PPAR-γ regulates inflammation and renin-angiotensin system activity in hypothalamic paraventricular nucleus and ameliorates peripheral manifestations of heart failure

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Abstract

Activation of peroxisome proliferator-activated receptor (PPAR)-γ, a nuclear transcription factor, has been shown to inhibit the production of proinflammatory cytokines and, in peripheral tissues, to down-regulate the renin-angiotensin system (RAS). PPAR-γ is expressed in key brain areas involved in cardiovascular and autonomic regulation. We hypothesized that activation of central PPAR-γ would reduce sympathetic excitation and ameliorate peripheral manifestations of heart failure (HF) by inhibiting central inflammation and brain RAS activity. Two weeks after coronary artery ligation, HF rats received an intracerebroventricular (ICV) infusion of the PPAR-γ agonist pioglitazone or vehicle for another 2 weeks. PPAR-γ expression in the paraventricular nucleus of hypothalamus (PVN), an important cardiovascular region, was unchanged in HF compared with sham-operated (SHAM) rats. However, PPAR-γ DNA binding activity was reduced, nuclear factor-kB activity was increased, and expression of proinflammatory cytokines and angiotensin II type-1 receptor was augmented in the HF rats. Mean blood pressure response to ganglionic blockade was greater, plasma norepinephrine levels, lung/body weight, right ventricle/body weight, and left ventricular end-diastolic pressure were increased and maximal left ventricular dP/dt was decreased. All these findings were ameliorated in HF rats treated with ICV pioglitazone, which increased PPAR-γ expression and DNA binding activity in PVN. The results demonstrate that cardiovascular and autonomic mechanisms leading to heart failure after myocardial infarction can be modulated by activation of PPAR-γ in the brain. Central PPAR-γ may be a novel target for treatment of sympathetic excitation in myocardial infarction-induced HF.

Keywords
peroxisome proliferator-activated receptor-γ; proinflammatory cytokines; renin-angiotensin system; nuclear factor-kB; autonomic regulation

INTRODUCTION

Central nervous system mechanisms activated by persistent altered neural and humoral signals from the periphery play an important role in the progression of heart failure (HF).¹⁻⁶ Previous studies from our laboratory⁷⁻⁹ and others¹⁰ have demonstrated that rats with

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DISCLOSURES
None
myocardial infarction (MI)-induced HF exhibit neurochemical abnormalities in the brain, as evidenced by upregulation of brain pro-inflammatory cytokines and renin-angiotensin system (RAS), both contributing to augmented sympathetic nervous activity. Interventions that attenuate proinflammatory cytokine or RAS activity in the brain can significantly reduce sympathetic nervous activity and ameliorate the peripheral manifestations of HF.7, 10

The peroxisome proliferator-activated receptor (PPAR-γ) belongs to the PPAR superfamily of nuclear hormone receptors that play an important role in regulating adipocyte differentiation, lipid metabolism and insulin resistance.11 The thiazolidinedione class of synthetic PPAR-γ agonists has commonly been used to increase insulin sensitivity12 in type 2 diabetes. In addition, systemic activation of PPAR-γ by its agonists has been shown to prevent the progression of multiple cardiovascular diseases such as hypertension, atherosclerosis and chronic kidney disease by reducing inflammation13–15 and down-regulating angiotensin II (ANG II)-induced ANG II type-1 receptor (AT₁R) expression.15–18 Limited studies have examined the effects of peripherally administered PPAR-γ agonists on the progression of HF, but the results have been controversial.19–21

In the brain, activation of PPAR-γ has been shown to inhibit the synthesis and release of pro-inflammatory cytokines in multiple central nervous system diseases in which massive inflammation plays a detrimental role.6, 22–24 Notably, PPAR-γ is expressed in several brain regions associated with cardiovascular and autonomic regulation, including the paraventricular nucleus of hypothalamus (PVN)25 and the rostral ventrolateral medulla.26 Inflammation in these regions of the brain has recently emerged as an important factor in the pathogenesis of hypertension and HF. In spontaneously hypertensive rats, activation of PPAR-γ decreases oxidative stress in the rostral ventrolateral medulla and reduces sympathetic vasomotor activity and blood pressure.26 There has been no study of the role of PPAR-γ in the brain in HF.

The present study sought to determine whether activation of brain PPAR-γ might modulate the HF-induced expression of inflammatory mediators and RAS in the brain, thereby reducing sympathetic excitation and ameliorating the peripheral manifestations of HF after MI. Since inflammation and RAS activity in the PVN contribute to sympathetic overactivity in HF rats,1, 2 changes in the neurochemical milieu of the PVN were examined as a measure of the effects of activating brain PPAR-γ.

METHODS

Animals

Adult male Sprague-Dawley rats weighing 250–300 g were obtained from Harlan Sprague-Dawley. They were housed in temperature (23±2°C) and light controlled animal quarters and were provided with rat chow ad libitum. These studies were performed in accordance with the “Guiding Principles for Research Involving Animals and Human Beings”.27 The experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Iowa.

Experimental protocols

Rats underwent coronary artery ligation to induce HF or sham coronary ligation (SHAM), and echocardiography to assess left ventricular (LV) function. They were divided into three experimental groups: SHAM rats that received no treatment (SHAM, n=26); HF rats that received intracerebroventricular (ICV) infusion of the selective PPAR-γ agonist pioglitazone (3 nmol/h, dissolved in 20% DMSO in artificial cerebrospinal fluid28, 29, HF+PIO, n=26); HF rats that received ICV vehicle (20% DMSO in artificial cerebrospinal fluid, HF+VEH, n=28). The pioglitazone dose was determined in a previous study29 to be optimal for in vivo
activation of central PPAR-γ in rats, with no effects on blood glucose. Cannulas for ICV infusion were implanted 1 week after coronary ligation, and osmotic minipumps to infuse pioglitazone or VEH were implanted at 2 weeks after coronary ligation.

Pioglitazone and VEH were infused for 2 weeks, beginning 2 weeks after coronary ligation. In some rats (n=8 for SHAM, n=10 for HF+VEH and HF+PIO), body weight and food intake were measured twice weekly over two consecutive 24-h periods, starting 1 week prior to coronary ligation, and an average value for each variable was reported for that time point. At the conclusion of the study protocol, a second echocardiogram and hemodynamic measurements, including systolic blood pressure (SBP), diastolic blood pressure (DBP), LV end-diastolic pressure (LVEDP), LV peak systolic pressure (LVPSP), maximum rate of rise of LV pressure (dP/dt max) and heart rate (HR), were obtained in these rats. They were then euthanized with an overdose of urethane to collect blood, cerebrospinal fluid (CSF), brain and heart tissues for biochemical, real-time PCR and anatomical studies. In some rats, brain and heart tissues were used for Western blot analysis (n=6 for each group) or for PPAR-γ and nuclear factor (NF)-κB activity assay (n=8 for SHAM and HF+VEH, n=6 for HF+PIO). In a separate group of conscious rats (n=4 for each group), the response of mean arterial pressure to ganglionic blockade was examined to evaluate sympathetic nerve activity.

Specific Methods
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RESULTS

PPAR-γ expression and activity in the PVN and LV
As shown in Figure 1, real-time PCR and Western blot analyses revealed the presence of PPAR-γ mRNA and protein in both PVN (Figure 1A and 1B) and LV (Figure 1D and 1E) from SHAM and HF+VEH rats. There were no differences in PPAR-γ mRNA and protein expression in the PVN and LV between the two groups. However, PPAR-γ DNA binding activity, detected by a quantitative assay, was significantly (p<0.05) lower in both PVN (Figure 1C) and LV (Figure 1F) of HF+VEH rats compared with SHAM rats. ICV pioglitazone treatment of HF rats increased PPAR-γ mRNA and protein expression as well as DNA binding activity in the PVN, but not in the LV.

Effects of central PPAR-γ activation on expression of inflammatory mediators and RAS in the PVN
The mRNA (Figure 2) and protein (Figure 3) expression of the proinflammatory cytokines interleukin (IL)-1β and tumor necrosis factor (TNF)-α, cyclooxygenase (COX)-2 and AT1R was significantly higher in the PVN of HF+VEH rats compared with SHAM rats. After ICV treatment with pioglitazone, mRNA and protein expression of IL-1β, COX-2 and AT1R was significantly reduced and TNF-α mRNA and protein expression was normalized in the PVN of HF rats. There were no differences in COX-1 and ANG II type-2 receptor (AT2R) mRNA (Figure 2) and protein (Figure 3) across the three experimental groups.

Effects of central PPAR-γ activation on (NF)-κB activation in the PVN
There was greater NF-κB p65 DNA binding activity in the nuclear extract (Figure 4A) and less cytoplasmic inhibitor of κB (IκB)-α protein expression (Figure 4B) in the PVN of HF+VEH rats compared with SHAM rats. Cytoplasmic IκB-α protein was higher and NF-κB p65 DNA binding activity was lower in the PVN of HF rats treated with pioglitazone (Figure 4A and 4B).
Effects of central PPAR-γ activation on indicators of sympathetic excitation

CSF prostaglandin E₂ (PGE₂), a product of COX-2 activity that acts centrally to stimulate the sympathetic nervous system, and plasma norepinephrine (NE), a marker of sympathetic nerve activity, were both higher in HF+VEH rats compared with SHAM rats (Figure 5A and 5B). CSF PGE₂ and plasma NE levels were significantly lower in the HF rats treated with pioglitazone.

In conscious rats, baseline mean blood pressure (MBP) before injection of hexamethonium bromide was not different among groups (Figure 5C). After infusion of hexamethonium bromide, HF+VEH rats exhibited greater decrease in MBP compared with SHAM rats (Figure 5D). The response of MBP to hexamethonium bromide was normalized in HF rats treated with ICV pioglitazone (Figure 5D).

Effects of central PPAR-γ activation on indices of HF

The echocardiographic data are shown in Table S2. HF rats assigned to treatment with pioglitazone or VEH were well-matched with regard to echocardiographically defined LV function. Echocardiography performed within 24 hours of coronary ligation showed that LV ejection fraction was lower and LV end-diastolic volume was higher in the rats subjected to coronary artery ligation (HF rats) compared with SHAM rats. Four weeks after coronary artery ligation, LV ejection fraction was still significantly lower and LV end-diastolic volume was higher in HF rats treated with pioglitazone or VEH than in SHAM rats. Treatment with ICV pioglitazone had no effect on LV ejection fraction, LV end-diastolic volume, or the ischemic zone as a percentage of LV circumference in HF rats.

As seen in Table 1, at four weeks after coronary artery ligation, the right ventricular (RV)/body weight (BW) and wet lung/BW ratios were substantially higher in HF+VEH rats compared with SHAM rats. SBP, LVPSP, and the LV dp/dt max were lower and LVEDP was higher in HF+VEH rats than in SHAM rats. HF rats treated with ICV pioglitazone had lower RV/BW, wet lung/BW ratios, LVEDP, and higher LV dp/dt max than HF+VEH rats, but these values were still significantly different from SHAM rats. SBP and LVPSP were not affected. There were no significant differences in BW, LV/BW, HR and DBP across the experimental groups.

Changes in BW and food intake

Figure S1 shows the time-course of changes in BW and food intake, beginning 1 week prior to coronary artery ligation. BW and food intake decreased in all three experimental groups early (at 1 and 2 weeks) after coronary artery ligation or SHAM operation, with HF rats exhibiting greater reductions than SHAM rats. Otherwise, there were no differences in BW and food intake among three experimental groups. BW and food intake did not differ between HF+VEH and HF+PIO groups at any time point.

DISCUSSION

The novel finding of this study is that activation of central PPAR-γ in rats with ischemia-induced HF inhibited the expression of inflammatory mediators and a key component of the brain renin-angiotensin system in PVN, reduced sympathetic nerve activity and ameliorated the peripheral manifestations of HF. To our knowledge, this is the first report that activation of PPAR-γ in the brain has beneficial effects on the severity of HF following MI.

We found no differences in PPAR-γ mRNA and protein levels between SHAM and HF rats, as previously reported by others in dog heart tissues, but PPAR-γ DNA binding activity was significantly decreased in both the PVN - the brain region we chose to examine - and in
the control non-infarct region of LV in HF rats, compared with SHAM rats. Thus, the transcriptional activity of PPAR-γ, rather than the actual amount of PPAR-γ, was reduced in the PVN of HF rats. The reduction in transcriptional activity of PPAR-γ in the PVN was accompanied by increased PVN NF-κB activity and increased PVN expression of proinflammatory cytokines and AT_1R. ICV infusion of pioglitazone increased PPAR-γ expression in the PVN of HF rats to a level higher than that of the SHAM rats, normalized PPAR-γ DNA binding activity in PVN, and inhibited PVN NF-κB activity and the PVN expression of proinflammatory cytokines and AT_1R. COX-2 and CSF PGE_2 were also reduced. As expected with the ICV route of administration, there was no effect of pioglitazone on PPAR-γ expression or PPAR-γ DNA binding activity in the non-infarct region of the LV.

A reduction in PPAR-γ DNA binding activity without changes in PPAR-γ expression has been reported by others in studies of rat cardiac fibroblasts and blood vessels treated with exogenous ANG II. In those studies, treatment with PPAR-γ agonists not only prevented that response but, as in the present study of PVN, significantly increased PPAR-γ expression and normalized DNA binding activity. How ANG II reduces PPAR-γ DNA binding activity without altering PPAR-γ expression remains unknown. It has been suggested that ANG II may inhibit PPAR-γ activity by increasing Bcr, a serine/threonine kinase which may reduce PPAR-γ activity by inducing phosphorylation of PPAR-γ at serine 82. In ischemia-induced HF, in which the systemic RAS and the brain RAS are activated, it is conceivable that an ANG II-dependent reduction in PPAR-γ DNA binding activity in the PVN and LV might occur. In the complex neurochemical milieu of the HF brain, other unrecognized mechanisms may also be involved.

HF rats treated with ICV pioglitazone had less sympathetic nerve activity, as evidenced by reduced plasma NE levels in anesthetized rats and a normal response of MBP to hexamethonium bromide in conscious rats. In addition, there was some improvement in LVEDP and LV dp/dt_max, less pulmonary vascular congestion and less RV remodeling. These findings are consistent with our previous experience with this model of HF, in which interventions that treat central inflammation or brain RAS activity reduce sympathetic activity and improve volume-and-pressure-dependent indices of heart function. Activation of brain PPAR-γ addresses both of those central regulatory systems simultaneously, and so may be an effective means of slowing the progression of heart failure following MI. As expected, there was no effect on infarct size or ejection fraction, measures of the degree of infarct-induced myocardial injury, following a large MI.

The ability of PPAR-γ to regulate inflammation and RAS activity in peripheral tissues is well established. Activation of PPAR-γ inhibits the synthesis and release of cytokines from cells that participate in inflammatory processes, prevents cytokine-induced COX-2 activity, and suppresses ANG II-induced AT_1R expression. Reducing PPAR-γ activity with RNA interference or pharmacological inhibitors abrogates the protective effects of PPAR-γ agonists.

The salutary effects of centrally administered pioglitazone may be explained by the ability of PPAR-γ to physically bind to NF-κB p65 and inhibit NF-κB activity. Potential downstream gene products of cytokine-induced NF-κB activation include angiotensinogen and the AT_1R, COX-2, and the proinflammatory cytokines IL-1β, TNF-α. Recent studies have suggested that NF-κB mediates “cross-talk” between the proinflammatory cytokines and brain RAS activity in HF rats and thereby contributes to neurohumoral excitation. The present data suggest that activation of PPAR-γ modulates both systems in the PVN by reducing NF-κB activity.
Central inflammatory mediators and the brain RAS are both implicated in sympathetic overactivity in HF, which was reduced by ICV pioglitazone. In addition, the brain RAS has important effects on thirst and sodium appetite, that may also be modulated by activating of PPAR-γ. The combined effects of brain RAS and renal sympathetic nerve activity to foster volume accumulation are important factors in the pathogenesis of HF and hypertension.

Limitations
In the present study, rats received ICV pioglitazone for only 2 weeks because of the size limitation of osmotic minipumps and the possibility that a repeat surgery to implant a second minipump might interfere with measurements of metabolic parameters. Over that treatment interval, there were no changes in PPAR-γ mRNA or protein expression or in PPAR-γ DNA binding activity in the non-infarcted heart of HF rats. In addition, there were no differences in food intake or body weight between HF rats treated with pioglitazone versus vehicle. These observations argue strongly against any peripheral effect of the ICV pioglitazone and in favor of an independent central influence of PPAR-γ on the central (i.e., neurochemical) and peripheral manifestation of heart failure.

Since pioglitazone can cross the blood brain barrier, it is conceivable that longer-term central administration of a PPAR-γ agonist might result in adverse peripheral side effects. Adverse effects associated with systemic administration of pioglitazone include increases in food intake and body weight and fluid accumulation secondary to an increase in renal sodium reabsorption, with peripheral edema and precipitations of heart failure. Over our short-term treatment interval, we observed no evidence of weight gain or worsening heart failure in the pioglitazone treated rats, and indices of volume status (i.e., lung/body weight, LVEDP) actually improved.

The present study was limited to an examination of the effects of a centrally administered PPAR-γ agonist on events in a single cardiovascular autonomic nucleus, the PVN. The PVN seemed an ideal window on the neurochemical effects of PPAR-γ because overexpression of proinflammatory cytokines and brain RAS activity in the PVN has been shown to drive sympathetic activity in HF rats. However, we recognize that other cardiovascular related nuclei – e.g., the rostral ventrolateral medulla and subfornical organ – that have been implicated in the autonomic dysfunction associated with HF may also express PPAR-γ. Activation of PPAR-γ in those regions may well have contributed to the beneficial peripheral effects of the centrally administered PPAR-γ agonist.

Perspectives
The present study demonstrates that PPAR-γ agonists, acting within the brain to quell NF-κB activity, can exert beneficial effects by suppressing the two major excitatory systems driving sympathetic activity in heart failure and hypertension – the brain RAS and the proinflammatory cytokines. (Figure 6) However, it will be a therapeutic challenge to selectively target brain PPAR-γ. Pioglitazone crosses the blood-brain barrier, but the ability of systemically administered pioglitazone to achieve centrally effective drug levels will likely be precluded by peripheral effects. New compounds designed to reduce systemic side effects and/or to selectively target the brain, or new brain-specific drug delivery systems, must be developed to take advantage of this mechanism. Nevertheless, the present study provides new insights into the mechanisms activating the sympathetic nervous system in heart failure, and identifies a novel potential target for therapeutic intervention in cardiovascular diseases characterized by augmented sympathetic drive.
Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References


Figure 1.
Expression of PPAR-γ mRNA, protein and DNA binding activity in the PVN (A, B and C) and non-infarcted left ventricle (LV; D, E and F) of SHAM rats and HF rats treated with VEH or PIO. Values are mean ± SEM (n = 6–10 for each group). mRNA data was expressed as a fold change relative to SHAM. *P < 0.05 vs. SHAM, †P < 0.05 HF+PIO vs. HF+VEH.
Figure 2.
Quantitative comparison of mRNA expression for proinflammatory cytokines IL-1β (A) and TNF-α (B), COX-2 (C), COX-1 (D), and RAS components AT$_1$R (E) and AT$_2$R (F), in the PVN of SHAM rats and HF rats treated with VEH or PIO. Values are mean ± SEM (n = 8–10 for each group) and expressed as a fold change relative to SHAM. *$P<0.05$ vs. SHAM, †$P<0.05$ HF+PIO vs. HF+VEH.
Figure 3.
Quantitative comparison of protein levels for proinflammatory cytokines IL-1β (A) and TNF-α (B), COX-2 (C), COX-1 (D), and RAS components AT₁R (E) and AT₂R (F), from the PVN of SHAM rats and HF rats treated with VEH or PIO. Representative Western blots are shown in G. Values are expressed as mean ± SEM (n= 6 for each group). *P < 0.05 vs. SHAM, †P < 0.05 HF+PIO vs. HF+VEH.
Figure 4.
Nuclear factor NF-κB p65 DNA binding activity from nuclear protein extract (A) and inhibitory protein IκB-α from cytosolic extract (B) of the PVN of SHAM rats and HF rats treated with VEH or PIO. Western blot data were normalized by β-actin. Values are mean ± SEM (n = 6–8 for each group). *P<0.05 vs. SHAM, †P<0.05 HF+PIO vs. HF+VEH.
Figure 5.
Levels of CSF PGE$_2$ (A) and plasma NE (B), baseline MBP (C) and peak decreases in MBP to hexamethonium bromide (D) in SHAM rats and HF rats treated with VEH or PIO. Values are mean ± SEM (n = 4–10 for each group). *$P<0.05$ vs. SHAM, †$P<0.05$ HF+PIO vs. HF +VEH.
Figure 6.
Schematic representation of PPAR-γ modulation of sympathetic activity in rats with heart failure. The PPAR-γ agonist activates PPAR-γ transcriptional activity, which inhibits NF-kB activity and reduces the production of inflammatory mediators and components of the brain renin-angiotensin system that drive the sympathetic nervous system.
Table 1

Anatomical and hemodynamic measurements

<table>
<thead>
<tr>
<th>Variables at 4 Weeks</th>
<th>SHAM (n=8)</th>
<th>HF+VEH (n=10)</th>
<th>HF+PIO (n=10)</th>
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</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>361 ± 6</td>
<td>353 ± 5</td>
<td>358 ± 4</td>
</tr>
<tr>
<td>LV/BW (mg/g)</td>
<td>2.07 ± 0.05</td>
<td>2.10 ± 0.08</td>
<td>2.13 ± 0.04</td>
</tr>
<tr>
<td>RV/BW (mg/g)</td>
<td>0.58 ± 0.02</td>
<td>1.02 ± 0.06*</td>
<td>0.78 ± 0.04*</td>
</tr>
<tr>
<td>Lung/BW (mg/g)</td>
<td>3.91 ± 0.13</td>
<td>9.85 ± 0.54*</td>
<td>6.83 ± 0.66*†</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>322 ± 7</td>
<td>325 ± 6</td>
<td>329 ± 8</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>126 ± 3</td>
<td>113 ± 2*</td>
<td>114 ± 3*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>91 ± 3</td>
<td>89 ± 2</td>
<td>90 ± 3</td>
</tr>
<tr>
<td>LVPSP (mmHg)</td>
<td>113 ± 2</td>
<td>100 ± 2*</td>
<td>103 ± 2*</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>4 ± 1</td>
<td>18 ± 2*</td>
<td>13 ± 2*†</td>
</tr>
<tr>
<td>LV dP/dt&lt;sub&gt;max&lt;/sub&gt; (mmHg/s)</td>
<td>8124 ± 342</td>
<td>4463 ± 173*</td>
<td>6013 ± 143*†</td>
</tr>
</tbody>
</table>

BW: body weight; LV: left ventricular; RV: right ventricular; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; LVPSP: LV peak systolic pressure; LVEDP: LV end-diastolic pressure; dP/dt<sub>max</sub>: maximum rate of rise of LV pressure. Values are expressed as mean ± SEM.

* P<0.05 versus SHAM
† P<0.05 HF+PIO versus HF+VEH.