusion within 3 h of the onset of symptoms exhibited significantly reduced mortality at one and six months, and event-free survival was enhanced compared to patients treated with placebo (Kloner et al 2006).

Given all of the experimental evidence supporting the cardioprotective effects of AR agonists administered either prior to ischemia or during reperfusion, there clearly needs to more research and development into the synthesis, screening, and testing of potent, selective AR agonists. Basic scientists must also utilize consistent experimental models to determine the specific contributions of the multiple AR subtypes and their mechanisms of action. Because animal efficacy studies do not always translate to human efficacy, preclinical models with high relevance to humans and that closely simulate the human condition should be designed. Finally, clinical trials must be better designed along the lines of the information learned from the multitude of preclinical studies and clinical studies performed to date.

6 Impact of Age and Disease

Ischemic heart disease occurs predominantly in the elderly population (affecting up to 50% of those over 65), and can be associated with multiple underlying disease states, including atherosclerosis, hyperlipidemia, hypertension, and diabetes. From a clinical perspective, it is thus essential that protective strategies derived from research into PC or other protective modalities are effective across age groups and in diseased hearts. Unfortunately, aging limits or even abrogates protection with PC (Abete et al. 1996; Fenton et al. 2000; Schulman et al. 2001), AR activation (Gao et al. 2000; Schulman et al. 2001; Headrick et al. 2003b; Willems et al. 2005), and other GPCR stimuli (Peart et al. 2007). Newly discovered postconditioning is also impaired (Przyklenk et al. 2008). These age-dependent failures may stem from ineffective activation of key components of downstream signaling cascades (Peart et al. 2007; Przyklenk et al. 2008). On the other hand, age-related failure of AR-dependent protection is not universally observed. For example, Kristo et al. (2005) found no age-related changes in functional AR sensitivity, and augmentation of the infarct-sparing actions of adenosine. Thus, adenosine's role in aged hearts as well as the efficacy of cardioprotection in these hearts by targeting ARs with adenosine or AR agonists are questions that remain open.

Disease states underlying or contributing to ischemic disorders (when intrinsic protective responses such as PC are more important) can also impair these responses. For example, Ghosh et al. (2001) showed failure of PC in diabetic human myocardium, which may also reflect abnormalities in distal signaling cascades. In terms of AR responses, Donato et al. (2007) showed not only involvement of A_1ARs (and the mito K_{ATP} channel) in ischemic PC in normal hearts, but confirmed the ability of this stimulus to limit ischemic injury in hypercholesterolemic hearts. Moreover, A_1 and A_3 AR-triggered PC responses appear to be preserved in hypertrophic myocardium (Hochhauser et al. 2007). Thus, the few studies to date do support the preservation of AR-mediated protection in animal models of some relevant disease states. Whether this extends to patients suffering from chronic forms of cardiovascular disease remains to be established. It is worth considering that combined effects of age and disease may well underlie the rather modest benefit obtained with adenosine in clinical trials (AMISTAD I and II) versus the profound protective responses observed in the laboratory.

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