Beneficial and Deleterious Effects of Rosiglitazone on Hypertension Development in Spontaneously Hypertensive Rats

Lingyun Wu, Rui Wang, Jacques de Champlain, and Thomas W. Wilson

The antihypertensive effect of the peroxisome proliferator-activated receptor (PPAR)γ agonist rosiglitazone has been reported in patients with diabetes or obesity. The correlation of PPARγ expression with blood pressure and the therapeutic application of rosiglitazone in spontaneously hypertensive rats (SHR) were investigated in the present study. Systolic blood pressure of 21-week SHR was significantly higher than that of age-matched Wistar-Kyoto rats (WKY) (225 ± 5 vs 144 ± 2 mm Hg, P < .05). Basal expression levels of PPARγ proteins in vascular tissues of 21-week SHR were significantly lower than that of age-matched 21-week WKY (P < .05). This reduced expression of PPARγ was not detected between 5- and 13-week SHR and age-matched WKY. Cardiac PPARγ expression was also not different among different age groups between SHR and WKY. Chronic treatment with rosiglitazone, but not PPARα agonist Wy14643, significantly retarded hypertension development and reversed abnormally faster heart rate in young SHR. An unfavorable effect of rosiglitazone treatment was the increased heart-to-body weight ratio accompanied by left ventricular hypertrophy. In conclusion, vascular PPARγ protein expression in adult SHR (21 weeks) is significantly decreased in comparison with the age-matched WKY. Chronic rosiglitazone treatment retards hypertension development, but the associated prohypertrophy effect calls for a cautious use of this thiazolidinedione in the treatment of insulin resistance syndrome associated with hypertension. Am J Hypertens 2004;17:749–756 © 2004 American Journal of Hypertension, Ltd.

Key Words: Rosiglitazone, hypertension, ventricular hypertrophy, peroxisome proliferator-activated receptor (PPAR)α, PPARγ.


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hypothesized that PPAR expression with the development of genetic hypertension in SHR has not been conducted. That rosiglitazone treatment attenuated angiotensin-induced hypertension by upregulating PPAR expression has also been observed. These observations as well as the general acknowledgment on the importance of PPAR in the homeostatic control of cardiovascular function prompted us to investigate the correlation of vascular PPAR expression with the development of genetic hypertension in the absence of diabetes or obesity. Accordingly, we hypothesized that PPAR expression could be altered in vascular tissues of SHR during the developmental stage of hypertension. A better understanding of this critical relationship between PPAR expression and hypertension development would help to reveal the pathogenesis of hypertension and to refine clinical strategies for the application of TZDs. To this end, the expression levels of PPAR and PPAR proteins were determined in various vascular tissues and heart from SHR and normotensive Wistar-Kyoto rats (WKY) at different ages (5, 13, and 21 weeks). Whether rosiglitazone possess a direct antihypertensive effect in SHR was examined. The effects of rosiglitazone and Wy14643 (a PPAR agonist) on the evolution of hypertension and tachycardia in SHR were investigated. Moreover, the effect of chronic treatment with rosiglitazone on the development of cardiac hypertrophy was determined.

Methods

Animal and Tissue Preparation

Studies were performed on male SHR and WKY (Charles River Laboratories, St. Constant, PQ, Canada). All experimental procedures followed the guidelines of the Canadian Council for Animal Care and approved by an Institutional Committee on Animal Care and Supply. The animals had free access to normal chow diet and drinking water.

A total of 72 animals aged 5, 13, and 21 weeks were used, of which 31 were WKY and 41 SHR. In Study 1, SHR at ages of 5, 13, and 21 weeks (n = 8 for each age group) and age-matched WKY (n = 8 for each group) were used. After measurement of BP and heart rate, hearts and different vascular tissues were excised and frozen immediately in liquid nitrogen. The frozen tissues were directly transferred to a special metal container and ground with a homogenizer (Mikro-Dismembrator USB; Braun Biotech International GmbH, Melsungen, Germany) for 1 min at 3000 rpm, repeated three times. The homogenized tissue powder was used for Western blot analysis. In Study 2, SHR of 5 weeks were divided into nontreated (n = 7), rosiglitazone-treated (n = 5), and Wy14643-treated (n = 5) groups. Rosiglitazone (150 mg/kg/d) and Wy14643 (50 mg/kg/d) were delivered by gavage at the same time every morning for 8 consecutive weeks. Age-matched WKY (n = 7) were used as the control. Systolic BP and heart rate (HR) were monitored before and after the treatment weekly, using tail-cuff plethysmography (Harvard Apparatus Ltd., Holliston, MA). Body weight was monitored weekly. At the end of 8-week treatments (age of 13 weeks), hearts, arteries, kidneys, and livers were excised and weighted immediately before further processing.

Western Blot Analysis

Samples containing equal amounts of proteins were separated on polyacrylamide gels using a minivertical electrophoresis system as described in our previous studies. After electrophoresis, proteins were transblotted onto polyvinylidifluoridene membranes. The membranes were then incubated with anti-PPAR antibody (Research Diagnostics Inc., Flanders, NJ) at a dilution of 1:100, anti-PPAR antibody (Research Diagnostics) at 1:200, anti-β-actin antibody (Sigma, Oakville, ON, Canada) at 1:5000, and antiactin antibody (Chemicon International, Temecula, CA) at 1:400 overnight at 4°C, respectively. The horseradish peroxidase-conjugated second antibodies were applied for 2 h at room temperature. The proteins were visualized using the Chemiluminescent Substrate Kit (Amersham Biosciences Inc., Baie d’Urfé, PQ, Canada) and quantified using the UN-SCAN-IT gel Automated Digitizing System (version 5.1; Silk Scientific Inc., Orem, UT). Protein levels were normalized relative to the expression levels of actin or β-actin (arbitrary units).

Determination of Left Ventricular Hypertrophy

A transverse section of left ventricles were removed with a razor blade. Similar sites were sampled from each rat treated with rosiglitazone or untreated control group. The tissue slices were fixed with 10% formalin in phosphate-buffered saline (PBS) and placed in paraffin blocks. Eight-micrometer thick cross sections were made from the blocked tissues and stained with hematoxylin-eosin. The sections were examined at a fixed magnification under an Olympus microscope (Markham, ON, Canada). The average thickness of the left ventricular wall was determined from a minimum of 10 sites of each ventricle. The measurements and analysis of ventricular thickness were carried out by a person without knowing the nature of treatment, using the image analysis software Scion Image (Scion Corp., Frederick, MD).

Chemicals and Statistical Analysis

Rosiglitazone was a gift from SmithKline Beecham (Glaxo-SmithKline, Worthing, West Sussex, UK) and Wy14643 was purchased from ChemSyn laboratories (Lenexa, KS). Data were expressed as means ± SEM and analyzed using

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the Student t test or repeated measures ANOVA, followed by Newman-Keuls analysis for multivariate. Differences between groups were considered statistically significant when \( P < .05 \).

## Results

### Basal Expression Levels of PPAR\(\gamma\) and PPAR\(\alpha\) in Heart and Vascular Tissues From SHR at Different Ages

Systolic BP increased progressively from age 5 through 21 weeks in untreated SHR (Table 1). There was no difference in BP or HR between SHR and WKY at age 5 weeks. Systolic BP of 13-week SHR was 189 ± 6 mm Hg and HR was 413 ± 8 beats/min, higher than that of age-matched WKY (140 ± 6 mm Hg and 366 ± 7 beats/min) (\( n = 8 \), respectively, \( P < .05 \)). The BP was 225 ± 5 mm Hg and HR 400 ± 9 beats/min in 21-week SHR (\( n = 8 \)) higher than in age-matched WKY (144 ± 2 mm Hg and 360 ± 14) (\( n = 8 \), \( P < .05 \)).

As shown in Fig. 1A, the basal expression levels of PPAR\(\gamma\) proteins in aorta, mesenteric, tail, and pulmonary arteries of 21-week SHR were reduced to 70% ± 5%, 49% ± 12%, 61% ± 7%, and 40% ± 10%, respectively, of control level in age-matched WKY (\( P < .05 \), \( n = 6 \) to 8 for each group). The expression levels of PPAR\(\gamma\) proteins in heart were not significantly different between SHR and WKY (\( P > .05 \), \( n = 6 \) for each group; Fig. 1B). No significant change in the basal expression levels of PPAR\(\alpha\) proteins in aorta, mesenteric, tail, and pulmonary arteries was observed between 21-week SHR and WKY (\( P > .05 \), \( n = 5 \) in each group; Fig. 2A), or in heart (\( P > .05 \), \( n = 5 \) in each group; Fig. 2B). In addition, there was no significant difference in the expression levels of PPAR\(\gamma\) and PPAR\(\alpha\) proteins in aorta, mesenteric, tail, and pulmonary arteries, as well as heart, between SHR aged 5 (data not shown) or 13 weeks (Table 2) and age-matched WKY (\( P > .05 \), \( n = 3 \) to 5 for each group).

### Effect of Rosiglitazone and Wy14643 on the Expression of PPAR\(\gamma\) and PPAR\(\alpha\) in Young SHR

The SHR at age 5 weeks were fed with rosiglitazone or Wy14643 for 8 weeks, and the expression levels of PPAR\(\gamma\) or PPAR\(\alpha\) proteins were determined and compared with that of age-matched untreated SHR groups. No significant changes in PPAR\(\gamma\) protein expression were observed in heart and arteries including aorta, mesenteric, pulmonary, and tail arteries between rosiglitazone treated and untreated SHR at age 13 weeks (Table 2). In addition, the expression levels of PPAR\(\alpha\) in heart and different arteries (aorta, mesenteric, pulmonary, and tail) were similar between untreated SHR and SHR treated with Wy14643 (Table 2).

### Effects of Rosiglitazone and Wy14643 on BP and Heart Rate in Young SHR

After a 3-week oral treatment with rosiglitazone, systolic BP of SHR (at age 8 weeks) became lower than that of untreated animal, and remained lower during the rest of the treatment. For instance, after an 8-week treatment with rosiglitazone, systolic BP of SHR (at age 8 weeks) became lower than that of untreated SHR, but not different from that of age-matched untreated SHR (\( n = 7 \)) at age 13 weeks (Table 2). There was no difference in HR between 5-week SHR and age-matched WKY (\( P > .05 \), \( n = 8 \) for each group; Table 1). The HR of 8-week-old SHR was significantly faster than that of age-matched WKY (\( P < .05 \)). At the age of 8 weeks (treatment week 3), HR was reduced in all groups (Fig. 3B). This HR reduction was more pronounced in rosiglitazone-treated animals. There was no significant difference in HR between rosiglitazone-

### Table 1. Baseline conditions of WKY and SHR at different ages

<table>
<thead>
<tr>
<th>Age</th>
<th>Animal</th>
<th>BP (mm Hg)</th>
<th>HR (beat/min)</th>
<th>BW (g)</th>
<th>HW/BW (mg/g)</th>
<th>KW/BW (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 wks</td>
<td>WKY (( n = 8 ))</td>
<td>110 ± 5.5</td>
<td>476 ± 19</td>
<td>110 ± 4.2</td>
<td>4.4 ± 0.1</td>
<td>8.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>SHR (( n = 8 ))</td>
<td>120 ± 4</td>
<td>486 ± 7</td>
<td>97 ± 6.1</td>
<td>4.4 ± 0.2</td>
<td>9.2 ± 0.2</td>
</tr>
<tr>
<td>13 wks</td>
<td>WKY (( n = 8 ))</td>
<td>140 ± 6*</td>
<td>366 ± 7</td>
<td>305 ± 3.8</td>
<td>3.3 ± 0.1</td>
<td>7.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>SHR (( n = 8 ))</td>
<td>189 ± 6*</td>
<td>413 ± 8*</td>
<td>250 ± 5.4*</td>
<td>3.8 ± 0.1*</td>
<td>7.1 ± 0.1</td>
</tr>
<tr>
<td>21 wks</td>
<td>WKY (( n = 8 ))</td>
<td>144 ± 2</td>
<td>360 ± 14</td>
<td>349 ± 18</td>
<td>3.0 ± 0.1</td>
<td>6.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>SHR (( n = 8 ))</td>
<td>225 ± 5*</td>
<td>400 ± 9*</td>
<td>308 ± 4.4*</td>
<td>4.2 ± 0.2*</td>
<td>6.6 ± 0.2</td>
</tr>
</tbody>
</table>

BP = blood pressure; HR = heart rate; BW = body weight; HW = heart weight; KW = kidney weight.

* \( P < .05 \), SHR versus WKY between the same age groups.
treated SHR and age-matched WKY group (P > .05), indicating that rosiglitazone reduced HR in SHR to a near normal level. Wy14634 had no effect on SHR HR (n = 5).

**Chronic Rosiglitazone Treatment Induced Body Weight Gain and Ventricular Hypertrophy**

Troglitazone-induced weight gain has been reported in clinical trials. We investigated the effect of rosiglitazone and Wy14643 on the body weight as well as cardiac hypertrophy of SHR. As shown in Fig. 4A, at the age of 5 weeks (treatment week 0) there was no significant difference in body weight between SHR and WKY. Starting at age 7 weeks, body weight was significantly decreased in SHR, compared to that of age-matched WKY group (P < .05, n = 7 in each group). After 2 weeks of treatment with rosiglitazone, body weight of SHR started to increase significantly and continuously, compared to that of age-matched untreated SHR (P < .05). In Wy14643-treated SHR, body weight gain was significant between treatment weeks 2 and 4 but subsided later on (Fig. 4A).

The heart-to-body weight ratio was slightly greater in SHR at age 13 weeks (Fig. 4B) and 21 weeks (Table 1) than in age-matched WKY. No difference in heart-to-body weight ratio was found between SHR (5 weeks) and age-matched WKY (Table 1). After a treatment period of 8 weeks with rosiglitazone, the heart-to-body weight ratio of treated SHR was significantly greater than that of untreated but age-matched SHR. Left ventricular wall thickness was slightly, but significantly increased in 13-week SHR (Fig. 4C) than in age-matched WKY (P < .05, n = 5). This greater left ventricular wall thickness was markedly amplified after chronic treatment of SHR with rosiglitazone but not with Wy14643 for 8 weeks in comparison with the untreated age-matched SHR (P < .05; n = 5 in each group).

**Effects of Chronic Treatment With Rosiglitazone and Wy14643 on Kidney Weight of Young SHR**

There is no significant increase in kidney-to-body weight ratio of SHR at age 13 weeks (Fig. 4D) and 21 weeks (Table 1), compared to that of age-matched WKY. No difference in

FIG. 1. Basal expression levels of PPARγ proteins in different arteries (A) and heart (B) from 21-week-old untreated SHR and WKY. The expression levels of PPARγ proteins were significantly lower in aorta (Ao), mesenteric arteries (MA), pulmonary arteries (PA), and tail arteries (TA), but not in heart, of untreated SHR than that of WKY. *P < .05 (SHR versus WKY). n = 6 for groups of aorta, pulmonary, and tail arteries and heart from both untreated SHR and WKY; n = 8 for groups of mesenteric arteries from both strains.
kidney-to-body weight ratio was found between SHR (5 weeks) and age-matched WKY (Table 1). After a treatment period of 8 weeks with rosiglitazone, the kidney-to-body weight ratio (Fig. 4D) and the liver-to-body weight ratio (data not shown) of treated SHR was not significantly changed, in comparison with that of untreated but age-matched SHR. However, an 8-week treatment of SHR (n = 5) with Wy14643 significantly increased the kidney-to-body weight ratio more than any other animal groups (P < .05).

**Discussion**

The present study yields several important and novel observations. The basal expression level of PPARγ proteins in vascular tissues was not different in prehypertensive 5-week SHR or in evolving hypertensive 13-week SHR from that of age-matched WKY. In contrast, the basal expression level of PPARγ proteins in vascular tissues was significantly decreased in older 21-week SHR than

**Table 2.** Expression levels of PPARα and PPARγ in various tissues from rosiglitazone or Wy14643 treated animals

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Animal</th>
<th>Aorta</th>
<th>MA</th>
<th>PA</th>
<th>TA</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARα</td>
<td>WKY (13 wks) (n = 5)</td>
<td>25 ± 4</td>
<td>32 ± 5</td>
<td>37 ± 4.6</td>
<td>40 ± 4</td>
<td>60 ± 5</td>
</tr>
<tr>
<td></td>
<td>SHR (13 wks) (n = 5)</td>
<td>22 ± 3</td>
<td>35 ± 4</td>
<td>34 ± 6</td>
<td>38 ± 5</td>
<td>62 ± 6</td>
</tr>
<tr>
<td>PPARα (Wy-treated)</td>
<td>SHR (13 wks) (n = 5)</td>
<td>25 ± 4</td>
<td>35 ± 4.5</td>
<td>34 ± 5</td>
<td>37 ± 4</td>
<td>62 ± 7</td>
</tr>
<tr>
<td>PPARγ</td>
<td>WKY (13 wks) (n = 5)</td>
<td>42 ± 6</td>
<td>37 ± 5</td>
<td>32 ± 6</td>
<td>38 ± 4</td>
<td>40.5 ± 6</td>
</tr>
<tr>
<td></td>
<td>SHR (13 wks) (n = 5)</td>
<td>36 ± 5</td>
<td>34 ± 4.5</td>
<td>30 ± 5</td>
<td>35 ± 6</td>
<td>35 ± 5</td>
</tr>
<tr>
<td>PPARγ (Rosig-treated)</td>
<td>SHR (13 wks) (n = 5)</td>
<td>37 ± 4</td>
<td>35 ± 4.6</td>
<td>37 ± 6</td>
<td>36 ± 4.7</td>
<td>40 ± 4.5</td>
</tr>
</tbody>
</table>

Wy = Wy14643; Rosig = rosiglitazone; MA = mesenteric arteries; PA = pulmonary arteries; TA = tail arteries.
Protein levels of PPARα or PPARγ were normalized relative to the expression levels of β-actin in arteries or to the expression levels of actin in heart, which were presented using arbitrary units.
that from age-matched WKY. It is likely that hypertension-related vascular damage or remodeling downregulates the expression of PPARγ proteins. Therefore, the decreased PPARγ expression in vascular tissues of older SHR seems to be secondary to the maintenance of chronic hypertension. However, the observed difference in PPARγ expression between old SHR and WKY may also represent one genetic trait of SHR, which manifests itself at older ages, independent of BP. In future studies, various antihypertension drugs that do not interfere with PPARγ expression or activity should be directly administered to SHR. If this maneuver lowers BP and simultaneously averts the decreased PPARγ expression in old SHR, hypertension-dependent changes in PPARγ expression would be demonstrated.

The PPARγ expression in heart of SHR in all age groups was not different from those of age-matched WKY, and PPARα expression in heart and vascular tissues was not altered in SHR at all ages. Diep and Schiffrin reported a higher basal level of PPARγ proteins in mesenteric artery of SHR (16 weeks), and higher basal mRNA levels of PPARγ and PPARα in mesenteric artery of SHR (6 and 16 weeks) than that of age-matched WKY. This report appears to be at variance with our observation, as we did not find any difference in the protein level of PPARγ in vascular tissues from 5- and 13-week SHR. The protein level of PPARγ in vascular tissues was, in fact, lower in 21-week SHR than in age-matched WKY. One major reason for this discrepancy might be that the expression level of PPARγ proteins was normalized against the level of β-actin in our study, whereas no housekeeping protein was included in the study by Diep and Schiffrin.

The antihypertensive effect of PPARγ agonist rosiglitazone has been reported in diabetic patients. In the present study, 8-week treatment with rosiglitazone attenuated significantly the development of hypertension in young SHR from age 5 to 13 weeks. This effect is in line with the antihypertensive effects of TZDs reported in other insulin-resistant animal models. Rosiglitazone treatment also reversed the increased HR of SHR to normal level (Fig. 3). We did not find any difference in the expression levels of PPARγ proteins in vascular tissues and heart from SHR before and after rosiglitazone treatment. This observation reinforces the concept that the effects of TZDs are mediated by the stimulation of PPARγ activity, rather than an effect on PPARγ protein expression. Once activated by TZDs, PPARs form heterodimers with the ligand-activated retinoic acid receptor (RXR). Through its DNA-binding domain, this heterodimer binds to PPAR-responsive elements (PPREs) of target gene and induces transcriptional activation of specific genes such as CD36 expression. It is, thus, reasonable to speculate that with or without an altered PPARγ expression, a reduced PPARγ activity would significantly contribute to the pathogenesis of hypertension in SHR. The hypotensive effect of rosiglitazone in SHR in our study is likely due to an increase in PPARγ activity. Using the same rationale, it is possible that a lower PPARγ activity might constitute a major pathogenic mechanism for the development or maintenance of hypertension in SHR. The pathophysiologic links between increased PPARγ activity and reduced BP in SHR merit further investigation.

In comparison with troglitazone that causes liver damage and weight gain, no increased liver-to-body weight ratio was found in our studies; hepatotoxicity has not been reported with rosiglitazone in animal or in T2DM during clinical trials. The SHR gain weight less rapidly than WKY (Fig. 4). Eight weeks of treatment with rosiglitazone restored SHR body weights significantly. Importantly, rosiglitazone treatment increased heart weight of SHR accompanied by a significant increase in left ventricular wall thickness. Successful management of insulin resistance syndromes including T2DM, hypertension, and obesity is a major challenge to basic researchers and clinicians. Rosiglitazone-induced cardiac hypertrophy could exert adverse effects on cardiovascular function in the chronic treatment of T2DM or hypertension. However, whether an
increased cardiac hypertrophy occurs in humans after long-term use of TZDs is unknown. The dose of TZDs between 20 and 200 mg/kg/d has been widely used in different animal models. Because a relatively high dose of rosiglitazone was used in the present study, whether the antihypertensive effect of rosiglitazone can be achieved at lower doses should be further examined. Very recently, Kermani and Garg have reported that rosiglitazone caused heart failure and pulmonary edema in six patients with T2DM after 1 to 16 months of treatment with rosiglitazone. Our studies provide the direct evidence that SHR have developed left ventricular hypertrophy after 8 weeks of treatment with rosiglitazone. Rosiglitazone-induced cardiomyocyte hypertrophy can cause left ventricular dysfunction or impaired left ventricular contractility, which may eventually lead to heart failure in patients. Because rosiglitazone and other TZDs such as pioglitazone are prescribed to several millions of people worldwide, cautious consideration of this side effect should be exercised when using rosiglitazone for the management of T2DM.

In summary, basal PPARγ expression level was found to be significantly lower in vascular tissues from older established hypertensive SHR (21 weeks). There was no change in PPARγ expression in vascular tissues from young and adult SHR (5 and 13 weeks) compared to that of age-matched WKY. Vascular PPARα expression was unaltered in SHR and WKY at different ages. The PPARγ agonist rosiglitazone retards the development of hypertension and reverses the abnormally faster HR in SHR. However, rosiglitazone-induced cardiac hypertrophy calls for additional caution regarding the chronic use of this TZD in the treatment of insulin-resistant syndrome.

References