Activation of Central PPAR-γ Attenuates Angiotensin II–Induced Hypertension

Yang Yu,* Bao-Jian Xue,* Shun-Guang Wei, Zhi-Hua Zhang, Terry G. Beltz, Fang Guo, Alan Kim Johnson, Robert B. Felder

Abstract—Inflammation and renin–angiotensin system activity in the brain contribute to hypertension through effects on fluid intake, vasopressin release, and sympathetic nerve activity. We recently reported that activation of brain peroxisome proliferator–activated receptor (PPAR)-γ in heart failure rats reduced inflammation and renin–angiotensin system activity in the hypothalamic paraventricular nucleus and ameliorated the peripheral manifestations of heart failure. We hypothesized that the activation of brain PPAR-γ might have beneficial effects in angiotensin II–induced hypertension. Sprague–Dawley rats received a 2-week subcutaneous infusion of angiotensin II (120 ng/kg per minute) combined with a continuous intracerebroventricular infusion of vehicle, the PPAR-γ agonist pioglitazone (3 nmol/h) or the PPAR-γ antagonist GW9662 (7 nmol/h). Angiotensin II+vehicle rats had increased mean blood pressure, increased sympathetic drive as indicated by the mean blood pressure response to ganglionic blockade, and increased water consumption. PPAR-γ mRNA in subfornical organ and hypothalamic paraventricular nucleus was unchanged, but PPAR-γ DNA-binding activity was reduced. mRNA for interleukin-1β, tumor necrosis factor-α, cyclooxygenase-2, and angiotensin II type 1 receptor was augmented in both nuclei, and hypothalamic paraventricular nucleus neuronal activity was increased. The plasma vasopressin response to a 6-hour water restriction also increased. These responses to angiotensin II were exacerbated by GW9662 and ameliorated by pioglitazone, which increased PPAR-γ mRNA and PPAR-γ DNA-binding activity in subfornical organ and hypothalamic paraventricular nucleus. Pioglitazone and GW9662 had no effects on control rats. The results suggest that activating brain PPAR-γ to reduce central inflammation and brain renin–angiotensin system activity may be a useful adjunct in the treatment of angiotensin II–dependent hypertension. (Hypertension. 2015;66:403-411. DOI: 10.1161/HYPERTENSIONAHA.115.05726.)

Key Words: angiotensin II □ brain □ inflammation □ peroxisome proliferator-activated receptors □ renin-angiotensin system

Numerous studies have demonstrated that inflammation and activation of the renin–angiotensin system (RAS) are involved in the pathogenesis of hypertension. Increased inflammation and RAS activity in the central nervous system contribute to the development and maintenance of hypertension in a variety of experimental models, in which pharmacological or genetic interventions that inhibit inflammation and RAS activity in the brain have been shown to reduce sympathetic activation and attenuate hypertension.

The peroxisome proliferator–activated receptor (PPAR)-γ belongs to the PPAR family of nuclear hormone receptors best known for their role in regulating various genes involved in glucose homeostasis, lipid metabolism, and adipocyte differentiation. Pioglitazone, a thiazolidinedione class synthetic PPAR-γ agonist, is used for treatment of patients with type II diabetes mellitus. However, pioglitazone has also been reported to reduce or prevent hypertension in animal models and in humans.

PPAR-γ is expressed in key brain areas involved in drinking, vasopressin release, and cardiovascular regulation, including the hypothalamic paraventricular nucleus (PVN) and the rostral ventrolateral medulla (RVLM). In the RVLM, activation of PPAR-γ attenuated angiotensin (ANG) II–induced hypertension in rats. However, the mechanism(s) by which activation of brain PPAR-γ lowers blood pressure remains poorly understood.

We recently reported that PPAR-γ DNA-binding activity was reduced in the PVN of rats with ischemia-induced heart failure, in association with increased expression of inflammatory markers and ANG II type 1 receptors (AT1R), and that central administration of pioglitazone ameliorated these molecular abnormalities and the peripheral manifestations of heart failure.
heart failure. The neurochemical profile of central nuclei regulating sympathetic nerve activity in animals with hypertension is similar to that in animals with heart failure. The present study examined the effects of centrally administered pioglitazone on ANG II–induced hypertension and on neurochemical mechanisms in 2 forebrain regions known to contribute to ANG II–induced hypertension: the subfornical organ (SFO) and the PVN. The results of this animal study suggest that centrally acting drugs with PPAR-γ agonist effects may confer additional benefits in antihypertensive therapy.

**Methods**

**Animals**

Adult male Sprague–Dawley rats weighing 250 to 300 g were purchased from Harlan (Indianapolis, IN). Rats were housed in temperature-controlled (23±2°C) and light-controlled animal quarters and were provided with standard rat chow (0.4% NaCl) ad libitum. The studies were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Iowa.

**Surgical Preparations**

All surgical procedures were performed under ketamine–xylazine (100 and 10 mg/kg, respectively) anesthesia and under sterile conditions. A telemetry transducer (TA11PA-C40; Data Science International) was implanted in a femoral artery for continuous monitoring of mean blood pressure (MBP) and heart rate. A cannula was implanted in a lateral ventricle for intracerebroventricular (ICV) drug infusion. Osmotic mini-pumps (model 2002; Alzet) were implanted subcutaneously for continuous systemic and ICV drug infusion.

**Drugs and Routes of Administration**

Hypertension was induced by slow infusion of ANG II (120 ng/kg per minute, SC) for 2 weeks, as previously described. A concomitant continuous ICV infusion of the PPAR-γ agonist pioglitazone (3 nmol in 0.5 µL/h), the PPAR-γ antagonist GW9662 (GW; 7 nmol in 0.5 µL/h), or the vehicle for pioglitazone (VEH; 20% dimethyl sulfoxide in artificial cerebrospinal fluid; 0.5 µL/h) was administered to control rats. The dose of pioglitazone was based on previous studies from our laboratory and from others showing that the optimal in vivo activation of central PPAR-γ in rats with no effect on blood glucose. The dose of GW was based on previous study. The ganglionic blocker hexamethonium bromide was administered (30 mg/kg, IP) to evaluate the sympathetic contribution to MBP, as previously described.

**Experimental Protocols**

1. MBP and heart rate were recorded by telemetry for 5 days at baseline and then for 2 weeks during subcutaneous infusion of ANG II combined with ICV VEH (ANG II+VEH, n=8), ICV pioglitazone (ANG II+PIO, n=8), or ICV GW9662 (ANG II+GW, n=6). Some age-matched untreated rats served as a time control (CON, n=6); others received ICV pioglitazone (CON+PIO, n=5) or ICV GW (CON+GW, n=5). One day before euthanasia, the MBP response to hexamethonium bromide was tested. At 2 weeks, the rats were euthanized, while deeply anesthetized with isoflurane or urethane to collect brain and heart tissue for measurement of PPAR-γ DNA-binding activity.

2. Additional studies were performed in identically treated ANG II+VEH (n=18), ANG II+PIO (n=18), ANG II+GW (n=15), CON (n=18), CON+PIO (n=15), and CON+GW (n=15) rats, without telemetry monitoring:
   a. Rats (n=6–8 from each group) were euthanized, while deeply anesthetized with isoflurane or urethane to obtain brain and heart tissues for mRNA measurement. Left ventricular (LV) weight:body weight (BW) ratio was determined in these animals.
   b. Rats (n=4 from each group) were deeply anesthetized with urethane and perfused with fixative for immunohistochemical study.
   c. Rats (n=6–8 from each group in protocol a. above) underwent twice weekly measurements of food and water intake and BW; measurements of food and water intake were made over 2 consecutive 24-hour periods, and an average value for each variable was reported for each time point.
   d. Rats (n=5–6 from each group) underwent a 6-hour water restriction and were then euthanized while deeply anesthetized with isoflurane to collect blood for the measurement of plasma arginine vasopressin (AVP); rats (n=6–8 from each group in protocol a. above) that did not undergo water restriction served as controls.

**Specific Methods**

Specific Methods are available in the online-only Data Supplement.

**Results**

**Effects of Pioglitazone and GW9662 on ANG II–Induced Hypertension and Sympathetic Activation**

ANG II+VEH rats experienced a gradual increase in MBP, from a baseline of 106±3 mmHg to a peak level of 142±4 mmHg at the end of week 2 (Figure 1A). The MBP response was attenuated in ANG II+PIO rats, but was augmented in ANG II+GW rats. MBP did not change from baseline in the CON, CON+PIO, and CON+GW rats. There were no significant changes in heart rate among groups (Figure 1B).

Hexamethonium caused a significant reduction in MBP in all 6 groups (Figure 1C). The decrease in MBP was significantly greater in ANG II+VEH rats than in CON rats. When compared with ANG II+VEH rats, ANG II+PIO rats had a significantly smaller depressor response, whereas ANG II+GW rats had a greater depressor response. CON+PIO and CON+GW rats had a depressor response similar to CON rats.

**Effects of Pioglitazone and GW9662 on ANG II–Induced PPAR-γ Expression and Activity in Brain and Heart Tissues**

There were no differences in PPAR-γ mRNA expression in the SFO, the PVN, or the LV between CON and ANG II+VEH rats (Figure 2A). However, PPAR-γ DNA-binding activity, detected by a quantitative assay, was reduced in the ANG II+VEH rats in SFO, PVN, and LV (Figure 2B). When compared with ANG II+VEH or CON rats, ANG II+PIO and CON+PIO rats had increased PPAR-γ mRNA expression (Figure 2A) and PPAR-γ DNA-binding activity (Figure 2B), whereas ANG II+GW and CON+GW rats had decreased PPAR-γ mRNA expression (Figure 2A) and PPAR-γ
DNA-binding activity in the SFO and PVN (Figure 2B) but not in the LV (Figure 2A and 2B).

Effects of Pioglitazone and GW9662 on ANG II–Induced RAS and Inflammatory Mediators in Brain Tissues

ANG II+VEH rats had significantly higher levels of mRNA for AT₁R, the proinflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), and cyclooxygenase (COX)-2 in SFO and PVN, compared with CON rats (Figure 3). Compared with ANG II+VEH rats, ANG II+PIO rats had lower levels of mRNA for AT₁R, IL-1β, and COX-2, and TNF-α mRNA was normalized. In contrast, ANG II+GW rats had higher levels of mRNA for AT₁R, TNF-α, and COX-2, but not for IL-1β. Of note, CON, CON+PIO and CON+GW had similar levels of mRNA for AT_1R, IL-1β, TNF-α, and COX-2. There were no differences across groups in ANG II type-2 receptor and COX-1 mRNA.

Effects of Pioglitazone and GW9662 on ANG II–Induced Neuronal Excitation in the PVN

Fra-like activity, a marker of chronic neuronal excitation, was examined in the PVN because of its recognized role as an integrative center for neurohumoral information and a source of presympathetic and vasopressinergic neurons. Compared with CON rats, ANG II+VEH rats expressed increased Fra-like activity throughout the PVN (Figure 4). Compared with ANG II+VEH, ANG II+PIO rats had less Fra-like activity and ANG II+GW rats had more Fra-like activity in the medial parvocellular, ventrolateral parvocellular, dorsal parvocellular, and posterior magnocellular regions of the PVN. No difference in Fra-like activity was observed between CON+PIO, CON+GW, and CON rats.

Effects of Pioglitazone and GW9662 on Volume Regulation and Food Intake

There were no differences across groups in baseline plasma AVP concentrations at 2 weeks (Figure 5A). However, after a

Figure 1. Mean blood pressure (MBP, A) and heart rate (HR, B), and peak changes (Δ) in MBP in response to ganglionic blockade (C), in angiotensin (ANG) II–infused rats treated with intracerebroventricular (ICV) vehicle (VEH), pioglitazone (PIO), or the PIO antagonist GW9662 (GW) and in age-matched normal control (CON) rats, untreated or treated with ICV PIO or GW. Values are mean±SEM (n=5–8 for each group). *P<0.05 vs baseline or CON; †P<0.05 vs ANG II+VEH. Bar over x-axis in A and B indicates duration of drug infusions.

Figure 2. The expression of peroxisome proliferator–activated receptor (PPAR)-γ mRNA (A) and PPAR-γ DNA-binding activity (B) in the subfornical organ (SFO), the hypothalamic paraventricular nucleus (PVN), and left ventricle (LV) of untreated control (CON) rats, CON rats treated with intracerebroventricular (ICV) pioglitazone (PIO) or PIO antagonist GW9662 (GW), and angiotensin (ANG) II–infused rats treated with ICV vehicle (VEH), PIO, or GW. Values are mean±SEM (n=5–8 for each group). mRNA data are expressed as a fold change relative to CON. *P<0.05 vs baseline or CON; †P<0.05 vs ANG II+VEH.
6-hour water restriction, plasma AVP concentration increased significantly in ANG II+VEH and ANG II+GW rats, but not in ANG II+PIO rats. An increase in baseline water consumption was observed in ANG II+VEH and ANG II+GW rats, compared with CON rats, but not in ANG II+PIO rats (Figure 5B). Notably, the increase in water consumption was greater in ANG II+GW rats than in ANG II+VEH rats. There were no differences across groups in food intake (Figure 5C) or BW (Figure 5D).

**Effects of Pioglitazone and GW9662 on Cardiac Remodeling**

Both ANG II–VEH and ANG II–GW rats experienced increases in LV weight and LV/BW ratio, compared with CON rats (Table). The LV/BW ratio was lower in the ANG II+PIO and higher in the ANG II+GW rats when compared with the ANG II+VEH rats. There were no differences in LV weight or LV/BW ratio among CON+PIO, CON+GW, and CON rats.

**Discussion**

The major findings of this study are as follows: (1) PPAR-γ DNA-binding activity is reduced in the SFO and in the PVN, 2 key forebrain regions regulating fluid volume and sympathetic drive, in rats with ANG II–induced hypertension; (2) ICV administration of the PPAR-γ agonist pioglitazone upregulates PPAR-γ mRNA and PPAR-γ DNA-binding activity in the SFO and in the PVN of rats with ANG II–induced hypertension; (3) ICV pioglitazone ameliorates ANG II–induced upregulation of RAS activity, inflammation, and neuronal excitation in the SFO and in the PVN, ANG II–induced sympathetic nerve activity, and ANG II–induced hypertension; (4) ICV pioglitazone reduces water consumption and the accentuated plasma AVP response to acute water restriction in rats with ANG II–induced hypertension.

Augmented brain RAS activity and central inflammation contribute to the activation of the sympathetic nervous system in hypertension and heart failure. In a previous study of rats with ischemia-induced heart failure, we found that the increased expression of excitatory/inflammatory mediators in the PVN was associated with reduced PPAR-γ DNA-binding activity, and that both could be ameliorated—along with the peripheral manifestations of heart failure—by central administration of pioglitazone. The present study reveals a similar favorable effect of the PPAR-γ agonist in the slow pressor model of ANG II hypertension. The ANG II–infusion protocol induced a reduction in PPAR-γ DNA-binding activity and an upregulation of mRNA for AT1R, TNF-α, IL-1β, and COX-2 in the SFO and in the PVN. A concomitant ICV infusion of pioglitazone ameliorated these ANG II–induced neurochemical changes and the ANG II–induced increases in blood

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**Figure 3.** mRNA expression for renin–angiotensin system components angiotensin (ANG) II type 1 receptors (AT1R; A), AT2R (B), proinflammatory cytokines interleukin (IL)-1β (C), tumor necrosis factor (TNF)-α (D), and cyclooxygenase (COX)-2 (E) and COX-1 (F), in subfornical organ (SFO) and paraventricular nucleus (PVN) of untreated control (CON) rats, CON rats treated with intracerebroventricular (ICV) pioglitazone (PIO) or PIO antagonist GW9662 (GW), and angiotensin (ANG) II–infused rats treated with ICV vehicle (VEH), PIO, or GW. Values are mean±SEM (n=6–8 for each group). Real-time polymerase chain reaction results are expressed as a fold change relative to CON. *P<0.05 vs baseline or CON; †P<0.05 vs ANG II+VEH.
pressure and sympathetic activity. The physiological significance of PPAR-γ as a modulator of the ANG II–induced central hypertensive state was confirmed by infusing the PPAR-γ antagonist GW9662, which exacerbated the ANG II–induced neurochemical changes and the hypertensive response.

The explanation for these favorable effects of activating PPAR-γ may reside in its ability to regulate oxidative stress and the activity of the nuclear transcription factors activator protein 1 and nuclear factor-kB. ANG II, acting on the AT1R, activates nicotinamide adenine dinucleotide phosphate oxidase–dependent superoxide production in SFO, the PVN, and other cardiovascular regions of the brain.24 Superoxide-dependent activation of activator protein 1 in the PVN has been implicated in the pathophysiology of renovascular hypertension,25 and superoxide-dependent activation of nuclear factor-xB has been implicated in ANG II–induced hypertension.26 ANG II upregulation of AT1R in the RVLM of heart failure rats has been attributed to activator protein 1,17 and potential gene products of nuclear factor-xB include the ANG II precursor protein angiotensinogen,26 the proinflammatory cytokines TNF-α and IL-1β,20 and COX-2.30 Ligand-activated PPAR-γ can alleviate oxidative stress31,32 and interfere with the activation or transcriptional functions of activator protein 1 and nuclear factor-kB,33–35 potentially inhibiting the effect of ANG II to upregulate brain RAS and the inflammatory mediators.27,36–38 In addition, PPAR-γ regulates microglial activation,39 which has recently been found to play an important role in the pathophysiology of neurogenic hypertension.7 Finally, there is evidence that the activation of PPAR-γ may induce the production of anti-inflammatory cytokines.40 All of these effects, considered in the context of the known central neurochemical abnormalities in hypertension, have potential for benefit.

The ANG II infusion had no effect on PPAR-γ mRNA in the SFO, the PVN, or the LV (a peripheral control). This result is consistent with findings of others that PPAR-γ DNA-binding activity is reduced without changes in PPAR-γ expression in cardiac fibroblasts and in blood vessels of rats treated with exogenous Ang II.41–43 Whether a reduction in PPAR-γ binding activity is a cause or a consequence43 of increased RAS activity and inflammation in the brain in hypertension and heart failure remains to be determined, but restoration of PPAR-γ activity is clearly associated with a suppression of these excitatory systems.

These findings are consistent with previous animal studies reporting that central administration of another thiazolidinedione, rosiglitazone, reduced the blood pressure response.
to centrally administered ANG II, and that oral dosing with pioglitazone (which crosses the blood–brain barrier) in a high-fructose diet model of metabolic syndrome reduced oxidative stress in the RVLM, sympathetic activation, and mean arterial pressure. The latter 2 studies focused on other effects of PPAR-γ activity and did not measure the indicators of RAS activity and inflammation reported here.

In clinical studies, treatment of patients with metabolic syndrome with thiazolidinediones has had relatively small effects on blood pressure although it could be argued that the brain levels achieved might not have been sufficient to produce such an effect. The combination of a PPAR-γ agonist with an AT1R blocker results in a facilitated effect on blood pressure, greater than seen with either agent alone. Although these effects were attributed to peripheral renal and vascular mechanisms, similar synergistic interactions between the PPAR-γ and AT1R have been suggested to occur in the brain. The present results support the general concept that PPAR-γ agonists may provide a significant additional benefit as adjunctive therapy for patients with hypertension.

Pioglitazone, the thiazolidinedione chosen for this study, is one of many naturally occurring and synthetic PPAR-γ agonists. Treatment with thiazolidinediones has been associated with several adverse effects, including increases in food intake and BW and fluid accumulation secondary to an increase in renal sodium reabsorption, with peripheral edema and precipitation of heart failure. However, pioglitazone may be safer than other thiazolidinediones and may even be beneficial from a cardiovascular standpoint. In the present study, over a 2-week treatment interval, fluid consumption and BW were not adversely affected by ICV pioglitazone. In fact, the ANG II+VEH rats and the ANG II+GW rats both consumed significantly more water than any of the pioglitazone-treated groups. Although the ANG II+VEH rats had normal circulating AVP levels at rest, they had an exaggerated AVP response to an acute fluid restriction—an apparent ANG II–induced increased sensitivity that was prevented by pioglitazone treatment. Previous work has demonstrated that a peripheral ANG II infusion upregulates AT1R in the SFO, a major regulatory center for thirst, and in the PVN, a central loci of AVP neurons. ANG II–induced upregulation of AT1R in these 2 centers may well account for these 2 findings.

Limitations

Pioglitazone, like other thiazolidinediones and some naturally occurring PPAR agonists, may have effects independent of activating PPAR-γ, including anti-inflammatory effects. To definitively attribute responses to activation of PPAR-γ, it would be necessary to demonstrate that the responses to pioglitazone were interrupted by a PPAR-γ agonist.

Table. Effects of Intracerebroventricular Pioglitazone and GW9662 on ANG II–Induced Cardiac Remodeling

<table>
<thead>
<tr>
<th>Group</th>
<th>BW, g</th>
<th>LV, mg</th>
<th>LV/BW, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON (n=8)</td>
<td>376±5</td>
<td>999±13</td>
<td>2.66±0.05</td>
</tr>
<tr>
<td>CON+PIO (n=5)</td>
<td>383±4</td>
<td>1010±27</td>
<td>2.64±0.07</td>
</tr>
<tr>
<td>CON+GW (n=5)</td>
<td>388±6</td>
<td>1008±12</td>
<td>2.60±0.06</td>
</tr>
<tr>
<td>ANG II+VEH (n=8)</td>
<td>373±5</td>
<td>1097±26</td>
<td>2.94±0.05*</td>
</tr>
<tr>
<td>ANG II+PIO (n=8)</td>
<td>387±6</td>
<td>1058±28</td>
<td>2.74±0.07†</td>
</tr>
<tr>
<td>ANG II+GW (n=6)</td>
<td>377±4</td>
<td>1173±25</td>
<td>3.11±0.05†</td>
</tr>
</tbody>
</table>

ANG II indicates angiotensin II; BW, body weight; CON, control; GW, the pioglitazone antagonist GW9662; LV, left ventricle; PIO, pioglitazone; and VEH, vehicle.

*P<0.05 vs CON.
†P<0.05 vs ANG II+VEH.
antagonist. That was not done in these studies. However, the observed reciprocal effect of GW9662 on the neurochemical, sympathetic, and blood pressure responses to ANG II strongly supports the argument that the pioglitazone effects are PPAR-γ mediated.

The present study examined the effects of a centrally administered PPAR-γ agonist on neurochemical events in 2 forebrain cardiovascular autonomic nuclei that have been shown to play a major role in ANG II-dependent hypertension. However, we recognize that the effects of ICV pioglitazone on blood pressure cannot be attributed solely to neurochemical changes in the SFO and the PVN. These 2 nuclei serve only as a window on the central effects of PPAR-γ agonists. The ICV infusion of pioglitazone would certainly have activated PPAR-γ in other cardiovascular regulatory centers as well—the RVLM, where a PPAR-γ agonist has been shown to modulate blood pressure, is an obvious additional potential site of action. However, the strikingly concordant effects of pioglitazone on the expression of RAS and inflammatory elements in SFO and PVN suggest that similar neurochemical changes are likely to occur in other cardiovascular related sites that express the PPAR-γ receptor.

In the rat model of ANG II–induced hypertension used in this study, the SFO senses circulating ANG II and signals presympathetic neurons. As we recently demonstrated, changes in the neurochemical milieu of the SFO can have significant effects on the neurochemical milieu downstream in the PVN. Accordingly, we cannot exclude the possibility that the effect of pioglitazone to reduce RAS activity and inflammation in the SFO may have contributed to the observed reduction in RAS activity and inflammation in the PVN. Arguing in favor of a local effect of pioglitazone in the PVN is the comparable increase in PPAR-γ activity in both nuclei.

Finally, the present study did not identify the specific cellular targets of the PPAR-γ agonist. Neurons, microglia, and astrocytes all express PPAR-γ, and one may speculate that the observed effects represent a collective response. For example, a PPAR-γ–mediated reduction in activated microglia and their release of proinflammatory cytokines may reduce the excitatory milieu surrounding presympathetic neurons, whereas a PPAR-γ–mediated reduction in AT,R may reduce the neuronal response to local levels of ANG II. Although additional studies may elucidate the relative role of the individual cell types involved, the response to ICV administration of the PPAR-γ agonist in the present study may simulate the more global central effect that a clinically administered agent might have on blood pressure regulation.

Perspectives
The present study demonstrates that a centrally administered PPAR-γ agonist reduces the activity of 2 major systems regulating sympathetic activity, body fluid homeostasis, and neurohormonal release in hypertension—the brain RAS and the proinflammatory cytokines—and ameliorates ANG II–dependent hypertension (Figure 6). The ability of a PPAR-γ agonist to suppress both mechanisms in this animal model suggests that antihypertensive agents with PPAR-γ agonist effects may have additional beneficial CNS effects in angiotensin-dependent hypertension and encourages the development of agents that more specifically target the central actions of this important receptor.

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Disclosures
None.

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**Novelty and Significance**

**What Is New?**

- Peroxisome proliferator-activated receptor (PPAR)-γ DNA-binding activity is reduced in the subfornical organ and the hypothalamic paraventricular nucleus, 2 key cardiovascular autonomic regions of the brain, in angiotensin II–induced hypertension.

- Central administration of a PPAR-γ agonist restores PPAR-γ DNA-binding activity, blunts the angiotensin II–induced neurochemical changes in the subfornical organ and the paraventricular nucleus, sympathetic nerve activity, and blood pressure.

**What Is Relevant?**

- PPAR-γ agonists act centrally and peripherally to modulate inflammation and the renin–angiotensin system.

- The development of agents that more specifically target central PPAR-γ may lead to more effective treatment of angiotensin-dependent hypertension.

**Summary**

Preventing the reduction in central PPAR-γ activity associated with angiotensin II–induced hypertension with a concomitant central infusion of a PPAR-γ agonist interferes with the upregulation of inflammatory mediators and angiotensin II type 1 receptors in the paraventricular nucleus that drive sympathetic excitation and the development of hypertension. Its ability to modulate both inflammation and brain renin–angiotensin system activity makes central PPAR-γ a logical target for drug development.
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to

Activation of Central PPAR-γ Attenuates Angiotensin II-Induced Hypertension

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Running Title: Brain PPAR-γ modulates ANG II-induced hypertension
**SPECIFIC METHODS**

**Implantation of ICV cannula and osmotic mini-pumps**

Using sterile surgical conditions under ketamine/xylazine anesthesia (100 mg/kg + 10 mg/kg, respectively, i.p.), a cannula was implanted 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), as described previously. The cannula was fixed to the cranium using small screws and dental cement. During the same surgery, an osmotic mini-pump (Alzet Osmotic Pump, Model# 2002, 0.5 µl/hr) for central infusion of pioglitazone, GW 9662 or vehicle was implanted under the skin on the back of the animal and connected to the cannula with silastic tubing. A second osmotic minipump (Alzet Osmotic Pump, Model# 2002, 0.5 µl/hr) was implanted under the skin on the back of the animal for systemic infusion of angiotensin II (ANG II). At the end of the study, the osmotic mini-pumps were removed to check residual volume to ensure that all of the drug was infused. Appropriate cannula placement was verified by sectioning the brain to check needle tracks.

**Dissection of brain and left ventricular tissues for molecular studies**

Subfornical organ (SFO) and hypothalamic paraventricular nucleus (PVN) tissues were obtained as previously described. Briefly, brain tissues were quickly removed, frozen in liquid nitrogen and stored at -80°C. The brain was cut into 500-µm coronal sections. The SFO and PVN regions were punched using a 15-gauge needle stub (ID: 1.5 mm). This method necessarily includes a small amount of surrounding tissue. The heart was removed, and the left ventricle was dissected free from the atria and right ventricular free wall and weighed.

**Quantification of mRNA expression**

The total RNA was extracted from the SFO, the PVN and heart tissues using TRI Reagent (Molecular Research Center, Inc). Following reverse transcription of total RNA, 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 (ANG II type-1 receptors [AT₁R] and ANG II type-2 receptor [AT₂R]) were analyzed by real-time PCR. 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, Foster City, CA). The values were normalized to GAPDH and the final concentration of mRNA was analyzed with the formula $x=2^{-∆∆CT}$, where $x=$fold difference relative to SHAM.
**PPAR-γ DNA binding assay**

Nuclear extracts for DNA binding were prepared from the SFO, the PVN and heart tissues using a Nuclear Extract Kit (Active Motif, Carlsbad, CA). PPAR-γ DNA binding activity was detected by TransAM PPAR-γ kits (Active Motif, Carlsbad, CA), according to the manufacturer's instruction.

**Immunohistochemistry**

Fra-like (Fra-LI; fos family gene) activity, a marker of chronic neuronal activation, was detected in the PVN using a rabbit polyclonal antibody (c-fos K-25; Santa Cruz Biotechnology) as previously described. In each animal, Fra-LI–positive neurons within a window (4x10⁴ μm²) superimposed over the posterior magnocellular, ventrolateral parvocellular, medial parvocellular and dorsal parvocellular subregions of PVN were counted manually in two representative 16-μm transverse sections approximately -1.8 mm from bregma and were averaged to obtain a value for data analysis. The subregions of PVN were defined as described in previous studies.

**Plasma AVP assay**

Plasma arginine vasopressin (AVP) levels were measured using a Vasopressin EIA kit (Enzo Life Sciences, Inc, Plymouth Meeting, PA), according to the manufacturer's instruction.

**Statistical Analysis**

All data are expressed as mean ± SE. The significance of differences in mean values was analyzed by one-way or two-way repeated-measure ANOVA followed by Fisher's post hoc test. P<0.05 was considered statistically significant.
REFERENCES


<table>
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<tr>
<th>Gene</th>
<th>Primers and Probes</th>
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<tr>
<td>PPAR-γ</td>
<td>Forward primer: 5′-CTTGGCCCATATTTATAGCTGATTATT-3′</td>
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