Phosphodiesterase 3A1 protects the heart against angiotensin II–induced cardiac remodeling through regulating transforming growth factor-β expression

Phosphodiesterase 3A1 protects the heart against angiotensin II-induced cardiac remodeling through regulating transforming growth factor-β expression

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One table and four figures are contained in this paper.

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Summary

Accumulating evidence suggests that there are direct interactions between ß-adrenergic and angiotensin II signaling pathways, and ß-blocker protects hearts against angiotensin II-induced cardiac remodeling. Phosphodiesterase 3A (PDE3A) regulates ß-adrenergic receptor/protein kinase A signaling by metabolizing cAMP. Therefore, we hypothesized that overexpressed PDE3A has cardioprotective effects against angiotensin II-induced cardiac remodeling by regulating angiotensin II signaling. In the present study, we used transgenic mice with cardiac-specific overexpressed PDE3A1. Continuous administration of angiotensin II caused cardiac hypertrophy in the wild-type mouse heart, but not in the transgenic mouse heart. Angiotensin II induced cardiac fibrosis in both wild-type and transgenic mice, but the extent of fibrosis was less in transgenic mice compared to wild-type mice. Moreover, basal expression levels of transforming growth factor-ß were lower in transgenic mouse hearts, and it remained lower levels after angiotensin II stimulation. These findings

suggest that PDE3A protects the heart from angiotensin II-induced cardiac remodeling through modulating the functional connection between angiotensin II and transforming growth factor-β.

Keywords

Angiotensin II; phosphodiesterase 3A; transforming growth factor-β; cardiac fibrosis

Introduction

Cardiac remodeling occurs in several clinical conditions, such as myocardial infarction, cardiomyopathy, and valvular heart diseases, leading to subsequent heart failure.[1)] Sympathetic nervous system and renin-angiotensin system (RAS) are important contributors in the development of cardiac remodeling. Sympathetic nerve ending secretes noradrenaline, and it binds to ß-adrenergic receptor (ß-AR) on cardiac myocytes to increase in cyclic adenosine 3' ,5'-monophosphate (cAMP) levels through activating adenylyl cyclases. Cyclic nucleotide is hydrolyzed by phosphodiesterases (PDEs), which constitute a superfamily of enzymes grouped into 11 broad families.[2)] In cardiac myocytes, there are at least 6 different families of PDEs, including PDE1, 2, 3, 4, 5, and 8.[3)] Although functional roles of PDEs are not understood rigorously, several studies using genetic engineering transgenic mice, PDE gene knock-out mice, or PDE inhibitors, revealed PDE functions in cardiac myocytes. For instance, PDE2 blunted ß-adrenergic cardiac inotropy by affecting cardiac L-type Ca^{2+}

current and it tightly coupled to the pool of adenylyl cyclases activated by β-AR stimulation.[4] PDE4D was related to hyperphosphorylation of salcolemmal ryanodine receptor, causing a "leaky" receptor.[5] PDE5 inhibitor elicited beneficial effects such as preventing ischemia-reperfusion injury and chronic pressure overload-induced cardiac remodeling.[6,7] PDE8 knockout hearts showed greater isoproterenol (ISO)-induced increases in Ca^{2+} transient, L-type Ca^{2+} currents, and Ca^{2+} spark activity.[8] Among those, PDE3 has been better understood of its physiological functions. The PDE3 gene family contains two subfamilies, PDE3A and PDE3B.[2] PDE3A is highly expressed in platelets, vascular smooth muscle cells, oocytes, and cardiac myocytes, whereas PDE3B is a major PDE in adipose tissue, liver, and pancreas.[2] PDE3A regulates β-adrenergic receptor/protein kinase A (PKA) signaling by metabolizing cAMP, and activated PKA phosphorylates L-type Ca^{2+} channels, phospholamban, troponin I, and myosin-binding protein C. Because these proteins are related to Ca^{2+} mobilization and Ca^{2+} sensitivity of contractile proteins, PDE3A is able to control cardiac inotropic and

lusinotropic effects through regulating PKA activity.[9] Moreover, PDE3A is associated with cardiac remodeling because PKA participates in proliferative signaling by phosphorylating transcriptional regulators such as cAMP responsive element binding protein (CREB) and cAMP responsive element modulator protein, both of which are associated with cardiac remodeling.[10,11]

RAS also plays important roles in cardiac remodeling, and angiotensin II (Ang II), effector molecule of RAS, upregulates expression level of transforming growth factor β (TGF-β).[12] TGF-β exerts potent and diverse effects on many different cell types and are involved in a wide variety of biological processes such as embryonic development, cell growth and differentiation, cell proliferation and survival, fibrosis and inflammatory responses.[13] In the heart, TGF-β is produced by both cardiac myocytes and cardiac fibroblasts. Although TGF-β1, -β2 and -β3 exhibit distinct patterns of regulation in infarcted and hypertrophic hearts, the specific role of these isoforms remains unknown.[13] TGF-β signals via binding to TGF-β type II receptor to activate type I receptor, and subsequently Smad transcription

factors and TGF-β activated-kinase 1, both of which contribute to cardiac remodeling and dysfunction.[13] Several studies have shown that there are direct interactions between β-AR and RAS signaling, for instance, the Ang II type 1 receptor blocker effectively blocks downstream signaling of β-AR.[14] Olmesartan inhibits isoproterenol-induced cardiac hypertrophy by repressing oxidative stress.[15]

Given the interactions between β-AR and Ang II signaling, we hypothesized that PDE3A regulates not only β-AR signaling, but also Ang II signaling and subsequent cardiac remodeling. To test this hypothesis, we used transgenic (TG) mice with cardiac-specific overexpressed PDE3A1, which are characterized by reduced heart rate, reduced left ventricular ejection fraction, lower response to isoproterenol stimulation, similar survival rate to wild-type (WT) mice, and high tolerance to ischemia/reperfusion injury through an anti-apoptotic effect.[16] In the present study, our data revealed that PDE3A1 prevents Ang II-induced cardiac hypertrophy and fibrosis via regulating Ang II/TGF-β axis.

Methods

Animals

The investigations conformed to the *Guide for the Care and Use of Laboratory Animals 8th edition* published by the US National Research Council. Our research protocol was approved by the institutional review board, and all animal experiments were conducted in accordance with the guidelines of Fukushima Medical University Animal Research Committee. TG mice generated with cardiac-specific overexpression of PDE3A1 have been described previously.[16] The TG mice express PDE3A1 mRNA 10-fold higher, and protein levels and enzyme activity are also 10-fold increased compared to WT mice.[16] Male PDE3A1 overexpressed TG mice and WT littermate mice at the age of 10 to 12 weeks were used for experiments.

Study protocol

To induce cardiac remodeling, either Ang II (800 ng/min per kg for 10 days) or vehicle was continuously infused subcutaneously using Alzet

osmotic mini-pumps (model 1002, Durect Corp, Cupertino, CA) in WT and TG mice. Mouse hearts were excised at 10 days after Ang II infusion. Excised hearts were washed with saline to remove blood, and whole hearts were weighed. Hearts were used for histological and immunoblotting analyses.

Measurements of blood pressure

At seven days after subcutaneous infusion of Ang II or saline, systolic blood pressure was measured by the tail-cuff method using programmable sphygmomanometer (BP-98A-L, Softron, Tokyo, Japan) under free from anesthesia as previously reported.[17)]

Histological analysis

Excised hearts were fixed in 4% buffered paraformaldehyde, and embedded in paraffin. Hearts were transversely sectioned (5 μm), deparaffinized and stained with hematoxylin-eosin or Elastica-Masson. The cardiomyocyte cross sectional area was measured in more than 200

cardiomyocytes per section for each animal. The fibrosis fraction was defined as a ratio of the Elastica-Masson stained blue area to the myocardial area.[18)]

Echocardiography

Echocardiography was performed in WT and TG mice at 10 days after Ang II or vehicle administration using Vevo 2100 echocardiography machine equipped with a 40 MHz frequency probe (VisualSonics, Toronto, Canada). For anesthesia, 1.5% isoflurane was used. M-mode image acquisition was performed at the level of cardiac papillary muscle. Anterior and posterior wall thickness, left ventricular dimensions at end-diastole and end-systole, and left ventricular ejection fraction were assessed using analysis software in Vevo 2100 Imaging System.[18)]

Western blotting

Heart lysates were prepared in a modified RIPA buffer containing the following: 50 mmol/L Tris-HCl pH 7.4, 1% NP-40, 0.1% SDS, 150 mmol/L

NaCl, 1 mmol/L PMSF, 1 mmol/L sodium orthovanadate, and protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO) as previous reports described.[18] Total protein lysates were separated using SDS-PAGE, transferred to a PVDF membrane and immunodetected with an anti-TGF-ß mouse monoclonal antibody (Cell Signaling, Beverly, MA), anti-α-tubulin antibody (Santa Cruz, Biotechnology, Dallas, TX). Blots were quantified using NIH image J software.

Statistics

Data are expressed as mean ± SEM. Comparisons between two groups were evaluated using student's t-test. One-way ANOVA followed Tukey's post-hoc test was used for multiple comparisons. P-values <0.05 were considered statistically significant.

Results

Overexpressed PDE3A1 attenuated Ang II-induced cardiac remodeling

To assess the effect of PDE3A1 on Ang II-induced cardiac remodeling, we performed continuous subcutaneous infusion of Ang II using osmotic mini-pumps. Echocardiographic and hemodynamic data at 10 days after Ang II or vehicle treatment are shown in Table 1. Ang II increased systolic blood pressure to similar levels in both WT and TG mice. We have already reported that the TG mice showed reduced cardiac function, characterized by enlarged left ventricular internal diameter and reduced left ventricular ejection fraction.[16] Consistent with our previous report, vehicle-TG mice displayed large left ventricular dimensions, lower left ventricular ejection fraction and slower heart rate compared to vehicle-WT mice. Interestingly, left ventricular wall thickness was increased in WT mice after Ang II stimulation, but not in TG mice, suggesting that Ang II-induced cardiac hypertrophy was attenuated in TG mice. After echocardiography, mice were sacrificed, and hearts were excised. As shown in Figure 1a, heart

size was enlarged in WT mice after Ang II, but Ang II-TG mice showed similar size compared to vehicle-TG mice. Although modest cardiac hypertrophy already occurred in vehicle-TG mouse hearts, Ang II failed to induce further cardiac hypertrophy in TG mice. Consistent with these data, heart weight to tibia length ratios (Figure 1b) and cardiomyocyte cross sectional area (Figure 1c) were increased in WT mice after Ang II, but not in TG mice.

Ang II-induced cardiac fibrosis was inhibited in TG mouse hearts

Cardiac fibrosis is well known characteristics of Ang II-induced cardiac remodeling.[19] To evaluate the extent of cardiac fibrosis, Elastica-Masson staining was performed. As shown in Figure 2, Ang II increased cardiac fibrosis in both WT and TG mice compared to same strain mice given vehicle. However, the TG mouse heart showed less fibrosis compared to the WT mouse heart after Ang II infusion.

Overexpressed PDE3A1 inhibited Ang II-induced TGF-β expression

It has been reported that TGF-β is an effector molecule of Ang II-induced cardiac hypertrophy and fibrosis.[12)] To investigate the mechanism by which PDE3A1 attenuated cardiac remodeling and fibrosis, we examined the protein expression levels of TGF-β in the myocardium. As shown in Figure 3, TGF-β protein expression levels were lower in the vehicle-TG mouse heart compared with the vehicle-WT mouse heart. Ang II stimulation increased TGF-β protein levels in WT mice, but it remained lower levels after Ang II stimulation in TG mice.

Discussion

In the present study, we demonstrated that continuous infusion of Ang II caused cardiac hypertrophy in WT mice, but not in TG mice. We also showed that Ang II induced cardiac fibrosis in both WT and TG mice, but the extent of fibrosis was less in the TG mouse heart compared with the WT mouse heart. Moreover, basal expression levels of TGF-ß, which is implicated as a downstream effector of Ang II,[12] was suppressed in TG hearts, and it remained lower levels after Ang II stimulation in TG hearts compared with WT hearts. These findings suggest that PDE3A protects the heart from Ang II-induced cardiac remodeling and fibrosis through modulating the functional connection between Ang II and TGF-ß.

Pivotal roles of TGF-ß in cardiac remodeling are well described in both experimental and clinical models.[20-23] Thus, regulating TGF-ß signaling is expected to be an attractive therapeutic target. Although the association between RAS and TGF-ß signaling is reported, ß-AR signaling pathways also regulate TGF-ß signaling.[12] Several reports have shown that ß-AR signaling

is enhanced by TGF-β, which serves as a downstream signaling of Ang II/TGF-β.[24-26] Considering overexpressed PDE3A1 behaves as like β-blocker by catabolizing cAMP to inhibit β-AR/PKA axis, lower TGF-β level in TG hearts implies that β-AR signaling may function as an upstream regulator of TGF-β expression. It has been reported that PDE3A expression levels are decreased in heart failure.[27] Conversely, TGF-β expression levels are upregulated in the failing heart.[22] These findings support the concept that repressing PDE3A would increase the expression levels of TGF-β, and subsequently enhance cardiac remodeling in the failing heart. Several molecules are reported as upstream regulators of TGF-β, such as nicotinamide adenine dinucleotide phosphate oxidase, protein kinase C, p38 mitogen-activated protein kinase (p38 MAPK), and activator protein-1.[28] There is little evidence of interaction between PDE3A and TGF-β, but a recent study has shown that A-kinase anchoring protein (AKAP)-Lbc enhanced p38 MAPK-mediated hypertrophic responses.[29] Considering the fact that AKAP-Lbc is tethered with PKA, PDE3A may affect p38

MAPK-induced TGF expression via PKA regulation (Figure 4). However, further studies are needed to elucidate more detailed molecular mechanisms between β-AR/TGF-β signaling.

Recent studies have demonstrated that PDE3A1 has cardioprotective effects through regulating cardiac apoptosis, which is regulated by PDE3A/inducible cAMP early repressor feedback loop.[30] In the present study, our findings would provide the novel mechanism of PDE3A1 for cardio-protection by modulating Ang II/TGF-β axis. It would be ideal to evaluate whether Ang II stimulation exaggerates cardiac remodeling in PDE3A knock-out mice in the future. Other PDE families might also contribute to cardiac remodeling. For example, PDE1, which is believed to be important in the crosstalk of second messenger Ca^{2+} and cyclic nucleotide signaling,[2] regulates both Ang II and isoproterenol-induced cardiomyocyte hypertrophy.[31] PDE4 also regulated β-AR signaling,[32] and $PDE4D^{-/-}$ mice developed progressive cardiomyopathy and accelerated heart failure after myocardial infarction.[5] Thus, cAMP regulation in the setting of heart failure

might be orchestrated by not only PDE3A but also other PDEs. Further studies are needed to elucidate roles of other PDEs in the situation of cardiac remodeling using PDE isoform specific inhibitors and/or genetically engineered mice.

Clinical perspective

In the clinical situation, a PDE3A inhibitor has been widely used for the treatment of acute decompensated heart failure, but long-term administration of it increases in mortality due to excess inotropic effects, subsequent arrhythmia, and sudden cardiac death. In the present study, we proposed a novel approach to protect the cardiac remodeling by overexpressing PDE3A in the heart. Although PDE3A activator is not available at this time, genetic engineering could express PDE3A in the cardiac tissues sufficiently.

Conclusions

PDE3A protects the heart from Ang II-induced cardiac remodeling

through modulating the functional connection between Ang II and TGF-ß.

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Figure legends

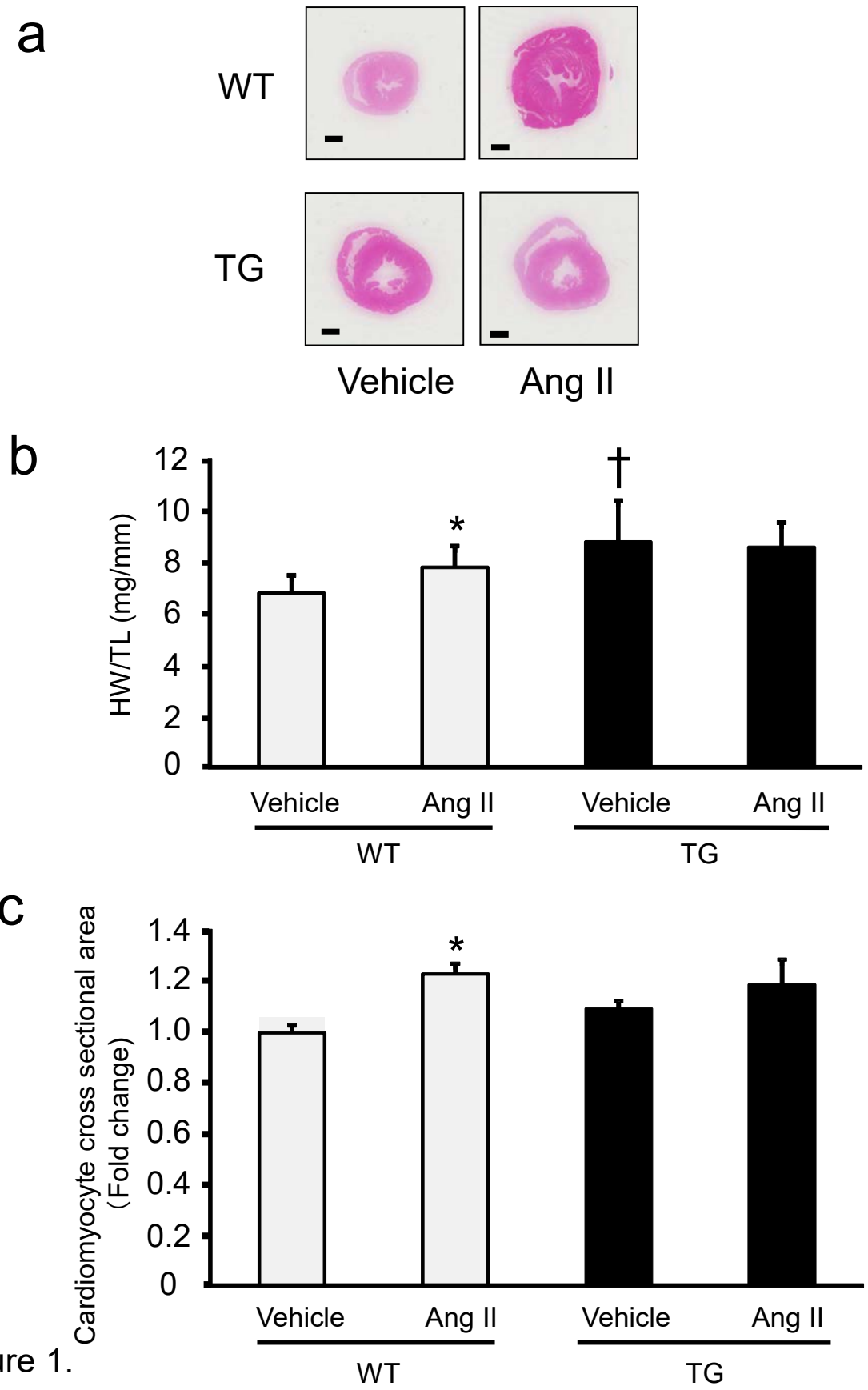
Figure 1. Effects of PDE3A on Ang II-induced cardiac hypertrophy. (a) Representative cross sectional images of the ventricle of WT or TG mice treated with Ang II or vehicle. Bars, 1 mm. (b) Quantitative data showing heart weight (HW) to tibia length (TL) ratio after Ang II or vehicle treatment. (c) Quantitative data showing cardiomyocyte cross sectional area of the left ventricle from either WT or TG mice treated with Ang II or vehicle. Values are mean ± SEM (n=5-7 in each group). *P<0.05 compared with same genotype mice given vehicle. †P<0.05 compared with WT.

Figure 2. Effects of PDE3A on Ang II-induced cardiac fibrosis. (a) Representative myocardial sections of Elastica-Masson stain of the left ventricle of WT or TG mice treated with Ang II or vehicle. Bars, 100 μm. (b) Quantitative data showing fibrosis fraction after Ang II or vehicle treatment. Values are mean ± SEM (n=4-5 in each group). **P<0.01 compared with

vehicle in the same genotype. †P<0.05 compared with WT.

Figure 3. Protein expression levels of TGF-β after Ang II treatment. (a) Representative immunoblotting of TGF-β in the left ventricle of WT or TG mice treated with Ang II or vehicle. (b) Quantitative data showing TGF-β expression levels normalized to α-tubulin. Values are mean ± SEM (n=6 in each group). *P<0.05 compared with vehicle in the same genotype. ††P<0.01 compared with WT.

Figure 4. A diagram presenting our hypothesis. PDE3A represses PKA activity through cAMP metabolism, resulting in inhibited PKA-mediated cardiac remodeling and reduction of TGF-β expression via AKAP-Lbc-mediated p38 MAPK activation.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TG
Vehicle
Ang II
b
HW/TL (mg/mm)
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10
8
6
4
2
0
*
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Vehicle
Ang II
Vehicle
Ang II
WT
TG
c
Cardiomyocyte cross sectional area
(Fold change)
1.4
1.2
1.0
0.8
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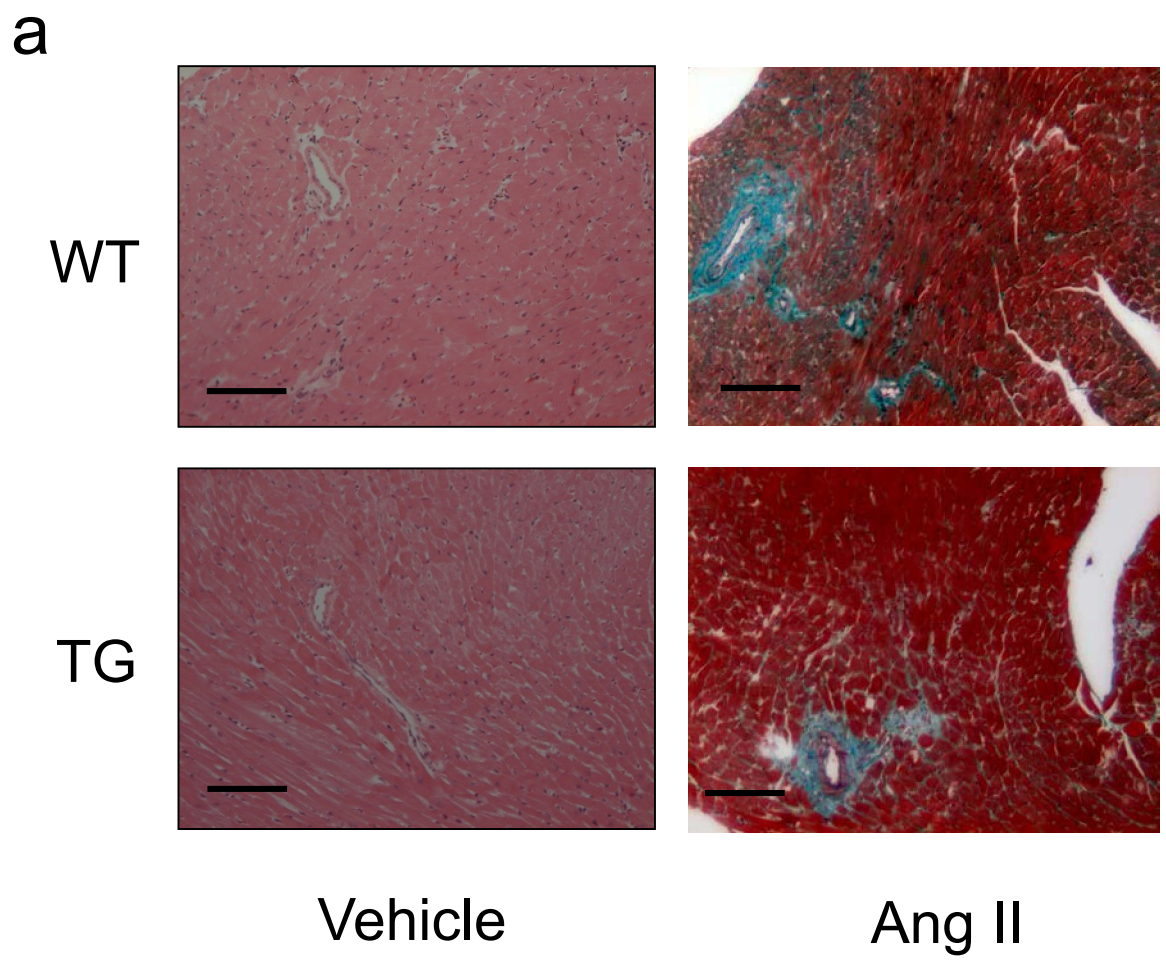
*
Vehicle
Ang II
Vehicle
Ang II
WT
TG

Figure 1.


a
WT
TG
Vehicle
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Fibrosis fraction (%)
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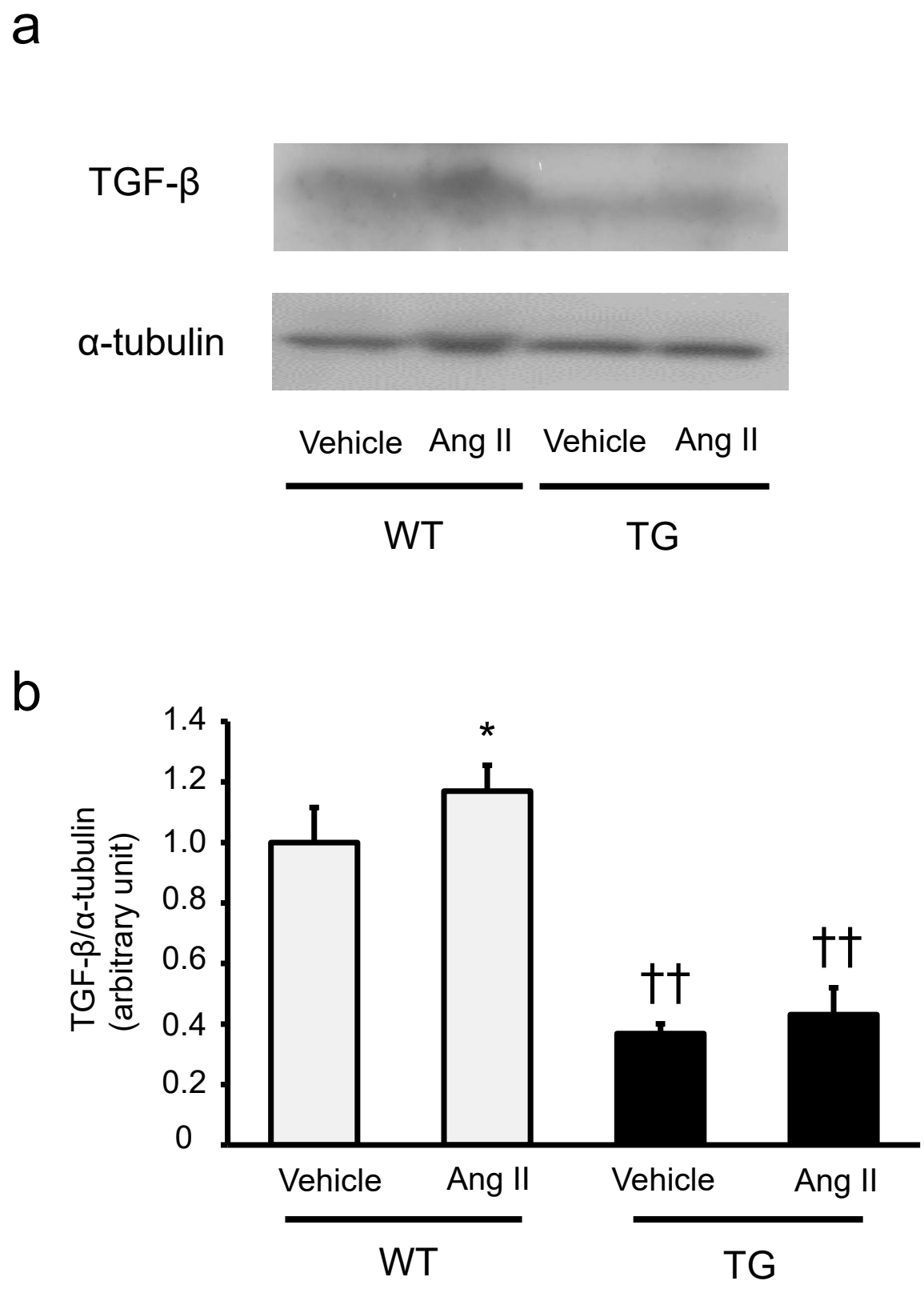
Vehicle
Ang II
Vehicle
Ang II
WT
TG

Figure 2.


a
TGF-β
α-tubulin
Vehicle
Ang II
Vehicle
Ang II
WT
TG
b
TGF-β/α-tubulin
(arbitrary unit)
1.4
1.2
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0.6
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Vehicle
Ang II
Vehicle
Ang II
WT
TG

Figure 3.

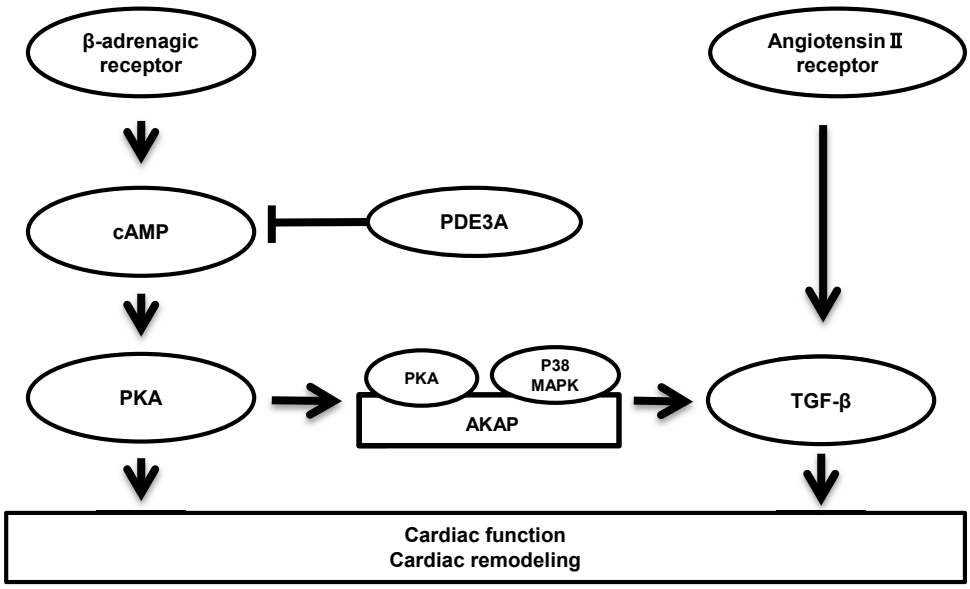
β-adrenagic receptor
Angiotensin Ⅱ receptor
cAMP
PDE3A
PKA
PKA
P38 MAPK
AKAP
TGF-β
Cardiac function
Cardiac remodeling

Figure 4.

Table 1. Comparisons of heart rate, systolic blood pressure, body weight, and echocardiographic parameters of WT and TG mice.

	WT	WT with Ang II	TG	TG with Ang II
HR, bpm	531.9 ± 41.0	698.4 ± 25.1*	347.7 ± 10.6††	414.5 ± 8.3**††
SBP, mmHg	108.6 ± 3.4	144.4 ± 13.8**	102.6 ± 2.4	136.5 ± 13.1**
AWth, mm	0.82 ± 0.07	1.24 ± 0.17**	0.93 ± 0.03	0.99 ± 0.09††
PWth, mm	0.83 ± 0.10	1.19 ± 0.04**	0.89 ± 0.06	0.99 ± 0.08††
LVDd, mm	3.59 ± 0.25	2.99 ± 0.16**	4.71 ± 0.13††	3.91 ± 0.56**††
LVDs, mm	2.67 ± 0.58	2.32 ± 0.12*	3.69 ± 0.02††	2.93 ± 0.37**††
LVEF, %	74.5 ± 13.8	77.6 ± 2.6	43.6 ± 1.4††	49.8 ± 6.9††
BW, g	28.2 ± 1.2	23.9 ± 1.9**	28.2 ± 1.5	24.4 ± 2.7**

Values are expressed as mean ± SEM from 6 to 7 mice.

HR, heart rate; SBP, systolic blood pressure; AWth, anterior wall thicknesss; PWth, posterior wall thickness; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic diameter dimension; LVEF, left ventricular ejection fraction; BW, body weight; WT, wild-type mice;TG, PDE3A1 overexpressed mice; AngII, angiotensin II. *$P<0.05$, **$P<0.01$ vs. same genotype mice given vehicle, †$P<0.05$, ††$P<0.01$ vs. WT mice.