Adenosine A_3 Receptor Deficiency Exerts Unanticipated Protective Effects on the Pressure-Overloaded Left Ventricle

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Background—Endogenous adenosine can protect the overloaded heart against the development of hypertrophy and heart failure, but the contribution of A_1 receptors (A_1R) and A_3 receptors (A_3R) is not known.

Methods and Results—To test the hypothesis that A_1R and A_3R can protect the heart against systolic overload, we exposed A_3R gene–deficient (A_3R knockout [KO]) mice and A_1R KO mice to transverse aortic constriction (TAC). Contrary to our hypothesis, A_3R KO attenuated 5-week TAC-induced left ventricular hypertrophy (ratio of ventricular mass/body weight increased to 7.6±0.3 mg/g in wild-type mice compared with 6.3±0.4 mg/g in KO mice), fibrosis, and dysfunction (left ventricular ejection fraction decreased to 43±2.5% and 55±4.2% in wild-type and KO mice, respectively). A_3R KO also attenuated the TAC-induced increases of myocardial atrial natriuretic peptide and the oxidative stress markers 3′-nitrotyrosine and 4-hydroxynonenal. In contrast, A_1R KO increased TAC-induced mortality but did not alter ventricular hypertrophy or dysfunction compared with wild-type mice. In mice in which extracellular adenosine production was impaired by CD73 KO, TAC caused greater hypertrophy and dysfunction and increased myocardial 3′-nitrotyrosine. In neonatal rat cardiomyocytes induced to hypertrophy with phenylephrine, the adenosine analogue 2-chloroadenosine reduced cell area, protein synthesis, atrial natriuretic peptide, and 3′-nitrotyrosine. Antagonism of A_3R significantly potentiated the antihypertrophic effects of 2-chloroadenosine.

Conclusions—Adenosine exerts protective effects on the overloaded heart, but the A_3R acts counter to the protective effect of adenosine. The data suggest that selective attenuation of A_3R activity might be a novel approach to treat pressure overload–induced left ventricular hypertrophy and dysfunction. **(*Circulation*. 2008;118:1713-1721.)**

Key Words: adenosine ■ free radicals ■ heart failure ■ hypertrophy ■ oxidative stress

Recently, we demonstrated that genetic deletion of CD73 (an ectonucleotidase that produces extracellular adenosine) exacerbated myocardial hypertrophy and heart failure resulting from left ventricular (LV) pressure overload produced by transverse aortic constriction (TAC),[1] suggesting that endogenous extracellular adenosine can protect against maladaptive hypertrophy. Adenosine exerts multiple functions through activation of individual adenosine receptor subtypes.[2–5] A_1 receptors (A_1R) and A_3 receptors (A_3R) are expressed in cardiomyocytes, and a substantial body of evidence indicates that adenosine can protect the heart during and after an ischemic insult.[6,7] Liao et al[8] demonstrated that the adenosine analogue 2-chloroadenosine (CADO) also attenuated pressure overload–induced LV hypertrophy through activation of the A_1R. Similar to A_1R, the A_3R are Gi protein–coupled receptors that have been shown to activate similar downstream signaling pathways.[9,10] A_3R activation has also been reported to protect the heart against ischemic[11,12] and doxorubicin-induced damage.[13] On the other hand, transgenic overexpression of the A_1R[14] or A_3R[15,16] promotes cardiac dilation and dysfunction, suggesting that these receptors may also exert adverse effects on cardiac function. Although we and others have demonstrated that adenosine protects against LV hypertrophy and maladaptive remodeling during pressure overload, the distinct contributions of the A_1R and A_3R to this protective effect are not known. Here we examined the effect of A_1R knockout (KO) mice and A_3R KO mice on TAC-induced ventricular hypertrophy in vivo and extended our examination to the roles of A_1R and A_3R in modulating hypertrophy in cultured neonatal rat cardiomyocytes, free from hemodynamic and neurohormonal factors that can influence the in vivo heart.

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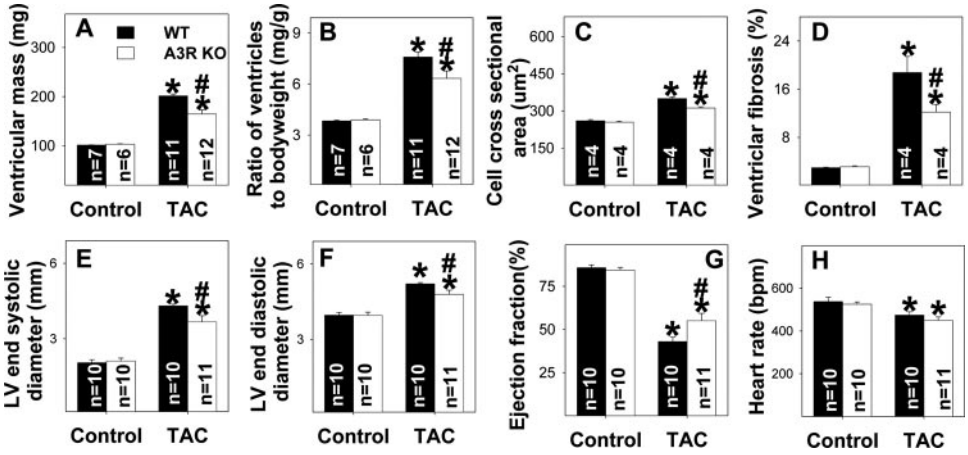

Figure 1. A_3R KO significantly attenuates chronic moderate TAC-induced ventricular hypertrophy (A, B), cardiac myocyte hypertrophy (C), ventricular fibrosis (D), increased LV end-systolic diameter (E), ventricular dilation (F), and decreased ejection fraction (G). Heart rate was not different between WT and A_3R KO mice under corresponding conditions (H). *$P<0.05$ compared with the corresponding control; #$P<0.05$ compared with WT-TAC.

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Methods

Mice

Male C57BL/6 (Taconic, Germantown, NY) body weight–matched A_3R KO mice[2] (crossed back to Taconic C57BL/6 mice at least 16 times), 8 to 12 weeks old, were used for TAC or control. A_1R KO mice (129 background) and their control wild-type (WT) mice were generated as described previously.[17] The CD73 KO strain and control WT mice were generated as described previously.[1,18] This study was approved by the Institutional Animal Care and Use Committee of University of Minnesota.

Minimally Invasive TAC Procedure

TAC of moderate (with the use of a 26-gauge needle to calibrate the degree of constriction) or severe (with the use of a 27-gauge needle) degree was created as described previously.[19] To ensure that similar pressure overload was produced in the KO and WT mice, the TAC procedure was performed on KO and corresponding WT mice on the same day by the same surgeon who was blinded regarding the genotype of the mice.

Echocardiography

Mice were anesthetized with 1.5% isoflurane. Echocardiographic images were obtained with a Visualsonics Veve 770 system as described previously.[19,20]

Sample Collection and Western Blots

Myocardial samples for protein analysis were flash-frozen in liquid nitrogen, weighed on an electronic balance, and stored in liquid nitrogen until transfer into a −80°C freezer where they were maintained until analysis. Samples for histological analysis were fixed in formaldehyde. Protein expression was analyzed by Western blots as described previously[19] with the use of antibodies against atrial natriuretic peptide (ANP) (Peninsula Biolabs), 3-nitrotyrosine, 4-hydroxynonenal (Millipore), cyclooxygenase-2 (COX-2), c-Jun N-terminal kinase (JNK), phosphorylated JNK (p-$JNK^{Thr183/Tyr185}$) (Santa Cruz Biotechnology, Santa Cruz, Calif), endothelial nitric oxide synthase (eNOS) (Transduction Laboratories), extracellular signal-regulated kinase (ERK), and p-$ERK^{Thr202/Tyr204}$, p-Akt^{Ser473}, and p-GSK-3$\beta^{Ser21/9}$ (Cell Signaling Technology, Danvers, Mass).

Histological Staining and Measurement of Fibrosis

Tissue sections (6 μm) from the central portion of the LV were stained with Sirius red (Sigma, St Louis, Mo) for fibrosis[19] and FITC-conjugated wheat germ agglutinin (AF488, Invitrogen, Carlsbad, Calif) to evaluate myocyte size. For mean myocyte size, the cross-sectional areas of at least 120 cells per sample and at least 4 samples per group were averaged.

Neonatal Rat Cardiomyocyte Isolation and Culture

Neonatal rat cardiomyocytes were isolated from 2-day-old Sprague-Dawley rats as described previously.[21] To induce hypertrophy, cells were treated with 50 μmol/L phenylephrine for 48 hours. The stable adenosine analogue CADO (5 μmol/L) was used to activate adenosine receptors (the affinities of CADO at rat A_1R and A_3R are 9.3 and 1890 nmol/L, respectively).[22] The selective inhibitors DPCPX and MRS1191 were used at 5 μmol/L to block A_1R and A_3R, respectively. It has been reported that 5 μmol/L MRS1911 selectively inhibits A_3R activation without affecting A_1R-dependent responses.[23] After treatment, cells were fixed with 4% paraformaldehyde and stained with rhodamine-conjugated phalloidin (5 U/mL in PBS, Invitrogen), DAPI, ANP (Peninsula Biolabs), and 3′-nitrotyrosine (Millipore), followed by Alexa Fluor 488– or Alexa Fluor 633–labeled secondary antibodies (Invitrogen). Protein synthesis was measured over 48 hours of treatment in 96 well plates by H^3-phenylalanine incorporation.

Data Analysis

All values are expressed as mean±SE. Kaplan-Meier survival analysis was performed with SigmaStat with the use of the Gehan-Breslow test. Two-way ANOVA was used to test for differences among treatment groups, followed by pairwise multiple comparisons of the Tukey test. Statistical significance was defined as $P<0.05$.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

A_3R KO Attenuated LV Hypertrophy and Dysfunction Produced by Moderate Pressure Overload

LV structure and function were not different between A_3R KO and WT mice under control conditions (Figure 1A to 1G), and histological staining of LV tissue showed no difference in cardiac myocyte size or relative fibrosis between A_3R KO and

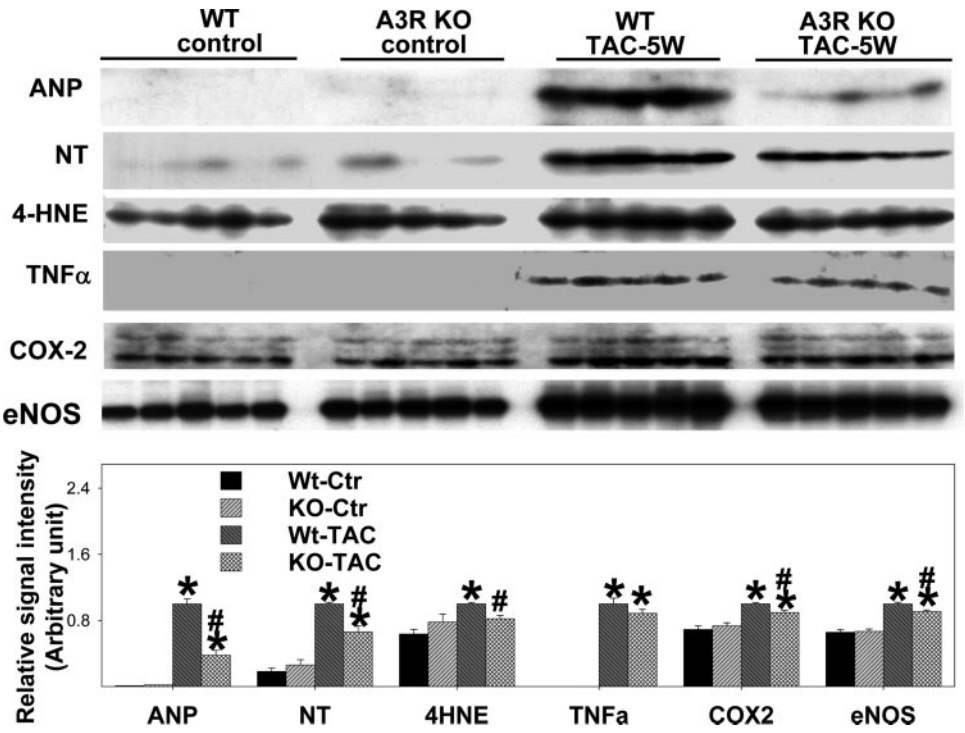

Figure 2. A_3R KO significantly attenuates moderate TAC-induced increases of ventricular ANP, nitrotyrosine (NT), 4-hydroxynonenal (4-HNE), and COX-2. TAC caused significant increases of ventricular TNF-α in both A_3R KO and WT mice. A_3R KO tended to decrease TNF-α after TAC, but this difference was not significant ($P=0.10$). Data are normalized to WT-TAC. *$P<0.05$ compared with the corresponding control (Ctr); #$P<0.05$ compared with WT-TAC. 5W indicates 5 weeks.

WT mice (Figure 1C, 1D). After 5 weeks of moderate TAC (with the use of a 26-gauge needle to calibrate the degree of TAC), ventricular weight and the ratio of ventricular weight to body weight were significantly lower in the A_3R KO mice than in WT mice (Figure 1A, 1B), indicating that loss of A_3R attenuated the TAC-induced myocardial hypertrophy. Histological staining showed that the A_3R KO hearts had significantly less TAC-induced increases of LV fibrosis and myocyte hypertrophy (Figure 1C, 1D; Figure I in the online-only Data Supplement). Thus, the lesser hypertrophy in the A_3R KO hearts after TAC resulted from both reduced myocyte size and decreased fibrosis. The TAC-induced mortality was not different between A_3R KO and WT mice (Figure II in the online-only Data Supplement).

Echocardiographic imaging of the heart 5 weeks after TAC demonstrated significant increases of LV end-systolic diameter and LV end-diastolic diameter in both A_3R KO and WT mice in comparison with mice of similar body weight without TAC (Figure 1E, 1F). However, TAC caused significantly less LV dysfunction in the A_3R KO mice, as demonstrated by a higher ejection fraction and a smaller LV end-systolic diameter (Figure 1E, 1G). Myocardial ANP (biochemical marker for LV dysfunction) was increased in both WT and A_3R KO mice 5 weeks after TAC, but this increase was significantly less in the A_3R KO mice (Figure 2). These data indicate that the presence of the A_3R exacerbated the LV hypertrophy and dysfunction in response to TAC.

Because recent studies using A_3R KO mice demonstrated that attenuation of A_3R signaling reduces the inflammatory response[2,24,25] in several pathological conditions, we examined myocardial tumor necrosis factor (TNF)-α and COX-2. TAC resulted in significant increases of TNF-α and COX-2 in the hearts of both WT mice and A_3R KO mice (Figure 2). However, the increase of COX-2 was significantly less in A_3R KO mice than in WT mice. The TAC-induced increase of TNF-α tended to be less in the A_3R KO mice ($P=0.10$). In addition, hearts from WT mice had higher levels of 3′-nitrotyrosine and 4-hydroxynonenal after TAC than did A_3R KO hearts, implying that the A_3R KO mice had lower levels of oxidative stress (Figure 2). eNOS uncoupling can be a source for increased oxidative stress,[19,26] and we have found that the increase of myocardial eNOS protein after TAC was related to the degree of LV dysfunction.[19] Consistent with our previous report, myocardial eNOS protein was significantly increased in the WT mice after TAC, and this response was attenuated in the A_3R KO mice (Figure 2).

Activation of mitogen-activated protein kinases (MAPKs) and the phosphoinositide 3–kinase (PI3K) signaling pathway is often associated with increased oxidative stress[27,28] and the development of LV hypertrophy or heart failure.[29–31] To examine signaling pathways related to the protective effect observed in the A_3R KO mice after TAC, total JNK and phosphorylated JNK, ERK, Akt, and GSK-3β were determined (Figure 3). Under control conditions A_3R KO had no effect on the myocardial content of total or phosphorylated ERK, JNK, Akt, or GSK-3β. TAC caused significant increases of p-ERK$^{Thr202/Tyr204}$ and p-JNK$^{Thr183/Tyr185}$ and the ratio to their total proteins in both KO and WT mice. However, A_3R KO significantly attenuated the TAC-induced increases of p-ERK$^{Thr202/Tyr204}$ and p-JNK$^{Thr183/Tyr185}$ (Figure 3), indicating that A_3R KO attenuated the TAC-induced activation of the

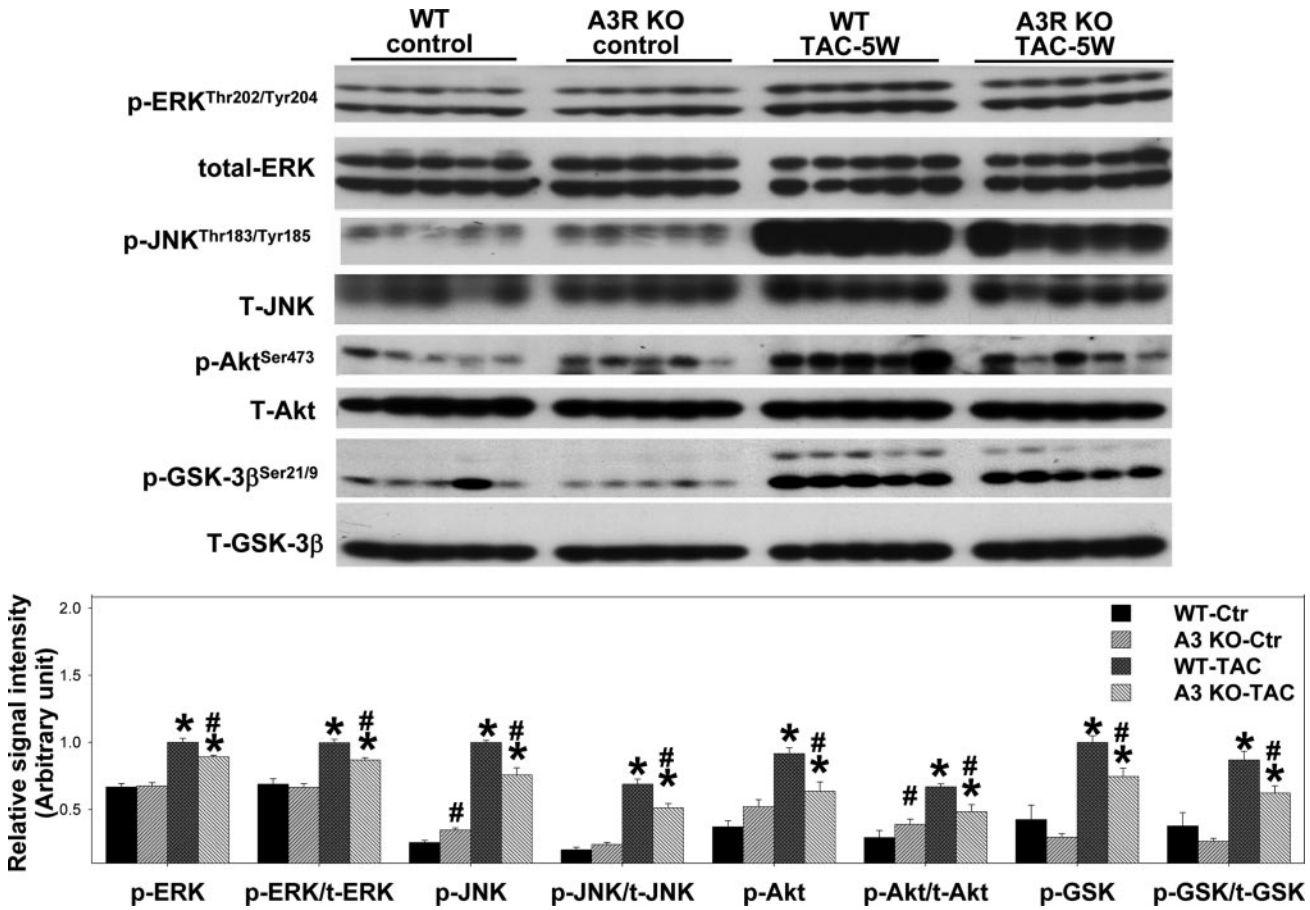

Figure 3. Ventricular p-ERK$^{Thr202/Tyr204}$, total-ERK, p-JNK$^{Thr183/Tyr185}$, total-JNK, p-Akt^{Ser473}, total-Akt, p-GSK-3$\beta^{Ser21/9}$, and total GSK-3β in A_3R KO and WT mice under control (Ctr) conditions and 5 weeks (5W) after moderate TAC. Data are normalized to WT-TAC. *$P<0.05$ compared with the corresponding control; #$P<0.05$ compared with WT-TAC.

MAPK signaling pathways. In addition, the TAC-induced increases of p-Akt^{Ser473} and p-GSK-3$\beta^{Ser21/9}$ were significantly attenuated in the A_3R KO mice, suggesting decreased signaling through the PI3K-Akt pathway.

A_1R KO Did Not Influence Ventricular Hypertrophy Produced by TAC but Exacerbated Mortality After Severe TAC

Although previous studies have demonstrated that either the adenosine analogue CADO[8,32] or endogenous adenosine[1] can protect the heart from pressure overload–induced LV remodeling, the specific contribution of A_1R activation has been controversial.[8,32] To determine whether A_1R KO might exacerbate the degree of hypertrophy and myocardial dysfunction later during systolic overload, we studied mice 4 weeks after moderate TAC (using a 26-gauge needle). This moderate systolic overload caused similar increases in the ratio of ventricular mass to body weight, LV end-diastolic diameter, LV end-systolic diameter, and LV wall thickness in A_1R KO and WT mice (Figure 4A to 4F). Moderate TAC of 4-week duration also caused similar decreases of LV ejection fraction in the 2 groups (Figure 4C). Although mortality tended to be higher in the A_1R KO group during the 4 weeks after moderate TAC (5 of 17 mice died) compared with WT mice (2 of 17 WT mice died), this difference was not significant (Figure 4H).

Because mice with severe LV dysfunction are more likely to die after TAC, the relatively higher TAC-induced mortality in the A_1R KO mice than in the WT mice might potentially have influenced ventricular weights of the surviving mice, that is, if the sicker A_1R KO mice died early after TAC, the residual surviving animals might underestimate the overall response to systolic overload. Because the TAC-induced death occurred predominantly during the first 2 days after TAC, we determined the degree of hypertrophy 2 days after severe TAC when comparable numbers of A_1R KO and WT mice survived. Compared with sham surgery, at 2 days after severe TAC the ratio of ventricular weight to body weight was similarly increased in WT (21±2.5%) and A_1R KO mice (22±3.5%), indicating that A_1R KO did not alter the acute hypertrophic response to severe pressure overload (Figure III in the online-only Data Supplement). Taken together, the data indicate that A_1R KO had no significant influence on TAC-induced ventricular hypertrophy or dysfunction.

Because the A_1R KO mice tended to have a higher mortality than their WT controls in this initial study, we subsequently examined whether this trend toward a higher mortality would be statistically significant when a more severe degree of systolic overload (using a 27-gauge needle) was applied. When TAC of severe degree was applied, the excess mortality in the A_1R KO animals did in fact become

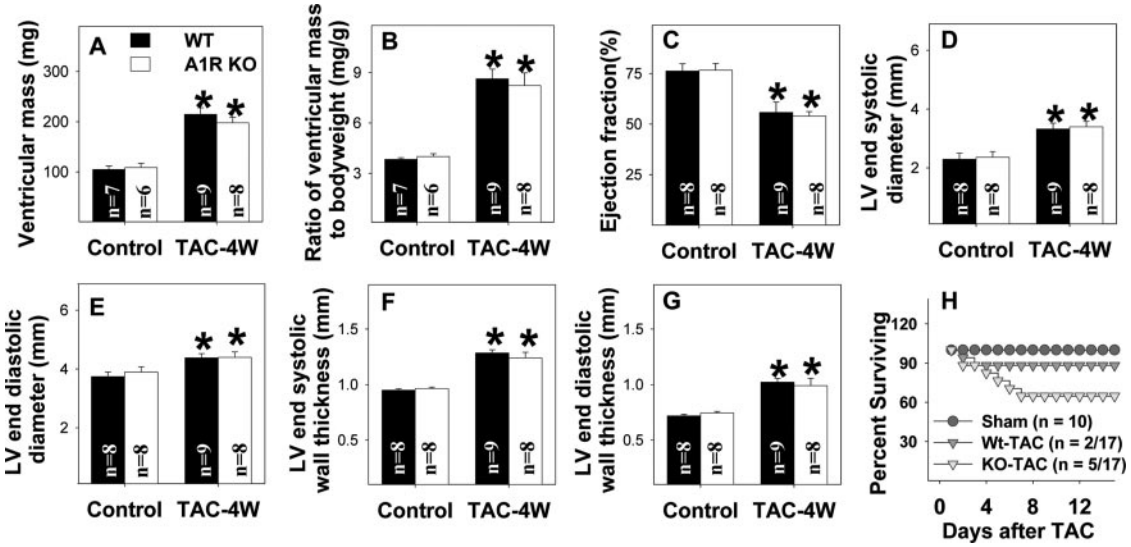

Figure 4. A_1R KO had no significant effect on the increase of ventricular mass (A), the ratio of ventricular mass to body weight (B), decrease of LV ejection fraction (C), increase of LV diastolic diameter (D, E), or LV wall thickness (F, G) produced by moderate TAC of 4-week (4W) duration. A_1R KO mice tended to have a higher mortality during 4 weeks after moderate TAC, but this difference was not significant (H). *$P<0.05$ compared with the corresponding control.

significant (Figure IV in the online-only Data Supplement). To understand the nature of the TAC-induced increase in mortality in the A_1R KO mice, ECG telemetry was performed in additional A_1R KO and WT mice. The results demonstrated that animals destined to die generally developed progressive sinus bradycardia with giant P waves that progressed to high-grade atrioventricular block with further bradycardia and death (Figure V in the online-only Data Supplement). Again, the degree of hypertrophy was not different between the surviving A_1R KO and WT mice after severe TAC.

Taken together, the data indicate that A_1R KO had no significant influence on TAC-induced ventricular hypertrophy or dysfunction but resulted in significantly greater mortality in mice subjected to severe TAC.

CD73 KO Exacerbated Oxidative Stress and Hypertrophy Produced by Moderate Pressure Overload

The reduction of extracellular adenosine production produced by CD73 KO significantly exacerbated the hypertrophy (Figure 5A, 5B), fibrosis (Figure 5D, 5E), myocyte

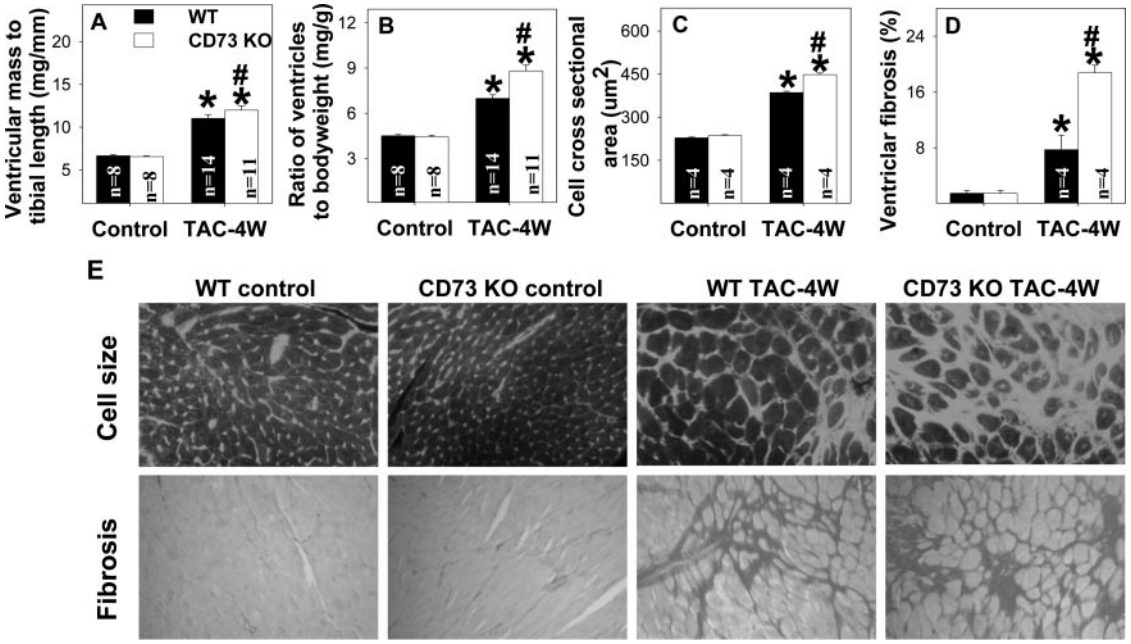

Figure 5. Disrupting extracellular adenosine production by CD73 KO exacerbated ventricular hypertrophy (A, B), cardiomyocyte hypertrophy (C, E), and ventricular fibrosis (D, E) produced by 4 weeks (4W) of moderate TAC. *$P<0.05$ compared with the corresponding control; #$P<0.05$ compared with WT-TAC.

hypertrophy (Figure 5C, 5E), LV dilation, and decrease of LV ejection fraction produced by moderate TAC of 4-week duration (Figure VI in the online-only Data Supplement). CD73 KO also exacerbated the TAC-induced increases of ventricular ANP and TNF-α (Figure VII in the online-only Data Supplement). In addition, CD73 KO exacerbated the TAC-induced increase of myocardial 3-nitrotyrosine (Figure VII in the online-only Data Supplement), indicating increased oxidative stress. To validate these findings, we examined the ability of the adenosine analogue CADO to rescue the increased ventricular hypertrophy produced by TAC in the CD73 KO mice. We found that CADO attenuated the myocardial hypertrophy produced by moderate TAC of 2-week duration in the CD73 KO mice (Figure VIII in the online-only Data Supplement).

The A_3R Antagonist MRS1911 Potentiates the Antihypertrophic Effect of CADO in Neonatal Cardiomyocytes

Understanding the role of A_1R and A_3R in the response of the cardiomyocytes to systolic overload in vivo may be complicated by effects of adenosine on blood flow, neurohormonal responses, and inflammatory or paracrine responses. Therefore, we sought to determine the role of A_1R and A_3R in isolated cardiomyocytes in the setting of saturating levels of the nonselective adenosine analogue CADO. We previously demonstrated that CADO or adenosine reduced phenylephrine-induced hypertrophy and ANP expression in neonatal cardiomyocytes.[1] To examine the role of A_1R and A_3R in mediating antihypertrophic effects of CADO, we treated cells with 50 μmol/L phenylephrine and 5 μmol/L CADO in the presence or absence of selective A_1R and A_3R antagonists and then measured cell area, protein synthesis, and the oxidative stress marker 3′-nitrotyrosine. Phenylephrine increased cardiomyocyte protein synthesis (Figure 6A, 6E), cell area (Figure 6B), ANP expression (Figure 6C), and 3′-nitrotyrosine production (Figure 6D, 6E) over 48 hours of treatment, whereas CADO significantly attenuated the phenylephrine-induced increases in these variables. Blocking A_1R with DPCPX slightly reversed the CADO-induced reductions of cell area (Figure 6B) and ANP levels (Figure 6C) in the phenylephrine-treated cells. Inhibition of A_1R caused a substantial increase in 3′-nitrotyrosine (Figure 6D), suggesting a role for A_1R in modulating oxidative stress in the hypertrophying myocytes. Inhibition of the A_3R with MRS1191 reduced protein synthesis, ANP expression, and 3′-nitrotyrosine production beyond the reduction caused by CADO alone (Figure 6). The reduction in 3′-nitrotyrosine by MRS1191 was confirmed by Western blot analysis (data not shown). The reduction in hypertrophy by the A_3R antagonist was associated with reduced sustained activation of the MAPKs ERK and JNK (Figure IX in the online-only Data Supplement). These results suggest that A_3R contributes to increased oxidative stress, higher sustained activation of ERK and JNK, and an increased hypertrophic response to phenylephrine.

Discussion

To the best of our knowledge, this is the first report assessing the effect of A_1R KO and A_3R KO on chronic pressure overload–induced ventricular hypertrophy and contractile function. The major new finding is that A_3R KO attenuated TAC-induced LV hypertrophy, fibrosis, oxidative stress, and dysfunction. Because adenosine has been reported to be cardioprotective in the setting of chronic pressure overload,[1,8] the finding that disruption of A_3R attenuated the TAC-induced ventricular hypertrophy and dysfunction is unexpected and intriguing. Our finding that the A_3R antagonist MRS1191 augmented the antihypertrophic effect of CADO in phenylephrine-treated isolated cardiomyocytes is in agreement with the concept that selective A_3R blockade can enhance the beneficial effect of adenosine. These results support the novel concept that the adenosine A_3R exerts adverse effects in the pressure-overloaded heart and suggest that A_3R blockade may have potential to protect the heart against pressure overload–induced oxidative stress, LV remodeling, and contractile dysfunction.

The effect of the A_1R on LV remodeling is controversial. CADO has been reported to attenuate TAC-induced LV hypertrophy in mice through A_1R activation.[8] Furthermore, an A_1R antagonist was reported to attenuate the antihypertrophic effect of CADO in vitro.[33] However, a subsequent study from the same group reported that A_1R blockade had no effect on infarct-induced cardiomyocyte hypertrophy or LV remodeling in rats.[32] We have observed that moderate A_1R overexpression in mice failed to exert a beneficial effect on myocardial infarct–induced ventricular remodeling (Y. Chen, PhD, unpublished data, 2007). It is unclear why A_1R blockade would attenuate the antihypertrophic effect of CADO, whereas A_1R KO had no effect on TAC-induced hypertrophy. Nevertheless, the present finding that A_1R KO exacerbated the death rate in mice exposed to severe TAC demonstrates that activation of the A_1R can exert some degree of cardioprotection in the pressure-overloaded heart.

Although no previous reports have directly examined the effect of A_3R KO on systolic overload–induced ventricular remodeling, there is evidence that A_3R signaling can affect cardiac structure and function. Thus, transgenic mice with cardiac-specific overexpression of A_3R developed a dilated cardiomyopathy characterized by increased ventricular mass, LV dilation, expression of biomarkers of hypertrophy, bradycardia, and systolic dysfunction,[15,16] suggesting that chronically augmented A_3R signaling in the heart is detrimental.

The MAPK and PI3K signaling pathways are often activated in response to extracellular stresses such as inflammation or oxidative stress[28] and have been shown to contribute to cardiac hypertrophy and heart failure. The increased myocardial oxidative stress after TAC in the present study, associated with activating phosphorylations of p-Akt^{Ser473}, p-ERK$^{Thr202/Tyr204}$, and p-JNK$^{Thr183/Tyr185}$ and inactivating phosphorylation of GSK3$\beta^{Ser21/9}$, is consistent with previous reports.[19,34] The decreases in TAC-induced oxidative stress, p-ERK$^{Thr202/Tyr204}$, p-JNK$^{Thr183/Tyr185}$, p-Akt^{Ser473}, and p-GSK3$\beta^{Ser21/9}$ as a result of A_3R KO likely contributed to the lesser degrees of fibrosis and cardiac myocyte hypertrophy in the A_3R KO mice. TAC-induced ventricular hypertrophy is associated with increased eNOS expression[19] and eNOS uncoupling,[26] so that attenuation of the increase of eNOS in the A_3R KO

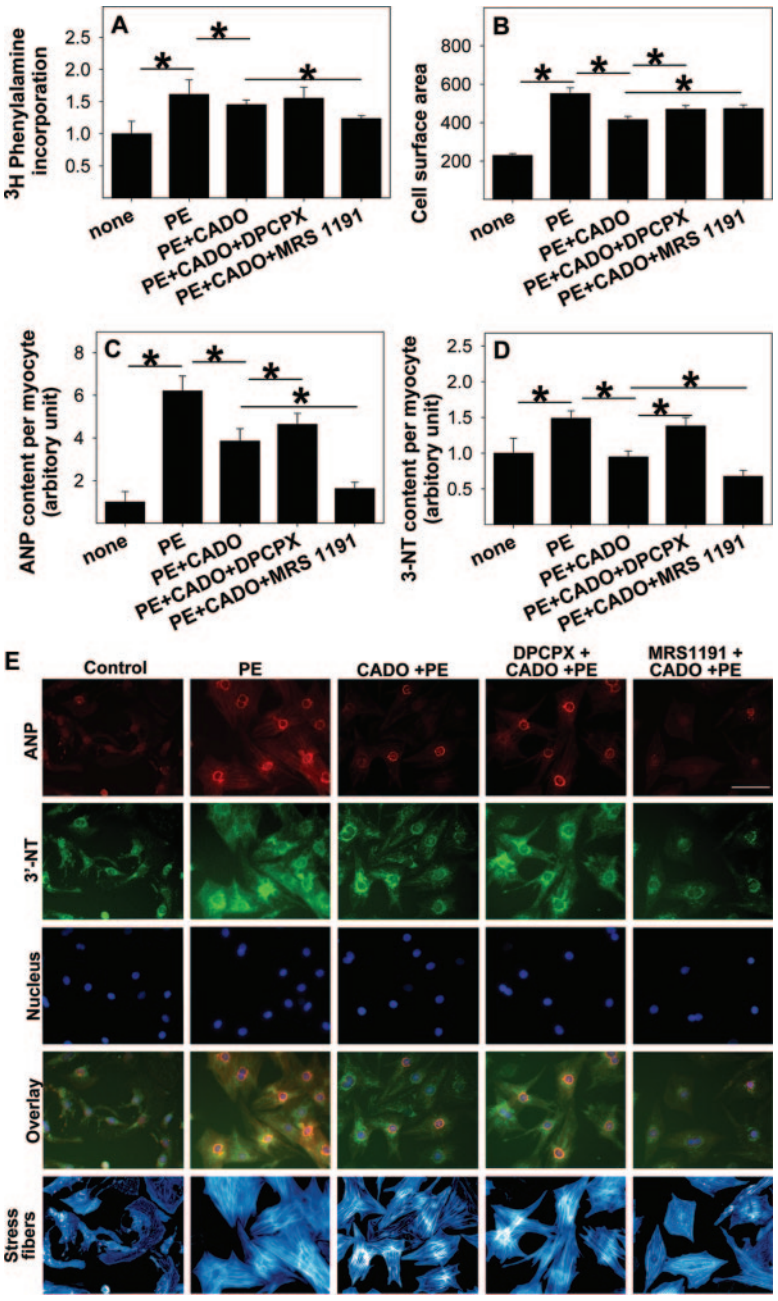

Figure 6. Addition of the A_3R antagonist MRS1191 to CADO-treated cells further attenuated the phenylephrine (PE)-induced increases of protein synthesis (A, E), and the expression of ANP (C, E) and 3′-nitrotyrosine (NT) (D, E) in cultured rat cardiomyocytes. MRS1191 did not further decrease the average cell area (B, E). *$P<0.05$ between the indicated groups.

mice after TAC may have contributed to the decreased oxidative stress in this strain.

The effect of A_3R on activation of myocardial PI3K/Akt signaling pathways in vivo has not been reported previously. However, the A_3R agonist 2-chloro-N^6-(3-iodobenzyl)adenosine-5′-*N*-methylcarboxamide (Cl-IB-MECA) or adenosine dose- and time-dependently increased p-Akt^{Ser473} in cultured neonatal rat cardiomyocytes[9] and A375 human melanoma cells,[35] which is consistent with our finding that A_3R KO attenuated the increase of myocardial p-Akt^{Ser473} and its downstream target p-GSK$^{Ser21/9}$ after TAC. Similarly, previous studies have reported that the A_3R agonist Cl-IB-MECA or adenosine can activate p-ERK$^{Thr202/Tyr204}$ in cultured cardiomyocytes[36] and tumor cell lines,[37] whereas the increase of p-ERK$^{Thr202/Tyr204}$ in response to A_3R agonist is PI3K/Akt dependent.[10] The finding that A_3R activation increased p-ERK$^{Thr202/Tyr204}$ in cultured cell lines[37] is conceptually consistent with our finding that A_3R KO attenuated the TAC-induced increase of p-ERK$^{Thr202/Tyr204}$. The effect of A_3R on p-JNK$^{Thr183/Tyr185}$ has not been reported previously.

The mechanism by which A_3R KO protected the heart against the LV hypertrophy and dysfunction produced by TAC is of considerable interest. A_3R are expressed in both cardiac myocytes and inflammatory cells. Our data demonstrating that antagonism of the A_3R further reduced oxidative stress and expression of ANP in CADO-treated cardiomyocytes indicates that the A_3R can contribute to oxidative stress and the hypertrophic response independent of the paracrine effects or inflammatory response that occur in vivo. The decrease in nitrotyrosine production in the isolated cardio-

myocytes was accompanied by decreases of ERK and JNK activation, similar to the reduced activation of these enzymes in A_3R KO mice. These results are consistent with numerous reports associating oxidative stress with activation of MAPK signaling.[28,38,39]

In addition to a direct role of A_3R on cardiomyocyte hypertrophy and oxidative stress, the A_3R has also been demonstrated to modulate the inflammatory response. Specifically, the A_3R appears important for mast cell degranulation,[2] neutrophil chemotaxis,[40] and infiltration of inflammatory cells.[24,25] It is possible that A_3R-mediated augmentation of the inflammatory response to the pressure overload produced by TAC could have exacerbated LV hypertrophy and dysfunction. Guo et al[41] demonstrated that inflammatory cell accumulation and infarct area were decreased in A_3R KO mice compared with WT mice 24 hours after ischemia/reperfusion injury, suggesting that the A_3R can promote an increased inflammatory response in the heart. Our finding that A_3R KO attenuated the TAC-induced increase of COX-2 and tended to decrease TNF-α after TAC supports a role for the A_3R in the TAC-induced myocardial inflammatory response.

The finding that A_3R KO enhanced the antihypertrophic effect of the CADO in neonatal cardiomyocytes suggests the possibility of interactions between A_3R and A_1R. There is some previous support for such interactions. Thus, Norton et al[42] demonstrated that adenosine $A_{2a}R$ antagonists enhanced A_1R-induced antiadrenergic responses in the heart, whereas $A_{2a}R$ agonists attenuated the antiadrenergic actions of A_1R activation. Although these investigators did not find interaction between A_3R activity and A_1R-mediated antiadrenergic effects in the heart, interaction between A_1R function and A_3R has been demonstrated in the hippocampus, where A_3R activation desensitized A_1R-dependent inhibition of excitatory neurotransmission by adenosine.[43] Although examination of potential interactions between adenosine receptors was beyond the scope of the present report, this is clearly an area in need of further study.

Unfortunately, there are no highly potent and selective A_3R antagonists available for mice. Therefore, a limitation of the present study is that the protective effect of A_3R KO on the pressure-overloaded heart could not be further confirmed by selective A_3R inhibition with pharmacological compounds in an in vivo model.

In summary, A_3R KO had no effect on LV structure or function in the unstressed heart but significantly attenuated TAC-induced LV hypertrophy, fibrosis, and dysfunction. Deletion of A_3R also attenuated the TAC-induced increases of ventricular oxidative stress, COX-2, and the phosphorylation of p-ERK$^{Thr202/Tyr204}$, p-JNK$^{Thr183/Tyr185}$, p-Akt^{Ser473}, and p-GSK-3$\beta^{Ser21/9}$, suggesting that A_3R-mediated increases of oxidative stress and/or inflammation exacerbate detrimental ventricular remodeling by activation of the MAPK and PI3K-Akt pathways. A_3R agonists are currently under development to treat tumors,[44] inflammation,[45] or cardiac injury. The present findings suggest that careful evaluation of the effect of selective A_3R agonists on ventricular hypertrophy and dysfunction in the overloaded or diseased heart will be of importance.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Adenosine A_3 receptors (A_3R) participate in cardioprotection against ischemia/reperfusion injury and are involved in regulation of cell growth, neutrophil chemotaxis, and activation of inflammatory cells. We examined whether the A_3R can facilitate the adaptation of the left ventricle to pressure overload produced by transverse aortic constriction. Contrary to our expectation, mice with genetic ablation of the A_3R developed less severe myocardial hypertrophy and left ventricular dysfunction in response to transverse aortic constriction than did wild-type mice, implying that A_3R activation during pressure overload has a deleterious effect on the heart. In support of these functional data, A_3R deletion decreased myocardial oxidative stress and the expression of proinflammatory cytokines. The findings suggest that selective A_3R inhibition might have potential for treatment of pressure overload–induced left ventricular hypertrophy and dysfunction.

Adenosine A_3 Receptor Deficiency Exerts Unanticipated Protective Effects on the Pressure-Overloaded Left Ventricle

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