PPARγ in Endothelial Cells Influences High Fat Diet-Induced Hypertension

Christopher J. Nicol, Masahiro Adachi, Taro E. Akiyama, and Frank J. Gonzalez

Background: Peroxisome proliferator-activated receptor γ (PPARγ) ligands improve human hypertension. However, the mechanism and site of this effect remains unknown, confounded by PPARγ expression in many cell types, including endothelial cells (ECs).

Methods: To evaluate the vascular role of PPARγ we used a conditional null mouse model. Specific disruption of PPARγ in ECs was created by crossing Tie2-Cre+ transgenic (T2T+) and PPARγ-boxed (fl/fl) mice to generate PPARγ (fl/fl)T2T+ (PPARγ E-null) mice. Conscious 8- to 12-week-old congenic PPARγ (fl/fl)Cre− (wild type) and PPARγ E-null mice were examined for changes in systolic blood pressure (BP) and heart rate (HR), untreated, after 2 months of salt-loading (drinking water), and after treatment for 3 months with high fat (HF) diet alone or supplemented during the last 2 weeks with rosiglitazone (3 mg/kg/d).

Results: Untreated PPARγ E-nulls were phenotypically indistinguishable from wild-type littermates. However, compared to similarly treated wild types, HF-treated PPARγ E-nulls had significantly elevated systolic BP not seen after normal diet or salt-loading. Despite sex-dependent baseline differences, salt-loaded and HF-treated PPARγ E-nulls of either sex had significantly elevated HR versus wild types. Interestingly, rosiglitazone improved serum insulin levels, but not HF diet-induced hypertension, in PPARγ E-null mice.

Conclusions: These results suggest that PPARγ in ECs not only is an important regulator of hypertension and HR under stressed conditions mimicking those arising in type 2 diabetics, but also mediates the antihypertensive effects of rosiglitazone. These data add evidence supporting a beneficial role for PPARγ-specific ligands in the treatment of hypertension, and suggest therapeutic strategies targeting ECs may prove useful.

Key Words: PPARγ, conditional null mice, endothelial cells, blood pressure, type 2 diabetes.

The peroxisome proliferator-activated receptors (PPARs), of which there are three family members (α, β, and γ), first discovered in the early 1990s, are members of the orphan nuclear receptor superfamily. PPARγ is expressed in a number of cell types, including adipocytes, colonic epithelial cells, and monocytes/macrophages, as well as in vascular smooth muscle cells (VSMCs) and endothelial cells (ECs). Known ligands for PPARγ include synthetic insulin-sensitizing thiazolidinedione compounds (TZDs), such as troglitazone, BRL-49653 (aka rosiglitazone), and pioglitazone, as well as natural compounds, such as 15-deoxy-D12,14-prostaglandin J2, 9- and 13-hydroxyoctadecanoic acid (9- and 13-HODE), 15-hydroxyeicosatetraenoic acid (15-HETE), linoleic acid, and the recently discovered putative endogenous PPARγ agonist, lysophosphatidic acid (LPA). Use of both natural and synthetic ligands has provided evidence associating PPARγ not only with adipocyte differentiation, but also the onset of type 2 diabetes and colon cancer. Expression of PPARγ in cells of the vascular system has led to numerous studies in an attempt to clarify the physiologic role of this receptor in ECs. Several reports have shown that the use of TZDs, such as troglitazone and rosiglitazone, is correlated with marked decreases in hypertension in humans resulting from the onset of insulin resistance in adult type 2 diabetic patients. Interestingly, the decrease in mean blood pressure (BP) was also found to be significantly associated with stabilization of plasma insulin levels. Together, these reports suggest that PPARγ activation by TZDs can normalize BP through their insulin-sensitizing effects. In contrast, the most common human PPARγ polymorphism,
a Pro:Ala change in codon 12 within the unique ligand-independent activation domain of PPARγ, has been correlated with increased systolic and diastolic BPs and body mass index (BMI) in a Utah population. More recently, two independent studies of Spanish and Swedish populations have similarly identified an increased risk of hypertension associated with the Pro/Pro polymorphic genotype. However, the nature and ability of either BMI or insulin resistance to influence the association between this variant and hypertension is controversial, as several studies have yielded conflicting results.

To directly evaluate the role of this nuclear receptor in the cardiovascular system, previously described PPARγ floxed (fl/fl)Cre− mice were bred with a transgenic mouse line expressing the Cre recombinase protein driven by the Tie2 promoter (T2T+), specifically disrupting PPARγ in endothelial (E) cells and generating PPARγ(fl/fl)T2T+ (henceforth referred to as PPARγ E-null) mice. The PPARγ E-nulls are viable and not phenotypically different from congenic PPARγ(fl/fl)Cre− (wild-type) littermate mice. However, after a high fat diet (HF) treatment, PPARγ E-null mice are markedly susceptible to increased systolic BP, a condition that is not improved by administration of rosiglitazone, suggesting a central role for PPARγ in the regulation of hypertension from within ECs.

Methods

Animals

All mice were housed and treated in accordance with protocols approved by the National Cancer Institute Animal Care and Use Committee. Mice were housed in microisolator cages on a 12-h light/dark cycle, with food and water provided ad libitum. Male and female PPARγ(fl/fl)Cre− (wild-type) were generated, as described previously, and crossed with Tie2-Cre+ transgenic mice (T2T+), as described elsewhere, to generate PPARγ(fl/fl)T2T+ (PPARγ E-null) mice. These mice are of mixed C57Bl6/N;Sv129;FVB/N background and were bred using sibling matings for at least 12 generations before the start of the study. Genotypes were confirmed by polymerase chain reaction (PCR) analysis as described previously.

Untreated and Salt-Loading Studies

Eight- to 12-week-old wild-type or congenic PPARγ E-null littermates were treated with AIN-93G-modified 35% high fat diet (F4048, BIO-SERV; Frenchtown, NJ) (HF) for 3 months. Two weeks before the end of the study, a set of mice were started on a blended HF diet containing the PPARγ-specific agonist rosiglitazone (Avandia, 3 mg/kg/d; Smith Kline Beecham, West Sussex, UK), as described previously. Nonfasted serum was obtained at the end of the study through the retro-orbital sinus. Serum was then frozen in liquid nitrogen and stored in a −80°C freezer until further analysis.

High Fat Diet Study

Eight- to 12-week-old wild-type or congenic PPARγ E-null littermates were treated with AIN-93G-modified 35% high fat diet (F4048, BIO-SERV; Frenchtown, NJ) (HF) for 3 months. Two weeks before the end of the study, a set of mice were started on a blended HF diet containing the PPARγ-specific agonist rosiglitazone (Avandia, 3 mg/kg/d; Smith Kline Beecham, West Sussex, UK), as described previously. Nonfasted serum was obtained at the end of the study through the retro-orbital sinus. Serum was then frozen in liquid nitrogen and stored in a −80°C freezer until further analysis.

Biochemical Assays

Nonfasted serum insulin was measured by radioimmunoassay (RIA) (SRI-13K, Linco Research Inc., St. Charles, MO) according to manufacturer’s instructions, as described previously.

BP Analysis

Systolic BP and heart rate (HR) were analyzed on conscious (nonanesthetized) mice, as previously described. Mice were trained on the apparatus for 1 week before the start of recording and measurements were made at the same time in the morning for 4 sequential days. One complete cycle was performed before recording values to allow the mice to acclimate before actual readings were taken.

Statistical Analysis

Statistical evaluation was performed using one-way, two-way, or repeated measures ANOVA as appropriate, followed by a Student Neuman-Keuls post-hoc test. A significance level of $P < .05$ was used for all analyses.

Results

To determine the role of PPARγ in ECs, PPARγ E-null mice were generated as described in the Methods section. Disruption of the PPARγ gene, after recombination of exon 2, was confirmed by Southern blotting (Fig. 1) and PCR analysis (data not shown). Note that the recombined allele was detected in both sexes and all tissues examined as expected, based on the wide distribution of blood vessels. Interestingly, recombination in the spleen appeared to be complete. The PPARγ E-null mice were born at the normal mendelian frequencies and without any outward phenotypic or biochemical differences in body weight or serum profiles compared to congenic PPARγ(fl/fl)Cre− (wild-type) littermates (data not shown).

To test for alterations in systolic BP and HR, conscious age-matched male and female wild-type and PPARγ E-null mice were examined using tail-cuff plethysmography. Before the start of analysis, control-treated mice were conditioned to the BP/recording apparatus by performing readings at the same time every morning for 7 days to
over the measured treatment period, albeit this was more pronounced among males (Fig. 2). Moreover, HR was significantly increased in both sexes of PPARγ E-null mice compared to wild-type controls when mean values were determined (*P < .05*) (Table 1).

To assess whether HF diet treatment would increase hypertension, systolic BP and HR were analyzed after 1 month of HF diet treatment (1 month). In fact, HF treatment significantly increased both systolic BP and HR in all genotypes and sexes examined as compared to respective untreated levels (*P < .001*) (Fig. 2A and Tables 1 and 2). Notably, HF-treated PPARγ E-null mice showed a consistent and significantly higher mean systolic BP compared to similarly treated wild-type mice, which was independent of gender (*P < .05*) (Fig. 2A and Table 2).

Similarly, HR were also significantly higher among the HF-treated PPARγ E-null mice of either sex compared to wild-type controls (*P < .05*) (Fig. 2B and Table 2). This pattern was repeated when systolic BP and pulse were examined at the end of 3 months of HF treatment (3 months) where wild-type, as well as one-half of randomly segregated female and male PPARγ E-null mice were maintained on HF diet alone, whereas the other half of PPARγ E-null mice were treated during the final 2 weeks of the study with HF diet supplemented with rosiglitazone (*P < .05*) (Fig. 3 and Table 2). Consistent with published reports in the literature, data from separate previously unpublished studies in conscious congenic wild-type mice on normal chow diets respectively 1 day prior, and 4 and 10 days after oral gavage administration of rosiglitazone (3 mg/kg/d) or its vehicle control (Table 3), or with conscious congenic male mice treated for 2 months on a HF diet alone or with 2-week dietary supplementation to minimize the influence of stress (data not shown). Because analysis of repeated measurements revealed no significant difference within any individual treatment group over the respective period of recordings, mean values of each group were averaged to obtain a representative physiologic measurement for that group. As shown in Fig. 2A and Table 1, no differences in systolic BP were observed between control-treated wild-type and PPARγ E-null mice of either sex. However, in contrast to control-treated female mice that exhibited no consistent difference in HR between genotypes, control-treated male PPARγ E-null mice had consistent significantly increased HR compared to their respective wild types, which was perhaps more apparent when respective means were compared (male PPARγ E-null versus wild type, mean bpm ± SEM: 539.5 ± 20.4 v 499.0 ± 16.1, *P < .05*) (Fig. 2B and Table 1). To determine whether PPARγ plays a role in salt loading-induced hypertension, these same groups of mice were continued on a 2% salt water treatment for 60 days. Salt-loading resulted in enhanced systolic BP and HR over respective baseline values for all genotypes and sexes examined (*P < .001*) (Fig. 2 and Table 1). However, unlike systolic BP, which was not significantly different between respective salt-loaded genotypes of either sex, HR showed a trend toward significantly increased levels among salt-loaded PPARγ E-nulls compared to respective wild-type mice.
of rosiglitazone (3 mg/kg/d) (systolic BP reported as mean mm Hg ± SEM of HF alone, n = 5 v HF + rosiglitazone, n = 5: 123.8 ± 4.7 v 110.6 ± 3.0) (P < .05), indicated that rosiglitazone was able to significantly decrease systolic BP. Interestingly, rosiglitazone treatment of PPARγ E-null mice did not alter the significant HF-mediated increase of systolic BP compared to wild-type controls, irrespective of sex (Fig. 3A and Table 2). Strikingly though, rosiglitazone did appear to significantly reduce HR among HF-treated female, but not male, PPARγ E-null mice to the levels of HF-treated wild types, a pattern that was consistent when overall means for the treatment period were determined and compared (P < .01) (Fig. 3B and Table 2).

To determine whether in fact rosiglitazone administration was being consumed and able to have an effect, nonfasted serum insulin levels were examined at the end of BP analysis studies. After 3 months of HF treatment alone, serum insulin levels were not different between female (mean ng/mL ± SD 8.22 ± 2.05 v 6.84 ± 1.16) and male (mean ng/mL ± SD 9.99 ± 2.82 v 8.40 ± 1.23) PPARγ E-null versus wild-type control mice treated with HF alone (Fig. 4). However, as expected HF + rosiglitazone treatment did significantly lower serum insulin in both PPARγ E-null female (1.48 ng/mL ± 1.08) and male (3.22 ng/mL ± 3.77) mice compared to either same sex wild-type (P < .05) or PPARγ E-null mice treated with HF alone (P < .01) (Fig. 4).

**Discussion**

Previous work has elucidated a clear role for PPARγ in adipogenesis, glucose and lipid homeostasis, and transport.1,13,18 This study shows, by using the first reported PPARγ conditional E-null mouse strain, that PPARγ in ECs plays an important role in HR and hyperlipidemic-mediated hypertension.

Because classic PPARγ null mice die in utero around gestational day 10.5 attributed to embryonic defects in placental vascularization and myocardial thinning,1,18–20 PPARγ floxed mice13 and an EC targeting strategy, previously characterized as active and sustained in mice from as early as embryonic day 7.5 through adulthood,14 were used to generate conditional PPARγ E-null mice, resulting in spontaneous disruption of PPARγ in cells of endothelial origin.14,21 Interestingly, recombination in the spleen of PPARγ E-null mice appeared to be complete, suggesting that immune cells might also be a major site of PPARγ disruption. Strikingly, these mice were viable at birth and

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Systolic BP (mm Hg)</th>
<th>Mean HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 2% Salt</td>
<td>Control 2% Salt</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>117.6 ± 2.3</td>
<td>547.4 ± 8.3</td>
</tr>
<tr>
<td>PPARγ E-null</td>
<td>116.7 ± 1.9</td>
<td>553.6 ± 7.9</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>124.4 ± 4.4</td>
<td>499.0 ± 16.1</td>
</tr>
<tr>
<td>PPARγ E-null</td>
<td>123.2 ± 3.0</td>
<td>539.5 ± 20.4*</td>
</tr>
</tbody>
</table>

**Table 1.** Mean systolic BP and HR for wild-type and PPARγ E-null mice

BP = blood pressure; HR = heart rate; PPARγ = peroxisome proliferator-activated receptor γ.

Mean values for each parameter were calculated by averaging the daily values for each animal determined over the 6 days of administration of normal drinking water (Control), and the first 3 and last 10 days of administration of 2% NaCl (2% salt water) as drinking water. Values shown are the means ± SEM. n = 7 each for female, and 3 and 6 for male wild-type and PPARγ E-null mice, respectively.

* Significantly different from respective same sex wild-type controls, P < .05.
indistinguishable phenotypically or biochemically from their congenic wild-type littermate controls, suggesting that disruption of PPARγ in ECs alone is insufficient to induce embryonic or early neonatal lethality as found in the classic PPARγ null mice. It is therefore likely that disruption of PPARγ in other vascular cell types such as VSMCs may, either alone or in cooperation with disruption of both alleles in endothelial and other cell types, contribute to the cardiovascular defects and lethality seen in classic PPARγ null embryos.

Because several reports using PPARγ ligands have suggested a role for this receptor in regulating BP in both humans and animals, it was hypothesized that PPARγ E-null mice may exhibit differences in hypertension as compared to wild-type controls. However, neither sex of untreated PPARγ E-null mice demonstrated any differences in mean systolic BP averaged over the course of the study. When mice were salt-loaded by the drinking water, a method previously shown to induce elevations in hypertension in mice, the results showed that compared to baseline, all groups had significant elevations in the mean systolic BP, averaged over the course of 8 weeks of treatment. However, there were no genotypic differences in mean systolic BP between PPARγ E-null and wild-type controls for either sex. Together, these data suggest that disruption of PPARγ in endothelial cells alone or in combination with long-term salt-loading is insufficient to cause significant elevations in systolic BP over wild-type controls.

In contrast, the observed increased mean HR of both untreated PPARγ E-null males and long-term salt-loaded PPARγ E-nulls of either sex suggests an important role in HR for PPARγ expressed in ECs. These effects are inconsistent with a study reporting no change in the HR of a small group of patients, with mild forms of both hyper- tension and type 2 diabetes, after treatment with the PPARγ ligand troglitazone. Reasons for this discrepancy may include strain differences or the inability to detect a significant decrease in HR after troglitazone treatment given the reportedly mild forms of the diseases and the small number of patients examined, although this remains speculative. However, consistent with the results presented here, a study comparing wild-type and whole body PPARγ

<table>
<thead>
<tr>
<th>Table 2.</th>
<th>HF diet effect on mean systolic BP and HR for wild-type and PPARγ E-null mice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
<td><strong>Mean Systolic BP (mm Hg)</strong></td>
</tr>
<tr>
<td></td>
<td>1 mo HF</td>
</tr>
<tr>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>4</td>
</tr>
<tr>
<td>PPARγ E-null</td>
<td>7</td>
</tr>
<tr>
<td>PPARγ E-null + Rosi</td>
<td>—</td>
</tr>
<tr>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>4</td>
</tr>
<tr>
<td>PPARγ E-null</td>
<td>11</td>
</tr>
<tr>
<td>PPARγ E-null + Rosi</td>
<td>—</td>
</tr>
</tbody>
</table>

Mean values for each parameter were calculated by averaging the daily values for each animal determined over the last 9 days leading up to treatment with 1 month of HF diet (HF), and during the final 2 weeks of treatment with 3 months of HF diet with or without rosiglitazone supplementation (HF + Rosi). Values shown are the means ± SEM.

** Significantly different from respective same sex wild-type controls, P < .01; *** significantly different from respective same sex wild-type controls, P < .001; ‡ significantly different from same sex PPARγ E-null HF alone group, P < .01.

<table>
<thead>
<tr>
<th>Table 3.</th>
<th>Rosiglitazone effect on mean systolic BP of wild-type mice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wild type</strong></td>
<td><strong>Mean systolic BP (mm Hg)</strong></td>
</tr>
<tr>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>7</td>
</tr>
<tr>
<td>Rosi</td>
<td>7</td>
</tr>
<tr>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>4</td>
</tr>
<tr>
<td>Rosi</td>
<td>4</td>
</tr>
</tbody>
</table>

Abbreviations as in Tables 1 and 2.

Female and male systolic BP were simultaneously measured, using tail-cuff plethysmography, in conscious wild-type mice on normal chow diets respectively 1 day before, and 4 and 10 days after oral gavage administration of the PPARγ ligand rosiglitazone (3 mg/kg/d) or its vehicle control (0.5% carboxymethyl cellulose). Values shown are the means ± SEM.

* Significantly different from respective pretreatment controls, P < .05.
PPARγ E-null model may be useful for understanding the mechanisms of hypertension. However, the apparent discrepancy regarding the lack of sex differences here with respect to hypertension may be due to inherent species differences between mouse and human studies. In contrast, untreated male, but not female, PPARγ E-null mice did have significantly increased HR versus wild-type controls, a difference that was masked in the presence of salt-loading or HF treatment. This suggests an underlying sex difference among PPARγ E-null mice that is overcome under stressed conditions, although the source of this effect is unclear and warrants further study.

Type 2 diabetes is often associated with an increase in hypertension, and is suggested to be linked to insulin resistance.6,33 Thus we sought to characterize whether an established mouse protocol of HF diet-mediated insulin resistance, previously shown to induce insulin resistance18 (and our unpublished observations) and essentially mimicking the human type 2 diabetic syndrome, would have an effect on our PPARγ conditional E-null mice. The results here suggest that PPARγ in ECs normally acts to prevent HF-induced hypertension. The difference in effects seen here between salt-induced and HF-mediated hypertension among PPARγ E-null mice suggests a differential susceptibility to the mechanisms involved. This pattern is consistent with recent reports of the α1D-adrenergic-receptor-deficient mouse, which is sensitive to central salt-loading-mediated, but not angiotensin II (ANG II)-mediated, hypertension.34 Previous studies have suggested that PPARγ agonists are capable of downregulating ANG II type 1 receptors35 and that activated PPARγ suppresses the type 1 ANG II gene at the transcriptional level.36 Whether the hypertensive effects seen after HF treatment may be mediated in whole or in part by an unregulated ANG II response among PPARγ E-null mice remains to be determined. Moreover, it is noted that salt and ANG II plasma levels are inversely related, with increased salt intake associated with suppression of the renin-angiotensin system,24 and hence a decreased dependence on ANG II-mediated regulation of BP. Thus, the salt-loading induced here may suppress levels of ANG II thereby masking the genotypic differences observed in hypertension during HF treatment and warrants further study. Alternatively, PPARγ has been shown to inhibit expression of a number of targets in the endothelium, including thrombin-mediated ET-1 production.29 Because plasma ET-1 levels have been shown to be elevated among type 2 diabetic patients,37,38 and given its vasoconstrictor and mitogenic effects as discussed, the unregulated expression of ET-1 among PPARγ E-null mice may contribute to the HF-mediated hypertensive effects observed in this study. However, a recent report39 suggests that thromboxane blockade decreases hypertension independent of ET-1, suggesting the latter may not play a critical role in our results. Interestingly, one report suggests that both PPARγ ligands and PPARγ overexpression were able to increase the secretion of nitric oxide (NO) from ECs derived from heterozygous mice observed no differences in BP either in untreated or pressure overloaded animals, whereas increases in the pressure overload-induced cardiac hypertrophy were more prominent among PPARγ heterozygous mice.28 Furthermore, although there are conflicting reports,27 several studies have shown that PPARγ ligands decrease cardiac hypertrophy,26,28 possibly through the PPARγ-dependent antagonistic action of transcription factors known to be involved in this condition. In support, PPARγ ligands also have been shown to suppress the AP-1-mediated induction of endothelin-1 (ET-1), the latter of which is capable of inducing cardiac hypertrophy.22,28,29 Hence, the increased HR observed here may be the result of increased cardiac hypertrophy induced by disruption of PPARγ in ECs of PPARγ E-nulls, and warrants future study.

Because several human studies have reported sex-dependent differences with respect to the susceptibility of developing hypertensive disorders,30–32 male and female mice in this study were examined separately. It was reported that the presence of the PPARγ Pro12Ala polymorphism, and more specifically the Pro/Pro genotype, was associated with significantly increased hypertensive risk among males but less so in females.9,10 A more recent study has further identified polymorphisms within the estrogen receptors ERα and ERβ associated with significantly elevated BP among males but not females.30 Thus, based on the literature, it was expected that male PPARγ E-null mice may exhibit more pronounced effects than their female counterparts. However, although untreated males of either genotype were observed to have a nonsignificant elevation in systolic BP when compared to respective untreated females, both sexes were observed to respond to a similar degree with respect to systolic BP after salt-loading or HF treatment. In addition, both sexes of PPARγ E-null mice developed significantly elevated hypertension after HF treatment as compared to their same sex wild-type controls. This suggests that both sexes of the
various species and tissue sites in a transcriptionally regulated, but in an endothelial nitric oxide synthase (eNOS)-independent manner. In light of the fact that NO contributes to vascular tone, platelet adhesion, and VSMC proliferation, the results shown here suggest that the elevation of systolic BP may be a direct result of disruption of the homeostatic mechanisms by which PPARγ-mediated NO secretion helps to inhibit hypertension.

The inability of the PPARγ-specific agonist rosiglitazone to reduce the HF-mediated hypertension among PPARγ E-null mice suggests its well-documented antihypertensive properties are exerted through activation of PPARγ in ECs. During the course of publishing this work, Ryan et al have recently reported that in a mouse model of lifelong hypertension and vascular dysfunction, rosiglitazone reduces BP in a manner that was independent of alterations in either eNOS, soluble guanylate cyclase, or genes thought to be important for vascular contraction such as angiotensin receptor 1 (AT1) and PPET-1 (precursor of the potent vasoconstrictor endothelin). It was further suggested that part of improvements seen in the vascular function and BP may be attributed to the PPARγ-independent effects of rosiglitazone, such as through the attenuation of inward calcium currents and enhancement of calcium-activated potassium currents resulting in vascular relaxation as previously identified in isolated VSMCs. Interestingly, these findings differ somewhat from the findings in other cell and tissue culture systems, and rat studies and may suggest that the differences may be intrinsic to the differences in the models of hypertension used, although it was suggested that activation of PPARγ may indirectly control expression of the formerly mentioned genes. However, perhaps most striking is the inability of rosiglitazone to reduce hypertension among PPARγ E-null mice of either sex in the present study, which suggests that PPARγ-dependent effects of rosiglitazone, as well as similar acting TZDs, within ECs play a direct and central role in improving HF-mediated hypertension. Nevertheless, the mechanism by which this occurs is still not clear.

In conclusion, a viable endothelial-specific PPARγ conditional null mouse strain was created to enable direct examination in vivo of the role of PPARγ expression in these cells. Treatment with a specific PPARγ agonist, rosiglitazone, was unable to lower the high fat diet-induced marked increase in systolic BP among PPARγ E-null mice. The present study demonstrates that the reduction in hypertension attributed to TZDs, such as rosiglitazone, is the result of action on PPARγ expressed within ECs, as distinct from its expression within other cells of not only the vascular system such as smooth muscle cells, but also other tissues such as adipocytes or skeletal muscle cells that also express PPARγ, which may play a role in the TZD-mediated insulin-sensitizing effects of these compounds. Therefore these studies have important clinical implications for the treatment of patients with hypertension resulting from exposure to a high fat diet or type 2 diabetes, and suggest a therapeutic strategy targeting ECs, and perhaps PPARγ directly, may prove useful. Together these results suggest that PPARγ in ECs may act as an important regulator of hypertension and HR under stressed conditions that mimic those arising in type 2 diabetic patients. These data also add further evidence in support of a beneficial role for PPARγ-specific ligands in the therapeutic treatment of hypertension, and suggest therapeutic strategies focusing on ECs may prove useful in the treatment and prevention of this condition.

Acknowledgment

We thank Dr. Jeffrey Kopp for advice and the generous use of plethysmographic apparatus during the course of this study.

References


