Peroxisome Proliferator-Activated Receptor-γ Regulates Inflammation and Renin-Angiotensin System Activity in the Hypothalamic Paraventricular Nucleus and Ameliorates Peripheral Manifestations of Heart Failure

Yang Yu, Zhi-Hua Zhang, Shun-Guang Wei, Robert M. Weiss, Robert B. Felder

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 downregulate the renin-angiotensin system. 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 renin-angiotensin system activity. Two weeks after coronary artery ligation, HF rats received an intracerebroventricular infusion of the PPAR-γ agonist pioglitazone or vehicle for another 2 weeks. PPAR-γ expression in the paraventricular nucleus of hypothalamus, an important cardiovascular region, was unchanged in HF compared with sham-operated rats. However, PPAR-γ DNA binding activity was reduced, nuclear factor-κB 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 of these findings were ameliorated in HF rats treated with intracerebroventricular pioglitazone, which increased PPAR-γ expression and DNA binding activity in the paraventricular nucleus of hypothalamus. 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. (Hypertension. 2012;59:00-00.) • Online Data Supplement

Key Words: peroxisome proliferator-activated receptor-γ, proinflammatory cytokines ■ renin-angiotensin system ■ nuclear factor-κB ■ autonomic regulation

Central nervous system mechanisms activated by persistently altered neural and humoral signals from the periphery play an important role in the progression of heart failure (HF).1–8 Previous studies from our laboratory and others have demonstrated that rats with myocardial infarction (MI)–induced HF exhibit neurochemical abnormalities in the brain, as evidenced by upregulation of brain proinflammatory cytokines and the renin-angiotensin system (RAS), both contributing to augmented sympathetic nervous activity. Interventions that attenuate the 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 sensitivity in type 2 diabetes mellitus. 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 inflammation and downregulating 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 proinflammatory cyto-
kines in multiple central nervous system diseases in which massive inflammation plays a detrimental role.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 emerged recently 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. Because inflammation and RAS activity in the PVN contribute to sympathetic overactivity in HF rats,²¹ 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 to 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, and echocardiography to assess left ventricular (LV) function. They were divided into 3 experimental groups: (1) sham rats that received no treatment (SHAM, n=26); (2) HF rats that received intracerebroventricular (ICV) infusion of the selective PPAR-γ agonist pioglitazone (PIO, 3 nmol/h, dissolved in 20% dimethyl sulfoxide in artificial cerebrospinal fluid);²⁸,²⁹ HF+PIO, n=26); and (3) HF rats that received ICV vehicle (VEH, 20% dimethyl sulfoxide in artificial cerebrospinal fluid; HF+VEH, n=28). The pioglitazone dose was determined in a previous study 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 vehicle were implanted at 2 weeks after coronary ligation.

Pioglitazone and vehicle were infused for 2 weeks, beginning 2 weeks after coronary ligation. In some rats (n=8 for sham and n=10 for HF+VEH or HF+PIO), body weight (BW) and food intake were measured twice weekly over 2 consecutive 24-hour periods, starting 1 week before 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, diastolic blood pressure, LV end-diastolic pressure (LVEDP), LV peak systolic pressure, maximum rate of rise of LV pressure (dP/dt max), and heart rate, were obtained in these rats. They were then euthanized with an overdose of urethane to collect blood, cerebrospinal fluid, brain, and heart tissues for biochemical, real-time PCR and anatomic 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 or HF+VEH and 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

Please see the online Data Supplement at http://hyper.ahajournals.org.
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 2 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) expressions of the proinflammatory cytokines interleukin-1β and tumor necrosis factor-α, cyclooxygenase (COX)-2, and AT1R were significantly higher in the PVN of HF+VEH rats compared with SHAM rats. After ICV treatment with pioglitazone, mRNA and protein expressions of interleukin-1β, COX-2, and AT1R were significantly reduced, and tumor necrosis factor-α mRNA and protein expression were normalized in the PVN of HF rats. There were no differences in COX-1 and Ang II type-2 receptor mRNA (Figure 2) and protein (Figure 3) across the 3 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**

Cerebrospinal fluid prostaglandin E2, a product of COX-2 activity that acts centrally to stimulate the sympathetic nervous system,30,31 and plasma norepinephrine, a marker of sympathetic nerve activity, were both higher in HF+VEH rats compared with SHAM rats (Figure 5A and 5B). Cerebrospinal fluid prostaglandin E2 and plasma norepinephrine levels were significantly lower in the HF rats treated with pioglitazone.

In conscious rats, baseline mean blood pressure before injection of hexamethonium bromide was not different among groups (Figure 5C). After infusion of hexamethonium bromide, HF+VEH rats exhibited a greater decrease in mean blood pressure compared with SHAM rats (Figure 5D). The response of mean blood pressure 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 (available in the online Data Supplement). HF rats assigned to treatment with pioglitazone or vehicle 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 vehicle 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.
differ between HF/VEH and HF/PIO groups at any time with HF rats exhibiting greater reductions than SHAM rats. 2 weeks) after coronary artery ligation or sham operation, decreased in all 3 of the experimental groups early (at 1 and 1 week before coronary artery ligation. BW and food intake the time course of changes in BW and food intake, beginning Figure S1 (available in the online Data Supplement) shows Changes in BW and Food Intake

As seen in the Table, at 4 weeks after coronary artery ligation, the right ventricular/BW and wet lung/BW ratios were substantially higher in HF/VEH rats compared with SHAM rats. Systolic blood pressure, LV peak systolic pressure, and the LV dp/dtmax were lower and LVEDP was higher in HF/VEH rats compared with SHAM rats. HF rats treated with ICV pioglitazone had lower right ventricular/BW ratio, wet lung/BW ratio, and LVEDP and higher LV dp/dtmax than HF/VEH rats, but these values were still significantly different from SHAM rats. Systolic blood pressure and LV peak systolic pressure were not affected. There were no significant differences in BW, LV/BW, heart rate, and diastolic blood pressure across the experimental groups.

Changes in BW and Food Intake

Figure S1 (available in the online Data Supplement) shows the time course of changes in BW and food intake, beginning 1 week before coronary artery ligation. BW and food intake decreased in all 3 of the 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 3 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 RAS 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 after MI.

We found no differences in PPAR-γ mRNA and protein levels between SHAM and HF rats, as reported previously 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 noninfarct 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 the PVN expression of proinflammatory cytokines and AT1R. ICV infusion of pioglitazone increased PPAR-γ expression in the PVN of HF rats compared with SHAM rats. Thus, the transcriptional activity of PPAR-γ in the PVN, and inhibited PVN NF-κB activity and the PVN expression of proinflammatory cytokines and AT1R. COX-2 and cerebrospinal fluid prostaglandin E2 were also reduced. As expected with the ICV route of administration, there was

Figure 3. Quantitative comparison of protein levels for proinflammatory cytokines interleukin (IL)-1β (A), tumor necrosis factor (TNF)-α (B), cyclooxygenase (COX)-2 (C), COX-1 (D), and renin-angiotensin system (RAS) components angiotensin (Ang) II type-1 receptor (AT1R; E) and Ang II type-2 receptor (AT2R; F), from the paraventricular nucleus of hypothalamus (PVN) of sham rats (SHAM) and heart failure (HF) rats treated with vehicle or pioglitazone. 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 rats that received intracerebroventricular (ICV) infusion of the selective peroxisome proliferator-activated receptor (PPAR)-γ agonist pioglitazone (HF+PIO) vs HF rats that received ICV vehicle (HF+VEH).
no effect of pioglitazone on PPAR-γ expression or PPAR-γ DNA binding activity in the noninfarct 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 that 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 may be that the suppression of Bcr expression or PPAR-γ expression or PPAR-γ activity by inducing phosphor-

Table. Anatomic and Hemodynamic Measurements

<table>
<thead>
<tr>
<th>Variables at 4 wk</th>
<th>SHAM (n = 6)</th>
<th>HF + VEH (n = 10)</th>
<th>HF + PIO (n = 10)</th>
</tr>
</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>LungBW, 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, bpm</td>
<td>322 ± 7</td>
<td>325 ± 6</td>
<td>329 ± 8</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>126 ± 3</td>
<td>113 ± 2*</td>
<td>114 ± 3*</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>91 ± 3</td>
<td>89 ± 2</td>
<td>90 ± 3</td>
</tr>
<tr>
<td>LPVSP, mm Hg</td>
<td>113 ± 2</td>
<td>100 ± 2*</td>
<td>103 ± 2*</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>4 ± 1</td>
<td>18 ± 2*</td>
<td>13 ± 2+†</td>
</tr>
</tbody>
</table>

LV dP/dt_max, mm Hg/s: 8124 ± 342, 4463 ± 173*, 6013 ± 143†

BW indicates body weight; LV, left ventricular; RV, right ventricular; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; LPVSP, LV peak systolic pressure; LVEDP, LV end-diastolic pressure; dP/dt_max, maximum rate of rise of LV pressure; SHAM, sham-operated control rats; HF + PIO, heart failure rats that received intracerebroventricular infusion of the selective peroxisome proliferator-activated receptor-γ agonist pioglitazone (HF + PIO) vs HF rats that received ICV vehicle (HF + VEH).

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 HF after MI. As expected,
there was no effect on infarct size or ejection fraction, measures of the degree of infarct-induced myocardial injury after a large MI.

The ability of PPAR-\(\gamma\) to regulate inflammation and RAS activity in peripheral tissues is well established. Activation of PPAR-\(\gamma\) inhibits the synthesis and release of cytokines from cells that participate in inflammatory processes, prevents cytokine-induced COX-2 activity,\(^{35-37}\) and suppresses Ang II–induced AT\(_R\) expression.\(^{15-18}\) Reducing PPAR-\(\gamma\) activity with RNA interference or pharmacological inhibitors abrogates the protective effects of PPAR-\(\gamma\) agonists.\(^{38,39}\)

The salutary effects of centrally administered pioglitazone may be explained by the ability of PPAR-\(\gamma\) to physically bind to NF-\(\kappa\)B p65 and inhibit NF-\(\kappa\)B activity.\(^{40-43}\) Potential downstream gene products of cytokine-induced NF-\(\kappa\)B activation include angiotensinogen,\(^{44}\) AT\(_R\),\(^{45}\) COX-2,\(^{46}\) and the proinflammatory cytokines interleukin-1\(\beta\) and tumor necrosis factor-\(\alpha\).\(^{47}\) Recent studies have suggested that NF-\(\kappa\)B mediates “cross-talk” between the proinflammatory cytokines and brain RAS activity in HF rats and thereby contributes to neurohumoral excitation.\(^{34,48}\) The present data suggest that activation of PPAR-\(\gamma\) modulates both systems in the PVN by reducing NF-\(\kappa\)B activity.

Central inflammatory mediators and the brain RAS are both implicated in sympathetic overactivity in HF,\(^{49}\) which was reduced by ICV pioglitazone. In addition, the brain RAS has important effects on thirst and sodium appetite\(^{50-55}\) that may also be modulated by activating of PPAR-\(\gamma\). The combined effects of brain RAS\(^{52}\) and renal sympathetic nerve activity\(^{53}\) 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-\(\gamma\) mRNA or protein expression or in PPAR-\(\gamma\) DNA binding activity in the noninfarcted heart of HF rats. In addition, there were no differences in food intake or BW 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-\(\gamma\) on the central (ie, neurochemical) and peripheral manifestations of HF.

Because pioglitazone can cross the blood-brain barrier, it is conceivable that longer-term central administration of a PPAR-\(\gamma\) agonist might result in adverse peripheral effects. Adverse effects associated with systemic administration of pioglitazone include increases in food intake and BW and in fluid accumulation secondary to an increase in renal sodium reabsorption, with peripheral edema and precipitation of HF.\(^{54-57}\) Over our short-term treatment interval, we observed no evidence of weight gain or worsening HF in the pioglitazone-treated rats, and indices of volume status (ie, lung/BW and LVEDP) actually improved.

The present study was limited to an examination of the effects of a centrally administered PPAR-\(\gamma\) agonist on events in a single cardiovascular autonomic nucleus, the PVN. The PVN seemed an ideal window on the neurochemical effects of PPAR-\(\gamma\), because overexpression of proinflammatory cytokines and brain RAS activity in the PVN has been shown to drive sympathetic activity in HF rats.\(^{7,8,58}\) However, we recognize that other cardiovascular-related nuclei, eg, the rostral ventrolateral medulla and subfornical organ, that have been implicated in the autonomic dysfunction associated with HF\(^{59,60}\) may also express PPAR-\(\gamma\). Activation of PPAR-\(\gamma\) in those regions may well have contributed to the beneficial peripheral effects of the centrally administered PPAR-\(\gamma\) agonist.

**Perspectives**

The present study demonstrates that PPAR-\(\gamma\) agonists, acting within the brain to quell NF-\(\kappa\)B activity, can exert beneficial effects by suppressing the 2 major excitatory systems driving sympathetic activity in HF and hypertension, the brain RAS and the proinflammatory cytokines (Figure 6). However, it will be a therapeutic challenge to selectively target brain PPAR-\(\gamma\). 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 adverse effects\(^{61,62}\) 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 HF and identifies a novel potential target for therapeutic intervention.
in cardiovascular diseases characterized by augmented sympathetic drive.

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Disclosures

None.

References


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to

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

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Running Title: Brain PPAR-γ and sympathetic nerve activity
SPECIFIC METHODS

Induction of heart failure

Rats underwent left coronary artery ligation under anesthesia (ketamine 90 mg/kg + xylazine 10 mg/kg, i.p.) to induce heart failure (HF), as previously described.\textsuperscript{1-3} In brief, the rats were endotracheally intubated and mechanically ventilated. The heart was rapidly exposed via a left thoracotomy, the pericardium was opened and the heart was exteriorized. The coronary ligation was made with a 6-0 silk suture passed through the superficial layers of myocardium between the pulmonary outflow tract and the left atrium. After coronary ligation, the heart was returned to the chest cavity, the lungs were reinflated and then the chest closed. For sham-operated rats (SHAM), the surgery was done in the same manner without ligating the coronary artery. Post-surgical animals were given benzathine penicillin (30,000 units IM) and buprenorphine (0.3 mg, SC) initially and then every 12 hours as needed for pain control.

Echocardiography

Echocardiography was performed under sedation (ketamine 25 mg/kg, i.p.) within 24 hours of coronary ligation to evaluate left ventricular (LV) function and assign rats to treatment groups, as previously described.\textsuperscript{2, 4} In some rats, echocardiography was repeated at 4 weeks to examine the effect of treatment on LV function. The size of the ischemic zone as a percent of LV circumference (\%IZ), LV ejection fraction (LVEF) and LV end-diastolic volume (LVEDV) were measured. Only animals with large infarctions (IZ $\geq$ 40\%) were used in the experimental groups in the study.

ICV cannula and implantation of osmotic mini-pumps

Sterile surgery was performed under anesthesia (ketamine 90 mg/kg + xylazine 10 mg/kg, i.p.) to implant a cannula into a lateral cerebral ventricle (stereotaxic coordinates: 1.5 mm lateral to midline, 1.0 mm caudal to bregma, and 3.5 mm ventral of dura). The cannula was fixed to the cranium with small screws and dental cement. An osmotic mini-pump (Alzet Osmotic Pump, Model# 2002, 0.5 $\mu$l/hr) was implanted subcutaneously behind the neck and connected to the cannula with silastic tubing. At the end of the study, the osmotic mini-pump was removed to check residual volume of the drug to ensure that all of the drug was infused into the animals and appropriate cannula placement was confirmed by sectioning the brain to check needle tracks.

Hemodynamic measurements

Rats were anesthetized with urethane (1.5 g/kg, IP), a Millar catheter was inserted into the right carotid artery for measurement of systolic blood pressure (SBP) and diastolic blood pressure (DBP), and then was advanced across the aortic valve into the left ventricle for measurement of LV end-diastolic pressure (LVEDP), LV peak systolic pressure (LVPSP), maximum rate of rise of LV pressure (dP/dt$_{max}$) and heart rate (HR) as described previously.\textsuperscript{2, 4}

Anatomical measurements

After hemodynamic measurements were obtained, the rats were euthanized with an overdose of urethane and the lungs and heart were removed. The wet lungs and right
ventricle (RV) were weighed to obtain lung/body weight and RV/body weight ratios as indices of pulmonary congestion and right ventricular remodeling, respectively.\textsuperscript{2, 4}

**Dissection of brain and heart tissue for molecular study**

The PVN and non-infarcted left ventricular (LV) tissues were obtained as previously described.\textsuperscript{3, 4} Briefly, brain tissue was cut into 500-μm coronal sections. The PVN and immediately surrounding hypothalamus were punched with a 15-gauge needle stub (ID: 1.5 mm). The LV was cut into three cross sections. Non-infarcted LV tissues were taken at least 1 mm away from the infarcted LV and were used for molecular study.

**Quantification of mRNA expression**

The total RNA was extracted from the PVN or heart tissues using TRI Reagent (Molecular Research Center, Inc). mRNA levels for PPAR-γ, proinflammatory mediators [interleukin (IL)-1β, tumor necrosis factor (TNF)-α, cyclooxygenase (COX)-2 and COX-1] and renin-angiotensin system components [angiotensin II (ANG II) type-1 receptors (AT\textsubscript{1}R) and ANG II type-2 receptor (AT\textsubscript{2}R)] were measured with TaqMan real-time PCR following reverse transcription of total RNA. The sequences for primers and probes used are summarized in Table S1. Primers and probes for TaqMan GAPDH were purchased from Applied Biosystems (Foster City, CA). Real-time PCR was performed using the ABI prism 7000 Sequence Detection System (Applied Biosystems, Carlsbad, CA). The values were normalized to GAPDH and the final concentration of mRNA was calculated using the formula $x=2^{-\Delta\Delta Ct}$\textsuperscript{5}, where $x=\text{fold difference relative to SHAM}$.

**Western blot analysis**

Protein was extracted from the PVN or heart tissues using cell lysis buffer (Cell Signaling Technology Inc, Beverly, MA). Protein level for PPAR-γ, inflammatory mediators and renin-angiotensin system components were measured with Western blotting analysis, as previously described,\textsuperscript{2, 4} using primary antibody to PPAR-γ, IL-1β, TNF-α, AT\textsubscript{1}R and AT\textsubscript{2}R (Santa Cruz Biotechnology Inc, Santa Cruz, CA), COX-2 and COX-1 (Cayman Chemical Co, Ann Arbor, MI). The density of the bands was quantified with NIH image software.

**Analysis of Blood and CSF**

The levels of norepinephrine (NE) in plasma and prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) in cerebrospinal fluid (CSF) were measured with commercial high sensitivity ELISA kits (Rocky Mountain Diagnostics Inc, Colorado Springs, CO; and R&D Systems Inc, Minneapolis, MN, respectively) as previously described.\textsuperscript{2, 4, 6}

**Ganglionic blockade with hexamethonium bromide**

Rats were anesthetized (ketamine 90 mg/kg + xylazine 10 mg/kg, i.p.), polyethylene catheters (PE-50) were inserted into the femoral artery and vein for blood pressure recording and for drug administration, respectively. The catheters were exteriorized at the back of the neck, and the rats were allowed to recover for 24 hours before blood pressure measurement. After a stable mean blood pressure (MBP) was obtained, 30 mg/kg hexamethonium bromide was administered into the femoral vein as
described by others. The maximum response of MBP to ganglionic blockade was used as
an index of peripheral sympathetic nervous system activity.\textsuperscript{7, 8}

**PPAR-\(\gamma\) and Nuclear factor (NF)-\(\kappa\)B p65 DNA binding assay**

Nuclear extracts for DNA binding and cytoplasmic extracts for NF-\(\kappa\)B inhibitory
protein (I\(\kappa\)B) were prepared from the PVN and heart tissues using a Nuclear Extract Kit
(Active Motif, Carlsbad, CA). PPAR-\(\gamma\) and NF-\(\kappa\)B p65 DNA binding activity were
detected by TransAM PPAR-\(\gamma\) and NF-\(\kappa\)B kits (Active Motif, Carlsbad, CA).
Cytoplasmic I\(\kappa\)B level was measured with Western blotting as described above, using a
polyclonal antibody to I\(\kappa\)B\(\alpha\) (Santa Cruz Biotechnology).

**Statistical Analysis**

All data are expressed as mean±SEM. The significance of differences in mean
values was analyzed by 2-way, repeated-measure ANOVA followed by post hoc
Fischer’s least-significant difference test. 1-way ANOVA followed by Fischer’s least
significant difference test was used to analyze echocardiographic parameters.
REFERENCES


Table S1. Sequences for primers and probes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers and Probes</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPAR-γ</td>
<td>Forward primer: 5’-CTTGGCCCATATTTATAGCTGTCATTATT-3’</td>
</tr>
<tr>
<td></td>
<td>Reverse primer: 5’-TGTCTCTCGATGGGCTTCAC-3’</td>
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<tr>
<td></td>
<td>Probe: 5’-TGGAGACCCTCCAGGCTTG-3’</td>
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<tr>
<td>IL-1β</td>
<td>Forward primer: 5’-CACCTCTCAAGCAGAGCACAG-3’</td>
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<tr>
<td></td>
<td>Reverse primer: 5’-GGGTCCATGGAATGCTCAAC-3’</td>
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<tr>
<td></td>
<td>Probe: 5’-TGTCCTCGATGGGCTTCAC-3’</td>
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<tr>
<td>TNF-α</td>
<td>Forward primer: 5’-CCAGGAGAAGTCAGCCTCCT-3’</td>
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<tr>
<td></td>
<td>Reverse primer: 5’-TCATACCAGGGGCTTGAGCTCA-3’</td>
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<tr>
<td></td>
<td>Probe: 5’-AGAGCCTGGCCTAAGGACACCCCT-3’</td>
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<tr>
<td>COX-2</td>
<td>Forward primer: 5’-GGCACAAATATGATGTTCGCA-3’</td>
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<td></td>
<td>Reverse primer: 5’-CCTCGCTTCTGATCTTGTTCA3’</td>
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<tr>
<td></td>
<td>Probe: 5’-TCTTTGCCCAGCAGTCTCAGGACTCAGTTCTTCA3’</td>
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<td>COX-1</td>
<td>Forward primer: 5’-GAGTCTCTCGCTCCAGTTTC-3’</td>
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<td>Reverse primer: 5’-AGGGAATGACTGGTGAGG-3’</td>
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<td></td>
<td>Probe: 5’-TGCTGCTGCTCAGCCTGCTGCT-3’</td>
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<td>AT1-R</td>
<td>Forward primer: 5’-GTAGCACAAGTCACCTGCACTCA-3’</td>
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<td>Reverse primer: 5’-GGTAGATGACGGCTGGCAAA-3’</td>
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<tr>
<td></td>
<td>Probe: 5’-CATCTGGCTTAATGGCGCTGGCC-3’</td>
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<td>AT2-R</td>
<td>Forward primer: 5’-CAATCTGGCTGTGGCTGACTT-3’</td>
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<td></td>
<td>Reverse primer: 5’-TGCAACCAGCTGGCTCA-3’</td>
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<tr>
<td></td>
<td>Probe: 5’-CAACCCTCTCTCTCTGGGAACACTATTACTCCTTATA-3’</td>
</tr>
</tbody>
</table>


Table S2. Echocardiographic measurements

| Variables at Baseline | SHAM  
(n=26)          | HF+VEH  
(n=28)          | HF+PIO  
(n=26)          |
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>LVEDV (ml)</td>
<td>0.33 ± 0.01</td>
<td>0.72 ± 0.05*</td>
<td>0.70 ± 0.06*</td>
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<tr>
<td>LVEF</td>
<td>0.76 ± 0.02</td>
<td>0.34 ± 0.04*</td>
<td>0.32 ± 0.03*</td>
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<tr>
<td>%IZ</td>
<td>----</td>
<td>45 ± 3*</td>
<td>46 ± 3*</td>
</tr>
</tbody>
</table>

| Variables at 4 weeks | SHAM  
(n=8)          | HF+VEH  
(n=10)          | HF+PIO  
(n=10)          |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>LVEDV (ml)</td>
<td>0.35 ± 0.02</td>
<td>1.20 ± 0.12*</td>
<td>1.17 ± 0.08*</td>
</tr>
<tr>
<td>LVEF</td>
<td>0.72 ± 0.03</td>
<td>0.30 ± 0.04*</td>
<td>0.33 ± 0.02*</td>
</tr>
<tr>
<td>%IZ</td>
<td>----</td>
<td>46 ± 2*</td>
<td>42 ± 4*</td>
</tr>
</tbody>
</table>

SHAM: sham-operated control; HF+VEH: vehicle-treated HF; HF+PIO: pioglitazone-treated HF. LVEDV: left ventricular end-diastolic volume; LVEF: left ventricular ejection fraction; %IZ: size of the ischemic zone as a percent of left ventricular circumference. Values are expressed as mean ± SEM. *P<0.05 versus SHAM at same week.
Figure S1. Time course of body weight (A) and food intake (B) in SHAM rats and HF rats treated with VEH or PIO. Arrows indicate time of coronary artery ligation to induce myocardial infarction (MI). Values are mean ± SEM (n = 8-10 for each group).