Oral Intake of Rosiglitazone Promotes a Central Antihypertensive Effect Via Upregulation of Peroxisome Proliferator-Activated Receptor-γ and Alleviation of Oxidative Stress in Rostral Ventrolateral Medulla of Spontaneously Hypertensive Rats

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Abstract—Rosiglitazone, a synthetic ligand of transcription factor peroxisome proliferator-activated receptor-γ (PPAR-γ), possesses a blood pressure–lowering effect beyond insulin sensitizing and glucose lowering. Oxidative stress in rostral ventrolateral medulla (RVLM), where sympathetic premotor neurons for the maintenance of neurogenic vasomotor tone are located, contributes to neural mechanisms of hypertension. Activation of PPAR-γ protects against oxidative stress in RVLM by upregulation of mitochondrial uncoupling protein 2 (UCP2). We tested the hypothesis that oral intake of rosiglitazone exerts a central antihypertensive effect by ameliorating oxidative stress in RVLM via transcriptional upregulation of UCP2 after PPAR-γ activation. In adult spontaneously hypertensive rats but not normotensive Wistar-Kyoto rats, oral intake of rosiglitazone for 1 week resulted in vasodepression and a reduction in the vasomotor components of the systemic arterial pressure spectrum, our experimental index for sympathetic vasomotor tone. These antihypertensive effects of rosiglitazone in spontaneously hypertensive rats were abrogated by microinjection bilaterally into RVLM of PPAR-γ small interfering RNA. Oral intake of rosiglitazone also upregulated UCP2 and ameliorated the heightened superoxide anion level in RVLM of spontaneously hypertensive rats. Protection against oxidative stress in RVLM by rosiglitazone was abrogated by PPAR-γ small interfering RNA or by antisense oligonucleotide against *ucp2* mRNA. Gene knockdown of *ucp2* in RVLM also reversed the antihypertensive effect of rosiglitazone. These results suggest that oral intake of rosiglitazone promotes a central antihypertensive effect by decreasing sympathetic vasomotor activity through a PPAR-γ–dependent protection against oxidative stress in RVLM via transcriptional upregulation of the mitochondrial UCP2. **(*Hypertension*. 2010;55:1444-1453.)**

Key Words: peroxisome proliferator-activated receptor ■ thiazolidinedione ■ mitochondrial uncoupling protein ■ oxidative stress ■ blood pressure

Peroxisome proliferator-activated receptor (PPAR)-γ is a member of the nuclear receptor superfamily of ligand-activated transcription factors that plays an important role in the regulation of adipocyte differentiation and lipid and carbohydrate metabolism.[1,2] Its well-characterized increase in insulin sensitivity prompted the use of PPAR-γ ligands of the thiazolidinedione (TZD) class, such as rosiglitazone, troglitazone, or pioglitazone,[3] for clinical treatment of type 2 diabetes mellitus.[4] Emerging evidence indicates that, in addition to its antihyperglycemic properties, TZD possesses beneficial effects in cardiovascular diseases, including hypertension, atherosclerosis, and heart failure.[1,5,6] With reference to hypertension, TZD decreases blood pressure in diabetic[7] and nondiabetic[8] hypertensive patients, and rosiglitazone reduces blood pressure and/or prevents the development of hypertension in animal models of insulin resistance and/or hypertension.[9–11]

Oxidative stress because of an imbalance of production over degradation of the reactive oxygen species (ROS), particularly superoxide anion ($O_2^{\cdot-}$), is associated with hypertension.[12,13] In addition to the documented degradative enzymes (eg, superoxide dismutase and catalase) and low molecular weight antioxidants (eg, ascorbic acid and glutathione), the mitochondrial uncoupling proteins (UCPs) have emerged as important natural antioxidants[14] in cellular ROS homeostasis. Of note is that overproduction of $O_2^{\cdot-}$ in the central nervous system contributes to neural mechanisms of hypertension by increasing sympathetic outflow to the peripheral blood vessels.[15,16]

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The blood pressure–lowering effect of TZD has been attributed to a reduction in ROS production in the vascular smooth muscle cells[11,17] and endothelial cells.[10,11,18] Whether TZD, in particular, rosiglitazone, possesses a central antihypertensive action is currently unknown. A recent report[19] on the neuroprotective effect of cerebral ischemia-induced brain infarction after oral intake of the PPAR-γ activator suggests a mechanism by which the PPAR-γ activator may exert its protective effect within the brain. Two pieces of observations further suggest that the rostral ventrolateral medulla (RVLM), where sympathetic premotor neurons for the maintenance of vasomotor tone are located,[20] presents itself as a reasonable candidate brain target for this PPAR-γ ligand. First, oxidative stress at the RVLM plays a pivotal role in the neural mechanism of hypertension.[15,16,21] Second, transcriptional upregulation of UCP2 by PPAR-γ reduces arterial pressure by ameliorating oxidative stress in the RVLM.[22] It follows that, on oral intake, rosiglitazone may exert a central antihypertensive effect by ameliorating oxidative stress in the RVLM via transcriptional upregulation of UCP2 after PPAR-γ activation. The present study was carried out to validate this hypothesis.

Materials and Methods

All of the experimental procedures were carried out in compliance with the guidelines of our institutional animal care committee. Adult (12-week–old) male spontaneously hypertensive rats (SHRs) or age-matched normotensive Wistar-Kyoto (WKY) rats, purchased from the Experimental Animal Center of the National Applied Research Laboratories (Taiwan) were used. Animals received oral administration of rosiglitazone, amlodipine, or saline (once per day between 12:00 and 1:00 PM via gastric gavage) for 1 week. The key experimental procedures included measurement of systemic arterial pressure (SAP) and heart rate (HR) under conscious condition by radiotelemetry,[22,23] computation of power density of vasomotor components (0 to 0.8 Hz) of the SAP spectrum as an index for sympathetic vasomotor tone,[24,25] detection of $O_2^{\cdot -}$,[22,23] microinjection into RVLM of small interfering RNA (siRNA) or antisense oligonucleotide (ASON), determination of mRNA or protein expression by real time RT-PCR or Western blot,[22,23] and characterization of metabolic indices.[26] Detailed procedures are provided in the expanded Materials and Methods section in the online Data Supplement at http://hyper.ahajournals.org.

Statistic Analysis

Data are expressed as mean±SEM. The statistical software SigmaStat (SPSS Inc) was used for data analysis. One-way or 2-way ANOVA with repeated measures was used, as appropriate, to assess group means, to be followed by the Scheffé multiple-range test for post hoc assessment of individual means. P value <0.05 was considered statistically significant.

Results

Oral Intake of Rosiglitazone Decreases SAP and Sympathetic Vasomotor Activity in SHRs

Our first series of experiments established the critical premise that rosiglitazone exerts an antihypertensive effect. Compared with saline control, oral intake of rosiglitazone (20 $mg \cdot kg^{-1} \cdot day^{-1}$) for 1 week resulted in a significant decrease in SAP in conscious SHRs that reached a maximum on day 7 after the commencement of treatment that was not found in WKY rats (Figure 1A). There was a concomitant reduction in power density of the vasomotor components of the SAP spectrum detected in animals maintained under propofol anesthesia on days 4 (Figure S1A, available in the online Data Supplement at http://hyper.ahajournals.org), 7 (Figure 1B), or 10 (Figure S1A) after rosiglitazone. Oral intake of rosiglitazone for 1 week, on the other hand, induced a minimal effect on HR in SHRs or WKY rats (Figure 1C). In control experiments, oral intake for 1 week of a calcium channel blocker amlodipine (16 $mg \cdot kg^{-1} \cdot day^{-1}$), which exhibits minimal central antihypertensive effects,[27] resulted in a hypotensive response that was comparable in the temporal profile to that of rosiglitazone in SHRs (Figure S1B), without a concomitant reduction in sympathetic vasomotor tone.

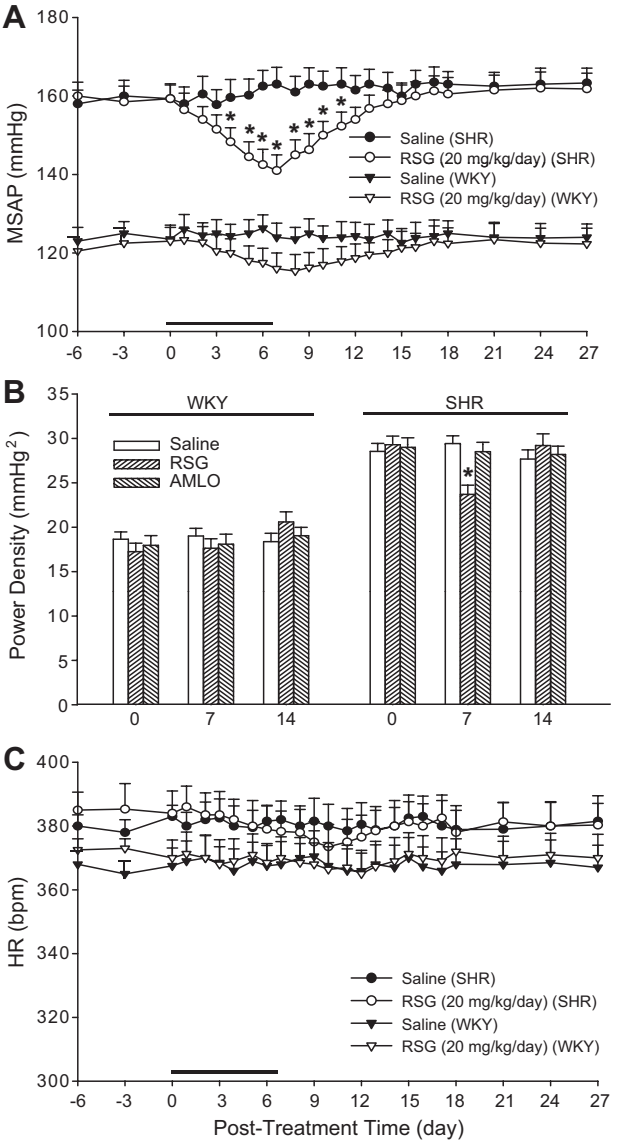


Figure 1. Temporal changes in MSAP (A), power density of vasomotor components of SAP spectrum (B), and HR (C) recorded from conscious (A and C) or anesthetized (B) SHRs or WKY rats after oral intake of rosiglitazone (RSG; 20 $mg \cdot kg^{-1} \cdot day^{-1}$), amlodipine (AMLO; 16 $mg \cdot kg^{-1} \cdot day^{-1}$), or saline for 1 week. Values are mean±SEM, n=5 to 7 animals per group. *$P<0.05$ vs saline group at corresponding time points in the Scheffé multiple-range analysis. Horizontal bar indicates the duration of drug treatment.

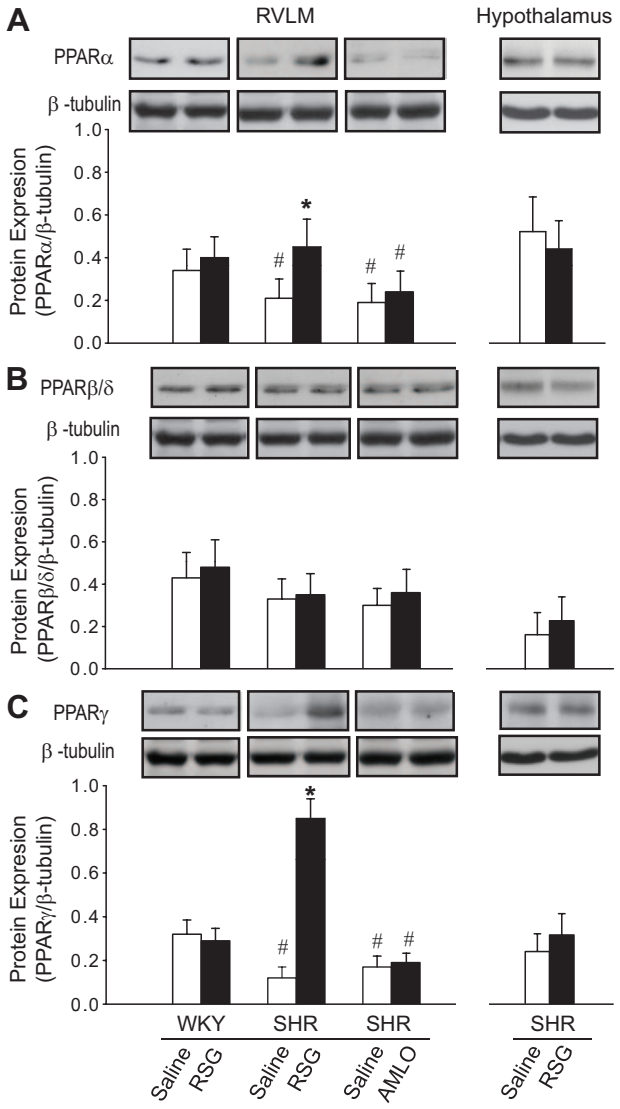


Figure 2. Representative gels (inset) or densitometric analysis of results from Western blots showing changes in expression of PPAR-α (A), -β/δ (B), -γ (C), and β-tubulin in tissue samples from RVLM or hypothalamus on day 7 after commencement of oral intake of rosiglitazone (RSG; 20 mg · kg^{-1} · day^{-1}), amlodipine (AMLO; 16 mg · kg^{-1} · day^{-1}), or saline for 1 week in SHRs or WKY rats. Values are mean±SEM of quadruplicate analyses on samples pooled from 4 to 5 animals in each group. *$P<0.05$ vs saline group, and #$P<0.05$ vs WKY group in the Scheffé multiple-range analysis.

Upregulation of PPARs in the RVLM After Oral Intake of Rosiglitazone

Our second series of experiments evaluated whether orally administered rosiglitazone exerts a central antihypertensive effect via upregulation of PPARs in RVLM. On day 7 after the commencement of oral intake of rosiglitazone (20 mg · kg^{-1} · day^{-1}), protein expression of PPAR-α or -γ, but not -β/δ, in the RVLM of SHRs was significantly increased (Figure 2). None of the PPAR isoforms in the hypothalamus of SHRs underwent significant alterations after the same treatment. Oral intake of amlodipine did not alter expression of the PPAR-α, -β/δ, or -γ isoform in the RVLM of SHRs. Likewise, rosiglitazone or amlodipine treatment also failed to alter expression of these PPAR isoforms in the RVLM of WKY rats.

Upregulation of PPAR-γ in the RVLM Underlies Central Antihypertensive Effects of Oral Intake of Rosiglitazone in SHRs

Our third series of experiments further ascertained a causal role for the upregulation of PPAR-α or -γ in the RVLM in its central antihypertensive effect of oral rosiglitazone. Microinjection bilaterally into the RVLM of PPAR-γ inhibitor GW9662 (0.5 nmol), but not PPAR-α inhibitor GW9471 (0.5 nmol), significantly reversed the antihypertensive effect (Figure 3A) and the depression of sympathetic vasomotor activity (Figure 3B) when measured in anesthetized SHRs on day 7 after the initiation of oral rosiglitazone. Furthermore, gene knockdown by microinjection bilaterally into the RVLM of PPAR-γ siRNA (0.1, 0.5, or 1.0 nmol) on day 4 after the commencement of rosiglitazone treatment abrogated the antihypertensive effect promoted by the PPAR-γ activator in a concentration-dependent manner (Figure 3C). The effectiveness of PPAR-γ siRNA (0.5 nmol) was confirmed by real-time RT-PCR and Western blot analyses, which showed a significant antagonism of rosiglitazone-induced upregulation of PPAR-γ mRNA and protein in the RVLM of treated SHRs (Figure S2A). PPAR-γ mRNA or protein in the RVLM of rosiglitazone-treated WKY rats was not changed, nor was it affected by PPAR-γ siRNA treatment (Figure S2B). Microinjection bilaterally into the RVLM of GW9662 (0.5 nmol), GW9471 (0.5 nmol), or PPAR-γ siRNA (1 nmol), on the other hand, had no effect on basal mean SAP (MSAP) in SHRs or WKY rats (data not shown). Treatment with PPAR-γ siRNA also did not affect the pressor response induced by microinjection bilaterally into the RVLM of L-glutamate (2 nmol), evaluated under anesthetic conditions 0, 12, 24, or 48 hours after silencing PPAR-γ transcription in SHRs (Figure S3).

Amelioration of Oxidative Stress in the RVLM of SHRs After Oral Intake of Rosiglitazone

As observed previously,[23] tissue level of $O_2^{\cdot-}$ in the RVLM of SHRs, which contributes to heightened sympathetic vasomotor tone and hypertension,[21,23] was significantly higher than that in WKY rats (Figure 4A). This augmented $O_2^{\cdot-}$ production in the RVLM of SHRs was reversed to a level comparable to that in WKY rats after oral intake of rosiglitazone, detected by the lucigenin-chemiluminescence method (Figure 4A) on day 4, 7, or 10, or by dihydroethedium fluorescence method (Figure 4B) on day 7 after the initiation of oral rosiglitazone. This induced alleviation of the elevated $O_2^{\cdot-}$ level in RVLM, measured on day 7, was antagonized by microinjection bilaterally of PPAR-γ siRNA (1 nmol) into the RVLM 3 days before measurement. Oral intake of rosiglitazone alone or together with PPAR-γ gene knockdown in RVLM by siRNA, on the other hand, exerted minimal effect on the $O_2^{\cdot-}$ level in the RVLM of WKY rats (Figure 4A and 4B). Likewise, amlodipine ingestion for 1 week did not alter the $O_2^{\cdot-}$ level in the RVLM of SHRs (Figure 4B).

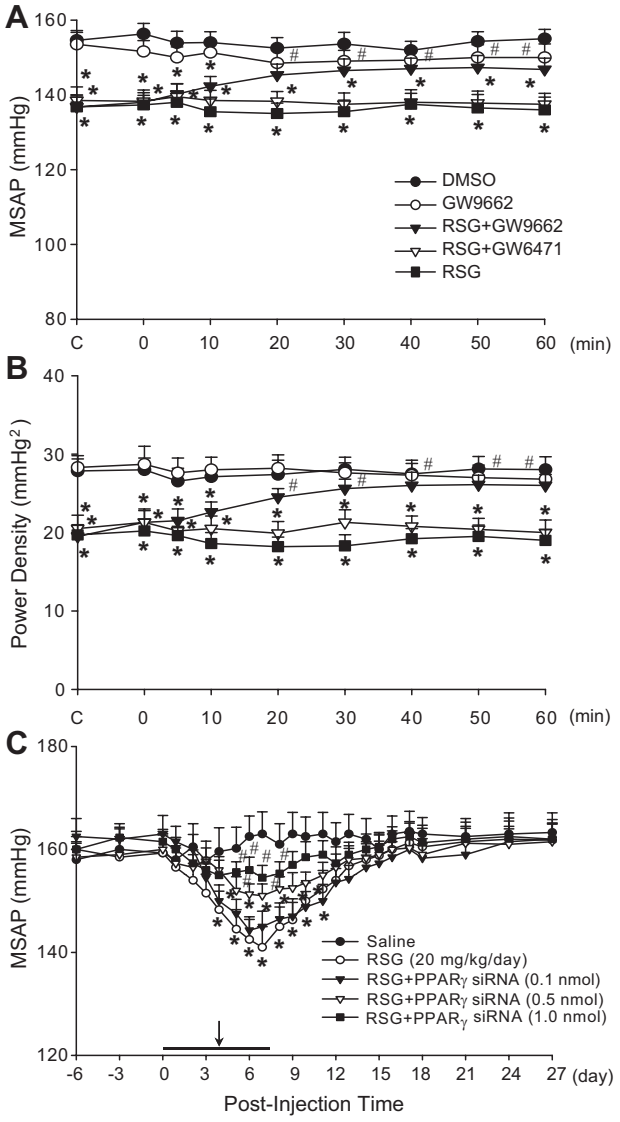


Figure 3. Temporal changes in MSAP (A) and power density of vasomotor components of SAP spectrum (B) recorded in anesthetized SHRs after microinjection bilaterally into RVLM of GW9662, GW6471, or 0.5% dimethyl sulfoxide (DMSO) on day 7 after commencement of oral intake of rosiglitazone (RSG; 20 $mg \cdot kg^{-1} \cdot day^{-1}$) for 1 week or time course changes in MSAP recorded by radiotelemetry in conscious SHRs (C) after initiation of oral administration of rosiglitazone (20 $mg \cdot kg^{-1} \cdot day^{-1}$) or saline for 1 week with additional treatment of microinjection bilaterally into RVLM (at arrow) of PPAR-γ siRNA (0.1, 0.5 or 1.0 nmol) on day 4 after rosiglitazone. Values are mean±SEM, n=6 to 8 animals in each group or quadruplicate analyses on samples pooled from 4 to 5 animals in each group. *$P<0.05$ vs DMSO (A and B) or saline (C) group, and #$P<0.05$ vs rosiglitazone group in the Scheffé multiple-range analysis. Horizontal bar indicates the duration of drug treatment and arrow denotes time during which microinjection was executed.

Oral Intake of Rosiglitazone Upregulates Mitochondrial UCP Expression in the RVLM of SHRs

PPAR-γ functions as a transcription factor to regulate expression of an array of downstream target genes, of which protein products of the mitochondrial *ucp* gene family have been reported to decrease $O_2^{\cdot -}$ production[14,23] and protect against tissue oxidative stress.[28] Our fifth series of experiments, therefore, investigated whether oral intake of rosiglitazone affects expression of mitochondrial UCPs in the RVLM. We detected basal expression of UCP2, 3, and 4, but not UCP1 or 5, in the RVLM of SHRs and WKY rats (Figure 5). The expression of UCP2 in the RVLM of SHRs, as well as UCP3 in the RVLM of SHRs or WKY rats, measured on day 7 after the beginning of oral treatment with rosiglitazone, was significantly upregulated (Figure 5). Oral intake of amlodipine, on the other hand, did not affect UCP2, 3, or 4 expression in the RVLM of SHRs or WKY rats.

Oral Intake of Rosiglitazone Protects Against Oxidative Stress in the RVLM in SHRs Via Transcriptional Upregulation of Mitochondrial UCP2

To establish a causal role for the upregulated mitochondrial UCP2 and UCP3 in rosiglitazone-induced protection against oxidative stress in the RVLM of SHRs, an ASON against UCP2 or UCP3 was microinjected bilaterally into the RVLM on day 4, and $O_2^{\cdot -}$ production was measured on day 7 after the commencement of rosiglitazone ingestion. Compared with sense oligonucleotide controls, pretreatment with UCP2 but not UCP3 ASON abrogated the reduction in $O_2^{\cdot -}$ production detected by the lucigenin-chemiluminescence method (Figure 6) after oral intake of rosiglitazone. Gene knockdown of *ucp2* or *ucp3* in the RVLM of WKY rats, on the other hand, did not affect the tissue level of $O_2^{\cdot -}$, which was not altered by rosiglitazone treatment.

Transcriptional Upregulation of Mitochondrial UCP2 in he RVLM Is Involved in a Central Antihypertensive Effect Induced by Oral Intake of Rosiglitazone in SHRs

Microinjection bilaterally on day 4 into the RVLM of an ASON against UCP2 (Figure 7A) but not UCP3 (Figure 7B) significantly antagonized the reduction in SAP or the power density of vasomotor components of SAP signals (Figure 7C) in SHRs, measured on day 7 after the initiation of oral rosiglitazone administration. UCP2 or UCP3 ASON alone had no discernible effect on baseline MSAP or the sympathetic vasomotor activity of SHRs. Real-time RT-PCR and Western blot analyses confirmed that UCP2 or UCP3 ASON effectively blunted the mRNA and protein expression of individual UCP (Figure S4).

Rosiglitazone Elicits a Central Antihypertensive Effect in SHRs via Activation of the PPAR-γ/UCP2 Pathway in RVLM

Our eighth series of experiments further ascertained that rosiglitazone elicits a central antihypertensive effect in SHRs via activation of the PPAR-γ/UCP2 pathway in RVLM. Microinjection bilaterally into the RVLM of a dose of rosiglitazone (1 nmol) that was ineffective by the intravenous route induced a significant reduction in MSAP (Figure S5A) measured under conscious condition by radiotelemetry and power density of the vasomotor components of SAP signals (Figure S5B) recorded in anesthetized SHRs or WKY rats. These cardiovascular depressive responses of rosiglitazone in SHRs, which were greater in amplitude and longer in duration

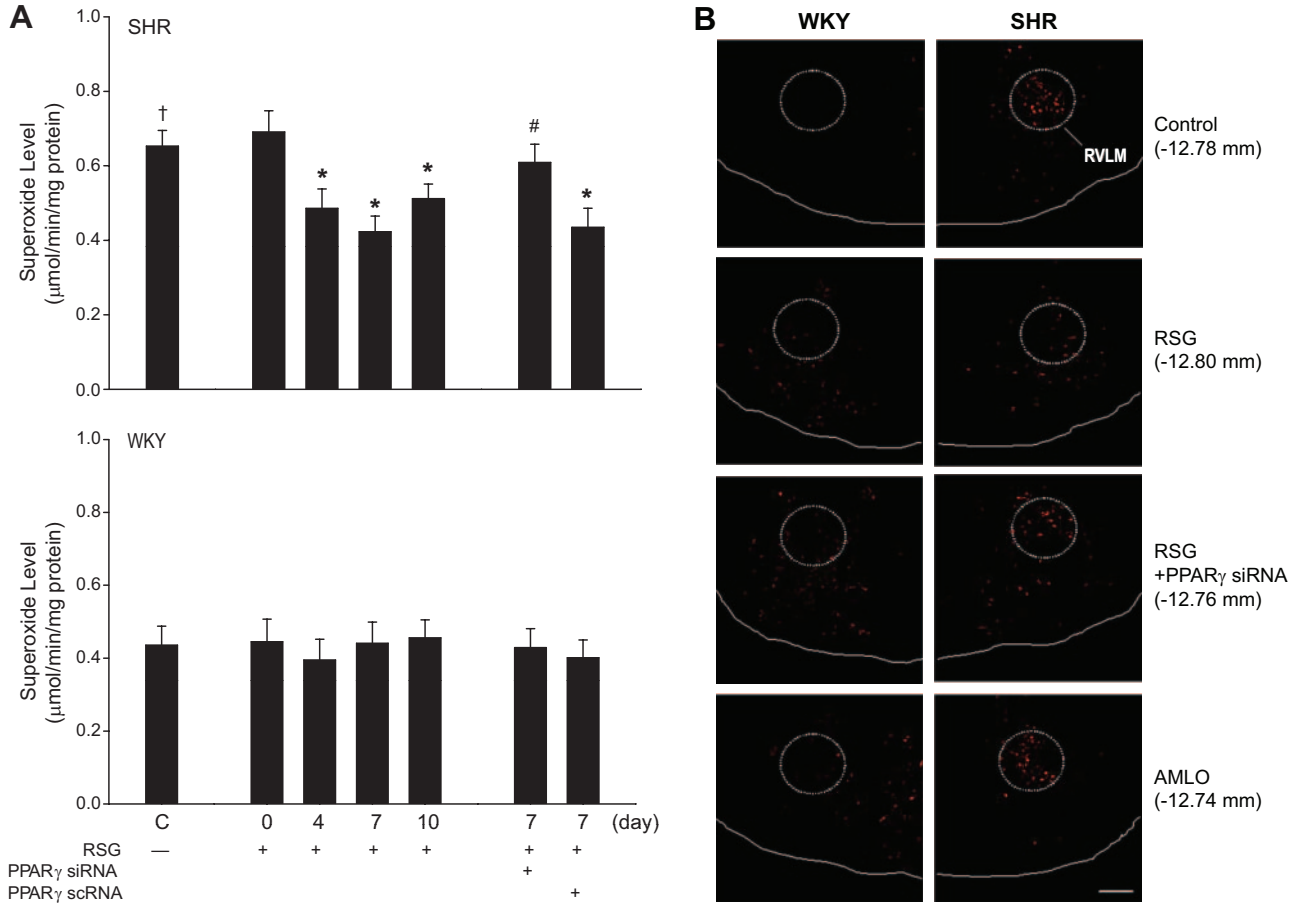


Figure 4. Temporal changes in tissue level of $O_2^{\cdot -}$ (A) or representative photomicrographs showing expression of red fluorescent ethidium bromide (B) in RVLM of SHRs or WKY rats on day 7 after commencement of oral intake of rosiglitazone (RSG; 20 $mg \cdot kg^{-1} \cdot day^{-1}$) or amlodipine (AMLO; 16 $mg \cdot kg^{-1} \cdot day^{-1}$) for 1 week, alone or with additional treatment of microinjection bilaterally into RVLM of PPAR-γ siRNA (1.0 nmol) or scramble (scRNA, 1.0 nmol) RNA on day 4 after rosiglitazone. Values are mean±SEM of quadruplicate analyses on samples pooled from 5 to 6 animals in each group. †$P<0.05$ vs WKY group, *$P<0.05$ vs baseline (C) group, and #$P<0.05$ vs rosiglitazone group in the Scheffé multiple-range analysis. The number on the right side of each photomicrograph denotes the distance caudal to bregma.

than those in WKY rats, were significantly blunted after gene knockdown of PPAR-γ in the RVLM by siRNA (1 nmol) or UCP2 ASON (100 pmol; Figure S5C and S5D). Microinjection of the same dose of rosiglitazone into areas outside the confine of the RVLM (eg, dorsolateral or ventromedial medulla, lateral reticular nucleus, or lateral paragigantocellular nucleus) resulted in minimal changes in the MSAP in SHRs or WKY rats (data not shown).

Effect of Oral Intake of Rosiglitazone on Body Weight, Food Intake, Food Efficiency, and Plasma Level of Insulin

Our final series of experiments measured various metabolic indices to confirm that, as a therapeutic agent for type 2 diabetes mellitus,[4] the dose of rosiglitazone used in the present study was within its therapeutic window. Oral intake of rosiglitazone (20 $mg \cdot kg^{-1} \cdot day^{-1}$) for 1 week significantly increased food intake and body weight in SHRs and WKY rats (Table S1). Although the food intake and body weight gain were greater in SHRs than in WKY rats, the increase in food efficiency, defined as grams of body weight gain per 100 grams of food ingested, was comparable in both strains of animals. SHRs were hyperinsulinemic when compared with age-matched WKY rats. Oral intake of rosiglitazone significantly reduced the fasting plasma insulin level in SHRs.

Discussion

The present study provided novel results to demonstrate that, on oral intake, rosiglitazone induces a central antihypertensive effect in SHRs via depression of sympathetic vasomotor activity through cellular mechanisms that involve amelioration of oxidative stress by upregulation of transcription factor PPAR-γ and increase in expression of mitochondrial UCP2 in the RVLM. To our knowledge, ours is the first report on the cellular and molecular mechanisms of a central antihypertensive effect of the oral administration of rosiglitazone.

Rosiglitazone is a synthetic, high-affinity PPAR-γ ligand of the TZD class[3] clinically used for management of type 2 diabetes mellitus because of its properties to enhance insulin-mediated glucose uptake and to improve insulin sensitivity.[1,4] Accumulating evidence indicates that rosiglitazone also possesses potent blood pressure–lowering effects in patients or

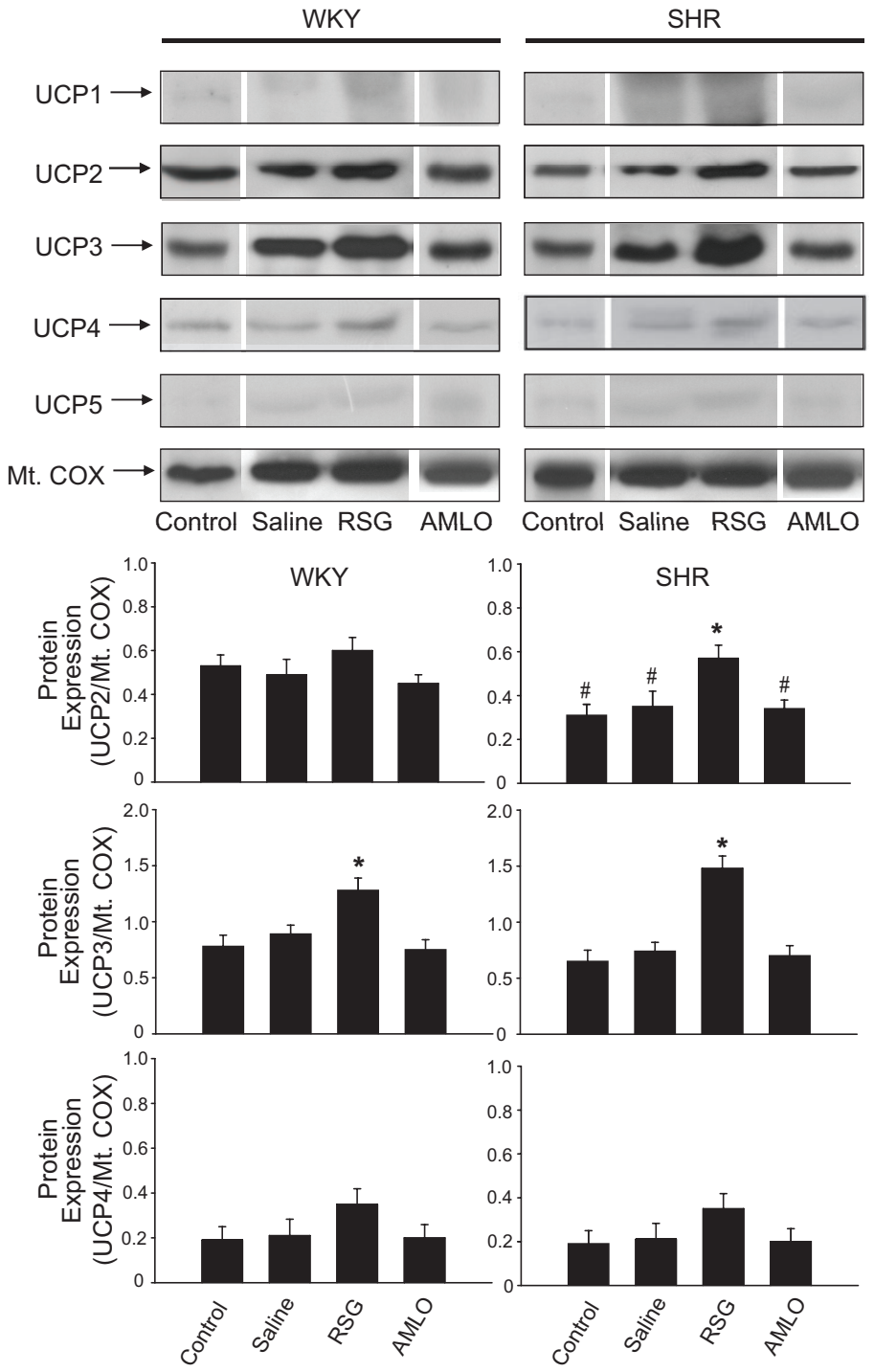


Figure 5. Representative gels and densitometric analysis of results from Western blots showing amount of UCP 1, 2, 3, 4, and 5 protein relative to mitochondrial cytochrome *c* oxidase (Mt. COX) detected from RVLM on day 7 after commencement of oral intake of rosiglitazone (RSG; 20 mg · kg^{-1} · day^{-1}), amlodipine (AMLO; 16 mg · kg^{-1} · day^{-1}), or saline for 1 week in SHRs or WKY rats. Values are mean±SEM of quadruplicate analyses on samples pooled from 4 to 5 animals in each group. *$P<0.05$ vs baseline control group, and #$P<0.05$ vs WKY group in the Scheffé multiple-range analysis.

animal models of both diabetes mellitus/metabolic and non-diabetes/metabolic syndrome,[7–11] although the underlying mechanisms are not fully elucidated. All hitherto implicated mechanisms, including the ability to inhibit the proliferation of arterial smooth muscle cells,[29] prevent augmented vasoconstriction to vasoactive compounds,[30] protect endothelial-dependent vasodilation,[31] or increase in NO production/availability,[32] depict peripheral effects of rosiglitazone. However, controversy still exists on the role of PPAR-γ in vascular smooth muscle cells and endothelial cells in hypertension. It is noted that endothelium-specific PPAR-γ knockout mice do not manifest an apparent hypertensive phenotype unless otherwise induced by challenges such as high-salt water or a high-fat diet.[33] Moreover, vascular smooth muscle

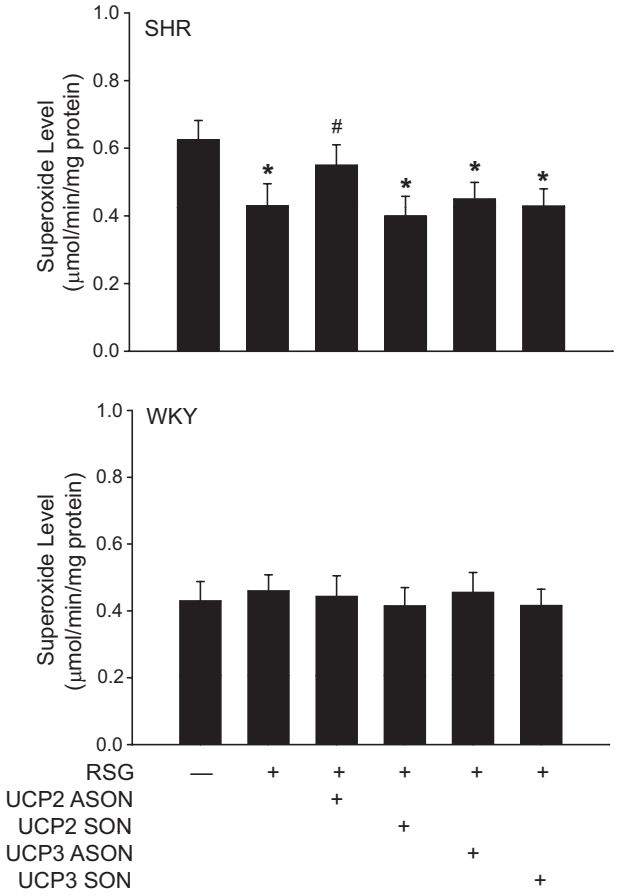
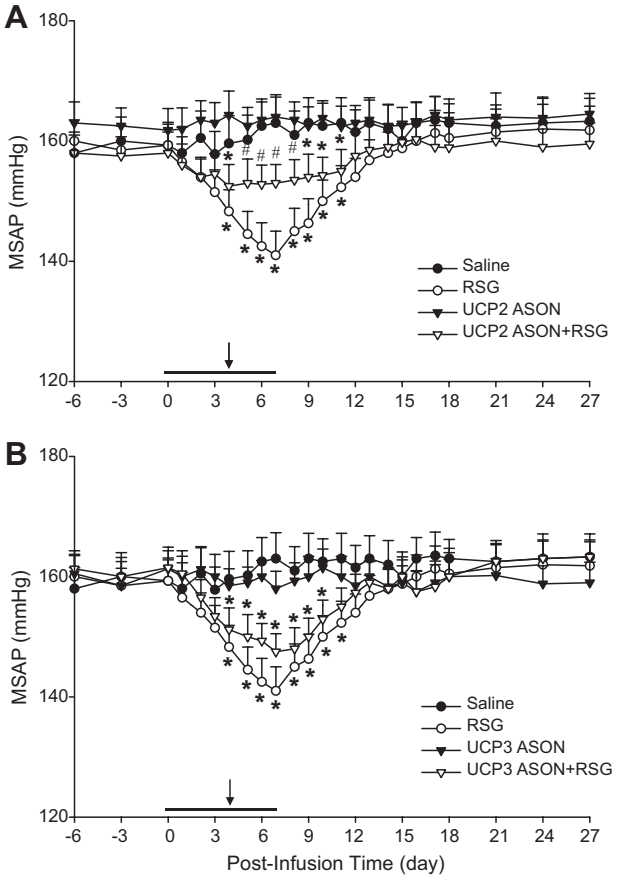


Figure 6. Temporal changes in tissue level of $O_2^{\cdot -}$ in RVLM of SHRs or WKY rats on day 7 after commencement of oral intake of rosiglitazone (RSG; 20 mg · kg^{-1} · day^{-1}) for 1 week, alone or with additional treatment of microinjection bilaterally into RVLM of ASON or sense (SON) oligonucleotide against UCP2 or UCP3 mRNA on day 4 after rosiglitazone. Values are mean±SEM of quadruplicate analyses on samples pooled from 5 to 6 animals in each group. *P<0.05 vs baseline group, and #P<0.05 vs rosiglitazone group in the Scheffé multiple-range analysis.



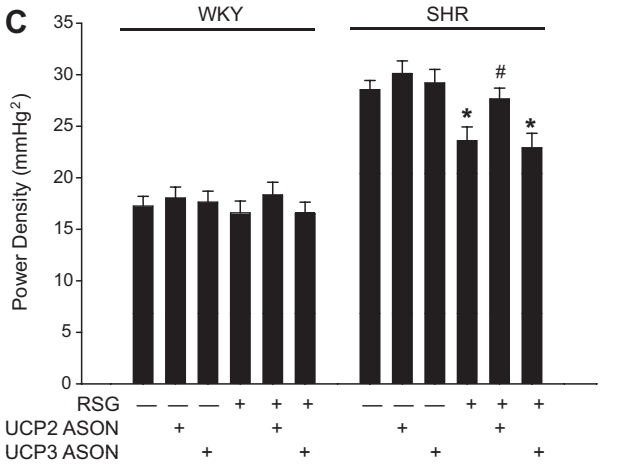


Figure 7. Temporal changes in MSAP (A and B) or power density of vasomotor components of SAP spectrum (C) detected on day 7 after commencement of oral intake of rosiglitazone (RSG; 20 mg · kg^{-1} · day^{-1}) or saline for 1 week, alone or with additional treatment of microinjection bilaterally into RVLM of UCP2 (A) or UCP3 (B) ASON on day 4 after rosiglitazone. Values are mean±SEM, n=5 to 7 animals per group, or quadruplicate analyses on samples pooled from 5 to 6 animals in each group. *P<0.05 vs saline group, and #P<0.05 vs rosiglitazone group in the Scheffé multiple-range analysis. Horizontal bar indicates the duration of drug treatment and arrow denotes time during which microinjection was executed.

cell–selective PPAR-γ gene deletion results in a paradoxical hypotensive phenotype.[34]

Results from the present study thus provided novel insights into the neural mechanism of rosiglitazone-induced hypotension. We found that oral intake of rosiglitazone for 1 week significantly decreased SAP in SHRs by reducing sympathetic vasomotor tone. This vasodepressor response may represent a central antihypertensive action of rosiglitazone at the RVLM for 4 reasons. First, the spectral components of SAP signals measured in the present study take origin from RVLM, and their power densities reflect the prevailing sympathetic vasomotor activity to the blood vessels.[24] We are aware that changes in baroreflex[35] or anesthesia[36] may influence the power of those low-frequency spectral signals. In this regard and in line with a recent report,[37] our preliminary results showed that SHRs exhibited an insignificant change in baroreflex after oral intake of rosiglitazone for 1 week. Furthermore, propofol infusion in our acute experiments provides satisfactory anesthetic maintenance while preserving the capacity of central cardiovascular regulation.[38]

Second, oral intake of amlodipine, a calcium channel blocker that induces hypotension via peripheral vasodilation,[27] resulted in a reduction in SAP in SHRs that was temporally similar to that elicited by rosiglitazone, without affecting sympathetic vasomotor tone. Third, blockade of PPARs in the RVLM by siRNA or its receptor antagonist

appreciably abrogates the antihypertensive effect on oral intake of rosiglitazone. Fourth, microinjection of rosiglitazone directly into the RVLM, at an intravenous dose that did not promote cardiovascular responses, duplicated the antihypertensive effects of oral intake of rosiglitazone in SHRs. The exhibition of hypotension that was greater in amplitude and longer in duration in SHRs compared with WKY rats further implicates a deficiency in the endogenous ligands to PPAR-γ in the RVLM under hypertensive condition. The site specificity of the central antihypertensive effect of rosiglitazone was confirmed when microinjection of this PPAR activator to areas adjacent to the confines of RVLM elicited minimal cardiovascular responses. Comparable magnitudes of vasodepression induced by rosiglitazone in WKY rats and Sprague-Dawley rats[22] further confirm the specificity of rosiglitazone effects in SHRs. It should be noted, however, that our results could not rule out the effect of PPAR activation on blood vessels or insulin sensitivity in the RVLM or exclude the participation of other brain sites in the central antihypertensive action of rosiglitazone.

Mechanistically, our results revealed that the central antihypertensive effect of rosiglitazone depends on activation of PPAR-γ in the RVLM. Oral intake of rosiglitazone upregulated PPAR-α and PPAR-γ protein expression in the RVLM of SHRs. This upregulation was not secondary to vasodepression, because SHRs that received oral intake of amlodipine exhibited no comparable alteration in PPAR-γ or PPAR-α protein expression in the RVLM. Antagonism by pharmacological blockade with specific inhibitors or gene knockdown with siRNA[39] further ascertained that selective activation of PPAR-γ in the RVLM underpins the central antihypertensive action of rosiglitazone. The lack of effect of siRNA treatment on L-glutamate–induced pressor response further ascertained its specificity in silencing the PPAR-γ mRNA. Our results also indicated that, despite being upregulated, PPAR-α in the RVLM is not involved in the central antihypertensive action of rosiglitazone. The role of PPAR-α and its agonist on blood pressure is still controversial. The PPAR-α agonist docosahexaenoic acid prevents the development of hypertension in angiotensin II–treated young rats,[40] but another agonist, clofibrate, is ineffective under normal salt condition.[41] In randomized, controlled trials, the PPAR-α agonist fenofibrate shows minor hypotensive effects in patients with type 2 diabetes mellitus.[42] The reason that expression of PPARs in the RVLM of WKY rats was not altered after oral intake of rosiglitazone is not immediately clear. Because no alteration in PPAR expression was obtained from the hypothalamus of SHRs, the likelihood of a strain-dependent effect of this PPAR activator is greatly reduced. The observation that siRNA affected PPAR-γ mRNA or the protein level in RVLM of SHRs only when activated by rosiglitazone is intriguing and suggests that the upregulated PPAR-γ was primarily derived from newly transcribed mRNA. This suggestion is supported by the lack of effects of PPAR-γ siRNA treatment in rosiglitazone-treated WKY rats, which did not exhibit upregulation of PPAR-γ mRNA or protein in the RVLM.

Oxidative stress in the RVLM plays a pivotal role in the neural mechanism of hypertension. An increase in $O_2^{\cdot-}$ production in RVLM induces sympathoexcitation and hypertension in normotensive WKY rats.[43] Overexpression of adenovirus encoding superoxide dismutase in the RVLM, on the other hand, ameliorates oxidative stress and promotes vasodepression in SHRs.[44] Our present results revealed that protection via activation of PPAR-γ against oxidative stress in the RVLM of SHRs contributes to the blood pressure–lowering effect by rosiglitazone. We observed that suppression of the augmented tissue level of $O_2^{\cdot-}$ in RVLM by oral intake of rosiglitazone but not amlodipine exhibited a temporal profile that coincided with its elicited cardiovascular depressive responses in SHRs. More importantly, silencing PPAR-γ transcription with siRNA rendered rosiglitazone ineffective. The design of the present study, however, did not allow us to decipher whether the $O_2^{\cdot-}$-producing cells affected by PPAR-γ siRNA were bulbospinal RVLM cells. The lack of effect of PPAR-γ siRNA on basal the $O_2^{\cdot-}$ level in RVLM of WKY rats implicates a minor role for endogenous PPAR-γ in the maintenance of redox balance, hence baseline SAP and sympathetic vasomotor tone, under normotensive conditions.

Glutamatergic and angiotensinergic transmission in the RVLM plays a significant role in the hypertensive state of SHRs.[45,46] Of interest is that transcriptional upregulation of mitochondrial UCP2 by PPAR-γ in response to elevated $O_2^{\cdot-}$ induced by angiotensin II at glutamate-sensitive loci in the RVLM plays an active role in feedback regulation of ROS production.[22] Intriguingly, the present study revealed that this novel mechanism also underpins the antioxidant effect of rosiglitazone on oral administration. Oral intake of this PPAR-γ activator resulted in upregulation of UCP2 in the RVLM of SHRs, as well as UCP3 in the RVLM of SHRs and WKY rats. Because gene knockdown of UCP3 did not affect the antioxidant effect of rosiglitazone, we reason that the protective effect of this PPAR-γ activator against oxidative stress in the RVLM of SHRs is primarily attributable to the upregulation of mitochondrial UCP2. This notion is corroborated by observations that knockdown of the *ucp2* but not *ucp3* gene rendered rosiglitazone ineffective in reducing sympathetic vasomotor activity and SAP.

UCP2 is a homologue of the UCP protein family of mitochondrial anion transporters that uncouple ATP synthesis from oxidative phosphorylation by causing proton leakage across the mitochondrial inner membrane, leading to energy dissipation and heat production.[47] More importantly, the resultant decrease in proton electrochemical gradient across the inner mitochondrial membrane mitigates the production of mitochondrial-derived ROS. It has been demonstrated that gene knockdown of *ucp2* increases mitochondrial membrane potential and $O_2^{\cdot-}$ production in murine endothelial cells.[48] Adenovirus-mediated overexpression of UCP2, on the other hand, decreases $O_2^{\cdot-}$ generation in human aortic endothelial cells.[49] We reported recently[22] that mitochondrial UCP2 is involved in ROS homeostasis in the RVLM. Our present results further suggest that oral intake of rosiglitazone promotes a long-term antihypertensive effect via transcriptional upregulation of mitochondrial UCP2 to protect brain RVLM tissues against oxidative stress. Because PPAR-γ and UCP2 are also expressed in brain endothelial[50] and vascular smooth mus-

cle cells,[51] the contribution of these sources of PPAR-γ/UCP2 signaling to the observed protection against oxidative stress by oral intake of rosiglitazone remains to be determined.

We confirmed that the dosage of rosiglitazone used in the present study was within its therapeutic range by showing a significant increase in insulin sensitivity in SHRs. Nonetheless, as a therapeutic agent against type 2 diabetes mellitus,[4] these observations may argue for the antihypertensive effect of rosiglitazone to be secondary to its antihyperinsulinemic action. This argument is deemed unlikely because P465L mutation in PPAR-γ in mice elicits a significant increase in blood pressure while maintaining normal blood glucose and insulin levels.[52] In addition, we observed recently that oral intake of a low dose (10 $mg \cdot kg^{-1} \cdot day^{-1}$) of rosiglitazone for 14 days induced a central antihypertensive effect without increasing insulin sensitivity (unpublished data). We also noted that food intake in SHRs that received rosiglitazone treatment was greater than that in WKY rats. Because feed efficiency was comparable between these 2 strains of animals, the central antihypertensive action of orally administered rosiglitazone may not be related to its dietary effect.

Perspectives

Recent studies[19,53] suggest that peripheral administration or oral ingestion of PPAR activators may exert central antihypertensive effects. Because of the pivotal role of oxidative stress in the neural mechanism of hypertension,[15,16,21] our demonstration that PPAR-γ–dependent transcriptional upregulation of the mitochondrial antioxidant UCP2 in RVLM underpins the central antihypertensive action of oral intake of rosiglitazone therefore opens a new vista for novel therapeutic strategies against hypertension. More importantly and in the spirit of translational medicine, our results are of particular clinical relevance because a significant proportion of patients with diabetes mellitus/metabolic syndrome is hypertensive,[54] and rosiglitazone is widely prescribed as an insulin sensitizer.[4]

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Disclosures

None.

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