

Research Article

Rosiglitazone Attenuated Endothelin-1-Induced Vasoconstriction of Pulmonary Arteries in the Rat Model of Pulmonary Arterial Hypertension via Differential Regulation of ET-1 Receptors

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Pulmonary arterial hypertension (PAH) is a fatal disease characterized by a progressive increase in pulmonary arterial pressure leading to right ventricular failure and death. Activation of the endothelin (ET)-1 system has been demonstrated in plasma and lung tissue of PAH patients as well as in animal models of PAH. Recently, peroxisome proliferator-activated receptor γ (PPAR γ) agonists have been shown to ameliorate PAH. The present study aimed to investigate the mechanism for the antivasoconstrictive effects of rosiglitazone in response to ET-1 in PAH. Sprague-Dawley rats were exposed to chronic hypoxia (10% oxygen) for 3 weeks. Pulmonary arteries from PAH rats showed an enhanced vasoconstriction in response to ET-1. Treatment with PPAR γ agonist rosiglitazone (20 mg/kg per day) with oral gavage for 3 days attenuated the vasocontractive effect of ET-1. The effect of rosiglitazone was lost in the presence of L-NAME, indicating a nitric oxide-dependent mechanism. Western blotting revealed that rosiglitazone increased ET_BR but decreased ET_AR level in pulmonary arteries from PAH rats. ET_BR antagonist A192621 diminished the effect of rosiglitazone on ET-1-induced contraction. These results demonstrated that rosiglitazone attenuated ET-1-induced pulmonary vasoconstriction in PAH through differential regulation of the subtypes of ET-1 receptors and, thus, provided a new mechanism for the therapeutic use of PPAR γ agonists in PAH.

1. Introduction

Pulmonary arterial hypertension (PAH) is characterized by a progressive increase of pulmonary vascular resistance, leading to right ventricular failure and death [1]. ET-1 plasma level was elevated in the patients and experimental models for PAH [2, 3]. Expression of ET-1 was increased in lung tissues of PAH patients, predominantly in pulmonary arteries [4, 5]. ET-1 has 2 major subtypes of receptors: ET-A receptor (ET_AR) is expressed on vascular smooth muscle cells (SMCs) and mediates vasoconstriction, whereas ET-B receptor (ET_BR) is predominantly expressed in endothelial cells (ECs), where it

primarily mediates vasodilatation and the clearance of ET-1. Expression of ET_AR was upregulated in the lung tissues and pulmonary arteries from PAH patients with a well-established pathophysiological role [6–8]. However, a role of ET_BR was rather controversial with the reports of unaltered, increased, or decreased expressions in the vessel tissues from various PAH conditions [9–15].

Emerging evidence suggests that peroxisome proliferator-activated receptor- γ (PPAR γ) agonists might have therapeutic role in treating PAH [16]. PPAR γ regulates the transcription of genes involved in glucose and lipid metabolism, inflammation, as well as vascular remodeling [17–19]. The

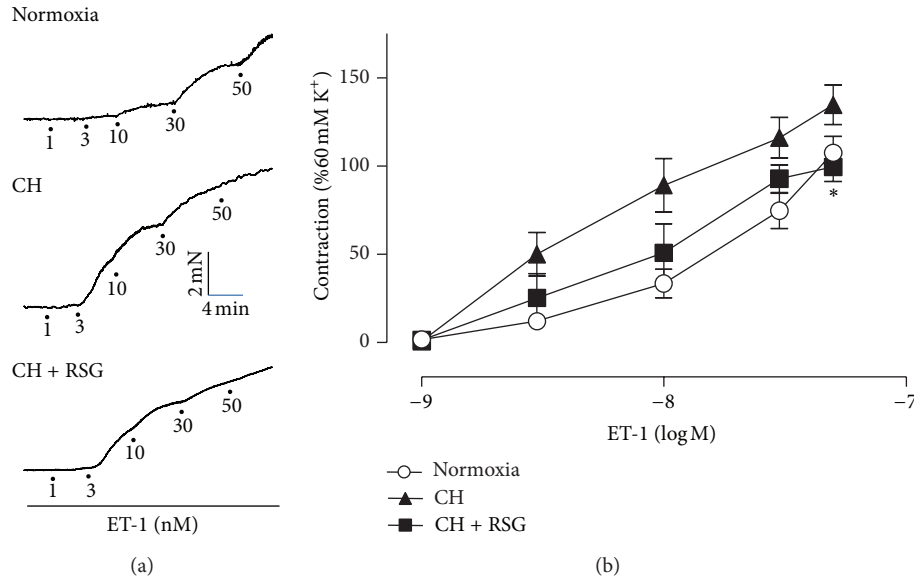


FIGURE 1: (a) Representative recordings of ET-1-induced contractions of pulmonary arteries from normoxia-, chronically hypoxic- (CH-), or rosiglitazone- (RSG-) treated CH-rats. (b) RSG ameliorated ET-1-mediated vasoconstriction in pulmonary arteries from the rats with PAH. Data were mean \pm SEM from 5 to 7 rats. * $P < 0.05$ CH + RSG versus CH group.

expression of PPAR γ was reduced in the lungs from the PAH patients and the rat models [20, 21]. Similarly, mice with deletion of PPAR γ in SMCs or ECs developed PAH. Pharmacological activation of PPAR γ ameliorated PAH. [21–25]. In ECs, PPAR γ activators inhibited thrombin- or oxidized low-density lipoproteins- (LDL-) induced ET-1 production [26, 27]. In particular, we recently observed that PPAR γ agonist rosiglitazone attenuated ET-1-induced vasoconstriction through upregulation of ET_BR in ECs [28]. However, whether the regulation of ET_BR accounts for the ameliorative effect of PPAR γ agonists in PAH arteries remains to be elucidated. In the present study, we examined the role of rosiglitazone on ET-1-induced vasoconstriction of pulmonary arteries in rat PAH models and the underlying mechanism.

2. Materials and Methods

2.1. Animals, Cell Culture, and Reagents. Male Sprague-Dawley rats were used and the experiments were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals with the approval by the institutional committee. Polyclonal rabbit anti-ET_BR antibody was from Abcam. Polyclonal rabbit anti-ET_AR was from Santa Cruz Biotechnology. ET-1 and N^G-nitro-L-arginine methyl ester (L-NAME) were from Sigma-Aldrich Co., rosiglitazone was from GlaxoSmithKline, and A192621 was from Abbott Laboratories.

2.2. Chronic Hypoxia Induced PAH in Rat. Rats were exposed to normobaric hypoxia (10% oxygen) or normoxia (21% oxygen) for 3 weeks and then treated with rosiglitazone (20 mg/kg per day) or water with oral gavage for 3 days.

2.3. Isometric Tension Measurement. Left lungs were removed and placed in oxygenated Krebs-Henseleit solution. Pulmonary arteries were carefully dissected from adjacent connective tissue and cut into several ring segments of ≈ 2 mm long for measuring isometric force. Organ chambers (Multi Myograph System, Danish Myo Technology A/S) were filled with (37°C) Krebs solution containing (in mmol/L) 119.0 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.0 MgCl₂, 25.0 NaHCO₃, 1.2 KH₂PO₄, and 11.0 D-glucose. The Krebs solution in the organ bath was initially open to room air, being bubbled with mixed 95% O₂ and 5% CO₂. Each ring was suspended between 2 tungsten wires (diameter, 40 μ m) in the chamber under optimal resting tension (2.5 mN as previously determined for the pulmonary arteries) and left for 90-minute equilibration. Vasoreactivity was measured to compare contractions in response to ET-1 (1 to 50 nmol/L) in the absence and presence of L-NAME (100 μ mol/L). The effects of antagonist of ET_BR were tested on ET-1-induced contractions.

2.4. Western Blot Analysis. Pulmonary arteries were dissected, frozen in liquid nitrogen, and homogenized in RIPA lysis buffer containing protease inhibitors. Protein lysates separated on 12.5% sodium dodecyl sulfate polyacrylamide gels (SDS-PAGE) and transferred to PVDF membranes, which were blocked with 5% nonfat milk in Tris-buffered saline-Tween (0.2%) (TBS-T) for 1h, incubated overnight with primary antibody and then horseradish peroxidase-(HRP-) conjugated secondary antibody, and visualized with ECL reagent.

2.5. Statistical Analysis. Results represent mean \pm SEM. Comparisons among groups involved ANOVA followed by

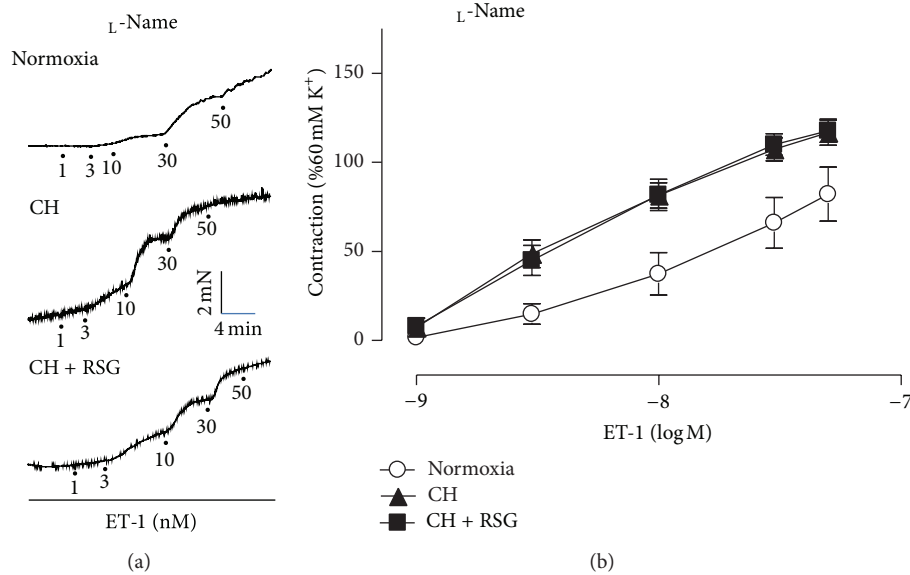


FIGURE 2: (a) Representative recordings of ET-1-induced contractions pretreated with L -NAME (100 μ mol/L) in pulmonary arteries from normoxia-, CH-, or RSG-treated CH-rats. (b) The effect of RSG on ET-1-mediated vasoconstriction was abrogated in the presence of L -NAME (100 μ mol/L). Data were mean \pm SEM from 5 to 7 rats.

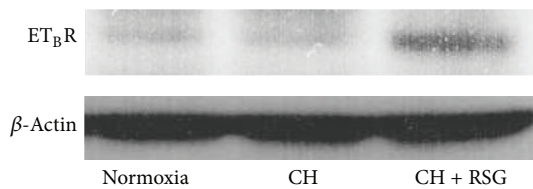


FIGURE 3: Rosiglitazone upregulated ET_BR expression in rats with PAH. Western blotting was performed with the protein samples extracted from the pulmonary arteries of normoxia-, CH-, or RSG-treated CH-rats. Data shown are representative of three independent experiments.

unpaired Student's t -test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Rosiglitazone Ameliorated ET-1-Mediated Vasoconstriction in Rats with PAH. To investigate the effect of rosiglitazone on vasoconstriction of pulmonary arteries induced by ET-1, pulmonary arteries from normoxia-, chronic hypoxia- (CH-), and rosiglitazone-treated CH-rats were dissected from groups of animals for isometric tension measurement responding to ET-1. The ET-1-induced contractions in pulmonary arteries were elevated in PAH rats compared to the normoxic rats. Treatment with PPAR γ agonist rosiglitazone (20 mg/kg per day) reversed the vasoconstrictive effect of ET-1 (Figure 1). However, this effect of rosiglitazone was abolished by the treatment with the inhibitor of endothelial nitric oxide synthase (eNOS) L -NAME, indicating a NO-dependent mechanism (Figure 2).

3.2. Rosiglitazone Increased ET_BR Protein Levels in Pulmonary Arteries from PAH Rats. To understand the mechanism for the effect of rosiglitazone on ET-1-induced vasoconstriction in pulmonary arteries, we examined the protein level of ET_BR with Western blotting. As shown in Figure 3, ET_BR protein level was unaltered in the pulmonary arteries from CH-induced PAH rats. However, rosiglitazone treatment increased the expression of ET_BR. In contrast, it reduced the expression of ET_AR (Supplemental Figure 2 available online at <http://dx.doi.org/10.1155/2014/374075>).

3.3. Inhibitory Effect of Rosiglitazone Is Abolished by ET_BR Antagonist. To examine the functional role of ET_BR in mediating the rosiglitazone effect on ET-1-induced vasoconstriction, pulmonary arteries were dissected from normoxia-, CH-, and rosiglitazone-treated CH-rats to measure the ET-1-responsive isometric tension in the presence or absence of A192621, a selective ET_BR antagonist. In normoxic and PAH rats, A192621 (10 nmol/L) did not significantly alter the ET-1-induced contraction (Figures 4(a) and 4(b)). However, in the rosiglitazone-treated pulmonary arteries, A192621 abolished the ameliorative effect on the ET-1-induced vasoconstriction (Figure 4(c)).

4. Discussion

The vascular effects of ET-1 are mediated by 2 pharmacologically distinct G protein-coupled receptors, ET_AR and ET_BR [29]. ET_AR is mostly expressed in SMCs and mediates the vasoconstrictive and proliferative effects of ET-1 [30]. However, ET_BR expressed in ECs mediates endothelial-dependent vasodilatation by stimulating the production of NO and prostacyclin, prevents apoptosis, and promotes the

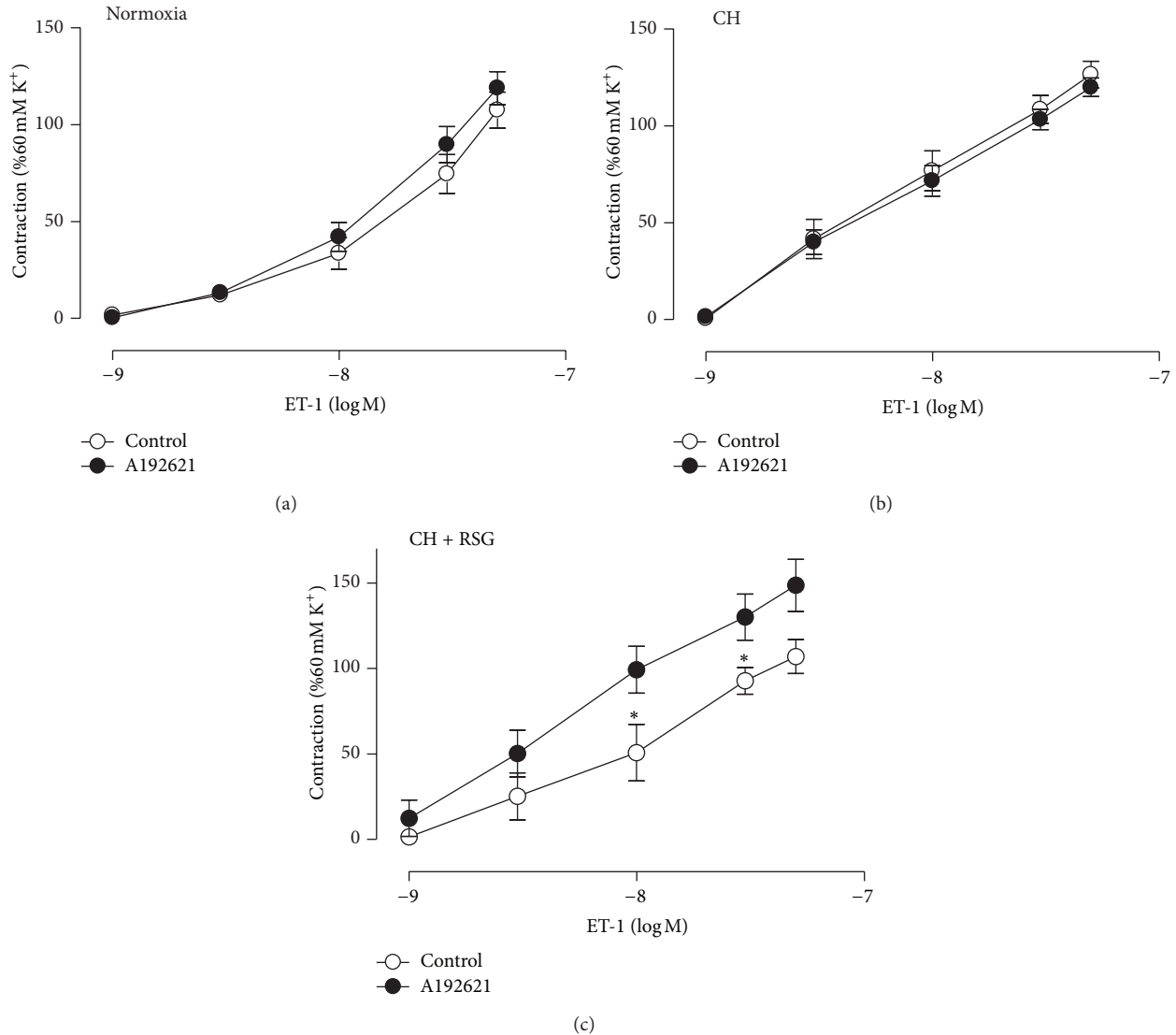


FIGURE 4: The ameliorative effect of RSG on ET-1-induced contractions was abrogated by ET_BR antagonist. Concentration-dependent contractions to ET-1 pretreated with ET_BR antagonist A192621 (10 nmol/L) in pulmonary arteries from normoxia- (a), CH (b), or RSG-treated CH (c) rats. Data were mean \pm SEM from 5 to 8 rats. * $P < 0.05$ versus control.

clearance of ET-1 [31, 32]. ET_BR is present in low densities on vascular smooth muscle cells where its activation induces vasoconstriction [33, 34]. Since ET_BR elicits vasodilation and vasoconstriction, its vascular functions in pulmonary arterial hypertension need to be further characterized. ET_BR-deficient rats developed exacerbated PAH after exposure to chronic hypoxia, characterized by elevated pulmonary arterial pressure, diminished cardiac output, increased right ventricular hypertrophy, and increased total pulmonary resistance. Plasma ET-1 level and mRNA of ET-converting enzyme-1 (ECE-1) were much higher in lungs from ET_BR-deficient rats compared with control rats. In ET_BR-deficient rats, the pulmonary vessels showed less endothelial NO synthase (eNOS) and NO production, supporting a role of NO in ET_BR-mediated vasodilation in the pulmonary vasculature [35]. Other studies in monocrotaline (MCT) induced

PAH rats also showed that ET_BR deficiency accelerated the progression of PAH and neointimal lesion [36, 37]. Although both ET_AR antagonist (ambrisentan) and dual ET_AR/ET_BR antagonist (bosentan) have been approved for treatment of PAH [38], selective antagonists for ET_AR and ET_BR appeared to have different effects on PAH. In a dog model for PAH, ET_BR antagonist RES-701-1 was found to increase pulmonary arterial pressure whereas sarafotoxin S6c, an ET_BR agonist, decreased pulmonary arterial resistance [39]. In addition, ET_BR antagonist also elevated ET-1 concentrations in both in vivo and in vitro studies [40]. These findings suggest that activation of ET_BR may play a protective role in the PAH.

In addition to three categories of FDA-approved treatments including prostanoids, ET-1 receptor antagonists, and phosphodiesterase 5 (PDE5) inhibitors, PPAR γ agonists thiazolidinediones (TZDs) including rosiglitazone and

pioglitazone have shown beneficial effects in animal models of PAH. In rodent PAH models induced by MCT or hypoxia and those associated with insulin resistance, TZDs were found to effectively reduce pulmonary arterial pressure and right ventricular hypertrophy [21, 22, 24, 25, 41]. Recently, we showed that rosiglitazone reversed pulmonary arterial remodeling and inhibited vasoconstriction in response to serotonin in the rat PAH models induced by MCT and hypoxia. Although the molecular mechanisms underlying the TZD effects on PAH development remain unclear, a generally accepted hypothesis is that TZDs may act via their receptor PPAR γ to modulate the expression of key genes involved in the pathogenesis of PAH such as ET-1, eNOS, p27^{KIP1}, adiponectin, apoE, MMP, and RhoA/ROCK. In this study, we provided in vivo evidence that rosiglitazone ameliorated ET-1-induced vasoconstriction in the pulmonary arteries of PAH rats (Figure 1). The ameliorative effect of rosiglitazone was mediated via differential regulation of ET-1 receptors. In particular, the upregulation of ET_BR might play a major role because rosiglitazone treatment increased the expression of ET_BR in the pulmonary arteries (Figure 3) and A192621, a selective antagonist of ET_BR, abrogated the effect (Figure 4). Conversely, rosiglitazone inhibited the induction of ET_AR in the pulmonary arteries of PAH rats (Supplemental Figure 2). It is conceivable that rosiglitazone may have the vasoprotective effects by altering the ratio of ET_{A/B} receptors. ET_BR in ECs may increase Ca²⁺ influx and the activation of eNOS, which leads to the production of NO and induction of vascular relaxation. This notion is corroborated with the result that the effect of rosiglitazone was abolished in the presence of L-NAME, an inhibitor of eNOS (Figure 2). Importantly, the induction of endothelial ET_BR is considered to be a PPAR γ -specific mechanism as we previously identified ET_BR to be a direct target gene of PPAR γ [28].

5. Conclusions

In conclusion, we demonstrated that rosiglitazone upregulated the expression of ET_BR, which mediated the decreased vasoconstriction in the rat models of PAH. This finding suggested a new mechanism for the protective role of PPAR γ in the development of PAH.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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