Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION

Genetic Deletion of the A_1 Adenosine Receptor Limits Myocardial Ischemic Tolerance

Melissa E. Reichelt, Laura Willems, Jose G. Molina, Chun-Xiao Sun, Janci C. Noble, Kevin J. Ashton, Jurgen Schnermann, Michael R. Blackburn and John P. Headrick

Circ Res. 2005;96:363-367; originally published online January 13, 2005;
doi: 10.1161/01.RES.0000156075.00127.C3
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571

Genetic Deletion of the A_1 Adenosine Receptor Limits Myocardial Ischemic Tolerance

Melissa E. Reichelt,* Laura Willems,* Jose G. Molina, Chun-Xiao Sun, Janci C. Noble, Kevin J. Ashton, Jurgen Schnermann, Michael R. Blackburn, John P. Headrick

Abstract—Adenosine receptors may be important determinants of intrinsic ischemic tolerance. Genetically modified mice were used to examine effects of global A_1 adenosine receptor (A_1AR) knockout (KO) on function and ischemic tolerance in perfused mouse hearts. Baseline contractile function and heart rate were unaltered by A_1AR KO, which was shown to abolish the negative chronotropic effects of 2-chloroadenosine (A_1AR-mediated) without altering A_2 adenosine receptor–mediated coronary dilation. Tolerance to 25 minutes global normothermic ischemia (followed by 45 minutes reperfusion) was significantly limited by A_1AR KO, with impaired contractile recovery (reduced by ≈25%) and enhanced lactate dehydrogenase (LDH) efflux (increased by ≈100%). Functional effects of A_1AR KO involved worsened systolic pressure development with little to no change in diastolic dysfunction. In contrast, cardiac specific A_1AR overexpression enhanced ischemic tolerance with a primary action on diastolic dysfunction. Nonselective receptor agonism (10 μmol/L 2-chloroadenosine) protected wild-type and also A_1AR KO hearts (albeit to a lesser extent), implicating protection via subtypes additional to A_1ARs. However, A_1AR KO abrogated effects of 2-chloroadenosine on ischemic contracture and diastolic dysfunction. These data are the first demonstrating global deletion of the A_1AR limits intrinsic myocardial resistance to ischemia. Data indicate the function of intrinsically activated A_1ARs appears primarily to be enhancement of postischemic contractility and limitation of cell death. **(*Circ Res*. 2005;96:363-367.)**

Key Words: adenosine ■ A_1 adenosine receptor ■ gene knockout ■ ischemia ■ reperfusion

The heart possesses protective or retaliatory mechanisms providing tolerance to ischemia/reperfusion. These may represent targets for therapeutic manipulation of ischemic tolerance, and conversely, alterations in these mechanisms could underlie changes in outcome with aging and/or disease. We and others have been studying the role of the purine nucleoside adenosine and its receptors in modulating injury during ischemia/reperfusion.[1,2] There are currently four known and potentially protective adenosine receptor (AR) subtypes encoded by distinct genes: the A_1, A_{2A}, A_{2B}, and A_3ARs.[1,2] All are G-coupled, with A_1, A_{2A}, and A_3ARs possessing higher affinities for adenosine, whereas the A_{2B}AR has a relatively low affinity. Although contentious,[2] the AR system may be an integral component of the hearts intrinsic protective arsenal, limiting damage during[3–5] and after ischemic challenge.[6] Although this is consistent with benefit via AR agonists,[1–3,7] effects of AR antagonism (to unmask responses to endogenous adenosine) are equivocal, with studies supporting[4–6,8–11] and refuting[12–15] a role for endogenous adenosine in dictating ischemic tolerance. Some of this controversy may stem from inherent limitations in pharmacological approaches to abrogating receptor-mediated responses: these can be hampered by potentially poor antagonist selectivity or potency, and/or potentiation of local agonist levels as a result of opening feedback loops linking "signal" (adenosine generation in this case) to tissue "response" (protection of cellular homeostasis).[16–18] An alternative approach involves selective gene deletion, which, coupled with complementary analysis of effects of transgenic overexpression, may facilitate assessment of the specific role of a protein in wild-type tissue.[19,20] In this study, we document for the first time the ability of genetic removal of A_1ARs to modify intrinsic tolerance to ischemia.

Materials and Methods

Investigations conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication no. 85-23, revised 1996).

Experimental Protocol

A_1AR knockout (A_1AR KO) mice (Jackson Laboratories, Bar Harbor, Me) were generated and genotyped as described previously,[21] with genotypes determined via PCR analysis of genomic DNA. All mice were on a mixed 129sv/C57BL/6J background and phenotypic comparisons were performed among littermates. Details of generation

Original received September 10, 2004; revision received January 4, 2005; accepted January 4, 2005.

From the Heart Foundation Research Center (M.E.R., L.W., K.J.A., J.P.H.), Griffith University, Southport, Australia; the Department of Biochemistry and Molecular Biology (J.G.M., C.-X.S., J.C.N., M.R.B.), University of Texas Health Science Center at Houston, Medical School, Houston; and the National Institute of Diabetes and Digestive and Kidney Diseases (J.S.), National Institutes of Health, Bethesda, Md.

*Both authors contributed equally to this study.

Correspondence to John Headrick, Heart Foundation Research Centre, Griffith University, Southport, QLD 4217, Australia. E-mail J.Headrick@griffith.edu.au

Circulation Research **is available at http://www.circresaha.org** **DOI: 10.1161/01.RES.0000156075.00127.C3**

of C57/BL/6J mice selectively overexpressing cardiac A_1ARs have been reported previously.[22] Hearts for this study were isolated from young (2 month) mice from the following groups: A_1AR KO (n=20); wild-type littermates (n=24); A_1AR overexpression (n=15); and wild-type C57/BL/6J (n=16).

All mice were anesthetized with 50 mg/kg sodium pentobarbitone administered intraperitoneally, a thoracotomy performed, and hearts excised into ice-cold perfusion fluid for cannulation and perfusion on a Langendorff perfusion system.[5,23] Hearts were stabilized at intrinsic rate a further 10 minutes before acquiring concentration-response curves for 2-chloroadenosine–mediated A_1AR and A_{2A}AR-dependent bradycardia/coronary dilation. Concentration-response curves were acquired in unpaced normoxic hearts from A_1AR KO (n=6), wild-type littermates (n=7), A_1AR overexpressing mice (n=7), and wild-type C57/BL/6J mice (n=7), as described previously.[24] Chronotropic and vasodilatory responses were scaled as percentage of baseline, and data were analyzed via nonlinear regression to acquire individual pEC_{50} values, as outlined previously.[24,25]

In ischemic studies, hearts were stabilized for 20 minutes at intrinsic heart rate before pacing at 420 bpm followed by a further 10 minutes stabilization period.[5,23] Baseline measurements were then made, and hearts were subjected to 25 minutes global normothermic ischemia followed by 45 minutes aerobic reperfusion. Coronary venous effluent was collected on ice for enzymatic analysis of lactate dehydrogenase (LDH) activity.[23] Total LDH efflux during reperfusion was expressed as international units (IU) per gm wet weight, and has been previously shown to correlate with measures of oncotic injury/infarction in this model.[26] Ischemic responses were assessed in A_1AR KO (n=7) and wild-type littermate (n=9) mice, and A_1AR overexpressing (n=8) and wild-type littermate (n=9) mice.

Effects of adenosinergic cardioprotection with 10 μmol/L of the nonselective agonist 2-chloroadenosine were also assessed in wild-type (n=8) and A_1AR KO hearts (n=7) subjected to 25 minutes ischemia and 45 minutes reperfusion. Based on concentration-response data in Figure 1, >3 μmol/L 2-chloroadenosine is required to maximally activate a functional A_1AR response (less for an A_2AR response). Thus, in an attempt to achieve near-maximal activation of all adenosine receptor subtypes in all murine lines studied, we used a 10 μmol/L agonist concentration. Although it is feasible prolonged treatment with high concentrations of 2-chloroadenosine might induce adenosine receptor-independent actions (because it is a substrate for nucleoside transporters), this is unlikely to be an issue in the current acute studies. The 10 μmol/L concentration is equivalent to or less than functional EC_{50} values for 2-chloroadenosine activation of adenosine receptor responses in cardiovascular and other cell types,[27–31] recent studies confirm this concentration induces receptor-mediated actions in cardiomyocytes and cardiac fibroblasts (mimicked by selective receptor agonists and/or blocked by adenosine receptor antagonists),[32–35] and our preliminary experiments (data not shown) confirmed acute chronotropic and vasodilatory responses to 10 μmol/L 2-chloroadenosine were sensitive to 100 μmol/L of the competitive receptor antagonist 8-sulfophenyltheophylline.

Statistical Analyses

All data are presented as mean±SEM. Baseline data, pEC_{50} values, final recoveries, and LDH efflux were analyzed via one-way ANOVA. Time course data were compared via two-way ANOVA for repeated measures. When significant differences were detected in ANOVA tests, a Newman-Keuls post hoc test was used for specific comparisons. A value of $P<0.05$ was considered significant in all tests.

Results

There were no differences in baseline contractile function or coronary flow between groups (Table). However, intrinsic heart rate was reduced in A_1AR-overexpressing hearts. Concentration-response analysis confirmed A_1AR KO abrogates A_1AR-mediated bradycardia without altering sensitivity of A_{2A}AR-mediated vasodilation (Figure 1). Conversely, A_1AR overexpression increased the sensitivity of A_1AR-mediated bradycardia without altering coronary responses. The pEC_{50} values for the different responses are provided in Table.

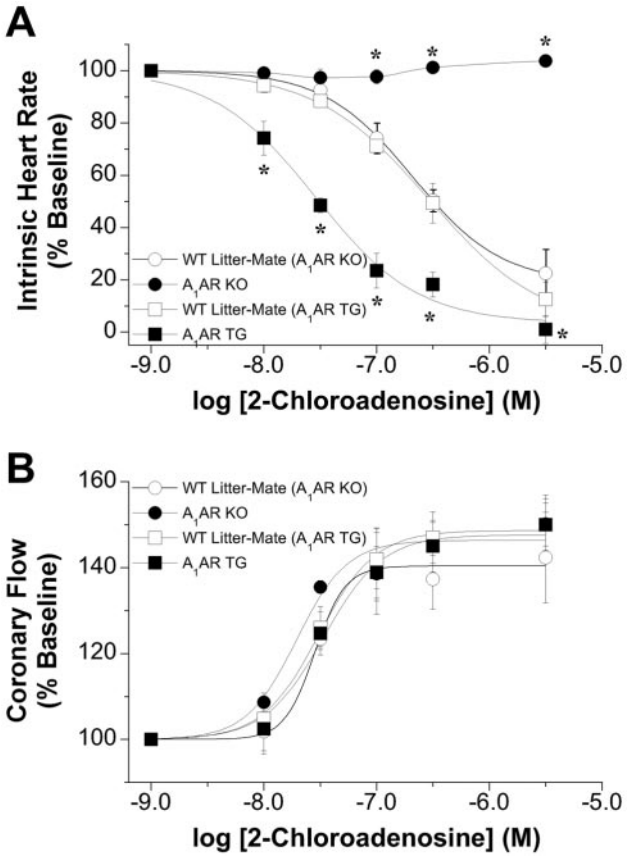

Figure 1. Effects of A_1AR KO and transgenic A_1AR overexpression on cardiac and vascular sensitivity to adenosine receptor agonism with 2-chloroadenosine. A, A_1AR-mediated bradycardia. B, A_{2A}AR-mediated coronary dilation. Responses are shown for hearts from A_1AR KO (n=6) and wild-type littermate (n=7) mice, and for hearts from transgenic (TG) mice overexpressing cardiac A_1ARs (n=7) and their wild-type littermates (n=7). Values are mean±SEM. *$P<0.05$ vs corresponding wild type.

Deletion of A_1ARs significantly reduced ischemic tolerance. Recovery profiles for hearts subjected to 25 minutes ischemia and 45 minutes reperfusion are depicted in Figure 2. Effects of A_1AR KO were evident in terms of reduced systolic pressure with little change in diastolic dysfunction (Figure 2). Thus, left ventricular pressure development was depressed (Figures 2 and 3). Deletion of the A_1AR did not modify the rate of contracture development during ischemia (time to reach 20 mm Hg diastolic pressure)[23] (Figure 3B), but significantly worsened cellular damage indicated by postischemic efflux of LDH (Figure 3C). Conversely, A_1AR overexpression enhanced ischemic tolerance (Figures 2 and 3), with the primary contractile effect being reduced diastolic dysfunction (Figures 2A and 3A). Overexpression of A_1ARs only improved systolic function during the initial minutes of reperfusion (Figure 2B). Ischemic contracture development was also reduced by A_1AR overexpression (Figure 3B), in contrast to lack of effect of A_1AR KO on this parameter.

Treatment of wild-type and A_1AR KO hearts with the nonselective agonist 2-chloroadenosine improved postischemic outcomes in both groups (Figure 3). However, the

Preischemic Functional Parameters and Sensitivities (pEC$_{50}$s) for 2-Chloroadenosine–Mediated Bradycardia (A$_1$AR-dependent) and Coronary Vasodilation (A$_2$AR-dependent) in Hearts From Each Experimental Group

Treatment	Heart Rate, bpm	LVDP, mm Hg	+dP/dt (mm Hg/s)	−dP/dt, mm Hg/s	Coronary Flow, mL/min/g	pEC$_{50}$ for A$_1$AR Bradycardia	pEC$_{50}$ for A$_{2A}$AR Dilation
WT littermate (n=17)	366±9	133±3	6543±195	4497±93	22.3±1.9	6.6±0.1 (n=7)	7.6±0.1 (n=7)
A$_1$AR KO (n=14)	379±7	130±3	6575±202	4194±112	22.2±1.1	No response (n=6)	7.7±0.1 (n=6)
A$_1$AR TG (n=8)	330±7*	130±7	6581±363	4115±202	20.8±1.0	6.5±0.1 (n=7)	7.5±0.1 (n=7)
WT littermate (n=9)	369±8	139±5	6702±308	4390±154	23.0±1.1	7.6±0.1* (n=7)	7.4±0.1 (n=7)

Values are mean±SEM. Function was measured after 30 minutes stabilization before ischemia, except for heart rate (measured after 20 minutes stabilization, before pacing). pEC$_{50}$ values represent −log molar concentrations of 2-chloroadenosine producing 50% of the maximal response. WT indicates wild type; TG, transgenic. *P<0.05 vs WT littermates.

protective actions of the agonist were significantly reduced in A$_1$AR KO versus wild-type hearts (Figure 3). Furthermore, beneficial effects of 2-chloroadenosine on diastolic dysfunction and ischemic contracture observed in wild-type hearts were abrogated by A$_1$AR KO (Figure 3B).

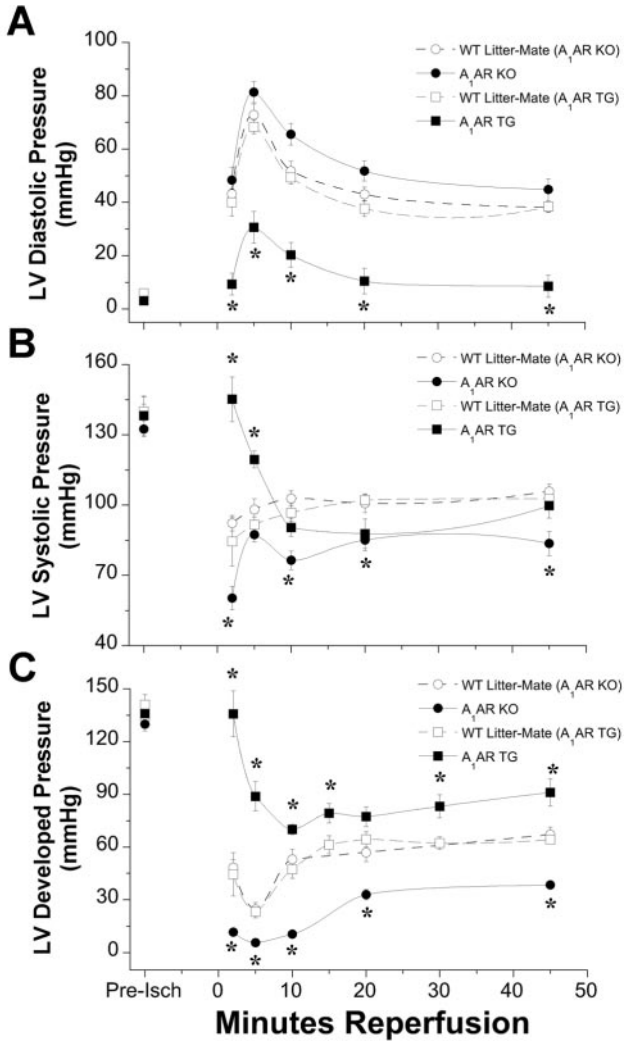

Figure 2. Effects of manipulation of A$_1$AR expression on postischemic recoveries for left ventricular diastolic pressure (A), systolic pressure (B), and developed pressure (C). Data are shown for recoveries in hearts from A$_1$AR KO (n=7) and wild-type (WT) littermate (n=9) mice, and for hearts from transgenic (TG) mice overexpressing cardiac A$_1$ARs (n=8) and their wild-type littermates (n=9). Values are mean±SEM. *P<0.05 vs corresponding wild type.

Discussion

The role of endogenous adenosine and adenosine receptors in determining intrinsic tolerance to ischemic insult remains controversial. Many studies do not observe effects of adenosine receptor antagonists on ischemic outcome in various species.[12–15] Moreover, there is even evidence A$_1$AR blockade actually improves outcome from ischemia.[36,37] In contrast, there is some support for a role for endogenously generated adenosine in protection of ischemic myocardium[4–6,8–11] and modulation of processes impinging on recovery from ischemia.[38,39] Several explanations may account for varied findings with antagonists, the most likely involving mixed selectivity and potency of agents used and the fact that an antagonist applied to any system in which the signal is coupled to the response (as with adenosine) will likely generate an elevation in the signal (ie, opening the feedback loop). This has been verified in prior work.[16–18] On the other hand, it is also important to note that generally observed cardioprotection with adenosine agonists[1–3,8,10,26,40] indicates the intrinsic adenosine response must normally be submaximally (if at all) engaged. In assessing potential roles of adenosine receptors, an alternate and relatively selective approach involves gene deletion of receptor protein. In this study, we provide the first evidence that genetic deletion of the A$_1$AR significantly limits the ability of mouse myocardium to withstand injury during ischemia/reperfusion (Figures 2 and 3). Conversely, cardiac A$_1$AR overexpression confers enhanced tolerance, as documented previously.[22] These data collectively provide strong support for a role of A$_1$ARs in determining intrinsic tolerance to ischemia/reperfusion.

Effects of A$_1$AR KO are evident in terms of reduced systolic dysfunction and oncotic injury, with little effect on diastolic dysfunction (Figures 2 and 3). This contrasts effects of A$_1$AR overexpression, which are manifest as reduced diastolic contracture with little change in systolic pressure (except during initial reperfusion). Thus, effects of receptor deletion do not mirror effects of receptor overexpression. Rather, data suggest responses mediated by a highly overexpressed receptor may be abnormal or "supraphysiological" and/or that functional effects of A$_1$ARs vary with the level of activation during insult. This is consistent with effects of 2-chloroadenosine, which did reduce postischemic diastolic dysfunction, an action ablated by A$_1$AR KO (Figure 3). These data indicate the A$_1$AR is responsible for "adenosinergic" reductions in diastolic dysfunction, but that the response is evident only with enhanced levels of agonism. Selective

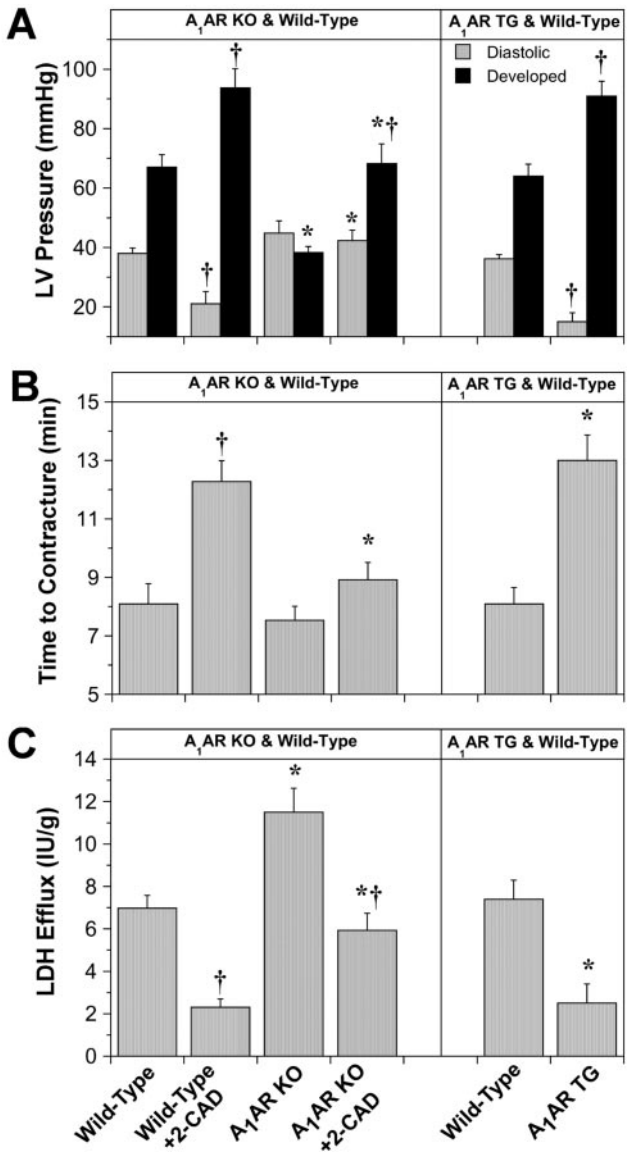

Figure 3. Effects of manipulation of A_1AR expression and the nonselective adenosine receptor agonist 2-chloroadenosine, on ischemic and postischemic outcomes. A, Final recoveries for left ventricular (LV) diastolic and developed pressure. B, Rate of ischemic contracture (time for diastolic pressure to reach 20 mm Hg during ischemia). C, Postischemic LDH efflux during 45 minutes reperfusion. Outcomes are shown for hearts from A_1AR KO (n=7) and wild-type (WT) littermate (n=9) mice, and for hearts from transgenic (TG) mice overexpressing cardiac A_1ARs (n=8) and their wild-type littermates (n=9). Effects of 10 μmol/L 2-chloroadenosine were assessed in hearts from A_1AR KO (n=7) and wild-type littermate (n=8) mice. Values are mean±SEM. *$P<0.05$ vs corresponding wild-type groups; †$P<0.05$ vs untreated hearts.

actions of A_1ARs on systolic versus diastolic function are consistent with prior observations regarding A_1AR antagonism, revealing that postischemic A_1AR activation improves systolic force without altering diastolic dysfunction, whereas intraischemic A_1AR activation limits diastolic dysfunction in addition to improving systolic force.[5] Extent (and timing) of A_1AR engagement likely dictates relative effects on diastolic versus systolic dysfunction.

Development of ischemic contracture was also unaffected by A_1AR KO, but was limited by A_1AR overexpression and 2-chloroadenosine. The latter response was again abrogated by A_1AR KO (Figure 3). Thus, adenosinergic limitation of ischemic contracture is A_1AR dependent but evident only with exaggerated receptor agonism or expression. This agrees with early work of Lasley et al[3] demonstrating A_1AR agonist–mediated protection against ischemic contracture in rat, and prior data demonstrating negligible effects of A_1AR blockade on contracture development in mice.[5]

Although 2-chloroadenosine–mediated protection against contracture and diastolic dysfunction is abrogated by A_1AR KO (Figure 3), supporting A_1AR-dependent effects of the agonist on diastolic function, the analogue still exerted some beneficial actions in A_1AR KO hearts. This is reflected in improved systolic function together with reduced LDH efflux (Figure 3). These effects, refractory to A_1AR KO, implicate a protective function for receptor subtypes distinct from A_1ARs. Prior evidence that exogenous A_3AR but not A_{2A}AR agonism is cardioprotective in the model studied here,[26,40] and that A_3AR protection selectively enhances systolic function and reduces cell death, argues for a potential role for this subtype in the remaining protection with 2-chloroadenosine. However, this remains to be directly assessed, and we cannot exclude a potential role for the less well-studied A_{2B}AR.

Two study limitations bear mention before closing. As with all gene deletion studies, adaptations may occur to compensate for life-long absence of a targeted protein. Although it may be fruitful to focus on such adaptations (see for example, Godecke et al[41] and Warth and Barhanin[42]), they also complicate interpretation of phenotypic outcomes. We have assessed, in part, obvious changes in other adenosine receptors, verifying that A_1AR deletion selectively abrogates an A_1AR response (bradycardia) without modifying A_{2A}AR sensitivity. However, we recognize the possibility of undetected compensatory changes contributing to the A_1AR KO phenotype. The second limitation relates to the fact that we focus on responses in the isolated buffer-perfused heart. This was deliberate, to assess more directly the myocardial phenotype, because A_1AR protection is primarily "direct" and mediated via cardiomyocyte receptors.[2,7] However, adenosinergic protection in vivo additionally involves modulation of blood cells and related inflammatory responses.[1,2,7] We therefore cannot ascertain potential effects of A_1AR deletion on these extracardiac responses. However, these responses are predominantly A_2AR-dependent[2,7] and thus not predicted to be substantially modified by A_1AR KO. In addition, intrinsic A_1AR-dependent protection during ischemia/reperfusion in vivo may involve actions of adenosine generated within neutrophils, platelets, and other blood-borne cells. Thus, this extracardiac component will be absent in the present model, potentially leading to an underestimation of the normal extent of A_1AR activation by endogenously generated adenosine.

In summary, the current analysis of the effects of A_1AR KO supports an important function of A_1ARs in dictating intrinsic myocardial resistance to ischemia/reperfusion. Effects of A_1AR deletion suggest these receptors normally play a role in enhancing postischemic contractility and limiting cell death, with little effect on abnormalities in diastolic

function. Finally, reduced yet significant protection via the nonselective agonist 2-chloroadenosine in A_1AR KO hearts implicates a significant cardioprotective response mediated via adenosine receptors additional to the A_1AR.

Acknowledgments

This work was supported by NIH AI-43572 (to M.R.B.) and National Health and Medical Research Council of Australia grant 231416 (to J.P.H.). J.P.H. was also the recipient of a career development fellowship from the National Heart Foundation of Australia. We are extremely grateful for the provision of mice overexpressing A_1ARs by Prof Paul Matherne and for the excellent technical assistance of Kirsten Holmgren.

References

1. Sommerschild HT, Kirkeboen KA. Adenosine and cardioprotection during ischaemia and reperfusion: an overview. *Acta Anaesthesiol Scand*. 2000;44:1038–1055.
2. Headrick JP, Hack B, Ashton KJ. Acute adenosinergic cardioprotection in ischemic-reperfused hearts. *Am J Physiol Heart Circ Physiol*. 2003;285: H1797–H1818.
3. . Lasley RD, Rhee JW, Van Wylen DG, Mentzer RM Jr. Adenosine A_1 receptor mediated protection of the globally ischemic isolated rat heart. *J Mol Cell Cardiol*. 1990;22:39–47.
4. Zhao ZQ, Nakanishi K, McGee DS, Tan P, Vinten-Johansen J. A_1 receptor mediated myocardial infarct size reduction by endogenous adenosine is exerted primarily during ischaemia. *Cardiovasc Res*. 1994;28:270–279.
5. Peart J, Headrick JP. Intrinsic activation of A_1 adenosine receptors during ischemia and reperfusion improves ischemic tolerance. *Am J Physiol Heart Circ Physiol*. 2000;279:H2166–H2175.
6. Zhao ZQ, McGee S, Nakanishi K, Toombs CF, Johnston WE, Ashar MS, Vinten-Johansen J. Receptor-mediated cardioprotective effects of endogenous adenosine are exerted primarily during reperfusion after coronary occlusion in the rabbit. *Circulation*. 1993;88:709–719.
7. Vinten-Johansen J, Thourani VH, Ronson RS, Jordan JE, Zhao ZQ, Nakamura M, Velez D, Guyton RA. Broad-spectrum cardioprotection with adenosine. *Ann Thorac Surg*. 1999;68:1942–1948.
8. Toombs CF, McGee S, Johnston WE, Vinten-Johansen J. Myocardial protective effects of adenosine. Infarct size reduction with pretreatment and continued receptor stimulation during ischemia. *Circulation*. 1992;86: 986–994.
9. Rynning SE, Brunvand H, Birkeland S, Hexeberg E, and Grong K. Endogenous adenosine attenuates myocardial stunning by antiadrenergic effects exerted during ischemia and not during reperfusion. *J Cardiovasc Pharmacol*. 1995;25:432–439.
10. Finegan BA, Lopaschuk GD, Gandhi M, Clanachan AS. Inhibition of glycolysis and enhanced mechanical function of working rat hearts as a result of adenosine A_1 receptor stimulation during reperfusion following ischaemia. *Br J Pharmacol*. 1996;118:355–363.
11. Seligmann C, Kupatt C, Becker BF Zahler S, Beblo S. Adenosine endogenously released during early reperfusion mitigates postischemic myocardial dysfunction by inhibiting platelet adhesion. *J Cardiovasc Pharmacol*. 1998;32:156–163.
12. Thornton JD, Thornton CS, Downey JM. Effect of adenosine receptor blockade: preventing protective preconditioning depends on time of initiation. *Am J Physiol*. 1993;265:H504–H508.
13. Kitakaze M, Minamino T, Funaya H, Node K, Shinozaki Y, Mori H, Hori M. Vesnarinone limits infarct size via adenosine-dependent mechanisms in the canine heart. *Circulation*. 1997;95:2108–2114.
14. Domenech RJ, Macho P, Velez D, Sanchez G, Liu X, Dhalla N. Tachycardia preconditions infarct size in dogs: role of adenosine and protein kinase C. *Circulation*. 1998;97:786–794.
15. Auchampach JA, Jin X, Moore J, Wan TC, Kreckler LM, Ge ZD, Narayanan J, Whalley E, Kiesman W, Ticho B, Smits G, Gross GJ. Comparison of three different A_1 adenosine receptor antagonists on infarct size and multiple cycle ischemic preconditioning in anesthetized dogs. *J Pharmacol Exp Ther*. 2004;308:846–856.
16. Heller LJ, Dole WP, Mohrman DE. Adenosine receptor blockade enhances isoproterenol-induced increases in cardiac interstitial adenosine. *J Mol Cell Cardiol*. 1991;23:887–898.
17. Headrick JP, Ely SW, Matherne GP, Berne RM. Myocardial adenosine, flow, and metabolism during adenosine antagonism and adrenergic stimulation. *Am J Physiol*. 1993;264:H61–H70.
18. Matherne GP, Berr SS, Headrick JP. Integration of vascular, contractile and metabolic responses to hypoxia: effects of maturation and adenosine. *Am J Physiol*. 1996;270:R895–R905.
19. James JF, Hewett TE, Robbins J. Cardiac physiology in transgenic mice. *Circ Res*. 1998;82:407–415.
20. Ehmke H. Mouse gene targeting in cardiovascular physiology. *Am J Physiol Regul Integr Comp Physiol*. 2003;284:R28–R30.
21. Sun D, Samuelson LC, Yang T, Huang Y, Paliege A, Saunders T, Briggs J, Schnermann J. Mediation of tubuloglomerular feedback by adenosine: evidence from mice lacking adenosine A_1 receptors. *Proc Natl Acad Sci U S A*. 2001;98:9983–9988.
22. Matherne GP, Linden J, Byford AM, Gauthier NS, Headrick JP. Transgenic A_1 adenosine receptor overexpression increases myocardial resistance to ischemia. *Proc Natl Acad Sci U S A*. 1997;94:6541–6546.
23. Headrick JP, Peart J, Hack B, Flood A, Matherne GP. Functional properties and responses to ischaemia-reperfusion in Langendorff perfused mouse heart. *Exp Physiol*. 2001;86:703–716.
24. Headrick JP, Gauthier NS, Morrison RR, Matherne GP. Chronotropic and vasodilatory responses to adenosine and isoproterenol in mouse heart: effects of adenosine A_1 receptor overexpression. *Clin Exp Pharmacol Physiol*. 2000;27:185–190.
25. Flood A, Headrick JP. Functional characterization of coronary vascular adenosine receptors in the mouse. *Br J Pharmacol*. 2001;133:1063–1072.
26. Peart J, Willems L, Headrick JP. Receptor and non-receptor-dependent mechanisms of cardioprotection with adenosine. *Am J Physiol Heart Circ Physiol*. 2003;284:H519–H527.
27. Collis MG, Brown CM. Adenosine relaxes the aorta by interacting with an A2 receptor and an intracellular site. *Eur J Pharmacol*. 1983;96:61–69.
28. Anand-Srivastava MB, Franks DJ. Stimulation of adenylate cyclase by adenosine and other agonists in mesenteric artery smooth muscle cells in culture. *Life Sci*. 1985;37:857–867.
29. Alexander SP, Losinski A, Kendall DA, Hill SJ. A comparison of A_2 adenosine receptor-induced cyclic AMP generation in cerebral cortex and relaxation of pre-contracted aorta. *Br J Pharmacol*. 1994;111:185–190.
30. Peakman MC, Hill SJ. Adenosine A_{2B}-receptor-mediated cyclic AMP accumulation in primary rat astrocytes. *Br J Pharmacol*. 1994;111:191–198.
31. Talukder MA, Morrison RR, Mustafa SJ. Comparison of the vascular effects of adenosine in isolated mouse heart and aorta. *Am J Physiol Heart Circ Physiol*. 2002;282:H49–H57.
32. Neely CF, DiPierro FV, Kong M, Greelish JP, Gardner TJ. A_1 adenosine receptor antagonists block ischemia-reperfusion injury of the heart. *Circulation*. 1996;94(suppl):II376–II380.
33. Forman MB, Vitola JV, Velasco CE, Murray JJ, Dubey RK, Jackson EK. Sustained reduction in myocardial reperfusion injury with an adenosine receptor antagonist: possible role of the neutrophil chemoattractant response. *J Pharmacol Exp Ther*. 2000;292:929–938.
34. Schreieck J, Richardt G. Endogenous adenosine reduces the occurrence of ischemia-induced ventricular fibrillation in rat heart. *J Mol Cell Cardiol*. 1999;31:123–134.
35. Arora RC, Armour JA. Adenosine A_1 receptor activation reduces myocardial reperfusion effects on intrinsic cardiac nervous system. *Am J Physiol Regul Integr Comp Physiol*. 2003;284:R1314–R1321.
36. Peart J, Flood A, Linden J, Matherne GP, Headrick JP. Adenosine-mediated cardioprotection in ischemic-reperfused mouse heart. *J Cardiovasc Pharmacol*. 2002;39:117–129.
37. Ikeda U, Kurosaki K, Shimpo M, Okada K, Saito T, Shimada K. Adenosine stimulates nitric oxide synthesis in rat cardiac myocytes. *Am J Physiol*. 1997;273:H59–H65.
38. Dubey RK, Gillespie DG, Jackson EK. Adenosine inhibits collagen and protein synthesis in cardiac fibroblasts: role of A_{2B} receptors. *Hypertension*. 1998;31:943–948.
39. Dubey RK, Gillespie DG, Zacharia LC, Mi Z, Jackson EK. A_{2b} receptors mediate the anti-mitogenic effects of adenosine in cardiac fibroblasts. *Hypertension*. 2001;37:716–721.
40. Liao Y, Takashima S, Asano Y, Asakura M, Ogai A, Shintani Y, Minamino T, Asanuma H, Sanada S, Kim J, Ogita H, Tomoike H, Hori M, Kitakaze M. Activation of adenosine A_1 receptor attenuates cardiac hypertrophy and prevents heart failure in murine left ventricular pressure-overload model. *Circ Res*. 2003;93:759–766.
41. Godecke A, Flogel U, Zanger K, Ding Z, Hirchenhain J, Decking UK, Schrader J. Disruption of myoglobin in mice induces multiple compensatory mechanisms. *Proc Natl Acad Sci USA*. 1999;96:10495–10500.
42. Warth R, Barhanin J. The multifaceted phenotype of the knockout mouse for the KCNE1 potassium channel gene. *Am J Physiol Regul Integr Comp Physiol*. 2002;282:R639–R648.