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Adenosine A3 receptor deficiency exerts unanticipated protective effects on the pressure overloaded left ventricle

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Abstract

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 A_1R and A_3R can protect the heart against systolic overload, we exposed A_3R gene deficient (A_3R KO) mice and A_1R KO mice to transverse aortic constriction (TAC). Contrary to our hypothesis, A_3R KO attenuated 5 weeks TAC-induced left ventricular (LV) hypertrophy (ratio of ventricular mass/body weight increased to 7.6±0.3 mg/g in wild type (Wt) mice as compared with 6.3±0.4 mg/g in KO), fibrosis and dysfunction (LV ejection fraction decreased to 43±2.5% and 55±4.2% in Wt and KO mice, respectively). A_3R KO also attenuated the TAC-induced increases of myocardial ANP and the oxidative stress markers 3′-nitrotyrosine (3′-NT) and 4-hydroxynonenal. In contrast, A_1R-KO increased TAC-induced mortality, but did not alter ventricular hypertrophy or dysfunction compared to Wt mice. In mice in which extracellular adenosine production was impaired by CD73 KO, TAC caused greater hypertrophy and dysfunction, and increased myocardial 3′-NT. In neonatal rat cardiomyocytes induced to hypertrophy with phenylephrine, the adenosine analogue 2-chloroadenosine (CADO) reduced cell area, protein synthesis, ANP and 3′-NT. Antagonism of A3R significantly potentiated the anti-hypertrophic effects of CADO.

Conclusions—Adenosine exerts protective effects on the overloaded heart, but A_3R act 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 LV hypertrophy and dysfunction.

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Clinical Perspective

Adenosine A3 receptors (A3R) 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 A3R can facilitate the adaptation of the left ventricle (LV) to pressure overload produced by transverse aortic constriction (TAC). Contrary to our expectation, mice with genetic ablation of the A3R developed less severe myocardial hypertrophy and LV dysfunction in response to TAC than did wild type mice, implying that A3R activation during pressure overload has a deleterious effect on the heart. In support of these functional data, A3R deletion decreased myocardial oxidative stress and the expression of pro-inflammatory cytokines. The findings suggest that selective A3R inhibition might have potential for treatment of pressure overload induced LV hypertrophy and dysfunction.

Keywords

hypertrophy; heart failure; oxidative stress; adenosine receptor

Introduction

Recently, we demonstrated that genetic deletion of CD73 (an ectonucleotidase that produces extracellular adenosine) exacerbated myocardial hypertrophy and heart failure resulting from 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. demonstrated that the adenosine analogue 2-chloroadenosine (CADO) also attenuated pressure overload induced LV hypertrophy through activation of the A_1R [8]. Similar to A_1R, the A_3R are Gi protein coupled receptors which 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 over-expression of the A_1R [14] or A_3R[15, 16] promotes cardiac dilation and dysfunction, suggesting these receptors may also exert adverse effects on cardiac function. While 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 KO and A_3R KO 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.

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), 8-12 weeks old, were used for TAC or control. A_1R KO (129 background) and their control wild type mice (Wt) were generated as previously described [17]. The CD73 KO strain and control Wt mice were generated as previously described 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 (using a 26G needle to calibrate the degree of constriction) or severe (using a 27G needle) degree was created as previously described [19]. To assure 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 as to the genotype of the mice.

Echocardiography

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

Sample collection and Western blots

Myocardial samples for protein analysis were flash frozen in liquid nitrogen, weighted 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 using Western blots as previously described[19] using antibodies against ANP (Penninsula Biolabs), 3-nitrotyrosine, 4-HNE (Millipore), cyclooxygenase-2 (COX-2), c-Jun N-terminal kinase (JNK), phosphorylated JNK (p-JNK $^{Thr183/Tyr185}$) (Santa Cruz Biotechnology), eNOS (Transduction Laboratories) extracellular signal-regulated kinase (ERK), and phospho-ERK$^{Thr202/Tyr204}$, phospho-Akt^{Ser473} and phospho GSK-3β$^{Ser21/9}$ (Cell Signaling).

Histological staining and measurement of fibrosis

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

Neonatal rat cardiomyocyte (NVM) isolation and culture

NVW were isolated from 2-day-old Sprague-Dawley rats as previously described[1]. To induce hypertrophy, cells were treated with 50μM phenylephrine for 48 hours. The stable adenosine analogue CADO(5μM) was used to activate adenosine receptors (the affinities of CADO at rat A_1R and A_3R are 9.3 nM and 1,890 nM, respectively) [22]. The selective inhibitors DPCPX and MRS1191 were used at 5μM to block A_1R and A_3R, respectively. 5μM MRS1911 has been reported to selectively inhibit A_3 receptor activation without affecting A_1 receptor dependent responses [23]. After treatment, cells were fixed with 4% paraformaldehyde and stained using Rhodamine conjugated Phalloidin (5 units/ml in PBS, Invitrogen), DAPI, ANP (Penninsula Biolabs) and 3′-NT (Millipore), followed by alexa fluor 488, or alexa fluor 633 labelled 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 ± standard error. Kaplan-Meier survival analysis was performed with SigmaStat using the Gehan-Breslow test. Two-way analysis of variance (ANOVA) was used to test for differences among treatment groups, followed with pairwise multiple comparisons of Tuke's 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-G), and histological staining of LV tissue showed no difference in cardiac myocyte size or relative fibrosis between A_3R KO and Wt mice (Figure 1C-D). After 5 weeks of moderate TAC (using a 26G 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 as compared with Wt mice (Figure 1A-B), indicating that loss of A_3 receptors 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-D, Figure S1). 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 S2).

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-F). 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_3 receptor 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 TNFα and COX-2. TAC resulted in significant increases of TNFα and COX-2 in the hearts of both Wt mice and in A_3R KO mice (Figure 2). However, the increase of COX-2 was significantly less in the A_3R KO mice as compared with Wt mice. The TAC-induced increase of TNFα tended to be less in the A_3R KO mice ($p=0.10$). In addition, hearts from Wt mice had higher levels of 3′-NT and 4-HNE 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 following TAC, and this response was attenuated in the A_3R KO mice (Figure 2).

Activation of mitogen-activated protein kinases (MAPK) and the 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 MAPK signaling pathways. In addition, the TAC-induced increases of p-Akt^{Ser473} and p-GSK-3β$^{Ser21/9}$ 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 following 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 26G 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-F). Moderate TAC of 4 weeks

duration also caused similar decreases of LV ejection fraction in the two groups (Figure 4C). Although mortality tended to be higher in the A_1R KO group during the 4 weeks following moderate TAC (5 out 17 mice died) as compared with Wt mice (2 out 17 wild type mice died), this difference was not significant (Figure 4H).

As 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. Since 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 wild type mice survived. As compared with sham surgery, at two days after severe TAC the ratio of ventricular weight to body weight was similarly increased in Wt (21± 2.5 %) and in A_1R KO mice (22 ± 3.5%), indicating that A_1R KO did not alter the acute hypertrophic response to severe pressure overload (Figure S3). Taken together, the data indicate that A_1R KO had no significant influence on TAC-induced ventricular hypertrophy or dysfunction.

Because the A1R KO mice tended to have a higher mortality than their wild type controls in this initial study, we subsequently went on to examine whether this trend toward a higher mortality would be statistically significant when a more severe degree of systolic overload (using a 27G needle) was applied. When TAC of severe degree was applied, the excess mortality in the A1R KO animals did in fact become significant (Figure S4). 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 S5). 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**-B), fibrosis (Figure 5D-E), myocyte hypertrophy (Figure 5C, 5E), LV dilation and decrease of LV ejection fraction produced by moderate TAC of 4 weeks duration (Figure S6). CD73 KO also exacerbated the TAC-induced increases of ventricular ANP and TNFα (Figure S7). In addition, CD73 KO exacerbated the TAC-induced increase of myocardial 3-nitrotyrosine (Figure S7), indicating increased oxidative stress. To validate these findings, we examined the ability of 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 weeks duration in the CD73 KO mice (Figure S8).

The A_3R Antagonist MRS1911 Potentiates the Anti-hypertrophic 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 non-selective adenosine analogue CADO. We previously demonstrated that CADO or adenosine reduced phenylephrine (PE) induced hypertrophy and ANP expression in neonatal cardiomyocytes [1]. To examine the role of A_1R and A_3R in mediating anti-hypertrophic effects of CADO, we treated cells with 50μM PE and 5μM 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′-NT. PE increased cardiomyocyte protein synthesis (Figure 6A, E), cell area (Figure 6B), ANP expression (Figure 6C) and 3′-NT production (Figure 6D-E) over 48 hours of treatment, while CADO significantly attenuated the PE-induced increases in these variables. Blocking A_1R with DPCPX slightly reversed the CADO induced reductions of cell area (Figure 6B), protein synthesis (Figure 6A) and ANP levels (Figure 6C) in the PE treated cells. Inhibition of A_1R caused a substantial increase in 3′-NT (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′-NT production beyond the reduction caused by CADO alone (Figure 6). The reduction in 3′-NT 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 MAP kinases ERK and JNK (Figure S9). These results suggest that A_3R contributes to increased oxidative stress, higher sustained activation of ERK and JNK, and an increased hypertrophic response to PE.

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. Since 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 anti-hypertrophic effect of CADO in PE 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 havepotential 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 A1R 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 (unpublished data). It is unclear why A_1R blockade would attenuate the anti-hypertrophic effect of CADO, while 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 over-expression 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 GSK3β$^{Ser21/9}$, 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-GSK3β$^{Ser21/9}$ as a result of A_3R KO likely contributed to the lesser degrees of fibrosis and cardiac myocyte hypertrophy in the A^3 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 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 previously reported. 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], while the increase of p-ERK$^{Thr202/Tyr204}$ in response to A_3R agonist is PI3K/Akt dependent [10]. The finding that A_3 receptor 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 previously reported.

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 cardiomyocytes 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 demonstrated that inflammatory cell accumulation and infarct area were decreased in A_3R KO mice as compared to wild type mice 24 hours after ischemia-reperfusion injury [41], 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 TNFα 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 demonstrated that adenosine $A_{2a}R$ antagonists enhanced A_1R-induced antiadrenergic responses in the heart, while $A_{2a}R$ agonists attenuated the antiadrenergic actions of A_1R activation[42]. 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-3β$^{Ser21/9}$, 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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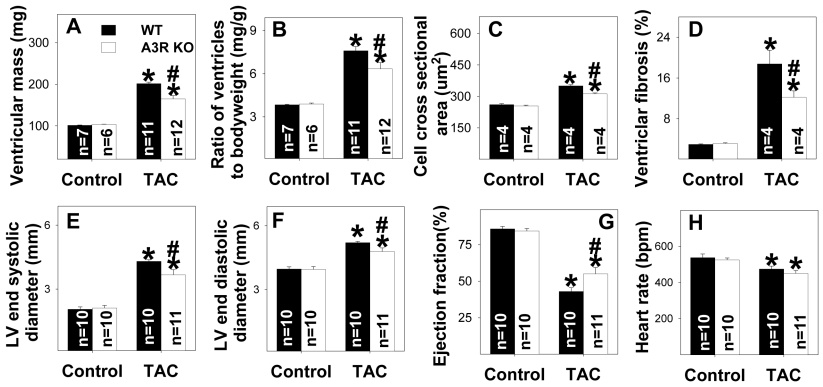

Figure 1.

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 under corresponding conditions (H). *P<0.05 compared to the corresponding control; #p<0.05 compared to Wt-TAC.

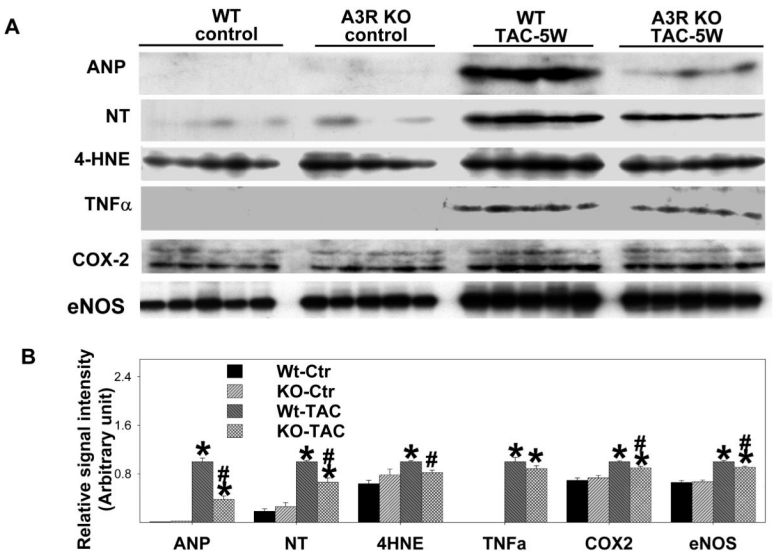

Figure 2.

A_3R KO significantly attenuates moderate TAC-induced increases of ventricular ANP, nitrotyrosine, 4-HNE and COX-2. TAC caused significant increases of ventricular TNFα in both A_3R KO and wild type mice. A_3R KO tended to decrease TNFα after TAC, but this difference was not significant (p=0.10). Data are normalized to Wt-TAC. *P<0.05 compared to the corresponding control; #p<0.05 compared to Wt-TAC.

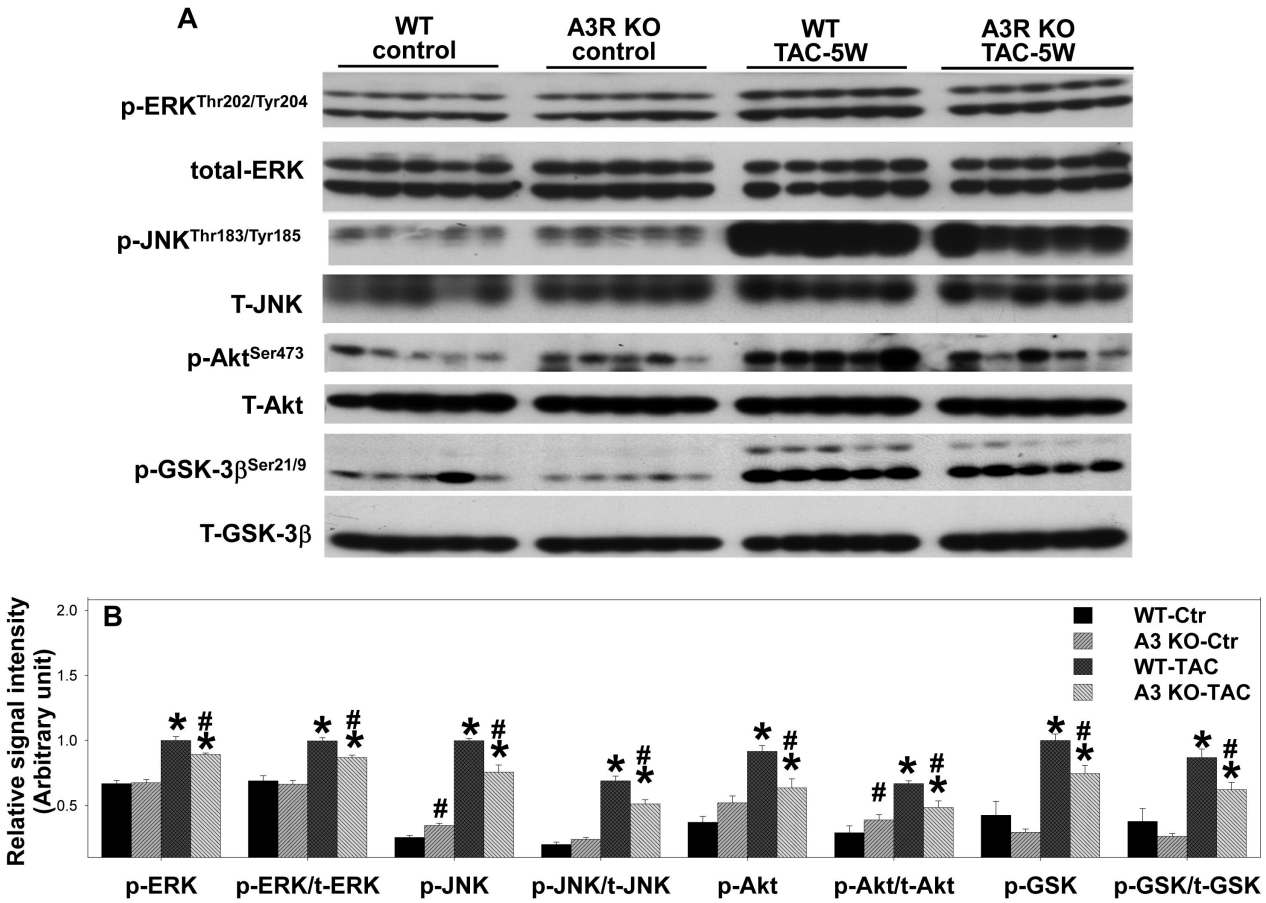

Figure 3.

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

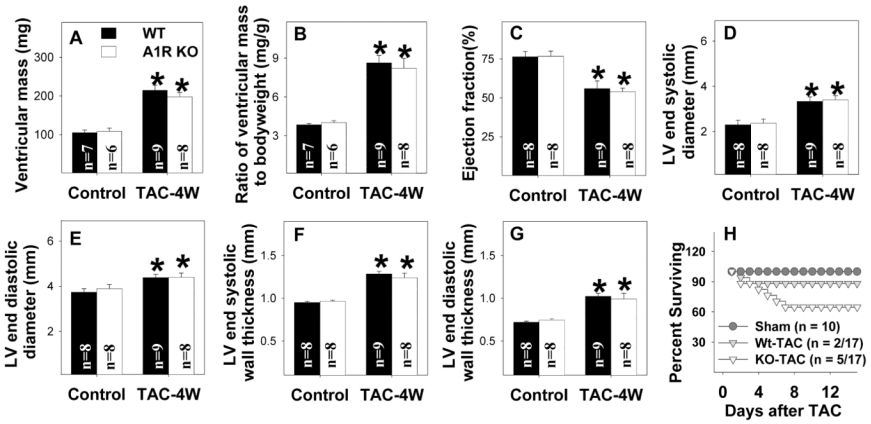

Figure 4.

A_1R KO had no significant effect 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 weeks duration. A_1R KO mice tended to have a higher mortality during four weeks following moderate TAC, but this difference was not significant (H)). *P<0.05 compared to the corresponding control.

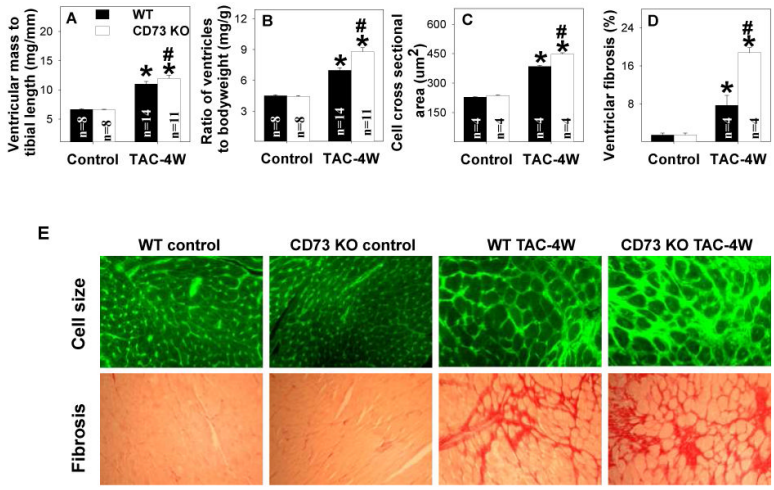

Figure 5.

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 of moderate TAC. *P<0.05 compared to the corresponding control; #p<0.05 compared to Wt-TAC.

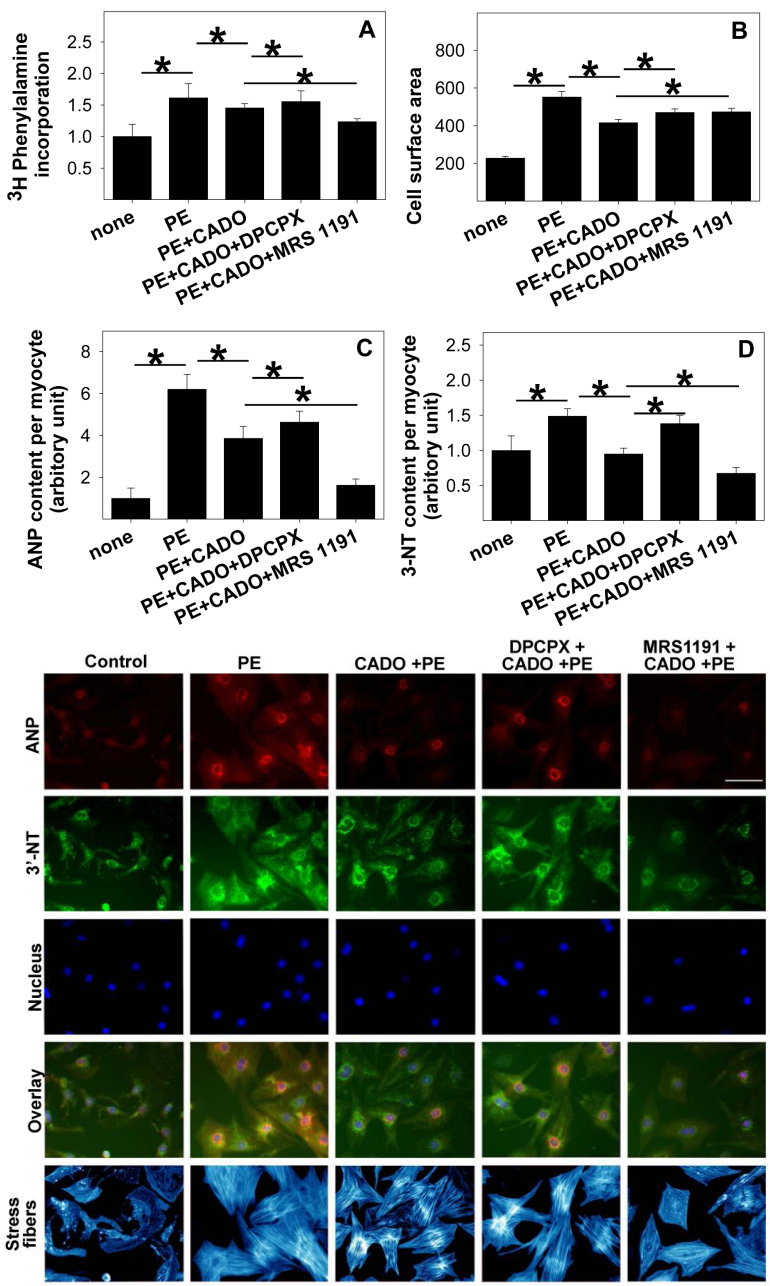

Figure 6.

Addition of the A3R antagonist MRS1191 to CADO treated cells further attenuated the PE-induced increases of protein synthesis (A,E), and the expression of ANP (C,E) and 3′-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.