Phosphodiesterase 3A1 Protects the Heart Against Angiotensin II–induced Cardiac Remodeling Through Regulation of Transforming Growth Factor-β Expression

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Summary

Accumulating evidence suggests that there are direct interactions between β-adrenergic and angiotensin II signaling pathways, and β-blockers protect the heart against angiotensin II-induced cardiac remodeling. Phosphodiesterase 3A (PDE3A) regulates β-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. We showed that 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-β were lower in transgenic mouse hearts, and it remained at lower levels after angiotensin II stimulation. These findings suggest that PDE3A protects the heart from angiotensin II-induced cardiac remodeling through its modulation of the functional connection between angiotensin II and transforming growth factor-β. (Int Heart J 2014; 55: 165-168)

Key words: Cardiac fibrosis

Cardiac remodeling occurs in several clinical conditions, such as myocardial infarction, cardiomyopathy, and valvular heart diseases, leading to subsequent heart failure.[1)] The sympathetic nervous system and renin-angiotensin system (RAS) are important contributors in the development of cardiac remodeling. Several studies have shown that there are direct interactions between β-adrenergic receptor (β-AR) and RAS signaling, for instance, an angiotensin II (Ang II) type 1 receptor blocker has been shown to effectively block the downstream signaling of β-AR.[2)] Olmesartan inhibits isoproterenol-induced cardiac hypertrophy by repressing oxidative stress.[3)] β-AR signaling is regulated by phosphodiesterases (PDEs) through hydrolyzing cyclic nucleotide. In cardiac myocytes, there are at least 6 different families of cAMP-hydrolyzing PDE, including PDE1, 2, 3, 4, 5, and 8.[4)] Each PDE has a different role based on its special distribution and cyclic nucleotide compartmentation.[5)] Among these PDEs, PDE3A regulates β-AR signaling by catalyzing cAMP, which plays a fundamental role in physiological cardiac performance.[5,6)] Therefore, 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 cardiac-specific overexpressed PDE3A1 mice, which are characterized by a reduced heart rate, reduced left ventricular ejection fraction, lower response to isoproterenol stimulation, and high tolerance to ischemia/reperfusion injury.[6)] In the present study, the data revealed that PDE3A1 prevents Ang II-induced cardiac hypertrophy and fibrosis via regulation of the Ang II/transforming growth factor-β (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 the Fukushima Medical University Animal Research Committee. Mice generated with cardiac-specific overexpression of PDE3A1 have been described previously.[6)] The mice express PDE3A1 mRNA 10-fold higher, and protein levels and enzyme activity are also increased 10-fold compared to those in WT heart. Male PDE3A1 overexpressed transgenic mice (TG) and wild-type littermate mice (WT) 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

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Table. Echocardiographic and Hemodynamic Data

	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**
AWd, mm	0.82 ± 0.07	1.24 ± 0.17**	0.93 ± 0.03	0.99 ± 0.09††
PWd, 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	2.04 ± 0.58	3.97 ± 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 indicates heart rate; SBP, systolic blood pressure; AWd, anterior wall end-diastolic thickness; PWd, posterior wall end-diastolic thickness; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; LVEF, left ventricular ejection fraction; BW, body weight; WT, wild-type mice; TG, PDE3A1 overexpressed mice; and Ang II, angiotensin II. $^{*}P < 0.05$, $^{**}P < 0.01$ versus same genotype mice given vehicle, $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ versus WT mice.

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 the whole hearts were weighed. Hearts were used for histological and immunoblotting analyses.

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 Masson's trichrome. The cardiomyocyte cross sectional area was measured in more than 200 cardiomyocytes per section for each animal. The fibrosis fraction was defined as the ratio of the Masson's trichrome stained blue area to the myocardial area.

Echocardiography: Echocardiography was performed in WT and TG mice at 10 days after Ang II or vehicle administration using a Vevo 2100 echocardiograph 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 the 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 a Vevo 2100 Imaging System.

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). Total protein lysates were separated using SDS-PAGE, transferred to a PVDF membrane and immunodetected with an anti-TGF-β mouse monoclonal antibody (Cell Signaling, Beverly, MA) and anti-α-tubulin antibody (Santa Cruz Biotechnology, Dallas, TX). Blots were quantified using NIH image J software.

Statistics: Data are expressed as the mean ± SEM. Comparisons between two groups were evaluated using Student's *t*-test. One-way ANOVA followed by Tukey's post-hoc test were used for multiple comparisons. $P < 0.05$ 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. 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.[6] Consistent with our previous report, vehicle-TG mice displayed large left ventricular dimensions, a 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, the mice were sacrificed and the hearts excised. As shown in Figure 1A, heart size was enlarged in WT mice after Ang II, but heart size was similar in Ang II-TG mice and 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 a well- known characteristic of Ang II-induced cardiac remodeling.[7] To evaluate the extent of cardiac fibrosis, Masson's trichrome staining was performed. As shown in Figure 2, Ang II increased cardiac fibrosis in both WT and TG mice compared to the same strain of 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: Accumulating evidence suggests that TGF-β is an effector molecule of Ang II-induced cardiac hypertrophy and fibrosis.[8] 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 they remained at lower levels after Ang II stimulation in TG mice.

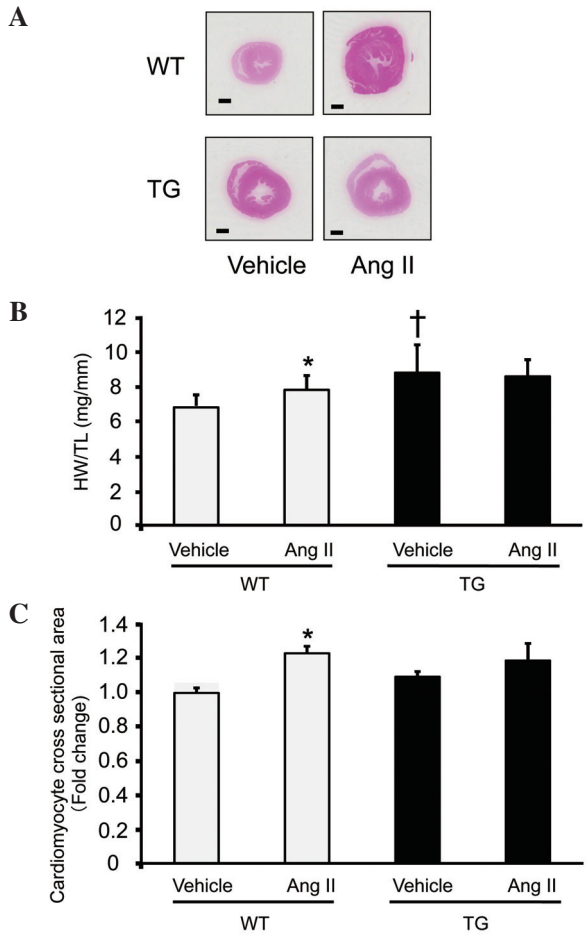

Figure 1. Effects of PDE3A on Ang II-induced cardiac hypertrophy. (**A**) Representative cross sectional images of the ventricles of WT or TG mice treated with Ang II or vehicle. Bars, 1 mm. (**B**) Quantitative data showing heart weight (HW) to tibia length (TL) ratio after Ang II or vehicle treatment. (**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. $^{\dagger}P < 0.05$ compared with WT.

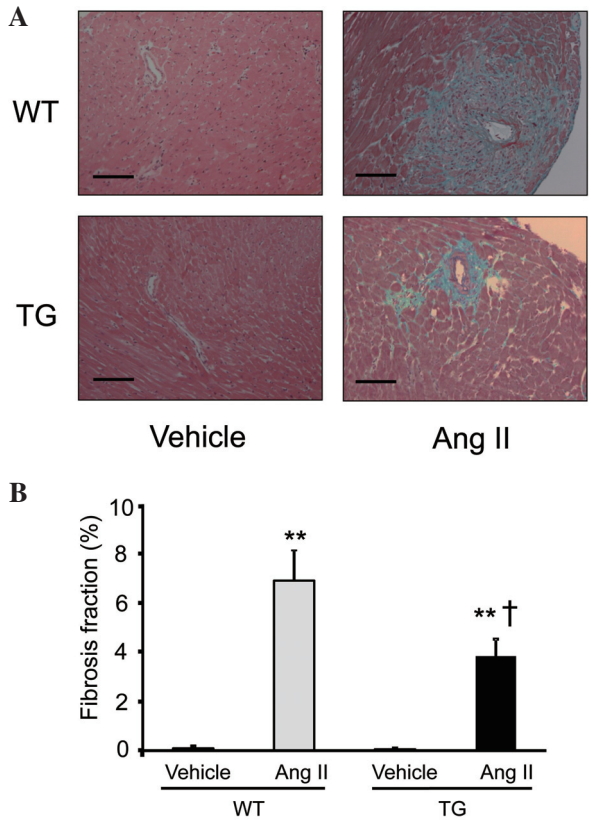

Figure 2. Effects of PDE3A on Ang II-induced cardiac fibrosis. (**A**) Representative myocardial sections of Masson's trichrome stain of the left ventricle of WT or TG mice treated with Ang II or vehicle. Bars, 100 μm. (**B**) Quantitative data showing fibrosis fraction after Ang II or vehicle treatment. Values are mean ± SEM (n = 4-5 in each group). $^{**}P < 0.01$ compared with vehicle in the same genotype. $^{\dagger}P < 0.05$ compared with WT.

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 than in the WT mouse heart. Moreover, basal expression levels of TGF-β, which is implicated as a downstream effector of Ang II,[8] were suppressed in TG hearts, and remained at 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 its modulation of the functional connection between Ang II and TGF-β.

The pivotal roles of TGF-β in cardiac remodeling are well described in both experimental and clinical models.[9-12] Thus, regulating TGF-β signaling is expected to be an attractive therapeutic target. Although the association between RAS and TGF-β signaling is well documented, β-AR signaling pathways also regulate TGF-β signaling.[8] Several reports have showed that β-AR signaling is enhanced by TGF-β, which serves as a downstream signaling of Ang II/TGF-β.[13-15] Considering overexpressed PDE3A1 behaves like a β-blocker by catabolizing cAMP to inhibit the β-AR/PKA axis, a 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.[16] Conversely, TGF-β expression levels are upregulated in the failing heart.[11] 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, and activator protein-1.[17] Further studies are needed to elucidate more detailed molecular mechanisms between TGF-β/β-AR signaling. Previous studies have demonstrated that PDE3A1 has cardioprotective effects through regulating cardiac apoptosis, which is regulated by a PDE3A/ICER feedback loop.[18] The present findings provide a novel mechanism of PDE3A1 for cardioprotection by modulating the Ang II/TGF-β axis. It would be ideal to evaluate whether Ang II

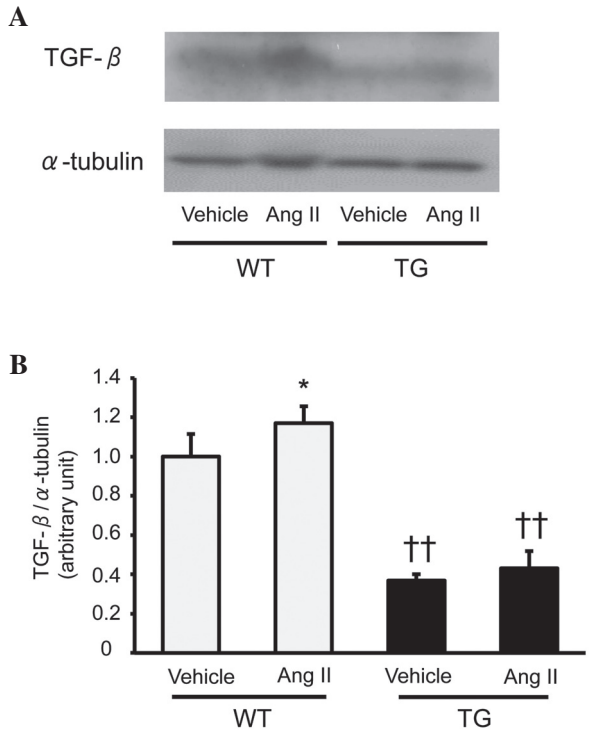

Figure 3. Protein expression levels of TGF-β after Ang II treatment. (**A**) Representative immunoblotting of TGF-β in the left ventricle of WT or TG mice treated with Ang II or vehicle. (**B**) Quantitative data showing TGF-β expression levels normalized to α-tubulin. Values are mean ± SEM ($n = 6$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

stimulation exaggerates cardiac remodeling in PDE3A knockout mice in the future. Other PDE families might also contribute to pathological cardiac remodeling. For example, PDE1, which is believed to be important in the crosstalk of second messenger Ca^{2+} and cyclic nucleotide signaling,[19] regulates both Ang II and isoproterenol-induced cardiomyocyte hypertrophy.[20] PDE4 also regulated β-AR signaling,[21] and PDE4D$^{-/-}$ mice developed progressive cardiomyopathy and accelerated heart failure after myocardial infarction.[22] 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 other roles for PDEs in the situation of cardiac remodeling using PDE inhibitors and transgenic mice.

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