Review

Adenosine-mediated cardioprotection in the aging myocardium

Laura Willems, Kevin J. Ashton, John P. Headrick*

Heart Foundation Research Centre, School of Health Science, Griffith University, Southport, QLD 4217, Australia

Received 28 September 2004; received in revised form 21 October 2004; accepted 2 November 2004
Available online 23 November 2004
Time for primary review 19 days

Abstract

With aging, it appears the heart’s ability to withstand injury declines markedly. Unfortunately, the incidence of ischemic disorders increases dramatically with age. Though the genesis of the ischemia-intolerant phenotype is incompletely understood (and likely multifactorial), it may involve changes in intrinsic cardioprotective responses. In this respect we and others have interrogated the role of the adenosine receptor (AR) system in dictating ischemic tolerance and the impact of age on AR-mediated cardioprotection. It is intriguing to note ARs impact on many processes implicated in myocardial aging: adenosine counters Ca2+ influx and oxidant injury, modifies substrate metabolism to improve tolerance, is pro-angiogenic, inhibits myocardial fibrosis, and can limit apoptosis. Thus, dysregulation of the AR system could contribute to many features of aged hearts (including ischemic intolerance). The latter is borne out by observations that AR-mediated protective responses decline with intrinsic tolerance and that transgenic manipulation of the AR system restores intrinsic tolerance and protective responses in aged hearts. Mechanisms underlying failure in adenosinergic protection remain undefined. Here we review data on the effects of aging on cardiovascular AR transcription and expression, generation of signal (adenosine formation), and protective signaling coupled to ARs.

© 2004 European Society of Cardiology. Published by Elsevier B.V. All rights reserved.

Keywords: Adenosine; Adenosine receptors; Aging; Cardioprotection; Cellular Signalling; Ischemia

1. Introduction

Despite a decline in mortality rates over the last decade, ischemic heart disease remains a leading cause of death and disability in developed countries, and is the single greatest cause of death in individuals over 65 years of age. Coronary artery disease affects 50% of those older than 65, contributing to an increased incidence of angina, myocardial infarction, arrhythmia, congestive heart failure, and sudden death. The age of hearts employed in cardiac transplant is also increasing in an attempt to expand the donor pool [1], and surgical ischemia-reperfusion in the older population is increasing with expanded use of thrombolysis, recanalization, and revascularization. Unfortunately, advanced age (≥70 years) is a significant risk factor for operative mortality during cardiac surgery in coronary artery bypass, and age is an adverse prognostic factor in morbidity and mortality following a myocardial infarction. Estimates in the USA indicate up to 80% of deaths from coronary artery disease occur in those over the age of 65 [2]. The problem is growing—it is anticipated the elderly population may nearly double (to 25%) within 25–30 years. Morbidity and mortality attributed to cardiovascular disease will thus increase dramatically. It is thus something of a paradox that most research into the pathophysiology and therapy of cardiovascular disease has been undertaken in young adult tissue/models. The initial exciting potential of such potent stimuli as preconditioning must now be tempered by emerging evidence of limited utility in the “at-risk” aged population [3–5]. Aged myocardium likely requires specific management strategies [4], not evident from studies in young tissue.

Aging-related ischemic intolerance has been documented in multiple species including humans [3–16].
Genesis of the intolerant phenotype is not understood, though is likely multi-factorial (a “single” molecular basis of intolerance seems improbable). Amongst other things, cytosolic \(\text{Ca}^{2+}\) handling may be impaired [7,9,17,18], energy and substrate metabolism altered [12], oxidant injury exaggerated [11,19,20], gene expression modified [11,17–20], membrane structure and function altered [21,22], mitochondrial function impaired [8,22–24], apoptosis potentially enhanced [25,26], capillary/arteriolar density reduced and myocardial fibrosis and hypertrophy enhanced [27]. These factors may all contribute to intolerance, and have attracted attention. One possibility, less extensively investigated, is that endogenous or intrinsic “cardioprotective” processes, such as the AR system, may be impaired.

2. Age-related failure of intrinsic cardioprotection—the adenosine receptor system

The heart possesses a range of retaliatory mechanisms designed to provide resistance to injurious stimuli. Of these, considerable evidence points to an important role for the adenosine receptor (AR) system [28–32]. The ARs were attributed with regulatory functions in the heart and vessels three-quarters of a century ago [33]. More recent data indicate that adenosine is an endogenous determinant of myocardial ischemic tolerance [34–40]. Adenosine acts through Group protein-coupled receptor (GPCR) subtypes—the \(\text{A}_1\), \(\text{A}_2\), \(\text{A}_3\) and \(\text{A}4\)ARs [32,34]—each encoded by a distinct adenosine receptor gene (Ador). Adenosine also enhances tolerance to ischemia via metabolic substrate effects [32,40,41]. Adenosinergic cardioprotection in ischemic-reperfused hearts involves reductions in oncotic [41–45] and apoptotic death [46], and improved functional outcomes [28–32,37,43]. Whether ARs specifically protect against reversible injury is difficult to ascertain, although AR agonism enhances post-ischemic contractility in models with minimal irreversible injury [37,43,47–49]. Recent work supports differential effects of acute adenosine [41,50] vs. transient adenosinergic preconditioning [51], consistent with multiple pathways of protection. In terms of cellular targets, adenosine appears to directly protect cardiomyocytes or myocardial tissue (likely via \(\text{A}_1\) and \(\text{A}_3\)ARs) [32,49,52], and additionally protects via limiting inflammation and injurious interactions between inflammatory cells and vascular and myocardial tissue [30,32,34,38,42,53]. Though anti-inflammatory and “extra-cardiac” responses are important in adenosinergic protection [30,53], and as noted below may be altered with aging, further detailed description of these processes is unfortunately beyond the scope of the current review.

The different cardioprotective effects of AR agonism have been verified in animal [28–32,53] and human tissue [52,54,55]. However, few studies have addressed the possibility that altered AR-mediated protection might underlie specific cardiovascular disorders, though there is evidence to support this. Hypertrophic hearts, for example, display abnormal adenosinergic signaling [56,57], and dysregulated adenosine formation [58]. In the context of aging, we and others have acquired data demonstrating significant age-related changes in AR signaling [59–63], and more recently failure in AR-mediated protection against cell death and contractile dysfunction [16,64,65]. Interestingly, ARs impact on many processes implicated in cardiovascular “aging”, regulating Ca\(^{2+}\) influx and oxidant injury [29,30,32,49], substrate metabolism [37], angiogenesis [67], myocardial fibrosis [68], and apoptotic processes [46]. Given evidence of a role for ARs in intrinsic cardioprotection [34–40], mediation of preconditioning [69,70], and modifying the above-mentioned processes, alterations in AR signalling could contribute both to ischemic intolerance and emergence of other features of aged myocardium. Data reveals failure in AR protection parallels the decline in intrinsic resistance to ischemia in aging mice (Fig. 1).

Several studies support impaired adenosine responses in aged heart [59–62,64,71–73], though findings regarding AR expression/signaling vary [16,60,71]. Our recent data verifies failure in adenosinergic protection with even moderate aging in mouse [16], consistent with findings of Gao et al. [64] and Schulman et al. [65] in aged rat hearts. Age limits effects of ARs on both contractile outcome and oncotic death during ischemia-reperfusion [16,64,65], and reduces protection via both pre-ischemic AR agonism [65] and adenosine treatment throughout ischemia-reperfusion [16,64]. The molecular basis for the global failure in adenosinergic cardioprotection remains undefined. However, evidence points to failed activation of signaling distal to mitochondrial ATP-sensitive K\(^+\) (mito K\(_{\text{ATP}}\) ) channels [16]. Failure in AR-mediated cardioprotection may occur at the level of ARs themselves.

![Fig. 1](https://example.com/fig1.png)
expression and coupling), generation of protective “signal” (adenosine formation), and/or at sites within signaling cascades triggered by ARs. Potential sites are depicted diagrammatically in Fig. 2.

3. Intrinsic cardioprotection via adenosine receptors: effects of aging

Endogenous adenosine may protect via one or all of the four characterized AR sub-types—A₁, A₂A, A₂B, and A₃ARs [28–32]. All are considered to be expressed within cardiovascular cells. Studies in different species verify endogenous adenosine contributes to intrinsic ischemic tolerance [34–40], and support cardioprotective roles for A₁ARs in vitro and in vivo [35,37,39], and for A₂ARs in vivo [34]. Anti-ischemic effects of A₁ARs appear direct (at cardiomyocytes), since similar protection is observed in isolated hearts, cardiomyocytes, and in vivo [28,32,35,37,49,52,54]. Protective A₂AR effects involve modulation of vascular function, platelet adhesion and neutrophil activation [34,35,42,53,74], and are absent in cardiomyocytes [75] and in vitro models lacking blood [37,45,76]. Nonetheless, cardiomyocyte Adora2a mRNA expression [77], and functional A₂AAR responses in cardiomyocytes [77–79] implicate a functional myocardial A₂AAR (of obscure function). There is currently no direct evidence for acute A₂BAR mediated cardioprotection, partially due to lack of selective A₂BAR agonists/antagonists.

In contrast to A₁ and A₂ARs, there is little evidence that intrinsically activated A₃ARs mediate protection—A₃AR antagonists have no effect on ischemic outcomes in myocytes [80] or hearts [81]. A₃AR overexpression enhances tolerance [82], implying an intrinsic function in cardioprotection. However, markedly elevated A₃AR expression will exaggerate sensitivity to endogenous adenosine and thus the role of the receptor in wild-types. As shown by Hill et al. [83], the A₃AR is over an order of magnitude less sensitive to adenosine than A₁ARs. Thus, A₁ARs will be intrinsically activated to a greater extent than A₃ARs during ischemia.

Given a demonstrable role of A₁AR and A₂ARs in determining ischemic tolerance, changes in expression/functionality could contribute to evolution of ischemic intolerance. In preliminary transcriptional analysis of moderately aged (16–18 month) mice under ischemic and normoxic conditions [84], we detect down-regulation of genes encoding heat-shock and stress-inducible proteins, contractile proteins, transcription factors and signal transducers. Similar age associated changes have been observed in cardiomyocytes [85]. Many genes identified have putative roles in ischemic tolerance, and in AR-mediated protection. Indeed, in a recent micro-array study, 15–20% of aging-related transcriptional changes were involved in...
GPCR signaling [73], and included changes in Adora1 mRNA.

In terms of A1ARs, work in mouse hearts verifies no change in normoxic expression [16], in agreement with earlier findings of Cai et al. [60] for rat, and consistent with a lack of effect of aging on Adora1 mRNA levels in mice [16,87]. The latter study [87] also detected ischemic repression of Adora1 in aged (but not young) hearts. If translated into repressed A1AR expression, this may contribute to poor outcome at later time points in aged vs. young tissue. Despite no change in baseline A1AR expression in rat [60] and mouse [16], age-related changes in Adora1 mRNA are observed, though findings vary. Arosio et al. [88] and Dobson et al. [73] observed elevations in Adora1 whereas Jenner et al. [89] reported a 5-fold reduction in Adora1 with age. Irrespective of effects of age on mRNA, it appears that changes do not translate into reduced A1AR responsiveness [90]. Consistent with impaired A1AR activation of downstream effectors, we show A1AR overexpression overcomes age-related failure in adenosinergic protection and restores ischemic tolerance to levels in young tissue [16].

While there is little evidence for alterations in cardiac A1AR density, the study of Cai et al. [60] revealed reduced coupling between A1ARs and G-proteins, leading to reduced A1AR responsiveness. Other work confirms the importance of changes in receptor–effector coupling, rather than A1AR expression, in dictating cardiac A1AR sensitivity [90]. Consistent with impaired A1AR activation of downstream effectors, we show A1AR overexpression overcomes age-related failure in adenosinergic protection and restores ischemic tolerance to levels in young tissue [16].

Despite no direct studies of age-related changes in Adora2A or Adora3 mRNA expression, it is interesting to focus on these subtypes for a moment, as there is unequivocal data indicating aging limits cardiovascular Adora2A sensitivity [59,63]. If such changes extend to A1ARs implicated in reducing infarct size and post-ischemic inflammatory responses in vivo [34,35,53,74], this form of cardioprotection may also decline with age. There is indirect evidence age impairs Adora2A expression, since Adora2A transcription is significantly reduced in rat myocardium [88,89]. On the other hand, Adora3 mRNA appears induced in rat [88] and mouse [87]. There are also no direct studies of effects of aging on cardiovascular expression of A1ARs, though recent work indicates Adora3 mRNA is repressed with age in murine myocardium [87]. It would be of interest to examine ischemia tolerance in models of cardiac A1AR overexpression [82].

At a post-translational level, impaired GPCR responsiveness in senescent cells (of non-cardiac origin) has been attributed to up-regulation of caveolin expression [91], and manipulation of caveolin expression restores cellular responsiveness to GPCR stimuli [91,92]. Lasley et al. have observed translocation of A1ARs from caveolae upon agonist stimulation [93], a form of compartmentalization that may explain lack of direct effects of A1AR agonism under certain conditions. Given these latter findings [93], and effects of age on caveolins [91], it is possible that age-related reductions in AR-mediated protection against ischemic injury [16,64,65] may be related to altered caveolin expression and receptor interactions.

4. Generation of the protective “trigger”—adenosine formation

Ischemic intolerance could result from impaired endogenous activation of ARs. During ischemia/hypoxia, [adenosine] increases by an order of magnitude or more [32,39,69,94,95], triggering AR responses including cardioprotection. Myocardial adenosine formation occurs primarily via intracellular and extracellular dephosphorylation of 5′-AMP via 5′-nucleotidases [32]. Adenosine is derived from cardiomyocytes, endothelial cells, and fibroblasts. However, endothelial-derived adenosine accounts for only 15–25% of basal release [96,97], and even less during ischemia/hypoxia [98]. Though problematic to measure relevant interstitial concentrations, a variety of methods (including microdialysis) suggest these are sufficient to activate cardiovascular receptors [39,69,94,95,99]. This is validated by actions of AR antagonists [34–36,39,69].

Few studies have assessed effects of aging and senescence on interstitial adenosine levels and handling. Early work by Ramani et al. [100] provided preliminary support that reduced adenosine contributes to ischemic intolerance. However, they assessed total tissue adenosine, which substantially overestimates physiologically relevant “free” levels due to existence of tissue adenosine in bound forms (e.g. with s-adenosylhomocysteine; coupled to contractile proteins). Other studies verify age-related impairment of maintenance of adenine nucleotides in ischemic hearts [12], which enhances 5′-AMP and is predicted to increase adenosine accumulation. This is indeed what is observed in aged rat hearts during normoxia, ischemia, and adrenergic agonism [59,72]. Elevated extracellular adenosine in aged rat hearts does not appear to stem from altered 5′-nucleotidase or adenosine kinase activities [72]. However, since these activities were assessed in tissue homogenates it remains feasible that in vivo activities may differ as a result of alterations in the intracellular milieu. The enzymes are subject to control by Mg2+, H+ and inorganic phosphate (P), and data supports enhanced ischemic acidosis and P accumulation, and impaired recovery of P and Mg2+ in aged hearts [12]. Importantly, elevations in adenosine in aged rat hearts cannot explain profound reductions in ischemic tolerance in this species. However, in contrast to rat tissues, recent work documents comparable normoxic adenosine in young and aged mouse hearts, and a decline in post-ischemic adenosine with aging [101]. Thus, changes in endogenous agonism may contribute to impaired ischemic tolerance in mice.

Changes in extracellular adenosine with age in some species hint at another potential explanation for ischemic intolerance with age. Bolling et al. [102] first presented the
notion of adenosine protecting the heart as “substrate” (for nucleotide pool repletion) vs. “signal” (via AR activity). Manipulation of nucleotide repletion and adenosine handling enhances ischemic tolerance in mature hearts [40,41,48,102], and protection with adenosine is reduced by adenosine kinase inhibition, confirming a role for phosphorylation [41]. In preliminary studies we have shown that treatment with adenosine deaminase or kinase inhibitors fails to modify ischemic tolerance in aged hearts [103]. Thus, the “substrate” role of adenosine may be modified with aging. Recent work demonstrates specific shifts in purine catabolism in aged hearts, which may predispose to ischemic intolerance [101]. Accumulation of salvageable adenosine is reduced while accumulation of poorly salvaged hypoxanthine and xanthine is enhanced. These changes may limit myocardial capacity to replete adenine nucleotides post-ischemia.

5. Cardioprotective signaling coupled to AR agonism

In addition to changes in AR expression, age may impair the signaling cascades coupled to ARs. It is interesting that other protective stimuli (e.g. ischemic preconditioning) may retain their ability to activate downstream protective proteins in aged myocardium, despite substantially impaired outcome [104]. Effects of A1AR overexpression indicate that protective signaling can be harnessed in aged tissue if AR expression is sufficiently enhanced [16], verifying existence of exploitable protective processes. Before considering repression of AR-triggered signaling, it is worth noting that not all studies document repressed AR signaling with age, and that different signaling coupled to the same AR sub-type may be differentially altered. For example, while A1AR-mediated cardioprotection is absent [16,64,65], A1AR-mediated bradycardia and anti-adrenergic effects may be enhanced with aging [30,62,73]. Preservation (or amplification) of contractile or electrophysiological responses to A1ARs, coupled with failure of A1AR mediated cardioprotection [16,64,65], clearly demonstrates different signaling paths are selectively modulated during aging.

Numerous investigations document impaired function of GPCRs with age, and these generalized effects have been reviewed [91]. There is also evidence of impaired cardioprotection via other GPCRs [66]. A recent study by Dobson et al. [73] found that 15–20% of transcriptional changes in aged myocardium relate to GPCR signaling, lending weight to the notion of generally impaired GPCR signaling. With respect to AR-mediated cardioprotection, signaling appears to involve G-protein mediated activation of phospholipases, PKC, mitochondrial (and possibly sarclolemal) K<sub>ATP</sub> channels, and potentially MAPK and PI3-kinase pathways [29–32]. Our own prior data, demonstrating conservation of protective responses to direct mito K<sub>ATP</sub> channel activation, indicate the lesion in adenosinergic protection lies proximal to these channels [16]. However, given the possibility that mito K<sub>ATP</sub> channels may present both a signaling inter-

mediate and potential end-effector, since there remain discrepancies in effects of PKC inhibition placing the kinase distal and proximal to mito K<sub>ATP</sub> channels [41,44,51,105,106], and since there is evidence that aging may impair protection via mito K<sub>ATP</sub> activation [65], it is worth considering effects of aging at all levels of signaling. It is also worth mentioning that "parallel" paths may contribute to AR-mediated protection [107,108], providing signaling redundancy. This, of course, raises the question of how aging abrogates AR-mediated protection [16,64,65], and favors a sufficiently distal lesion to impact on multiple convergent paths (e.g. MAPK, PKC, PI3-kinase), and/or a lesion in the initial steps in AR signaling (e.g. G-protein function and phospholipase activity).

5.1. Phospholipase activation

The A<sub>1</sub> and A<sub>3</sub>ARs couple to pertussis-sensitive G<sub>i</sub> and G<sub>q</sub> family proteins. Liang et al. demonstrate (in chick myocytes) that A1AR and A3AR mediated protection involves G<sub>i</sub>-dependent activation of PLC activity (a short-lived response) and RhoA-dependent activation of phospholipase D (PLD; a more long-lived response), respectively [109]. The A<sub>2A</sub> and A<sub>2B</sub>ARs activate adenylyl cyclase via G<sub>q</sub> protein, and A<sub>2B</sub>ARs also trigger PLC activity via G<sub>q</sub> protein [110]. Though not specifically addressed in cardiomyocytes, there is evidence that aging and/or cellular senescence impair PLC and IP3 activation [111,112], together with PLD activation [112,113]. Impaired activity of PLC and particularly PLD may not only involve altered upstream activation but also reduced expression [112]. No study has directly addressed the relevance of reduced phospholipase signaling to changes in anti-ischemic actions of adenosine.

5.2. PKC activity

Activation of PKC is considered crucial in adenosinergic protection, and is often thought to occur upstream of mito K<sub>ATP</sub> channels [114]. This is consistent with failure of PKC inhibition to modulate protection via mito K<sub>ATP</sub> channel activation [44], and abrogation of PKC-mediated protection with a mito K<sub>ATP</sub> channel blocker [115]. On the other hand, PKC inhibition has been shown to eliminate protection via mito K<sub>ATP</sub> channel openers [41,51,105,106], placing PKC downstream. To reconcile such differences, PKC could act up- and downstream of mito K<sub>ATP</sub> channels (e.g. modulating mito K<sub>ATP</sub> function and subsequently being modified by mito K<sub>ATP</sub> channel-dependent signaling, including ROS generation) (Fig. 2).

The impact of age on PKC signaling has received some attention. Tani et al. [116,117] observe impaired PKC activation/translocation and altered PKC-ε expression with age. Data of Przyklenk et al. [118] indicate protective PKC signaling paths differ substantially in aged vs. young tissues, with a requisite role of PKC-ε in young but not aged tissues. If PKC does play a central role in AR-mediated protection...
[29,31,32], and is a point of convergence in signaling, these changes in activation/ translocation could certainly contribute to failure in AR-mediated cardioprotection with age.

5.3. K$_{ATP}$ channels

Stimulation of mito K$_{ATP}$ channel opening is required, under most conditions studied, for expression of the anti-ischemic actions of AR. The A$_1$AR can activate both sarcolemmal [119] and mitochondrial ATP sensitive K$^+$ channels [32,41,44,51], the A$_2$AR also activates mito K$_{ATP}$ channels [120,121], and acute and preconditioning responses to AR agonism appear mito K$_{ATP}$ channel mediated [41,44,49,51,120,121]. However, there remains support for a role for sarcolemmal K$_{ATP}$ channels [122,123], despite the shift in focus to mitochondrial channels. Failure in K$_{ATP}$ channel activation could certainly limit AR-mediated protection, however mixed data exist regarding ability of mito K$_{ATP}$ channel activation to protect aged tissue. In mice a mito K$_{ATP}$ activator retains its ability to protect against ischemic injury despite lack of AR-mediated protection [16]. This suggests failure distal to ARs yet proximal to mito K$_{ATP}$ channels. In contrast, Schulman et al. [65] observed failure in both AR and diazoxide-mediated preconditioning in aged rat hearts, implying failure in signaling distal to the mito K$_{ATP}$ channel. Differing observations could reflect different signaling with acute protection vs. preconditioning [41,51], and may be species-related. Complicating interpretation, there is evidence that effects of the commonly employed K$_{ATP}$ opener diazoxide and inhibitor 5-hydroxydenacoyl acid may be unrelated to mito K$_{ATP}$ channel modulation [124,125]. If these agents indirectly target ROS generation and mitochondrial function, as suggested by Hanley and colleagues [124], or act via sarcolemmal K$_{ATP}$ channels, as suggested by Suzuki et al. [125], then it may be these sites as opposed to mito K$_{ATP}$ channels themselves, that are altered with aging. Indeed, strong evidence indicates protection attributed to mito K$_{ATP}$ channels requires sarcolemmal K$_{ATP}$ activity [122,125–127]. Direct reductions in sarcolemmal K$_{ATP}$ channel expression and functionality do occur with aging (reviewed in Ref. [128]), and may thus lead to ischemic intolerance and failed AR cardioprotection, in agreement with intolerant phenotypes evident with reduced K$_{ATP}$ channel expression [126,127].

5.4. MAPK Signaling

The mitogen-activated protein kinase (MAPK) family has been implicated in cardioprotective responses to adenosine [108,129]. Fredholm et al. show all ARs couple to MAPKs in non-cardiac cells [130], and preliminary studies support a role for p38 MAPK in A$_1$AR mediated cardioprotection [131]. As noted by Gabai and Sherman [132], MAPK signaling may favor either cell death or survival depending on the balance of differing elements and pathways. Thus, subtle changes in MAPK signals with age may have profound effects on cellular outcome from ischemia and on effects of protective stimuli. Aging substantially reduces MAPK (JNK and ERK) activity/levels in rat myocardium [133,134]. Findings in the few studies of aged human hearts are equivocal, with studies generally undertaken in diseased groups of only moderate age (e.g. Refs. [135–137]). Changes appear to involve impaired activity in the absence of changes in protein content, and may reflect impaired levels of upstream receptor activation and/or changes in caveolin function [91,92]. Studies of effects of aging on transcriptional responses to oxidative stress reveal impairment of early response genes dependent on MAPK signaling and stress-response genes (e.g. Gadd45, JunB) [86].

5.5. PI3-kinase

Agonism of ARs can activate myocardial PI3-kinase via transactivation of tyrosine kinase [138]. A key target of PI3-kinase is Akt, which promotes cell survival and is implicated in cardioprotection [139]. There is evidence of repressed PI3-kinase expression in aged cardiac tissue [140], in other tissues [141]. Immunohistochemical data verifies a decline in PI3-kinase expression and activity [142]. Additionally, Akt is repressed in aged cardiac tissue [142]. Such observations suggest the PI3-kinase/Akt pathway may well be of limited activity in aged myocardium, contributing to impaired intrinsic tolerance and adenosinergic protection. It should be noted, however, that preliminary data do not support essential roles for PI3-kinase/Akt signaling in A$_1$AR mediated protection against cardiomyocyte death [46], or in adenosine mediated preconditioning [143]. Thus, the relevance of repressed PI3-kinase/Akt signaling to impaired AR-mediated protection remains to be defined.

5.6. Nitric oxide signaling

There is evidence supporting adenosinergic protection via modulation of nitric oxide (NO) bioavailability. Adenosine-mediated preconditioning may trigger changes in NO synthase (NOS) activity [144], and acute A$_1$AR-mediated cardiac responses appear to be at least partially NO-dependent [145]. In mice, age-related changes in NO bioavailability parallel changes in adenosine-mediated protection [64]. However, there is also evidence that NO generation and iNOS activity are enhanced in aged mouse hearts [146]. Data for rats are equivocal. Recent work reveals impaired mitochondrial NOS activity in aging myocardium [147], while Ishihata et al. [148] observed no differences in basal NO release, though stimulated release was markedly reduced. Ziemer et al. [149] detected increases in eNOS activity in aged rat heart whereas Chou et al. [150] report decreased eNOS activity with age. Precise changes in NO bioavailability with age, and their role in ischemic intolerance, remain to be more clearly defined. Nonetheless, impaired mitochondrial NO generation [147] and parallel changes in NO generation and cardioprotection...
[64] give credence to the possibility that alterations in NO signaling may contribute to impaired cardioprotection.

5.7. Anti-oxidant defense

There is evidence that A1AR agonism protects through improving anti-oxidant defense [31,32]. Adenosine receptor activation reduces mitochondrial radical formation [49] and oxidant injury [151,152], and increases cellular anti-oxidant capacity [153]. Moreover, cardioprotection via mito KATP channel-dependent pathways may involve inhibition of reactive oxygen species (ROS) generation [154]. Given the central role of oxidant damage in both reversible and irreversible injuries, these effects may contribute to the resistant phenotype observed with adenosine receptor activation. Aging is associated with increased oxidant generation and impaired anti-oxidant defences [11,155,156]. The documented elevations in radical generation and repression of endogenous anti-oxidant systems with age would be consistent with impairment of the beneficial effects of the AR system on radical generation [49] and anti-oxidant defences [153].

6. Relevance of failed adenosinergic cardioprotection

As already noted, the substantial evidence for intrinsic protection via ARs, coupled with parallel reductions in AR-mediated cardioprotection and intrinsic ischemic tolerance (Fig. 2), supports the idea that changes in adenosinergic protection may contribute to the ischemia-intolerant aged phenotype. This is further supported by the observation that enhanced A1AR expression renders myocardium comparable to young myocardium in terms of ischemic tolerance [16]. Changes in the AR system may additionally impact on post-ischemic events, with evidence that adenosine may regulate remodeling. Findings of Dubey et al. [68] indicate A2BARs normally inhibit cardiac fibroblast growth, and this group proposes A2BARs play a key role in regulating cardiac remodeling associated with fibroblast proliferation. This has yet to be more directly tested, although the normal role of A2BARs in regulating collagen synthesis and fibroblast growth is supported by other recent studies [157]. Since aging is associated with repression of A2BAR responses in vascular tissue [63], it is possible a decline in fibroblast A2BAR responses could enhance mitogenic/ fibrotic activity in aged tissue. Aging does impact on remodeling in experimental models and is associated with more advanced basal myocardial fibrosis [158,159].

Finally, age-related reductions in AR-mediated cardioprotection may be highly relevant to modest outcomes in recent clinical trials of adenosinergic therapies. Several placebo-controlled clinical trials have been conducted, including Acute Myocardial Infarction Study of Adenosine Trial (AMISTAD) I and II, Attenuation by Adenosine of Cardiac Complications (ATTACC), and ADMIRE (AmP579 Delivery for Myocardial Infarction REduction). In AMISTAD, infarct size was reduced and LV systolic function improved by adenosine, primarily in patients with anterior MI localization [160]. Morbidity and mortality were unaffected. In ATTACC, LV systolic function was unaffected, and there was a trend towards improved survival in patients again with anterior MI localization [161]. In ADMIRE, final infarct size was unaffected by the A1/A2 agonist studied (AMP579), though again there was a trend towards greater myocardial salvage in patients with anterior MI [162]. The disparity between these modest effects and more profound protection in animal models [28–32,34,35,42–44,53] may relate to the age of the patients. In ADMIRE the age range was 31 to 85, with similarly broad ranges and advanced ages in AMISTAD and ATTACC. Based on animal data (Fig. 1), failure in adenosinergic protection may occur well prior to senescence (by middle age), explaining lack of efficacy of adenosine-based therapies in clinical trials.

7. Conclusions

The adenosine receptor system and adenosine metabolism play important roles in determining cardiac outcome from ischemic insult. Despite advances in knowledge regarding the role of adenosine and ARs in protecting young tissues, it is now clear these responses are substantially impaired (or eliminated) with aging. These changes themselves may be important in the genesis of the ischemia-intolerant phenotype with aging. Origins of this dysfunction remain to be identified, but are likely to be multi-factorial (Fig. 2). Evidence does implicate failure in signaling as opposed to AR expression and adenosine handling itself. Clarification of changes in cardioprotective signaling may not only provide insight into the genesis of ischemic intolerance, but ultimately permit manipulation of myocardial tolerance and post-ischemic outcome in older subjects at greatest risk of ischemic events.

Acknowledgment

Dr. Headrick is the recipient of a Career Research Fellowship from the National Heart Foundation of Australia.

References


Finegan BA, Lopaschuk GD, Gandhi M, Clanahan AS. Inhibition of glycolysis and enhanced mechanical function of working rat hearts as a result of adenosine A1 receptor stimulation during reperfusion following ischaemia. Br J Pharmacol 1996;118:355–63.


Miura T, Liu Y, Kita H, Ogawa T, Shimamoto K. Roles of mitochondrial ATP-sensitive K+ channels and PKC in anti-infarct


Lasley RD, Smart EJ. Cardiac myocyte adenosine receptors and caveolae. Trends Cardiovasc Med 2001;11:259–63.


Lasley RD, Smart EJ. Cardiac myocyte adenosine receptors and caveolae. Trends Cardiovasc Med 2001;11:259–63.


Lasley RD, Smart EJ. Cardiac myocyte adenosine receptors and caveolae. Trends Cardiovasc Med 2001;11:259–63.


