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

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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 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/min) 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-1 β , 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.

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Conflict(s) of Interest/Disclosure(s)

None

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Keywords

peroxisome proliferator-activated receptor- γ ; inflammation; renin-angiotensin system; brain; autonomic regulation

INTRODUCTION

Numerous studies have demonstrated that inflammation and activation of the renin-angiotensin system (RAS) are involved in the pathogenesis of hypertension.^{1, 2} 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.^{3–8}

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.⁹ Pioglitazone (PIO), a thiazolidinedione (TZD) class synthetic PPAR- γ agonist, is used for treatment of patients with type II diabetes mellitus.¹⁰ However, PIO has also been reported to reduce or prevent hypertension¹¹ in animal models^{12–15} and in humans.^{16–18}

PPAR- γ is expressed in key brain areas involved in drinking, vasopressin release and cardiovascular regulation, including the hypothalamic paraventricular nucleus (PVN)¹⁹ and the rostral ventrolateral medulla (RVLM).²⁰ In the RVLM, activation of PPAR- γ attenuated angiotensin (ANG) II-induced hypertension in rats.²⁰ 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 PIO ameliorated these molecular abnormalities and the peripheral manifestations of heart failure.²¹ 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 effect of centrally administered PIO on ANG II-induced hypertension and on neurochemical mechanisms in two 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 anti-hypertensive therapy.

METHODS

Animals

Adult male Sprague-Dawley rats weighing 250–300 g were purchased from Harlan (Indianapolis, IN). Rats were housed in temperature- (23 $\pm$ 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 mg/kg 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 (HR). A cannula was implanted in a lateral ventricle for intracerebroventricular (i.c.v.) drug infusion. Osmotic mini-pumps (model 2002, Alzet) were implanted subcutaneously for continuous systemic and i.c.v. drug infusion.

Drugs and Routes of Administration

Hypertension was induced by slow infusion of ANG II (120 ng/kg per min, s.c.) for 2 weeks, as previously described.^{3, 4} A concomitant continuous i.c.v. infusion of the PPAR- γ agonist PIO (3 nmol in 0.5 μ l/hr), the PPAR- γ antagonist GW9662 (GW, 7 nmol in 0.5 μ l/hr) or the vehicle for PIO (VEH, 20% dimethyl sulfoxide in artificial cerebrospinal fluid; 0.5 μ l/hr) was administered in the ANG II infused rats; the same PIO and GW infusions were administered to control rats. The dose of PIO was based on previous studies from our laboratory²¹ and from others showing optimal *in vivo* activation of central PPAR- γ in rats with no effect on blood glucose.²² The dose of GW was based on a previous study.²³ The ganglionic blocker hexamethonium bromide was administered (30 mg/kg, i.p.) to evaluate the sympathetic contribution to MBP, as previously described.³

Experimental Protocols

1. MBP and HR were recorded by telemetry for 5 days at baseline and then for 2 weeks during s.c. infusion of ANG II combined with i.c.v. VEH (ANG II+VEH, n=8), i.c.v. PIO (ANG II+PIO, n=8) or i.c.v. GW (ANG II+GW, n=6). Some age-matched untreated rats served as a time control (CON, n=6); others received i.c.v. PIO (CON+PIO, n=5) or i.c.v. GW (CON+GW, n=5). One day prior to sacrifice, the MBP response to hexamethonium bromide was tested. At 2 weeks, the rats were euthanized while deeply anesthetized with isoflurane 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:
 - i. 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 to body weight (BW) ratio was determined in these animals.
 - ii. Rats (n=4 from each group) were deeply anesthetized with urethane and perfused with fixative for immunohistochemical study.

- iii. Rats (n=6–8 from each group in Protocol i above) underwent twice weekly measurements of food and water intake and BW; measurements of food and water intake were made over two consecutive 24-hour periods, and an average value for each variable was reported for each time point.
- iv. 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 i above) that did not undergo water restriction served as controls.

Specific Methods

Please see the online Data Supplement.

RESULTS

Effects of PIO 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 HR among groups (Figure 1B).

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

Effects of PIO 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 left ventricle (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). 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 (Figures 2A and 2B).

Effects of PIO 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 pro-inflammatory cytokines tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β) 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-1 β 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 IL-1 β . Of note, CON, CON+PIO and CON+GW had similar levels of mRNA for AT₁R, IL-1 β , TNF- α and COX-2. There were no differences across groups in ANG II type-2 receptor and COX-1 mRNA.

Effects of PIO and GW9662 on ANG II-induced Neuronal Excitation in the PVN

Fra-like immunoreactivity (Fra-LI), 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-LI throughout the PVN (Figure 4). Compared with ANG II+VEH, ANG II+PIO rats had less Fra-LI and ANG II+GW rats had more Fra-LI in the medial parvocellular, ventrolateral parvocellular, dorsal parvocellular and posterior magnocellular regions of the PVN. No differences in Fra-LI were observed between CON+PIO, CON+GW and CON rats.

Effects of PIO 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 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 ANG II+VEH rats. There were no differences across groups in food intake (Figure 5C) or body weight (Figure 5D).

Effects of PIO 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 1). The LV/BW ratio was lower in the ANG II+PIO and higher in the ANG II+GW rats, as 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: (1) PPAR- γ DNA binding activity is reduced in the SFO and the PVN, two key forebrain regions regulating fluid volume and sympathetic drive, in rats with ANG II-induced hypertension; (2) i.c.v. administration of the PPAR- γ agonist PIO upregulates PPAR- γ mRNA and PPAR- γ DNA binding activity in the SFO and the PVN of rats with ANG II-induced hypertension; (3) i.c.v. PIO ameliorates ANG II-induced upregulation of RAS activity, inflammation and neuronal excitation in the SFO and the PVN, ANG II-induced sympathetic nerve activity, and ANG II-induced hypertension; (4) i.c.v. PIO 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 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 PIO. 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-1 β and COX-2 in the SFO and the PVN. A concomitant i.c.v. infusion of PIO ameliorated these ANG II-induced neurochemical changes and the ANG II-induced increases in blood 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 (AP-1) and nuclear factor kappaB (NF- κ B). ANG II, acting upon the AT₁R, activates NADPH oxidase dependent superoxide production in SFO, the PVN and other cardiovascular regions of the brain.²⁴ Superoxide-dependent activation of AP-1 in the PVN has been implicated in the pathophysiology of renovascular hypertension,²⁵ and superoxide-dependent activation of NF- κ B 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 AP-1,²⁷ and potential gene products of NF- κ B include the ANG II precursor protein angiotensinogen,²⁸ the pro-inflammatory cytokines TNF- α and IL-1 β ,²⁹ and COX-2.³⁰ Ligand-activated PPAR- γ can alleviate oxidative stress^{31, 32} and interfere with the activation and/or transcriptional functions of AP-1 and NF- κ B,^{31, 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 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 consequence³⁸ 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 TZD, rosiglitazone, reduced the blood pressure response to centrally administered ANG II,²⁰ and that oral dosing with PIO (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.⁴⁴ The latter two 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 TZDs has had relatively small effects on blood pressure,^{45, 46} though 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.^{47, 48} 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.⁴⁹ The present results support the general concept that PPAR- γ agonists may provide a significant additional benefit as adjunctive therapy for patients with hypertension.

PIO, the TZD chosen for this study, is one of a number of naturally occurring and synthetic PPAR- γ agonists. Treatment with TZDs has been associated with several adverse effects, including increases in food intake and body weight and fluid accumulation secondary to an increase in renal sodium reabsorption⁵⁰, with peripheral edema and precipitation of heart failure.⁵¹ However, PIO may be safer than other TZDs, and may even be beneficial from a cardiovascular standpoint.^{10, 51} In the present study, over a 2-week treatment interval, fluid consumption and body weight were not adversely affected by i.c.v. PIO. In fact, the ANG II +VEH rats and the ANG II+GW rats both consumed significantly more water than any of the PIO-treated groups. While 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 PIO treatment. Previous work⁵² has demonstrated that a peripheral ANG II infusion upregulates AT₁R in the subfornical organ, a major regulatory center for thirst, and in the PVN, a central loci of AVP neurons. ANG II-induced upregulation of AT₁R in these two centers may well account for these two findings.

Limitations of the study

PIO, like other TZDs and some naturally occurring PPAR agonists, may have effects independent of activating PPAR- γ ,⁵³ including anti-inflammatory effects.⁵⁴ To definitively attribute responses to activation of PPAR- γ , it would be necessary to demonstrate that the responses to PIO were interrupted by a PPAR- γ 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 PIO 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.^{4, 5, 55–57} However, we recognize that the effects of i.c.v. PIO 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 i.c.v. infusion of PIO 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,²⁰ is an obvious additional potential site of

action. However, the strikingly concordant effects of PIO 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 and neuroendocrine effector neurons in the PVN. As we recently demonstrated,⁵⁸ 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 PIO 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 PIO 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 ,⁵⁹ 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 pro-inflammatory cytokines may reduce the excitatory milieu surrounding presympathetic neurons, while a PPAR- γ mediated reduction in AT₁R may reduce the neuronal response to local levels of ANG II. While further studies may elucidate the relative role of the individual cell types involved, the response to i.c.v. 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 two 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 anti-hypertensive 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Ferrario CM, Strawn WB. Role of the renin-angiotensin-aldosterone system and proinflammatory mediators in cardiovascular disease. *Am J Cardiol.* 2006; 98:121–128. [PubMed: 16784934]
2. Harrison DG, Guzik TJ, Lob HE, Madhur MS, Marvar PJ, Thabet SR, Vinh A, Weyand CM. Inflammation, immunity, and hypertension. *Hypertension.* 2011; 57:132–140. [PubMed: 21149826]
3. Xue B, Beltz TG, Yu Y, Guo F, Gomez-Sanchez CE, Hay M, Johnson AK. Central interactions of aldosterone and angiotensin II in aldosterone- and angiotensin II-induced hypertension. *Am J Physiol Heart Circ Physiol.* 2011; 300:H555–564. [PubMed: 21112947]
4. Yu Y, Xue BJ, Zhang ZH, Wei SG, Beltz TG, Guo F, Johnson AK, Felder RB. Early interference with p44/42 mitogen-activated protein kinase signaling in hypothalamic paraventricular nucleus attenuates angiotensin II-induced hypertension. *Hypertension.* 2013; 61:842–849. [PubMed: 23438934]
5. Cardinale JP, Sriramula S, Mariappan N, Agarwal D, Francis J. Angiotensin II-induced hypertension is modulated by nuclear factor- κ B in the paraventricular nucleus. *Hypertension.* 2012; 59:113–121. [PubMed: 22106405]
6. Huang BS, White RA, Bi L, Leenen FH. Central infusion of aliskiren prevents sympathetic hyperactivity and hypertension in Dahl salt-sensitive rats on high salt intake. *Am J Physiol Regul Integr Comp Physiol.* 2012; 302:R825–832. [PubMed: 22262304]
7. Shi P, Diez-Freire C, Jun JY, Qi Y, Katovich MJ, Li Q, Sriramula S, Francis J, Summers C, Raizada MK. Brain microglial cytokines in neurogenic hypertension. *Hypertension.* 2010; 56:297–303. [PubMed: 20547972]
8. Yamazato M, Yamazato Y, Sun C, Diez-Freire C, Raizada MK. Overexpression of angiotensin-converting enzyme 2 in the rostral ventrolateral medulla causes long-term decrease in blood pressure in the spontaneously hypertensive rats. *Hypertension.* 2007; 49:926–931. [PubMed: 17325232]
9. Moore-Carrasco R, Poblete Bustamante M, Gonzalez Guerra O, Leiva Madariaga E, Mujica Escudero V, Aranguel Arellano C, Palomo I. Peroxisome proliferator-activated receptors: Targets for the treatment of metabolic illnesses (review). *Mol Med Rep.* 2008; 1:317–324. [PubMed: 21479412]
10. Abbas A, Blandon J, Rude J, Elfar A, Mukherjee D. PPAR- γ agonist in treatment of diabetes: Cardiovascular safety considerations. *Cardiovasc Hematol Agents Med Chem.* 2012; 10:124–134. [PubMed: 22471957]
11. Usuda D, Kanda T. Peroxisome proliferator-activated receptors for hypertension. *World J Cardiol.* 2014; 6:744–754. [PubMed: 25228953]
12. Dobrian AD, Schriver SD, Khraibi AA, Prewitt RL. Pioglitazone prevents hypertension and reduces oxidative stress in diet-induced obesity. *Hypertension.* 2004; 43:48–56. [PubMed: 14638618]
13. Nakamoto M, Ohya Y, Shinzato T, Mano R, Yamazato M, Sakima A, Takishita S. Pioglitazone, a thiazolidinedione derivative, attenuates left ventricular hypertrophy and fibrosis in salt-sensitive hypertension. *Hypertens Res.* 2008; 31:353–361. [PubMed: 18360056]
14. Dovinova I, Barancik M, Majzunova M, Zorad S, Gajdosechova L, Gresova L, Cacanyiova S, Kristek F, Balis P, Chan JY. Effects of PPAR γ agonist pioglitazone on redox-sensitive cellular signaling in young spontaneously hypertensive rats. *PPAR Res.* 2013; 2013:541871. [PubMed: 24454335]
15. Dorafshar AH, Moodley K, Khoe M, Lyon C, Bryer-Ash M. Pioglitazone improves superoxide dismutase mediated vascular reactivity in the obese Zucker rat. *Diab Vasc Dis Res.* 2010; 7:20–27. [PubMed: 20368229]
16. Schondorf T, Forst T, Hohberg C, Pahler S, Link C, Roth W, Pflutzner A, Lubben G, Link C, Pflutzner A. The IRIS III study: Pioglitazone improves metabolic control and blood pressure in patients with type 2 diabetes without increasing body weight. *Diabetes Obes Metab.* 2007; 9:132–133. [PubMed: 17199730]
17. Derosa G, Fogari E, Cicero AF, D'Angelo A, Ciccarelli L, Piccinni MN, Pricolo F, Salvadeo SA, Gravina A, Ferrari I, Fogari R. Blood pressure control and inflammatory markers in type 2 diabetic

- patients treated with pioglitazone or rosiglitazone and metformin. *Hypertens Res.* 2007; 30:387–394. [PubMed: 17587750]
18. Abe M, Okada K, Kikuchi F, Matsumoto K. Clinical investigation of the effects of pioglitazone on the improvement of insulin resistance and blood pressure in type 2-diabetic patients undergoing hemodialysis. *Clin Nephrol.* 2008; 70:220–228. [PubMed: 18793563]
 19. Moreno S, Farioli-Vecchioli S, Ceru MP. Immunolocalization of peroxisome proliferator-activated receptors and retinoid X receptors in the adult rat CNS. *Neuroscience.* 2004; 123:131–145. [PubMed: 14667448]
 20. Chan SH, Wu CA, Wu KL, Ho YH, Chang AY, Chan JY. Transcriptional upregulation of mitochondrial uncoupling protein 2 protects against oxidative stress-associated neurogenic hypertension. *Circ Res.* 2009; 105:886–896. [PubMed: 19762685]
 21. Yu Y, Zhang ZH, Wei SG, Weiss RM, Felder RB. Peroxisome proliferator-activated receptor- γ regulates inflammation and renin-angiotensin system activity in the hypothalamic paraventricular nucleus and ameliorates peripheral manifestations of heart failure. *Hypertension.* 2012; 59:477–484. [PubMed: 22083161]
 22. Glatz T, Stock I, Nguyen-Ngoc M, Gohlke P, Herdegen T, Culman J, Zhao Y. Peroxisome-proliferator-activated receptors gamma and peroxisome-proliferator-activated receptors beta/delta and the regulation of interleukin 1 receptor antagonist expression by pioglitazone in ischaemic brain. *J Hypertens.* 2010; 28:1488–1497. [PubMed: 20410836]
 23. Morgenweck J, Abdel-Aleem OS, McNamara KC, Donahue RR, Badr MZ, Taylor BK. Activation of peroxisome proliferator-activated receptor gamma in brain inhibits inflammatory pain, dorsal horn expression of Fos, and local edema. *Neuropharmacology.* 2010; 58:337–345. [PubMed: 19891980]
 24. Chan SH, Chan JY. Angiotensin-generated reactive oxygen species in brain and pathogenesis of cardiovascular diseases. *Antioxid Redox Signal.* 2013; 19:1074–1084. [PubMed: 22429119]
 25. Burmeister MA, Young CN, Braga VA, Butler SD, Sharma RV, Davisson RL. In vivo bioluminescence imaging reveals redox-regulated activator protein-1 activation in paraventricular nucleus of mice with renovascular hypertension. *Hypertension.* 2011; 57:289–297. [PubMed: 21173341]
 26. Kang YM, Ma Y, Zheng JP, Elks C, Sriramula S, Yang ZM, Francis J. Brain nuclear factor-kappa B activation contributes to neurohumoral excitation in angiotensin II-induced hypertension. *Cardiovasc Res.* 2009; 82:503–512. [PubMed: 19246475]
 27. Zucker IH, Schultz HD, Patel KP, Wang W, Gao L. Regulation of central angiotensin type 1 receptors and sympathetic outflow in heart failure. *Am J Physiol Heart Circ Physiol.* 2009; 297:H1557–1566. [PubMed: 19717736]
 28. Brasier AR, Jamaluddin M, Han Y, Patterson C, Runge MS. Angiotensin II induces gene transcription through cell-type-dependent effects on the nuclear factor-kappaB (NF-kappaB) transcription factor. *Mol Cell Biochem.* 2000; 212:155–169. [PubMed: 11108147]
 29. Siebenlist U, Franzoso G, Brown K. Structure, regulation and function of NF-kappa B. *Annu Rev Cell Biol.* 1994; 10:405–455. [PubMed: 7888182]
 30. Tsatsanis C, Androulidaki A, Venihaki M, Margioris AN. Signalling networks regulating cyclooxygenase-2. *Int J Biochem Cell Biol.* 2006; 38:1654–1661. [PubMed: 16713323]
 31. Liu X, Luo D, Zheng M, Hao Y, Hou L, Zhang S. Effect of pioglitazone on insulin resistance in fructose-drinking rats correlates with AGEs/RAGE inhibition and block of NADPH oxidase and NF kappa B activation. *Eur J Pharmacol.* 2010; 629:153–158. [PubMed: 20004657]
 32. Chan SH, Wu KL, Kung PS, Chan JY. Oral intake of rosiglitazone promotes a central antihypertensive effect via upregulation of peroxisome proliferator-activated receptor-gamma and alleviation of oxidative stress in rostral ventrolateral medulla of spontaneously hypertensive rats. *Hypertension.* 2010; 55:1444–1453. [PubMed: 20404217]
 33. Genolet R, Wahli W, Michalik L. PPARs as drug targets to modulate inflammatory responses? *Curr Drug Targets Inflamm Allergy.* 2004; 3:361–375. [PubMed: 15584886]
 34. Chinetti G, Fruchart JC, Staels B. Peroxisome proliferator-activated receptors (PPARs): Nuclear receptors at the crossroads between lipid metabolism and inflammation. *Inflamm Res.* 2000; 49:497–505. [PubMed: 11089900]

35. Schiffrin EL. Peroxisome proliferator-activated receptors and cardiovascular remodeling. *Am J Physiol Heart Circ Physiol*. 2005; 288:H1037–1043. [PubMed: 15374828]
36. Guo F, Chen XL, Wang F, Liang X, Sun YX, Wang YJ. Role of angiotensin II type 1 receptor in angiotensin II-induced cytokine production in macrophages. *J Interferon Cytokine Res*. 2011; 31:351–361. [PubMed: 21235392]
37. Li XC, Zhuo JL. Nuclear factor-kappaB as a hormonal intracellular signaling molecule: focus on angiotensin II-induced cardiovascular and renal injury. *Curr Opin Nephrol Hypertens*. 2008; 17:37–43. [PubMed: 18090668]
38. Tham DM, Martin-McNulty B, Wang YX, Wilson DW, Vergona R, Sullivan ME, Dole W, Rutledge JC. Angiotensin II is associated with activation of NF-kappaB-mediated genes and downregulation of PPARs. *Physiol Genomics*. 2002; 11:21–30. [PubMed: 12361987]
39. Bernardo A, Minghetti L. PPAR-gamma agonists as regulators of microglial activation and brain inflammation. *Curr Pharm Des*. 2006; 12:93–109. [PubMed: 16454728]
40. Meier CA, Chicheportiche R, Juge-Aubry CE, Dreyer MG, Dayer JM. Regulation of the interleukin-1 receptor antagonist in THP-1 cells by ligands of the peroxisome proliferator-activated receptor gamma. *Cytokine*. 2002; 18:320–328. [PubMed: 12160520]
41. Hao GH, Niu XL, Gao DF, Wei J, Wang NP. Agonists at PPAR-gamma suppress angiotensin II-induced production of plasminogen activator inhibitor-1 and extracellular matrix in rat cardiac fibroblasts. *Br J Pharmacol*. 2008; 153:1409–1419. [PubMed: 18278065]
42. Benkirane K, Viel EC, Amiri F, Schiffrin EL. Peroxisome proliferator-activated receptor gamma regulates angiotensin II-stimulated phosphatidylinositol 3-kinase and mitogen-activated protein kinase in blood vessels in vivo. *Hypertension*. 2006; 47:102–108. [PubMed: 16344371]
43. Alexis JD, Wang N, Che W, Lerner-Marmarosh N, Sahni A, Korshunov VA, Zou Y, Ding B, Yan C, Berk BC, Abe J. Bcr kinase activation by angiotensin II inhibits peroxisome-proliferator-activated receptor gamma transcriptional activity in vascular smooth muscle cells. *Circ Res*. 2009; 104:69–78. [PubMed: 19023129]
44. Wu KL, Chao YM, Tsay SJ, Chen CH, Chan SH, Dovinova I, Chan JY. Role of nitric oxide synthase uncoupling at rostral ventrolateral medulla in redox-sensitive hypertension associated with metabolic syndrome. *Hypertension*. 2014; 64:815–824. [PubMed: 24958506]
45. Kelly AS, Bank AJ. The cardiovascular effects of the thiazolidinediones: A review of the clinical data. *J Diabetes Complications*. 2007; 21:326–334. [PubMed: 17825758]
46. Sarafidis PA, Lasaridis AN. Actions of peroxisome proliferator-activated receptors-gamma agonists explaining a possible blood pressure-lowering effect. *Am J Hypertens*. 2006; 19:646–653. [PubMed: 16733240]
47. Namikoshi T, Tomita N, Satoh M, Haruna Y, Kobayashi S, Komai N, Sasaki T, Kashihara N. Pioglitazone enhances the antihypertensive and renoprotective effects of candesartan in Zucker obese rats fed a high-protein diet. *Hypertens Res*. 2008; 31:745–755. [PubMed: 18633187]
48. Kong X, Ma MZ, Qin L, Zhang Y, Li XY, Wang GD, Su Q, Zhang DY. Pioglitazone enhances the blood pressure-lowering effect of losartan via synergistic attenuation of angiotensin II-induced vasoconstriction. *J Renin Angiotensin Aldosterone Syst*. 2014; 15:259–270. [PubMed: 23676251]
49. Saavedra JM. Angiotensin II AT(1) receptor blockers as treatments for inflammatory brain disorders. *Clin Sci (Lond)*. 2012; 123:567–590. [PubMed: 22827472]
50. Zanchi A, Maillard M, Jornayvaz FR, Vinciguerra M, Deleaval P, Nussberger J, Burnier M, Pechere-Bertschi A. Effects of the peroxisome proliferator-activated receptor (PPAR)-gamma agonist pioglitazone on renal and hormonal responses to salt in diabetic and hypertensive individuals. *Diabetologia*. 2010; 53:1568–1575. [PubMed: 20414637]
51. Rizos CV, Elisaf MS, Mikhailidis DP, Liberopoulos EN. How safe is the use of thiazolidinediones in clinical practice? *Expert Opin Drug Saf*. 2009; 8:15–32. [PubMed: 19236215]
52. Wei SG, Yu Y, Zhang ZH, Felder RB. Angiotensin II upregulates hypothalamic AT1 receptor expression in rats via the mitogen-activated protein kinase pathway. *Am J Physiol Heart Circ Physiol*. 2009; 296:H1425–1433. [PubMed: 19286949]
53. Mandrekar-Colucci S, Sauerbeck A, Popovich PG, McTigue DM. PPAR agonists as therapeutics for CNS trauma and neurological diseases. *ASN Neuro*. 2013; 5:e00129. [PubMed: 24215544]

54. Thal SC, Heinemann M, Luh C, Pieter D, Werner C, Engelhard K. Pioglitazone reduces secondary brain damage after experimental brain trauma by PPAR-gamma-independent mechanisms. *J Neurotrauma*. 2011; 28:983–993. [PubMed: 21501066]
55. Sriramula S, Cardinale JP, Lazartigues E, Francis J. ACE2 overexpression in the paraventricular nucleus attenuates angiotensin II-induced hypertension. *Cardiovasc Res*. 2011; 92:401–408. [PubMed: 21952934]
56. Young CN, Cao X, Guraju MR, Pierce JP, Morgan DA, Wang G, Iadecola C, Mark AL, Davisson RL. ER stress in the brain subfornical organ mediates angiotensin-dependent hypertension. *J Clin Invest*. 2012; 122:3960–3964. [PubMed: 23064361]
57. Lob HE, Schultz D, Marvar PJ, Davisson RL, Harrison DG. Role of the NADPH oxidases in the subfornical organ in angiotensin II-induced hypertension. *Hypertension*. 2013; 61:382–387. [PubMed: 23248154]
58. Wei SG, Yu Y, Zhang ZH, Felder RB. Proinflammatory cytokines upregulate sympathoexcitatory mechanisms in the subfornical organ of the rat. *Hypertension*. 2015; 65:1126–1133. [PubMed: 25776070]
59. Bernardo A, Minghetti L. Regulation of Glial Cell Functions by PPAR-gamma Natural and Synthetic Agonists. *PPAR Res*. 2008; 2008:864140. [PubMed: 18464925]

Novelty and Significance

1) What Is New

- PPAR- γ DNA binding activity is reduced in the subfornical organ (SFO) and the hypothalamic paraventricular nucleus (PVN), two key cardiovascular autonomic regions of the brain, in angiotensin (ANG) II-induced hypertension.
- Central administration of a PPAR- γ agonist restores PPAR- γ DNA binding activity, blunts the ANG II-induced neurochemical changes in the SFO and the PVN, sympathetic nerve activity, and blood pressure.

2) What Is Relevant?

- PPAR- γ agonists act centrally as well as 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.

3) Summary

Preventing the reduction in central PPAR- γ activity associated with ANG II-induced hypertension with a concomitant central infusion of a PPAR- γ agonist interferes with the upregulation of inflammatory mediators and ANG II type 1 receptors in the PVN 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.

Figure 1.

Mean blood pressure (MBP, A) and heart rate (HR, B), and the peak changes () in MBP in response to ganglionic blockade (C), in ANG II-infused rats treated with intracerebroventricular (i.c.v.) VEH, PIO or GW9662, and in age-matched normal control (CON) rats, untreated or treated with i.c.v. PIO or GW9662. Values are mean $\pm$ SEM (n = 5–8 for each group). *P < 0.05 vs. baseline or CON; †P < 0.05 vs. ANG II + VEH.

Figure 2.

The expression of 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 i.c.v. PIO or GW9662, and ANG II-infused rats treated with i.c.v. VEH, PIO or GW9662. 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

Figure 3.

mRNA expression for renin-angiotensin system components AT₁R (A), AT₂R (B), proinflammatory cytokines IL-1 β (C) and TNF- α (D), and COX-2 (E) and COX-1 (F), in SFO and PVN of untreated Control (CON) rats, CON rats treated with i.c.v. PIO or GW9662, and ANG II-infused rats treated with i.c.v. VEH, PIO or GW9662. Values are mean $\pm$ SEM (n = 6–8 for each group). Real-time PCR results are expressed as a fold change relative to CON. * P < 0.05 vs. baseline or CON; † P < 0.05 vs. ANG II+VEH.

Figure 4.

Expression of Fra-like immunoreactivity (Fra-LI) in the PVN of Control (CON) rats, untreated or treated with i.c.v. PIO or GW9662 and ANG II-infused rats treated with i.c.v. VEH, PIO or GW9662. (A) Representative sections from each group showing Fra-LI positive PVN neurons. Dark dots indicate Fra-LI positive neurons. (B) Quantification of Fra-LI positive neurons in 4 different regions of the PVN. Values are expressed as mean $\pm$ SEM (n=4 for each group). * P < 0.05 vs. baseline or CON; † P < 0.05, vs. ANG II+VEH. mp, medial parvocellular; vlp, ventrolateral parvocellular; dp, dorsal parvocellular; pm, posterior magnocellular.

Figure 5.

PPAR- γ effects on plasma AVP levels at baseline and following a 6-hour water restriction (A), consumption of water (B) and food (C) and body weight (D) in Control (CON) rats, untreated or treated with i.c.v. PIO or GW9662, and ANG II-infused rats treated with i.c.v. VEH, PIO or GW9662. Values are mean $\pm$ SEM ($n = 5-8$ for each group). * $P < 0.05$ vs. baseline or CON; † $P < 0.05$, vs. ANG II+VEH.

Figure 6.

Schematic diagram showing mechanisms by which manipulations of PPAR- γ in cardiovascular regulatory regions of the brain might affect ANG II-induced hypertension. SNA, sympathetic nerve activity; AP-1, activator-protein 1; NF- κ B, nuclear factor kappa B; PIO, pioglitazone; GW, the PIO antagonist GW9662; ROS, reactive oxygen species.

Table 1

Effects of I.C.V. Pioglitazone and GW9662 on ANG II-induced Cardiac Remodeling

Group	BW (g)	LV (mg)	LV/BW (mg/g)
CON (n=8)	376 ± 5	999 ± 13	2.66 ± 0.05
CON + PIO (n=5)	383 ± 4	1010 ± 27	2.64 ± 0.07
CON + GW (n=5)	388 ± 6	1008 ± 12	2.60 ± 0.06
ANG II + VEH (n=8)	373 ± 5	1097 ± 26 [*]	2.94 ± 0.05 [*]
ANG II + PIO (n=8)	387 ± 6	1058 ± 28	2.74 ± 0.07 [†]
ANG II + GW (n=6)	377 ± 4	1173 ± 25 [*]	3.11 ± 0.05 ^{*†}

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

^{*} P < 0.05 vs. CON,

[†] P < 0.05 vs. ANG II+VEH.