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Effects of Cardiac Overexpression of the Angiotensin II Type 2 Receptor on Remodeling and Dysfunction in Mice Post-Myocardial Infarction RR

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Abstract

Activation of the angiotensin II type 2 receptors (AT_2R) has been considered cardioprotective. However, there are controversial findings regarding the role of overexpressing AT_2R in the heart. Using transgenic mice with different levels of AT_2R gene overexpression in the heart (1, 4 or 9 copies of the AT_2R transgene, Tg^1, Tg^4 or Tg^9), we studied the effect of AT_2R overexpression on left ventricular remodeling and dysfunction post-myocardial infarction (MI). Tg^1, Tg^4, Tg^9 and their wild-type (WT) littermates were divided into 1) sham MI, 2) MI + vehicle, and 3) MI + AT_2R antagonist. Treatments were started 4 weeks after MI and continued for 8 weeks. AT_2R protein and mRNA expression in the heart was significantly increased in transgenic mice and the increase positively correlated with copies of the transgene. AT_1R mRNA and protein expression remained unchanged in Tg^1 and Tg^4 but slightly higher in Tg^9 mice. Systolic blood pressure and cardiac phenotypes did not differ among strains under basal conditions. MI caused myocardial hypertrophy, interstitial fibrosis, ventricular dilatation and dysfunction associated with increased protein expression of Nox2 and transforming growth factor (TGF)-β_1 expression. These pathological responses were diminished in Tg^1 and Tg^4 mice. Moreover, the protective effects of AT_2R were abolished by AT_2R antagonist and also absent in Tg^9 mice. We thus conclude that whether overexpression of AT_2R is beneficial or detrimental to the heart is largely dependent on expression levels and possibly *via* regulations of Nox2 and TGF-β_1 signaling pathways.

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Keywords

angiotensin II type 1 receptor; angiotensin II type 2 receptor; myocardial infarction; transgenic mice

INTRODUCTION

Angiotensin II (Ang II), the principal effector of the renin-angiotensin system (RAS), binds to two distinct receptors, AT_1R and AT_2R. AT_1R is ubiquitous and abundant in adult tissues and known to be responsible for most of the detrimental cardiovascular effects of Ang II, whereas expression of AT_2R is high in the fetus, rapidly reduced after birth, and up-regulated in response to pathological stimuli such as hypertension and myocardial ischemia.[1,2] AT_2R has been suggested to exert cardioprotective properties most likely *via* kinin/nitric oxide (NO) mediated mechanisms.[3–6] Using an animal model of heart failure induced by myocardial infarction (MI), we and others demonstrated that activation of AT_2R contributes to the cardioprotective effects of Ang II receptor blocker (ARB).[7–9] Studies using *in vivo* gene-transfer techniques or transgenic mice with AT_2R specifically overexpressed in the heart showed that activation/overexpression of AT_2R attenuated pressure- or ischemia-induced cardiac remodeling and dysfunction.[10–13] Likewise, deletion of AT_2R enhanced mortality and aggravated cardiac dysfunction.[14] These data support a cardioprotective role of AT_2R.

However, controversial findings have been reported. D'Amore et al found that in cultured neonatal cardiomyocytes overexpression of AT_2R caused hypertrophy that correlated positively with the level of AT_2R expression.[15] Yan et al[16] and Nakayama et al[17] showed that overexpressing AT_2R specifically in ventricular cardiomyocytes decreased cardiac contractility and caused dilated cardiomyopathy, and the severity of cardiac dysfunction correlated positively with copy numbers of the AT_2R transgene. Because AT_2 remains at a very low level in the normal adult heart, we hypothesize that whether activation/overexpression of AT_2R is beneficial or detrimental to the heart largely depends on its level of expression. Overexpression at high amounts, associated with increased AT_1, could be detrimental *via* a signaling mechanism similar to AT_1. For this, we used transgenic mice with different levels of AT_2R overexpression (1, 4 or 9 copies of the AT_2R transgene, Tg^1, Tg^4 or Tg^9) specifically in ventricular cardiomyocytes to study 1) whether mice with low copy numbers of AT_2R transgene (Tg^1 or Tg^4) had less severe cardiac hypertrophy and remodeling and better preserved left ventricular (LV) function post-MI and these beneficial effects were diminished by AT_2R blockade, and 2) whether AT_2R overexpression at high levels (Tg^9) worsened LV remodeling and dysfunction post-MI, which involves increased oxidative stress and $TGF\beta_1$-mediated fibrotic and inflammatory responses.

MATERIALS AND METHODS

Animals

Male transgenic mice with overexpression of 1, 4 or 9 copies of the AT_2R transgene (Tg^1, Tg^4 or Tg^9) were kindly provided by Dr. Xinhua Yan (Tufts University - St. Elizabeth's

Medical Center of Boston) and bred in our mutant mouse facility. The AT_2R transgene was driven by the myosin light chain promoter and expressed specifically at ventricular cardiomyocytes. Their wild-type (WT) littermates were used as controls. Mice were housed in an air-conditioned room with a 12-hour light/dark cycle and given standard chow with free access to tap water. This study was approved by the Institutional Animal Care and Use Committee (IACUC) of Henry Ford Health System. All studies were conducted in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals.

Induction of myocardial infarction and experimental protocols

When mice were 11–12 weeks of age, MI was created by ligating the left anterior descending coronary artery as described previously.[8] Four weeks after surgery, each strain of mice (WT, Tg^1, Tg^4, and Tg^9) was divided into 3 groups: 1) sham MI; 2) MI + vehicle; and 3) MI + AT_2-antagonist (AT_2-ant; PD123319, 20 mg/kg/day (provided by Pfizer Inc) *via* osmotic minipump (Alzet, Cupertino, CA). Treatment was started 4 weeks after MI to avoid effects on infarct size (IS) and healing and continued for 8 weeks.

Systolic blood pressure (SBP) and cardiac function were measured in conscious mice as described previously[6,18,19].

Histopathological study

Heart weight, myocyte cross-sectional area (MCSA) and interstitial collagen fraction (ICF) were measured as described previously[6,18,19].

Real-time PCR of AT_1R and AT_2R mRNA expression and Western blot for AT_2R, endothelial nitric oxide synthase (eNOS), Nox2 and TGF-β_1 protein expression

Detailed methods are available in online-only Data Supplements.

Data analysis

All data are expressed as mean ± SE. Student's two-sample *t*-test was used to compare differences between strains or between treatments within strains. When multiple comparisons were performed, Hochberg's step-up procedure was used to adjust *p*-values. The family-wise type I error rate was set at 0.05.

RESULTS

AT_2R and AT_1R protein and mRNA expression

AT_2R protein and mRNA expression in the heart was very low in WT mice but significantly increased in Tg^1, Tg^4 and Tg^9 mice. The levels of increase correlated positively with copies of the transgene (Fig 1, left panels). AT_1R expression was similar in WT, Tg^1 and Tg^4 mice but was higher in Tg^9 mice, though the increase did not reach statistical significance (Fig. 1, right panels).

Early and late mortality

The early mortality rate (within 24 hours after MI) was similar among the strains. During the first week of MI, 36% of the WT mice, 11% of the Tg^1 mice, 23% of the Tg^4 mice and 31% of the Tg^9 mice died, mostly from cardiac rupture ($p < 0.01$ WT *vs* Tg^1 and $p < 0.05$ Tg^9 *vs* Tg^1). Thereafter, there was no difference in mortality among groups (see Fig S1. in Online Data Supplement).

SBP and heart rate (HR)

Basal SBP and HR were similar among groups and remained unchanged in sham-operated mice. MI caused a decrease in SBP similarly in all strains, although the change did not reach statistical significance (Table 1). Blockade of AT_2R had no effect on SBP in all groups. HR was not affected by MI or treatment with AT_2R antagonist.

Body and tissue weight and infarct size

Body and organ weights were similar among strains with sham MI. MI significantly increased heart weight in all strains but no strain difference was detected. MI *per se* also increased lung weight in WT and Tg^9 strains but not in Tg^1 and Tg^4 groups. However, this protective effect on lung congestion was reversed by blockade of AT_2R in Tg^1 mice (Table 1). MI did not affect liver weight; although blockade of AT_2R caused significant liver congestion, no strain difference was seen (Table 1). Infarct size was similar in all strains with or without AT_2R antagonist (Table 1).

Cardiac function and remodeling

Overexpression of AT_2R with different copy numbers had no effect on cardiac morphology and function under basal conditions, since left ventricular diastolic dimension (LVDd) and ejection fraction (EF) were similar among WT, Tg^1, Tg^4 and Tg^9 mice with sham MI (Fig. 2). MI caused LV dilatation and dysfunction in all strains as evidenced by increased LVDd and decreased EF; however, these detrimental cardiac effects were less severe in Tg^1 and Tg^4 mice compared to WT controls. The cardioprotective effects observed in Tg^1 and Tg^4 mice were completely blunted by AT_2R blockade (Fig. 2).

Myocyte hypertrophy and cardiac interstitial fibrosis

MCSA and ICF were similar among WT, Tg^1, Tg^4, and Tg^9 groups receiving sham operations. MI increased MCSA and ICF in all strains; however, the hypertrophic and fibrotic responses were less severe in Tg^1 and Tg^4 compared to WT controls or Tg^9 mice (Fig. 3). Blockade of AT_2R reversed the anti-hypertrophic and anti-fibrotic effects observed in Tg^1 and Tg^4 mice. MCSA and ICF did not differ between WT and Tg^9 mice treated with either vehicle or AT_2R antagonist (Fig. 3).

Protein expression of eNOS, Nox2 and TGF-β_1 in the heart

Protein expression of eNOS, Nox2 and TGF-β_1 was determined in all strains (since there was no difference between Tg^1 and Tg^4, only data from Tg^1 are presented in the figures). eNOS, Nox2 and TGF-β_1 expression did not differ among strains with sham MI. MI increased eNOS expression in Tg^1 and Tg^4 mice and this effect was completely blocked by

AT_2R antagonist (Fig. 4). AT_2R antagonist also tended to decrease eNOS expression in Tg^9 mice compared to vehicle treated mice of same strain, but the difference did not reach statistical significance. Nox2 expression was increased in all groups after MI; however, the increase was less prominent in Tg^1 and Tg^4 compared to WT and Tg^9 groups (Fig. 5). AT_2R antagonist reversed the effect observed in Tg^1 and Tg^4 but had no effect in WT. AT_2R antagonist tended to lower Nox2 expression in Tg^9 mice but the difference was not statistically significant. TGF-β_1 protein expression was also increased in all groups, but the increase was insignificant in Tg^1 and Tg^4 and this effect was blunted by AT_2R antagonist (Fig. 6). Blockade of AT_2R did not affect TGF-β_1 expression in WT and Tg^9 groups.

DISCUSSION

Our present study shows that transgenic mice with cardiac-specific AT_2R overexpression had a significant increase in AT_2R mRNA and protein expression, which positively correlated with copies of the transgene. Furthermore, overexpression of AT_2R at excessive levels as observed in mice with 9 copies of the transgene (Tg^9) tended to increase AT_1R expression. Under basal conditions, overexpression of AT_2R either at high or low levels did not affect blood pressure, cardiac morphology and function. When subjected to MI, mice with a low number of AT_2R transgene copies (Tg^1 and Tg^4) had a lower mortality rate and less severe LV remodeling and dysfunction; these effects were abolished by AT_2R antagonist. In mice with higher copies of the transgene (Tg^9) and increased AT_1R expression, the cardioprotective effect of AT_2R vanished. These data suggest that under normal conditions overexpression of AT_2R specifically in ventricular cardiomyocytes does not play an important role in the regulation of blood pressure, cardiac morphology or function. However, in response to cardiac injury such as MI, overexpression of cardiac AT_2R protects the heart against adverse remodeling and dysfunction, but these protective effects largely rely on the AT_2R expression levels. Indeed, low or moderate overexpression of AT_2R is beneficial, but excessively overexpressing AT_2R could be detrimental to the heart.

Two major Ang II receptors, AT_1R and AT_2R, have been identified. AT_1R is widely expressed in adult organs and mediates the major effects of Ang II. Conversely, AT_2R is reportedly expressed at high levels in fetus and its expression decreases rapidly after birth and remains low in the adult heart, but relatively abundant in the vasculature.[1] In response to pathological stimuli such as hypertension, cardiac hypertrophy and/or ischemia, AT_2R is upregulated in both humans and experimental animals, probably as a compensatory mechanism in response to increased renin-angiotensin activity.[1,2] It has been reported that activation of AT_2R counteracts AT_1-mediated actions, which in most cases is believed to be cardiac beneficial.[20] We and others previously demonstrated that activation of AT_2R contributes to the cardioprotective effects of ARB, possibly *via* release of kinins and NO.[3,4,6,8,21] Since AT_2R is upregulated in cardiovascular disease (CVD)[1,2] and activation of AT_2R by blocking AT_1R by ARB leads to cardioprotection, it is clinically relevant to overexpress AT_2R at levels that do not overly exceed the physiological limits and to develop and study new AT_2R ligands for the treatment of cardiovascular disease. Indeed, the cardioprotective role of AT_2R is further supported by a number of studies involving activation and/or overexpression of AT_2 in cardiomyocytes, cardiac tissue and blood

vessels.[10,12,13] In the present study we found that mice with low or moderate overexpression of AT_2R had less severe cardiac hypertrophy, fibrosis and chamber dilatation coupled with better preserved cardiac function compared to WT. MI-induced increase in Nox2 and TGF-β_1 protein expression were also attenuated in Tg^1 and Tg^4 mice. None of these protective effects were seen in Tg^9 mice Furthermore, the protective effects observed in Tg^1 and Tg^4 were abolished by the AT_2R antagonist. These data demonstrate that overexpression of AT_2R at low or moderate level is cardioprotective and those effects are AT_2R specific.

However, conflicting results regarding AT_2R overexpression in cardiomyocytes have been reported as well. Yan et al[16] and Nakayama et al[17] found that ventricle-specific expression of the AT_2R receptor (9 and 18 transgene copies, driven by the myosin light chain promoter) caused cardiac hypertrophy and aggravated the development of dilated cardiomyopathy and heart failure. Furthermore, the severity of cardiac dysfunction correlated positively with the copy numbers of the AT_2R transgene. Using cultured neonatal myocytes transfected with an adenovirus encoding the AT_2R gene, D'Amore et al[15] found that increased AT_2R did not inhibit Ang II-induced myocyte hypertrophy but rather aggravated the hypertrophic response. In the present study using transgenic mice with different levels of AT_2R overexpression in the heart, we found that blood pressure and cardiac morphology and function did not differ among WT and Tg^1, Tg^4 and Tg^9 strains subjected sham operation. Although it is a little surprising that overexpression of AT_2R, particularly at an excessive level considered far beyond the physiological levels, did not affect the cardiac phenotypes, we speculate that AT_2R is saturated under basal conditions, and thus overexpression of AT_2R without a change in the endogenous ligand would not affect the function. Also, in the present study AT_2R is specifically overexpressed in the heart and activation of the receptor may initiate mainly local or paracrine effects, which may have little or no effect on systemic hemodynamics.

Although we have no definite answer for these disparate results of AT_2R overexpression, based on our findings and others we believe that the levels of AT_2R overexpression may play a major role as to whether overexpression of AT_2R is beneficial or detrimental to the heart. In studies showing that AT_2R overexpression in the heart is beneficial, the levels of AT_2R were about 20–35% relative to AT_1,[3,22] whereas in studies showing that overexpression of AT_2R is detrimental or non-cardioprotective, levels of AT_2R were often similar to or greater than AT_1R.[15,16] For example in Yan's study,[16] mice with a high copy number of the AT_2R transgene (18 copies) developed dilated cardiomyopathy, and mice with an even higher copy number (34 copies) developed overt heart failure and died prematurely. Unfortunately, AT_1R expression was not reported in this study. Qi et al[12] recently reported that moderate cardiac-specific AT_2R overexpression suppressed AT_1R expression. In the present study, we measured both AT_2R and AT_1R expression and showed that overexpression of AT_2R at low or moderate levels did not affect AT_1R expression but at a high level it tended to increase AT_1R and blunted the cardioprotective effects of AT_2R. At the present time we have no explanation as to why overexpression of AT_2R increases AT_1R expression but this result may warrant further investigation. Nevertheless, our data indicate that levels of AT_2 overexpression are important determinants for whether AT_2 overexpression in myocytes is beneficial or detrimental.

Several signaling mechanisms have been explored as to the cardioprotective actions of AT_2R.[23] An increase in NO release *via* kinins has drawn great attention.[3,8] We recently *in vitro* transfected mouse coronary artery endothelial cells with the AT_2R gene and found that overexpression and activation of AT_2R increases bradykinin release through prolylcarboxypeptidase (a plasma prekallikrein activator), leading to enhanced NO production.[5] In the present study, overexpression of AT_2R in mouse heart at low or moderate levels upregulated eNOS expression and attenuated the cardiac remodeling and dysfunction post-MI and these effects were not seen in Tg^9 mice. These results suggest that overexpression of AT_2R exerts cardioprotective effects post-MI, possibly through activation of eNOS and release of NO.

Anti-oxidative stress could be another potential mechanism responsible for AT_2R-mediated cardioprotection. It is well known that reactive oxygen species (ROS) generated by an NADPH oxidase-dependent pathway play a pivotal role in the pathophysiology of heart failure. The $gp91^{phox}$ (Nox2)-containing NADPH oxidase is the major source of ROS in the heart.[24] Studies show that vascular overexpression of AT_2R blunted Ang II-induced increase in Nox2 mRNA expression and vascular injury,[25] whereas deletion or blockade of AT_2R augmented oxidative stress, NADPH oxidase activity and renal and vascular injury.[26,27] In the present study, we found that MI-induced a significant increase in Nox2 protein expression in the heart but this increase was attenuated in mice with low overexpression of AT_2R, which was reversed by AT_2R blockade. However, in mice with high overexpression of the AT_2R (Tg^9) MI-induced increase of Nox2 protein remained high as seen with WT mice. These data may indicate that overexpression of AT_2R protects the heart against ischemia-induced injury partly through reducing oxidative stress and this protection is inversely related to the overexpression levels of AT_2R.

Increased TGF-β_1 expression/signaling contributes to the fibrotic remodeling and tissue damage.[28,29] Overexpression/activation of AT_2R has been shown to exert anti-fibrotic and anti-growth functions *via* suppressing TGF-β_1. For example, Hashimoto et al[30] reported that vascular overexpression of AT_2R ameliorated 5/6 nephrectomy-induced upregulation of TGF-β_1 and glomerular injury. In renovascular hypertension, activation of AT_2R decreased renal TGF-β_1 associated with reduction of inflammation.[31] Habashi et al[32] showed that loss of AT_2R accelerates aortic aneurysm formation and rupture in mouse model of Marfan syndrome most likely through TGF-β_1-mediated activation of extracellular signal-regulated kinase. In the current study we showed that transgenic mice with 1 or 4 copies of the AT_2R gene had less degree of cardiomyocyte hypertrophy, cardiac fibrosis and upregulation of TGF-β_1, which were not seen in Tg^9 mice. These data indicate that overexpression of AT_2R in the heart at low or moderate levels might be responsible for the reduced cardiac hypertrophy, fibrosis and dysfunction post-MI, in part mediated *via* reduced TGF-β_1 signaling.

In summary, the present study demonstrates that AT_2R overexpression specifically in the heart does not seem to play an essential role in cardiac hemodynamics, morphology, and function under basal conditions. However, low or moderate levels of overexpression (Tg^1, Tg^4) of AT_2R appear to protect the heart from maladaptive remodeling and dysfunction post-MI. These beneficial effects involve upregulation of eNOS and downregulation of

TGF-β_1 and oxidative stress signaling pathways, which disappeared when AT_2R overexpressed at an excessive level. Thus we conclude that whether overexpression of AT_2 is beneficial or detrimental to the heart post-MI is largely dependent on levels of its expression, possible *via* regulation of eNOS, Nox2 and TGF-β_1 signaling pathways.

PERSPECTIVE

AT_2R expression is upregulated in patients with cardiovascular disease, probably as a compensatory mechanism. Activation of AT_2R also reportedly contributes to the cardioprotective effect of ARB. However, there are conflicting reports regarding the role of AT_2R in the heart. Thus, understanding the role of AT_2R and defining the mechanism(s) underlying AT_2R-mediated cardioprotection could help to develop new therapeutic strategies, such as using an AT_2R agonist alone or in combination with ACEi or ARB to treat patients with cardiovascular disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Novelty and Significance

What is new

There are conflicting reports regarding the role of AT_2R in the heart. We demonstrate for the first time that low or moderate overexpression of AT_2R in the heart is beneficial, but high levels may be detrimental.

What is relevant

AT_2R expression is upregulated in patients with cardiovascular disease, probably as compensatory mechanisms. Defining the role of AT_2R and the mechanism(s) underlying AT_2R-mediated cardioprotection could lead to new therapeutic strategies such as specific activation/overexpression of AT_2R either alone or combined with ACEi or ARB to treat hypertension, ischemic heart disease and end-organ damage, which has high clinical relevance.

Summary

Low or moderate levels of AT_2R overexpression protects the heart from maladaptive remodeling and dysfunction post-MI. An excessive increase in AT_2R abolishes such protection. We thus conclude that whether overexpression of AT_2R is beneficial or detrimental to the heart is largely dependent on expression levels. The beneficial effects involve upregulation of eNOS and downregulation of TGF-β_1 and oxidative stress signaling pathways.

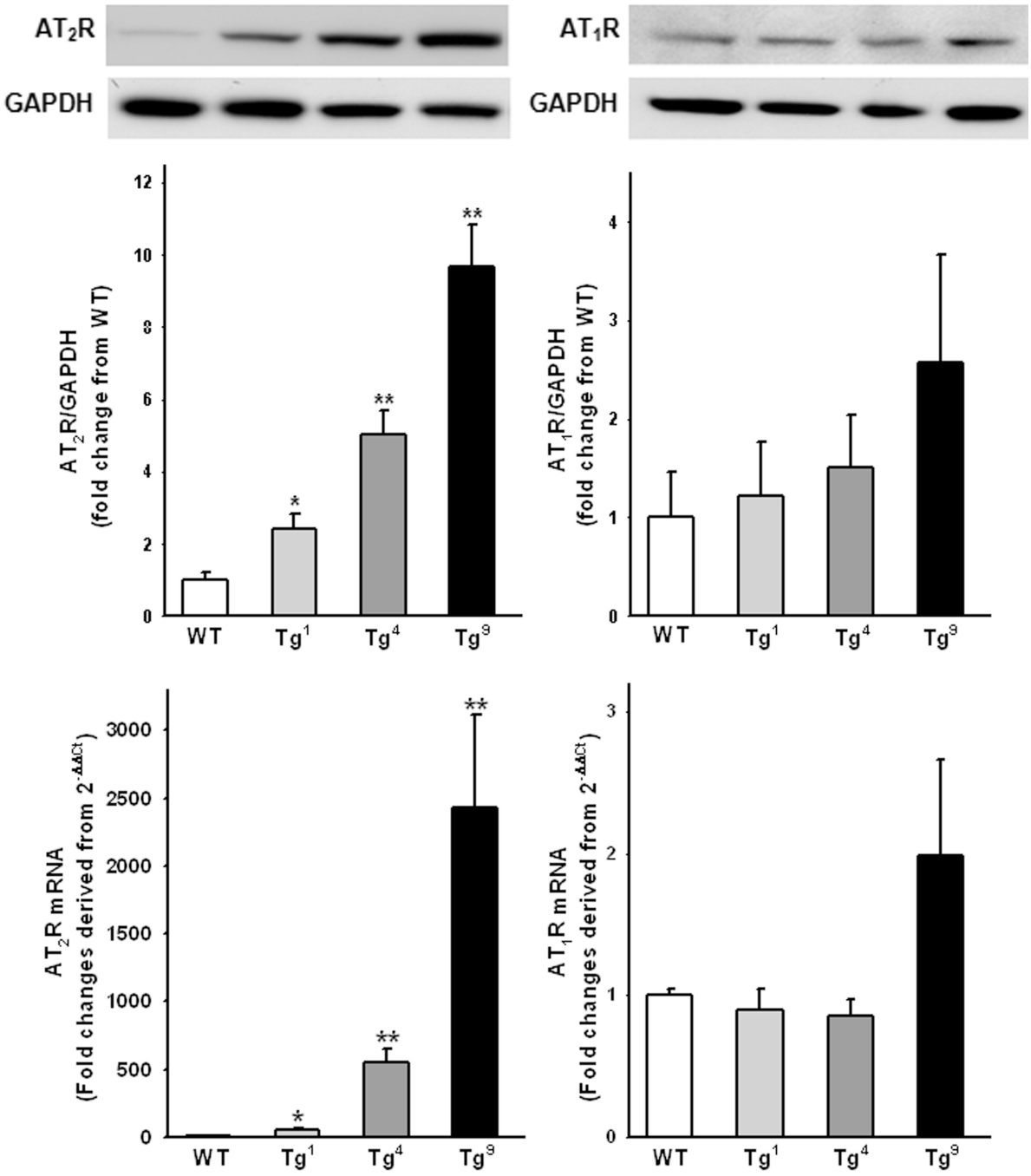

Figure 1.

AT_2R and AT_1R mRNA (top) and protein (bottom) expression in mice with 1, 4 or 9 copies of the AT_2R transgene (Tg^1, Tg^4 or Tg^9). Data are presented as fold change relative to wild-type (WT) controls. $n = 4 – 6$ in each group. *: $p < 0.05$, **: $p < 0.01$ Tg^1, Tg^4 or Tg^9 *vs* WT controls.

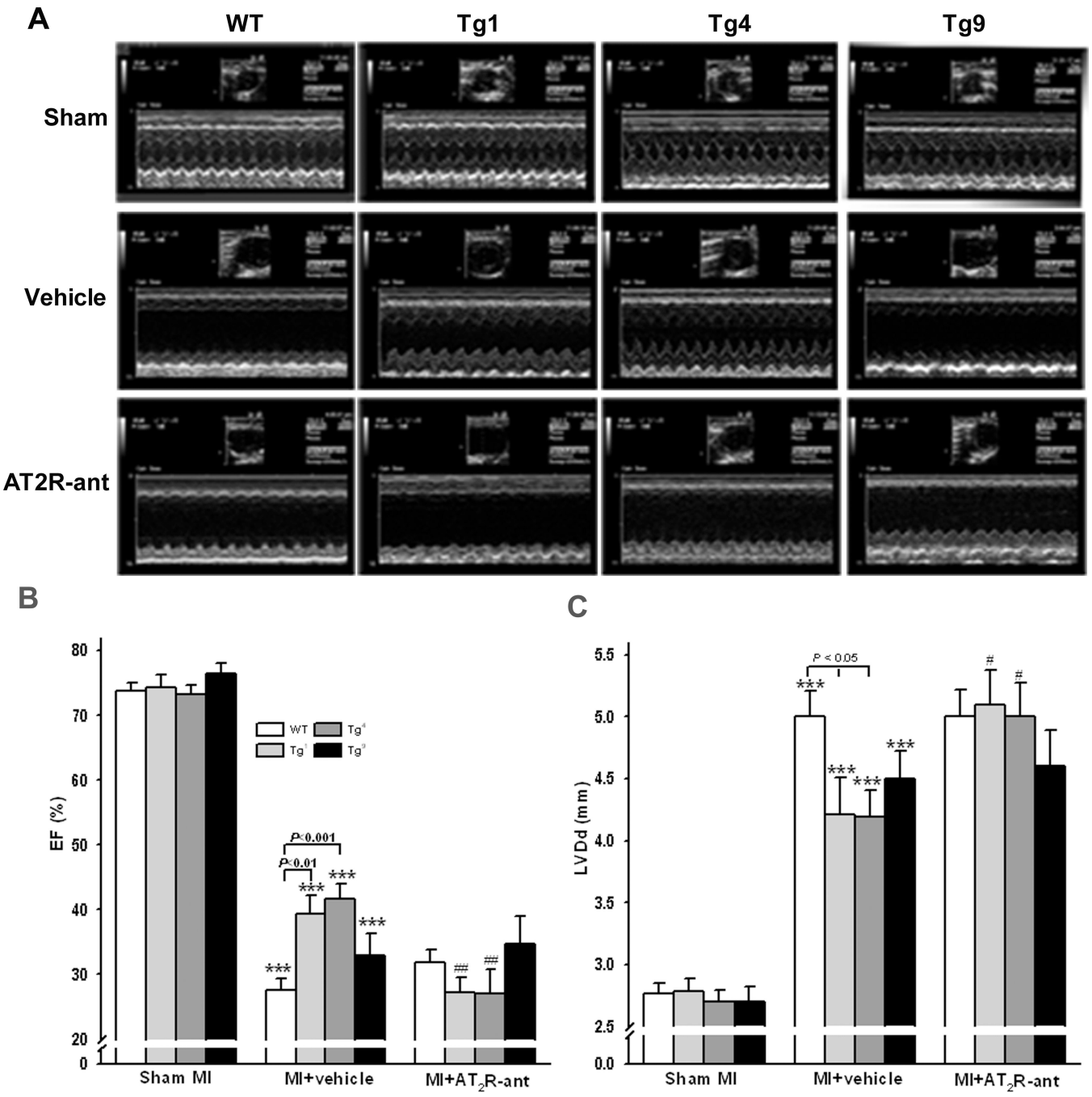

Figure 2.

Effect of cardiac-specific overexpression of AT_2R with or without AT_2R antagonist (AT_2R-ant, PD123319) on left ventricular (LV) ejection fraction (EF) and diastolic dimension (LVDd) post MI. A: representative echocardiograms; B and C: quantitative analysis of EF and LVDd, respectively. ***: $p < 0.001$ MI + vehicle *vs* sham groups within strains; #: $p < 0.05$, ##: $p < 0.01$ MI + AT_2R-ant *vs* MI + vehicle within strains.

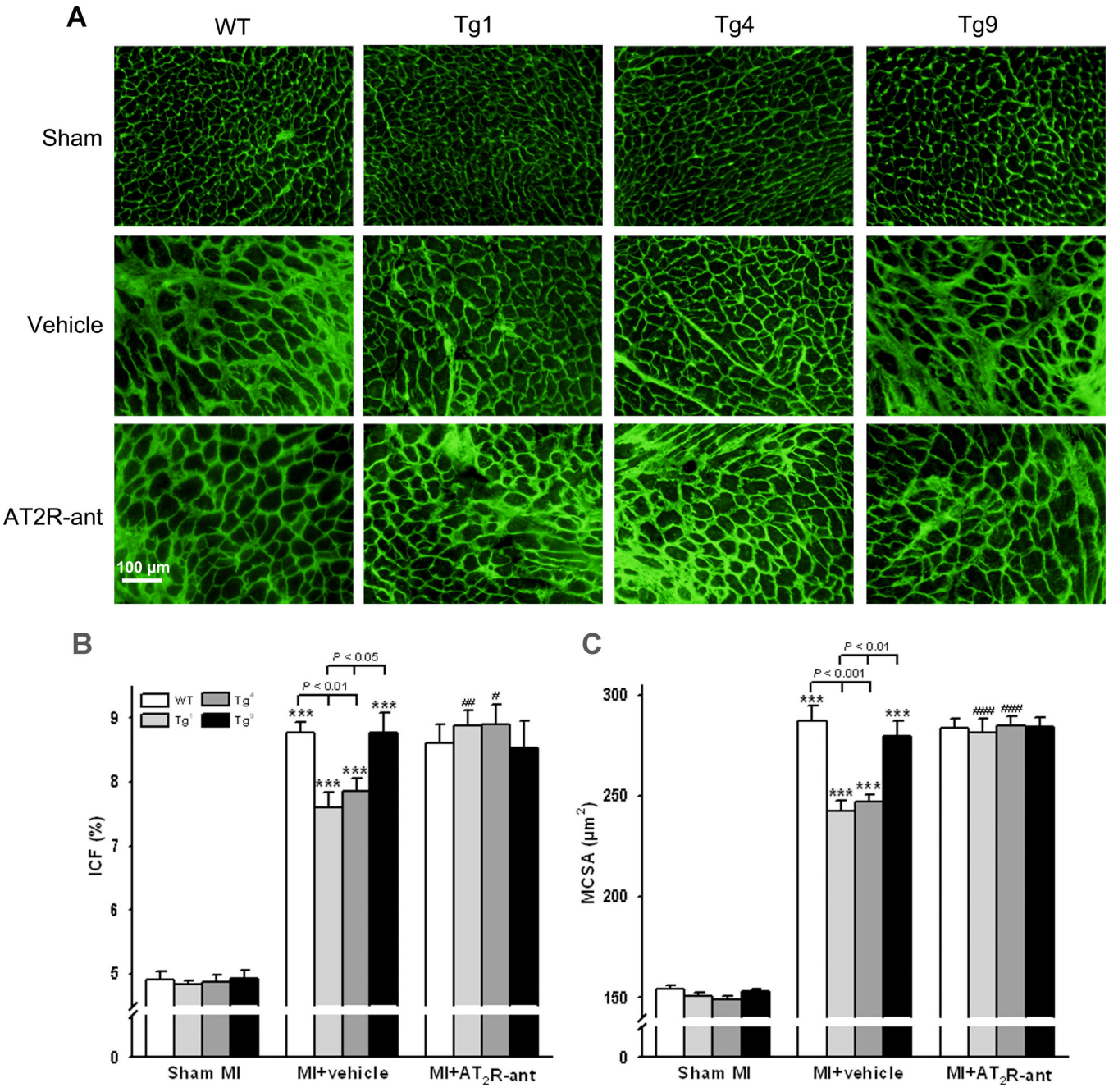

Figure 3.

Effect of cardiac-specific overexpression of AT_2R with or without AT_2R-ant on myocyte cross-sectional area (MCSA) and interstitial collagen fraction (ICF) post- MI. A: representative images of MCSA and interstitial collagen deposition; B and C: quantitative analysis of MCSA and ICF, respectively. ***: $p < 0.001$ MI + vehicle *vs* sham groups within strains; #: $p < 0.05$, ##: $p < 0.01$, ###: $p < 0.001$ MI + AT_2R-ant *vs* MI + vehicle within strains.

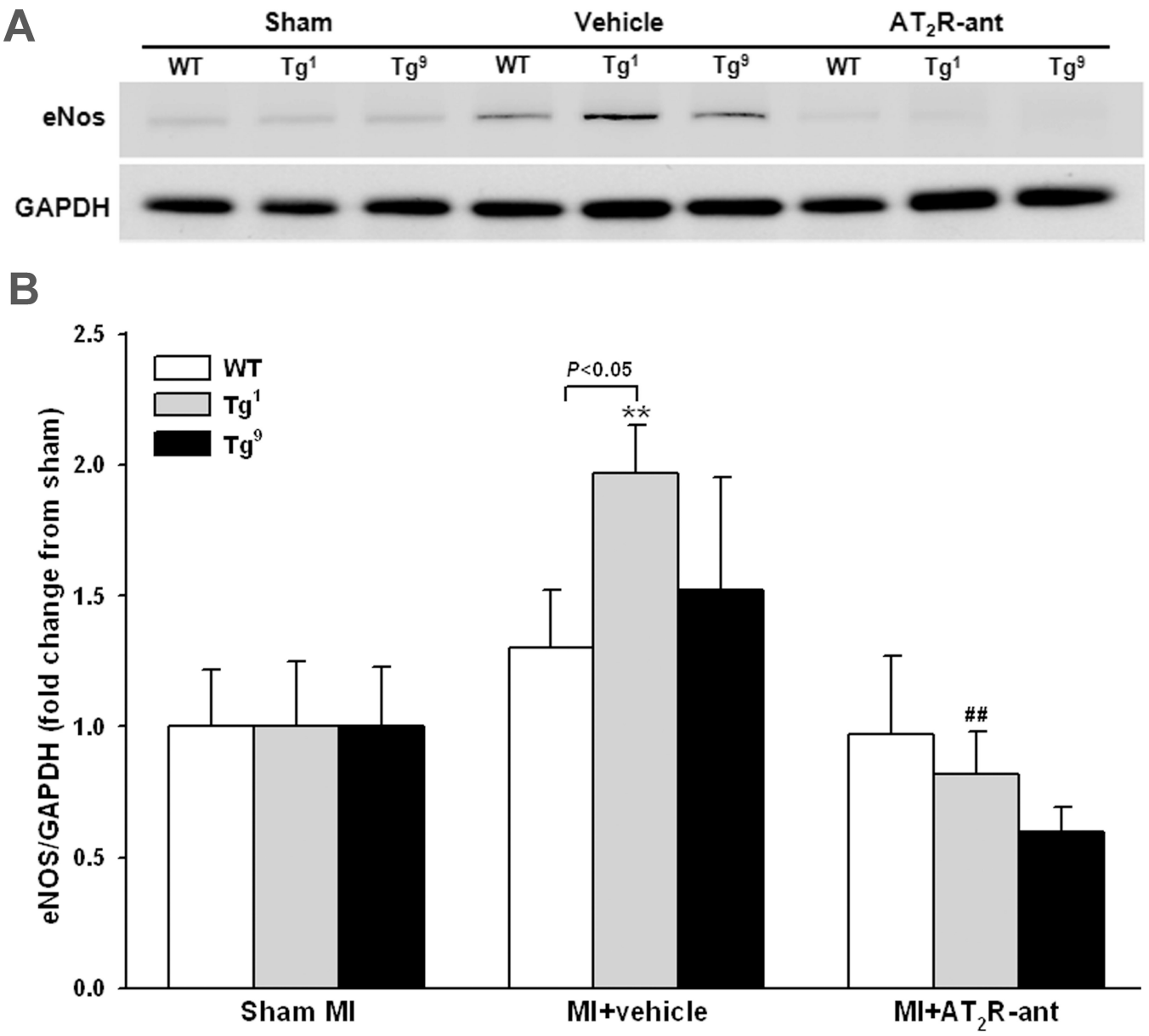

Figure 4.

Effect of cardiac-specific overexpression of AT_2R with or without AT_2R-ant on endothelial NOS (eNOS) protein expression post-MI. A: Representative Western blots of eNOS and GAPDH. B: Semi-quantitative analysis of eNOS protein corrected by GAPDH and expressed as fold change relative to sham controls within strain. $n = 8$ in each group. **: $p < 0.01$ MI + vehicle *vs* sham groups within strains; ##: $p < 0.01$ MI + AT_2R-ant *vs* MI + vehicle within strains.

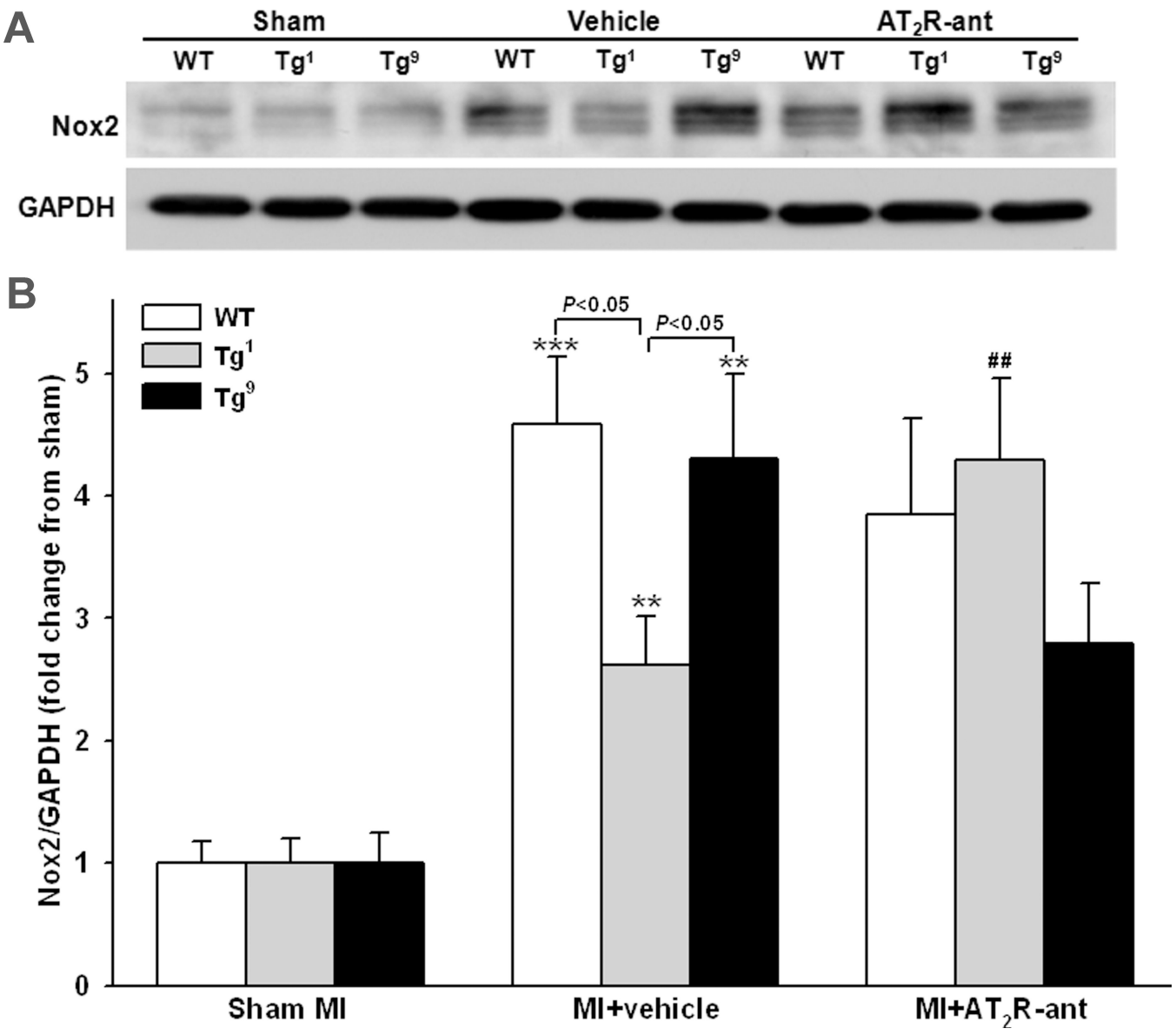

Figure 5.

Effect of cardiac-specific overexpression of AT_2R with or without AT_2R-ant on Nox2 protein expression post-MI. A: Representative Western blots of Nox2 and GAPDH. B: Semi-quantitative analysis of Nox2 protein corrected by GAPDH and expressed as fold change relative to sham controls within strain. $n = 8$ in each group. **: $p < 0.01$, ***: $p < 0.001$ MI + vehicle *vs* sham groups within strains; ##: $p < 0.01$ MI + AT_2R-ant *vs* MI + vehicle within strains.

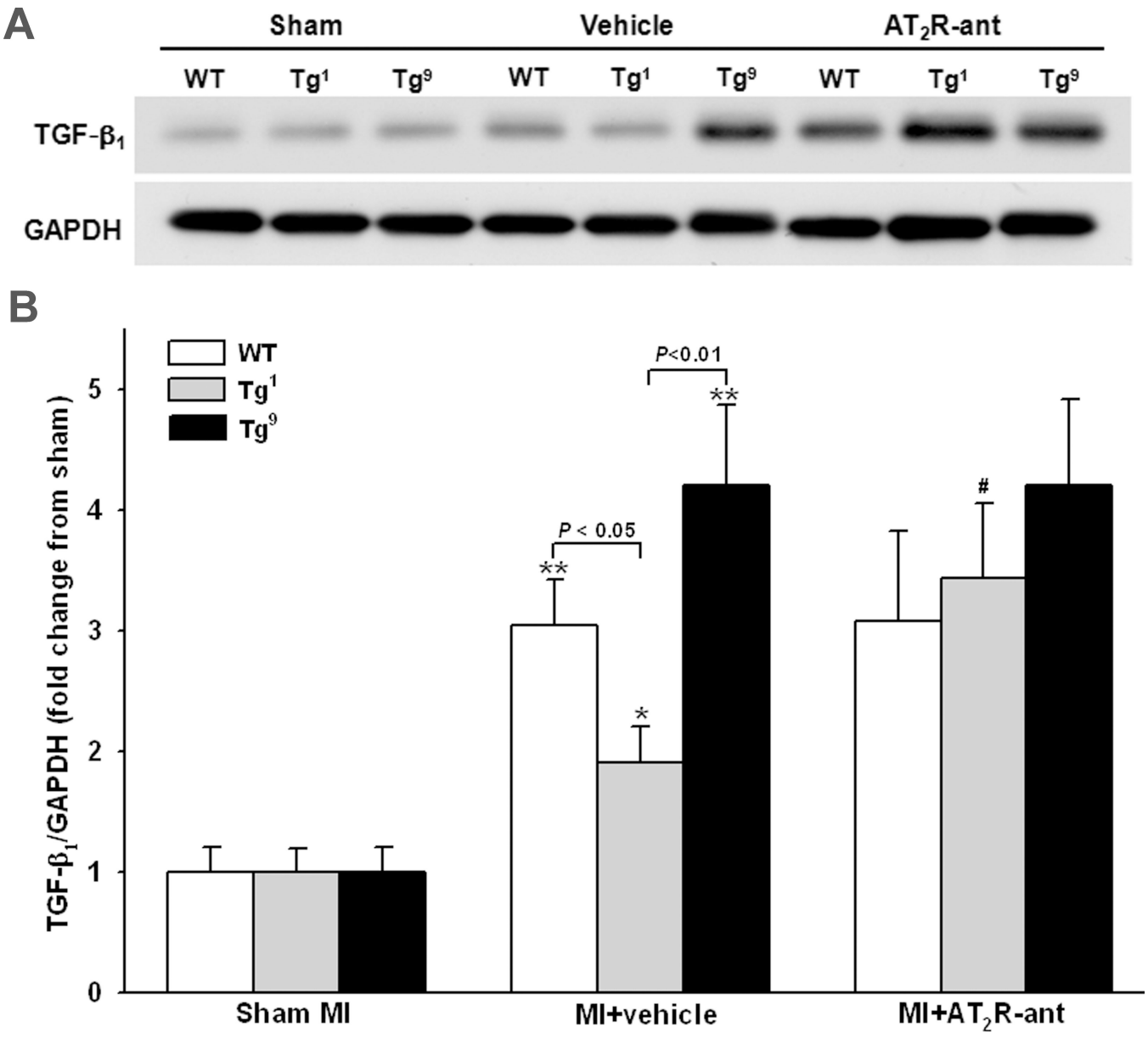

Figure 6.

Effect of cardiac-specific overexpression of AT_2R with or without AT_2R-ant on TGF-β_1 protein expression post-MI. A: Representative Western blots of TGF-β_1 and GAPDH. B: Semi-quantitative analysis of TGF-β_1 protein corrected by GAPDH and expressed as fold change relative to sham controls within strain. $n = 8$ in each group. *: $p < 0.05$, **: $p < 0.01$ MI + vehicle *vs* sham groups within strains; #: $p < 0.05$ MI + AT_2R-ant *vs* MI + vehicle within strain.

Table 1

Effect of cardiac specific overexpression of AT_2R on SBP, HR, tissue weight and infarct size post-MI.

Group	WT			Tg^1			Tg^4			Tg^9		
	Sham *n* = 11	MI		Sham *n* = 9	MI		Sham *n* =12	MI		Sham *n* = 10	MI	
Parameters		Veh *n* = 14	AT_2R-ant *n* = 12		Veh *n* = 12	AT_2R-ant *n* = 14		Veh *n* = 8	AT_2R-ant *n* = 8		Veh *n* = 11	AT_2R-ant *n* = 11
BW (g)	31.9±1	33.0±1	31.2±1	33.7±2	33.9±1	31.9±1	31.4±1	32.7±1	31.9±1	30.0±2	28.2±2	28.8±2
SBP (mmHg)	110±3	102±3	107±3	111±4	104±2	107±4	110±3	103±4	96±5	114±3	107±2	106±2
HR (beats/min)	665±13	698±12	667±12	674±12	671±17	653±14	657±18	675±13	642±30	681±11	681±9	674±9
LVW (mg/10 g)	30.7±1	44.3±2*	45.4±2	28.6±2	38.5±2*†	45.1±2‡	28.7±1	40.4±2*	44.2±2	32.5±1	45.9±2*¶	42.8±1
THW (mg/10 g)	41.0±1	61.4±3*	61.0±3	39.6±2	51.7±3*†	62.0±3‡	38.9±1	56.3±3*	60.7±3	41.8±1	59.3±2*¶	55.1±2
Lungs (mg/10 g)	55.0±3	61.7±5	65.5±6	55.5±4	55.2±3	71.1±5‡	57.0±2	57.5±6	57.1±2	54.7±3	65.0±4*¶	64.1±4
Liver (mg/10 g)	445±17	426±10	482±16‡	436±20	407±8	438±9‡	421±10	391±16	488±26‡	396±15	399±14	452±16‡
IS (%)	--	33.3±2	33.1±4	--	30.2±8	34.8±2	--	31.3±2	33.9±2	--	30.6±2	33.9±5

WT: wild-type littermates; Tg^1, Tg^4 and Tg^9: mice with AT_2R specifically overexpressed in ventricular cardiomyocytes (1, 4 or 9 copies of the AT_2R transgene); MI: myocardial infarction; Veh: vehicle; AT_2R-ant: angiotensin II type 2 receptor antagonist, PD123319; BW: body weight; SBP: systolic blood pressure; HR: heart rate; LVW: left ventricular weight corrected by body weight; THW: total heart weight corrected by body weight; lung and liver weight corrected by body weight; IS: infarct size.

*: $p < 0.05$, MI+ vehicle *vs* sham groups within strain;

†: $p < 0.05$, Tg^1, Tg^4 or Tg^9 *vs* WT within treatments;

‡: $p < 0.05$, AT_2R *vs* vehicle within strain;

¶: $p < 0.05$, Tg^9 *vs* Tg^1 or Tg^4.