Table 1
Physiological data.

	VEH	TLM	T+G	T+A	GW
SBP (mmHg)	240 ± 28	228 ± 17	229 ± 16	231 ± 19	243 ± 21
HR (bpm)	338 ± 30	331 ± 26	340 ± 29	343 ± 30	329 ± 37
BW (g)	282 ± 15	280 ± 14	288 ± 17	276 ± 19	291 ± 22
Calorie intake (Kcal/day)	77 ± 5	74 ± 8	72 ± 4	78 ± 6	74 ± 9
Water intake (ml/day)	32 ± 4	29 ± 4	30 ± 5	30 ± 3	28 ± 5

Data are expressed as the mean ± SEM.
SBP, systolic blood pressure; HR, heart rate; BW, body weight; VEH, vehicle; TLM, telmisartan; T+G, telmisartan+GW9662; T+A, telmisartan+ANA-12; GW, GW9662; $n=5$ for each.

quadrant, where the platform had been located during training, and the time spent in the other quadrants were measured. In the visible-platform test which was performed at day 6, the platform was elevated above the water surface and placed in a different position.

Statistical analysis

All values are expressed as mean ± SEM. Comparisons between any two mean values were performed using Bonferroni's correction for multiple comparisons. ANOVA was used to compare all the parameters in all groups. Differences were considered to be statistically significant at a p-value of <0.05.

Results

Physiological data

Systolic blood pressure and heart rate were not changed in TLM, T+G, GW, T+A, and VEH after the treatments (Table 1). Body weight, dairy calorie intake, and water intake were also not different in all groups (Table 1).

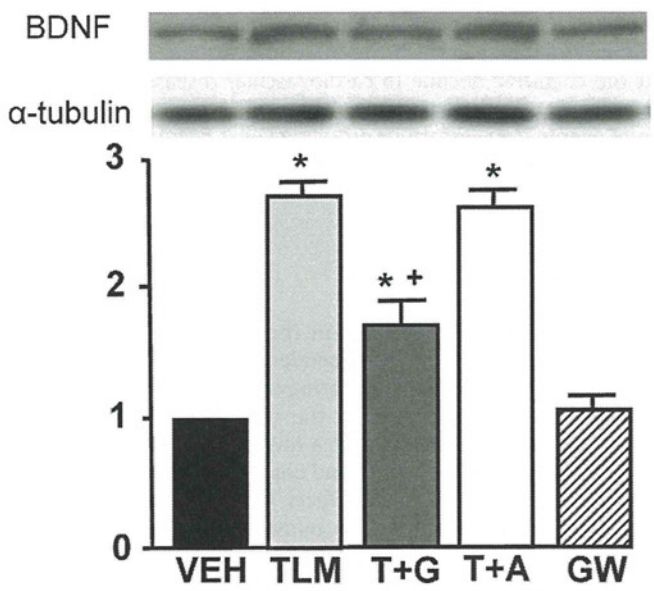

Fig. 1. Expression of BDNF in the hippocampus in each group. BDNF/α-tubulin expression was expressed relative to that in VEH which was assigned a value of 1. $^{*}p<0.05$ versus VEH, $^{+}p<0.05$ in T+G versus TLM, $n=5$ for each. BDNF, brain-derived neurotrophic factor; VEH, vehicle; TLM, telmisartan; T+G, telmisartan+GW9662; T+A, telmisartan+N-[2-[[(hexahydro-2-oxo-1H-azepin-3-yl) amino] carbonyl] phenyl]-benzothiophene-2-carboxamide (ANA-12); GW, GW9662.

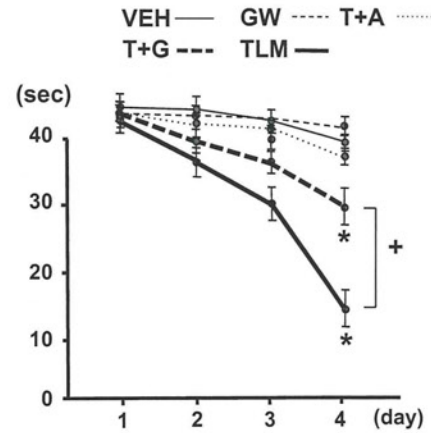

Fig. 2. Escape latency in the hidden platform test of Morris water maze. $^{*}p<0.05$ versus VEH, $^{+}p<0.05$ in T+G versus TLM, $n=5$ for each. VEH, vehicle; TLM, telmisartan; T+G, telmisartan+GW9662; T+A, telmisartan+N-[2-[[(hexahydro-2-oxo-1H-azepin-3-yl) amino] carbonyl] phenyl]-benzothiophene-2-carboxamide (ANA-12); GW, GW9662.

Expression of BDNF in the hippocampus

The expression of BDNF in the hippocampus was significantly higher in TLM than in VEH (Fig. 1). The up-regulation of BDNF in the hippocampus in TLM was attenuated in T+G, but not in T+A (Fig. 1). However, the expression of BDNF in the hippocampus was not different between GW and VEH (Fig. 1).

Morris water maze test

In the hidden platform test, escape latency was significantly lower in TLM than in VEH to a greater extent than in T+G (Fig. 2), and was not different between in VEH, GW, and T+A (Fig. 2). In the probe test, TLM resulted in significantly more time in the target quadrant as compared with VEH, GW, and T+A to a greater extent than in T+G (Fig. 3). In the visible platform test, there were no significant differences in escape latency among all of the groups.

Discussion

In the present study, we have demonstrated two major findings. First, telmisartan has a protective effect on the cognitive decline via up-regulation of BDNF/TrkB in the hippocampus of SHRSPs without depressor effect. Second, co-administration of a PPAR-gamma antagonist with telmisartan partially attenuated the telmisartan-mediated protective effect on the cognitive decline. These results suggest that telmisartan has a possibility of protective effect against cognitive decline via activation of BDNF/TrkB through blockade of AT_1R and part activation of PPAR-gamma in the hippocampus of SHRSPs independent of blood pressure-lowering effect.

In the hippocampus, BDNF protects against ischemic cell damage [32]. Angiotensin II blocks long-term potentiation in the

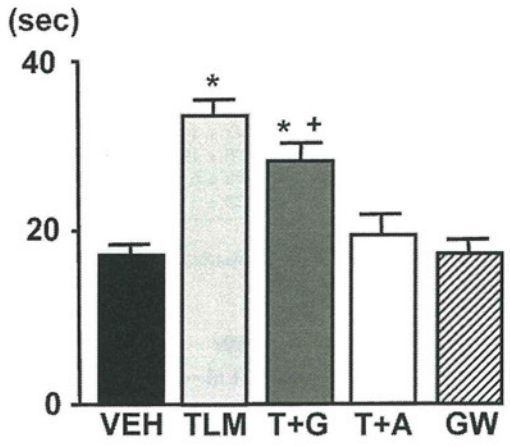

Fig. 3. Time in the target quadrant of the probe test of Morris water maze. $^{*}p < 0.05$ versus VEH, $^{+}p < 0.05$ in T+G versus TLM, $n = 5$ for each. VEH, vehicle; TLM, telmisartan; T+G, telmisartan+GW9662; T+A, telmisartan+N-[2-[[(hexahydro-2-oxo-1H-azepin-3-yl) amino] carbonyl] phenyl]-benzothiophene-2-carboxamide (ANA-12); GW, GW9662.

hippocampus [40–44], and induces superoxide-dependent down regulation of BDNF [27]. In the present study, low-dose telmisartan caused the protective effect against cognitive decline with the increase in BDNF expression in hippocampus of SHRSPs, and the effects were attenuated by TrkB antagonist. These results suggest that telmisartan has a protective effect on the cognitive decline via up-regulation of BDNF/TrkB in the hippocampus of SHRSPs without a depressor effect. Among ARBs, candesartan at sub-hypotensive and renin–angiotensin system blocking dose affords neuroprotection after focal ischemia, associated with increased activity of the BDNF [28]. Interestingly, ramipril at sub-hypotensive, hypotensive, and renin–angiotensin system blocking doses showed no significant neuroprotective effects [28]. Oxidative stress and/or antioxidant deficiency cause cognitive decline [45], and oxidative stress in hippocampus impairs cognitive function [46]. Combining the previous studies with our results in the present study, we consider that the telmisartan-induced up-regulation of BDNF/TrkB is caused by the blockade of AT_1R-induced superoxide in the hippocampus, and that ARBs have a potential to be preferable agents for the treatment of hypertension with the protection against cognitive decline via up-regulation of BDNF/TrkB in the hippocampus.

We also demonstrated that, in the present study, telmisartan-induced protection against cognitive decline via up-regulation of BDNF/TrkB in the hippocampus was partially attenuated by co-administration of PPAR-gamma antagonist with telmisartan. In a previous study, low-dose telmisartan without depressor effect protected against focal brain ischemia partly through activation of PPAR-gamma as well as AT_1R blockade [12]. Telmisartan improves memory impairment and reduces neural apoptosis in hippocampus via a PPAR-gamma-dependent anti-apoptotic mechanism in rats with repeated cerebral ischemia [8]. In other studies, co-administration of PPAR-gamma antagonist had no effect on the losartan-mediated reduction in ischemic area [8,12]. Our results are comparable with those previous studies, and suggest that telmisartan could exert protective effects against cognitive decline via up-regulation of BDNF/TrkB in the hippocampus through AT_1R blockade and partly PPAR-gamma stimulation. Interestingly, in the present study, PPAR-gamma antagonist alone did not change cognitive performance and the expression of BDNF in the hippocampus. There is a possibility that AT_1R blockade has a synergistic effect of PPAR-gamma activation. If so, ARB with partial PPAR-gamma agonist, telmisartan, has a potential to be a preferable agent for the treatment of hypertension with the protection against cognitive decline via up-regulation of BDNF/TrkB in the hippocampus.

The protective effect against cognitive decline is not specific in telmisartan among ARBs. Candesartan has a positive effect on cognitive decline in hypertensive patients [47] or diabetic model [48], and also significantly reduced the incidence and progression of dementia [49]. In SHRSPs, candesartan improves hippocampal CA1 neuron cell reduction, and superoxide production in the hippocampus [50]. In the brain, AT_1R-induced superoxide decreases BDNF [27]. Both telmisartan and candesartan are reported to reduce oxidative stress via blockade of AT_1R in the brain [51–53]. Although candesartan was not examined in the present study, we consider that the protective effect against cognitive decline via up-regulation of BDNF/TrkB in the hippocampus is also caused by candesartan, not only telmisartan among ARBs, through the blockade of AT_1R in the hippocampus. However, the change in permeability of the blood–brain barrier by ARBs has not been well assessed to date. Ischemic brain damage enhances blood–brain barrier permeability and penetration of ARBs into the brain, and blood–brain barrier is disrupted in SHRSPs [54,55]. Telmisartan is expected to readily shift to organs compared with other ARBs, due to its high lipid solubility [56,57]. Moreover, telmisartan is a unique ARB with a partial PPAR-gamma agonistic property [16]. From the results obtained in the present study, AT_1R blockade with PPAR-gamma agonist is considered to be preferable to the protection against cognitive decline via up-regulation of BDNF/TrkB in the hippocampus.

Although the present study could demonstrate a beneficial effect of low-dose telmisartan on cognitive function, depressor dose of telmisartan could not provide positive effect on cognition in previous clinical studies [14,15]. This discrepancy could not be due to the difference in the dose of telmisartan, because the beneficial effects in the present study were obtained with the low and not depressor dose of telmisartan. We could not fully clarify the reasons of the discrepancy in the present study. We used the Morris water maze test in SHRSPs to evaluate cognitive function instead of the shuttle avoidance test. A spatial working memory task, such as Morris water maze test, depends on hippocampus function [58,59]. Because we focused on cognitive performance via BDNF/TrkB in the hippocampus of SHRSPs, we used the Morris water maze test. However, it has not been determined whether other cognitive function tests could obtain similar beneficial effects in other models, such as Alzheimer, diabetes, or cardiovascular disease models. We consider that the cognitive decline in cardiovascular diseases has various clinical backgrounds, and that multi-targeted therapy by combination of agents is necessary to protect against cognitive decline. In these aspects, AT_1R blockade with PPAR-gamma agonist, telmisartan, might be considered to be preferable among ARBs.

Limitations

There are several limitations in the present study. First, we could not determine the dose dependency of telmisartan and not demonstrate the direct data indicating that telmisartan penetrates blood–brain barrier and reaches the hippocampus. Telmisartan used in the present study was at a low and not depressor dose, and we consider that the higher and depressor dose of telmisartan would provide more beneficial effects. It is necessary in a further study to determine whether the telmisartan-induced depressor effect is synergistic to the present results or not, and to measure the concentration of telmisartan in the hippocampus. Second, we did not quantify superoxide in the hippocampus, and did not determine whether telmisartan reduced superoxide in the hippocampus. Furthermore, we examined only cognitive function and BDNF expression in the hippocampus in the present study, and we did not examine the brain damage in the other areas and vascular inflammation. Previously many studies have already demonstrated

that ARBs could prevent brain damage [5,8,10–12] and vascular inflammation [30–32]. Telmisartan also has benefits in SHRSP [34], and anti-oxidant effects in the brain [35,51]. Because of these previous studies, we consider that the benefits of ARBs on brain damage and vascular inflammation are established, and focused on only cognitive function and BDNF expression in the hippocampus in the present study. Third, we did not perform histochemical experiments to determine the expression of PPAR-gamma and changes in CA1 neuron in the hippocampus, and performed only pharmacological inhibition of PPAR-gamma or BDNF/TrkB in the hippocampus. Although previous studies suggested the expression of PPAR-gamma in the hippocampus of cerebral-ischemia models [8,60] and GW9662 or ANA-12 have been used as reasonable agents to inhibit PPAR-gamma or TrkB [8,9,37,39,61], It would strengthen the results of the present study to determine the expression of PPAR-gamma and changes in CA1 neuron in the hippocampus and to do the specific PPAR-gamma or BDNF/TrkB-targeting methods (such as gene transfer methods) locally in the hippocampus.

Conclusion

Telmisartan has a possibility of protective effect against cognitive decline via activation of BDNF/TrkB through blockade of AT_1R and part activation of PPAR-gamma in the hippocampus of SHRSPs independent of blood pressure-lowering effect, which might not be a class effect of ARBs. These results could provide a new aspect that telmisartan may be more effective to prevent cognitive decline compared with other ARBs, and might contribute to improve quality of life in hypertensive patients.

Conflict of interest

None.

Acknowledgments

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Original Article

Sympathoinhibitory effects of telmisartan through the reduction of oxidative stress in the rostral ventrolateral medulla of obesity-induced hypertensive rats

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Objectives: Sympathetic nervous system (SNS) activity is critically involved in the development and progression of obesity-induced hypertension. Angiotensin II type 1 receptor (AT_1R)-induced oxidative stress in the rostral ventrolateral medulla (RVLM), a vasomotor center in the brainstem, activates the SNS in hypertensive rats. The aim of the present study was to determine whether oral administration of an AT_1R blocker (ARB) inhibits SNS activity via antioxidative effects in the RVLM of rats with dietary-induced obesity.

Methods and results: Obesity-prone rats fed a high-fat diet were divided into groups treated with either telmisartan obesity-prone (TLM-OP), or losartan obesity-prone (LOS-OP), or vehicle obesity-prone (VEH-OP). SBP, SNS activity, and oxidative stress in the RVLM were significantly higher in obesity-prone rats than in obesity-resistant rats. Body weight, visceral fat, blood glucose, serum insulin, and plasma adiponectin concentrations were significantly lower in TLM-OP and LOS-OP than in VEH-OP, and plasma adiponectin concentrations were significantly higher in TLM-OP than in LOS-OP. Although SBP was reduced to similar levels both in TLM-OP and LOS-OP, both oxidative stress in the RVLM and SNS activity were significantly lower in TLM-OP than in LOS-OP or VEH-OP.

Conclusion: Orally administered telmisartan inhibited SNS activity through antioxidative effects via AT_1R blockade in the RVLM of obesity-prone rats. AT_1R and oxidative stress in the RVLM might be novel treatment targets for obesity-induced hypertension through sympathoinhibition, and telmisartan might be preferable for obesity-induced hypertension.

Keywords: angiotensin II, brain, hypertension, obesity, sympathetic nervous system

Abbreviations: ARBs, angiotensin II receptor blockers; AT_1R, angiotensin II type 1 receptor; MetS, metabolic syndrome; NAD (P) H, nicotinamide adenine dinucleotide phosphate; PPAR, peroxisome proliferator-activated receptor; RVLM, rostral ventrolateral medulla; SNS, sympathetic nervous system; TBARS, thiobarbituric acid-reactive substances

INTRODUCTION

Metabolic syndrome (MetS) is characterized by the presence of central obesity, impaired fasting glucose, dyslipidemia, and obesity-induced hypertension [1–3]. Previous studies indicated that sympathetic nervous system (SNS) activity is involved in the development and progression of obesity-induced hypertension [4–8]. SNS activity is mediated by the rostral ventrolateral medulla (RVLM), a major vasomotor center in the brainstem, and the functional integrity of the RVLM is essential for the maintenance of basal vasomotor tone [9,10]. Neurons in the RVLM contribute to elevated sympathetic outflow in rats with dietary-induced obesity [11]. We previously demonstrated that oxidative stress in the RVLM produced by activation of angiotensin II type 1 receptors (AT_1R) increases SNS activity [12–14]. In obesity-induced hypertension, oxidative stress is increased and is associated with the development and progression of hypertension in various organs [15–18]. Oxidative stress in the hypothalamus contributes to the progression of obesity-induced hypertension through central sympathoexcitation [19]. Moreover, AT_1R-induced oxidative stress in the RVLM induces sympathoexcitation in rats with obesity-induced hypertension [20]. Direct microinjection of AT_1R blockers (ARBs) into the RVLM or intracerebroventricular infusion of ARBs inhibits SNS activity in hypertensive rats [13,21–23]. Orally administered telmisartan inhibits SNS activity by blocking AT_1R in the brain of hypertensive rats [14]. Further, orally administered telmisartan inhibits the central responses to angiotensin II, and peripherally administered telmisartan penetrates the blood–brain barrier in a dose-dependent and time-

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dependent manner to inhibit the centrally mediated effects of angiotensin II [24]. Although other ARBs also inhibit the central actions of angiotensin II in the brain [24–29], these effects might differ depending on the pharmacokinetics and properties of each drug [24]. It is not known, however, whether orally administered telmisartan blocks AT_1R in the RVLM in obesity-induced hypertension, and if so, whether these are class effects of ARBs.

In the present study, we divided obesity-induced hypertensive rats into three treatment groups receiving either telmisartan, losartan, or vehicle. In hypertensive patients, renin–angiotensin inhibitors, such as angiotensin-converting enzyme inhibitors and ARBs, are preferable for the treatment of hypertension in patients with MetS [30,31]. SNS activity was determined by 24-h urinary norepinephrine excretion and oxidative stress in the RVLM was measured using the thiobarbituric acid-reactive substances (TBARS) method. We also examined the activity of nicotinamide adenine dinucleotide phosphate [NAD (P) H] oxidase, which is the key AT_1R-activated component in the production of oxidative stress in the RVLM [13,32]. Furthermore, we microinjected losartan, angiotensin II, a superoxide dismutase mimetic (tempol), or NAD (P) H oxidase inhibitor (apocynin) into the RVLM of each group.

METHODS

Animals

This study was reviewed and approved by the committee on the ethics of Animal Experiments, Kyushu University Graduate School of Medical Sciences, and conducted according to the Guidelines for Animal Experiments of Kyushu University. Male Sprague–Dawley rats (Charles River Laboratories, Kingston, New York, USA) weighing 350–425 g were individually housed in a temperature-controlled room (22–23°C) with a 12-h/12-h light–dark cycle (lights on at 0700 h). The rats were placed on a moderate high-fat diet (32% kilocalorie from fat; Research Diets, New Brunswick, New Jersey, USA) for 13 weeks. After 5 weeks, rats fed the moderately high-fat diet were classified as obesity-prone or obesity-resistant based on the body weight distribution, as described previously [11,20]. Briefly, a body weight histogram was constructed to show the distribution of the rats; rats falling within the upper third of the weight distribution were classified as obesity prone ($n = 40$) and those falling within the lower third were classified as obesity resistant ($n = 10$) [11,20].

Oral administration of telmisartan or losartan to obesity-prone rats

Of the 40 obesity-prone rats, 10 rats were used for the obesity-prone-only group, and the remaining 30 rats were divided into three groups and treated with either telmisartan obesity-prone (TLM-OP, $n = 10$), losartan obesity-prone (LOS-OP, $n = 10$), or vehicle obesity-prone (VEH-OP, $n = 10$). The TLM-OP group was orally administered telmisartan (5 mg/kg per day; Sigma Aldrich, St. Louis, Missouri, USA) dissolved in 0.3% methylcellulose with a pellet once daily. The LOS-OP group was orally administered losartan (30 mg/kg per day; Sigma Aldrich) in the drinking water for 12 weeks. Food was given to all groups daily 2–3 h before lights-off. Doses of telmisartan and losartan were selected to produce comparable antihypertensive effects based on earlier reports [33,34].

Measurement of blood pressure, heart rate, and sympathetic nervous system activity

SBP and heart rate were measured using the tail-cuff method (BP-98A; Softron, Tokyo, Japan). We calculated the 24-h urinary norepinephrine excretion as an indicator of SNS activity, as described previously [12,13].

Microinjection of losartan, angiotensin II, tempol, or apocynin into the rostral ventrolateral medulla

In the acute experiments, drugs were microinjected under anesthesia (sodium pentobarbital, 50 mg/kg intraperitoneally followed by 2 mg/kg per hour intravenously) in five rats from each group. Blood pressure and heart rate were measured through a catheter inserted into the femoral artery as described previously [12,13]. Prior to the microinjection, blood samples were collected from the arterial line. Each drug was microinjected after blood pressure and heart rate recovered to the basal levels. To inhibit AT_1R, oxidative stress, and NAD (P) H oxidase in the RVLM locally, we microinjected losartan (1 nmol), tempol (100 pmol), and apocynin (1 nmol), respectively, into the bilateral RVLM of obesity-prone and obesity-resistant rats, and in TLM-OP, LOS-OP, and VEH-OP rats. Furthermore, we microinjected angiotensin II into the bilateral RVLM of obesity-prone and obesity-resistant rats, and into TLM-OP, LOS-OP, and VEH-OP rats at the end of this study. After all of the microinjections, we measured the visceral fat, including retroperitoneal, epididymal, and total mesenteric fats.

Measurement of thiobarbituric acid-reactive substances in the rostral ventrolateral medulla

To obtain the RVLM tissues, the rats were deeply anesthetized with sodium pentobarbital (100 mg/kg intraperitoneally) and perfused transcardially with PBS (150 mol/l NaCl, 3 mmol/l KCl, and 5 nmol/l phosphate; pH 7.4, 4°C). The brains were removed quickly, and 1-mm thick coronal sections were obtained with a cryostat at -7 ± 1°C. The RVLM was defined according to a rat brain atlas as described previously [12], and obtained using a punch-out technique. The RVLM tissues were homogenized in 1.15% KCl (pH 7.4) and 0.4% sodium dodecyl sulfate, 7.5% acetic acid adjusted to pH 3.5 with NaOH. Thiobarbituric acid (0.3%) was added to the homogenate. The mixture was maintained at 5°C for 60 min, followed by heating to 100°C for 60 min. After cooling, the mixture was extracted with distilled water and *n*-butanolpyridine (15 : 1) and centrifuged at 1600 *g* for 10 min. The absorbance of the organic phase was measured at 532 nm. The amount of TBARS was determined by absorbance, as described previously [12,13].

Measurement of nicotinamide adenine dinucleotide phosphate oxidase activity

NAD (P) H-dependent superoxide production in the RVLM was measured using a lucigenin luminescence assay as

TABLE 1. Metabolic profiles

			OP		
	OP	OR	TLM	LOS	VEH
n	5	5	5	5	5
Body weight (g)	788 ± 43*	602 ± 38	795 ± 28+	812 ± 30+	887 ± 32
Visceral fat (g)	68 ± 8*	32 ± 11	57 ± 9+	65 ± 10+	86 ± 12
Caloric intake (kcal/day)	113 ± 9*	80 ± 7	106 ± 10	102 ± 11	109 ± 8
Fasting BG (mg/dl)	92 ± 5*	81 ± 6	86 ± 4+	88 ± 3+	96 ± 5
Fasting BI (ng/ml)	1.2 ± 0.2*	0.3 ± 0.1	0.6 ± 0.1+	0.8 ± 0.2+	1.4 ± 0.2
Adiponectin (μg/ml)	1.7 ± 0.2*	2.5 ± 0.3	2.2 ± 0.2+,#	1.8 ± 0.2+	1.5 ± 0.2
Serum TG (mg/dl)	94 ± 7*	64 ± 8	71 ± 9+	76 ± 8+	98 ± 7
Serum FFA (μmol/l)	688 ± 72*	326 ± 85	524 ± 103+	562 ± 93+	724 ± 84

BG, blood glucose; BI, blood insulin; FFA, free fatty acid; LOS, losartan; OP, obesity-prone rats; OR, obesity-resistance rats; TG, triglyceride; TLM, telmisartan; VEH, vehicle.
*$P < 0.05$ vs. OR in OP
+$P < 0.05$ vs. VEH in TLM or LOS
#$P < 0.05$ vs. LOS in TLM

described previously [13,32]. Quantification of NAD (P) H oxidase activity was expressed relative to that in obesity-resistant rats, which was assigned a value of 1.

Statistical analysis

All values are expressed as mean ± SEM. Comparisons between any two mean values were performed using Bonferroni's correction for multiple comparisons. Analysis of variance was used to compare all the parameters in the obesity-prone, obesity-resistant, TLM-OP, LOS-OP, and VEH-OP groups. Differences were considered to be statistically significant at a P value of less than 0.05.

RESULTS

Total body weight, visceral fat weight, and metabolic profiles of obesity-prone, obesity-resistant, and obesity-prone after treatment

Body weight, visceral fat weight, fasting blood glucose, and serum insulin were significantly higher in obesity-prone than in obesity-resistant, and significantly lower in both TLM-OP and LOS-OP than in VEH-OP after 12 weeks of treatment (Table 1). In TLM-OP and LOS-OP, the increases in body weight and visceral fat were attenuated during treatment (Table 1). Mean daily caloric intake was significantly higher in obesity-prone than in obesity-resistant. Mean daily caloric intake did not differ, however, between obesity-prone, TLM-OP, LOS-OP, and VEH-OP (Table 1). Plasma adiponectin concentrations were significantly lower in obesity-prone than in obesity-resistant, and significantly higher in both TLM-OP and LOS-OP than in VEH-OP after 12 weeks of treatment (Table 1). Plasma adiponectin concentrations were significantly higher in TLM-OP than in LOS-OP (Table 1).

Blood pressure, heart rate, urinary norepinephrine excretion of obesity-prone, obesity-resistant, and obesity-prone after treatment

SBP and heart rate were significantly higher in obesity-prone than in obesity-resistant, and significantly lower in both TLM-OP and LOS-OP than in VEH-OP after 12 weeks of treatment (Fig. 1a and b). SBP was not significantly different between TLM-OP and LOS-OP. Heart rate was significantly lower in TLM-OP than in LOS-OP (Fig. 1a and b). Urinary norepinephrine excretion was significantly higher in obesity-prone than in obesity-resistant, and significantly lower in TLM-OP than in VEH-OP after 12 weeks of treatment (Fig. 2). Urinary norepinephrine excretion did not differ significantly between LOS-OP and VEH-OP (Fig. 2).

Thiobarbituric acid-reactive substances levels and nicotinamide adenine dinucleotide phosphate oxidase activity in the rostral ventrolateral medulla of obesity-prone, obesity-resistant, and obesity-prone after treatment

TBARS levels in the RVLM were significantly higher in obesity-prone than in obesity-resistant, and significantly lower in TLM-OP than in VEH-OP after 12 weeks of treatment (Fig. 3a). TBARS levels in the RVLM were not significantly different between LOS-OP and VEH-OP (Fig. 3a). NAD (P) H oxidase activity in the RVLM was significantly higher in obesity-prone than in obesity-resistant, and significantly lower in TLM-OP than in VEH-OP after 12 weeks of treatment (Fig. 3b).

Effects of microinjection of angiotensin II or losartan into the rostral ventrolateral medulla of obesity-prone, obesity-resistant, and obesity-prone after treatment

In the anesthetized condition, basal mean arterial pressure (MAP) levels were significantly higher in obesity-prone

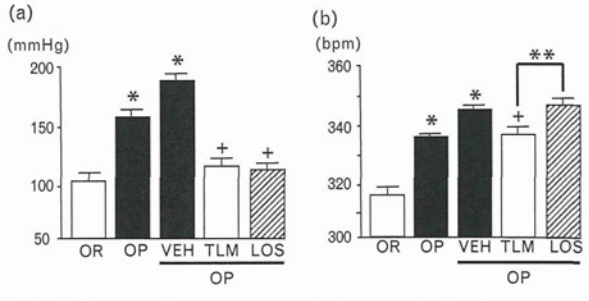

FIGURE 1 (a) SBP and (b) heart rate in obesity-prone (OP) rats, obesity-resistant (OR) rats, telmisartan (TLM)-treated OP rats, losartan (LOS)-treated OP rats, and vehicle (VEH)-treated OP rats ($n = 5$ per group). *, $P < 0.05$ vs. OR in OP or VEH; +, $P < 0.05$ vs. VEH in TLM or LOS; **, $P < 0.05$ vs. TLM in LOS.

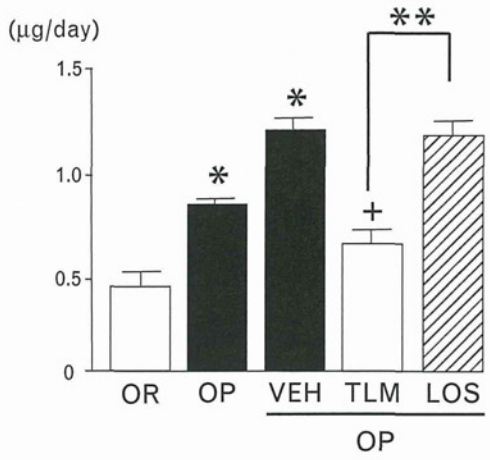

FIGURE 2 Twenty-four hour urinary norepinephrine excretion in obesity-prone (OP) rats, obesity-resistant (OR) rats, telmisartan (TLM)-treated OP rats, losartan (LOS)-treated OP rats, and vehicle (VEH)-treated OP rats ($n=5$ per group). *, $P<0.05$ vs. OR in OP or VEH; +, $P<0.05$ vs. VEH in TLM or LOS; **, $P<0.05$ vs. TLM in LOS.

than in obesity-resistant, and were significantly lower in both TLM-OP and LOS-OP than in VEH-OP (Table 2). Basal MAP levels were not significantly different between TLM-OP and LOS-OP (Table 2). Pressor effects caused by microinjection of angiotensin II into the RVLM were significantly greater in obesity-prone than in obesity-resistant (Fig. 4a; ΔMAP/basal MAP 11 ± 1 vs. $1\pm1\%$, $n=5$ for each, $P<0.01$) and significantly smaller in TLM-OP than in VEH-OP after 12 weeks of treatment (Fig. 4a; ΔMAP/basal MAP 5 ± 1 vs. $15\pm2\%$, $n=5$ for each, $P<0.01$). Pressor effects were not different between LOS-OP and VEH-OP (Fig. 4a; ΔMAP/basal MAP 17 ± 1 vs. $15\pm2\%$, $n=5$ for each).

Depressor effects caused by the microinjection of losartan into the RVLM were significantly greater in obesity-prone than in obesity-resistant (Fig. 4b; ΔMAP/basal MAP -10 ± 1 vs. $0\pm1\%$, $n=5$ for each, $P<0.01$), and significantly smaller in TLM-OP than in VEH-OP after 12 weeks of treatment (Fig. 4b; ΔMAP/basal MAP -8 ± 1 vs. $-15\pm2\%$, $n=5$ for each, $P<0.01$). Depressor effects were not different between LOS-OP and VEH-OP (Fig. 4b; ΔMAP/basal MAP -17 ± 2 vs. $-15\pm2\%$, $n=5$ for each).

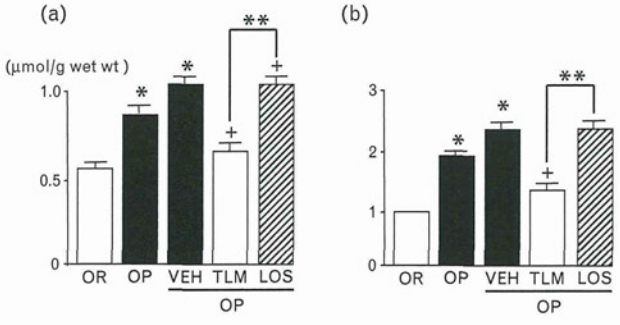

FIGURE 3 (a) Thiobarbituric acid-reactive substances levels and (b) nicotinamide adenine dinucleotide phosphate oxidase activity in the rostral ventrolateral medulla of obesity-prone (OP) rats, obesity-resistant (OR) rats, telmisartan (TLM)-treated OP rats, losartan (LOS)-treated OP rats, and vehicle (VEH)-treated OP rats ($n=5$ per group). *, $P<0.05$ vs. OR in OP or VEH; +, $P<0.05$ vs. VEH in TLM or LOS. **, $P<0.05$ vs. TLM in LOS.

Effects of microinjection of tempol or apocynin into the rostral ventrolateral medulla of obesity-prone, obesity-resistant, and obesity-prone after treatment

Depressor effects caused by microinjection of tempol into the RVLM were significantly greater in obesity-prone than in obesity-resistant (Fig. 5a; ΔMAP/basal MAP -9 ± 1 vs. $0\pm1\%$, $n=5$ for each, $P<0.01$), and significantly smaller in TLM-OP than in VEH-OP after 12 weeks of treatment (Fig. 5a; ΔMAP/basal MAP -7 ± 1 vs. $-19\pm2\%$, $n=5$ for each, $P<0.01$). Depressor effects were not different between LOS-OP and VEH-OP (Fig. 5a; ΔMAP/basal MAP -19 ± 1 vs. $-19\pm2\%$, $n=5$ for each).

Depressor effects caused by microinjection of apocynin into the RVLM were significantly greater in obesity-prone than in obesity-resistant (Fig. 5b; ΔMAP/basal MAP -8 ± 1 vs. $-1\pm1\%$, $n=5$ for each, $P<0.01$), and significantly smaller in TLM-OP than in VEH-OP after 12 weeks of treatment (Fig. 5b; ΔMAP/basal MAP -5 ± 1 vs. $-12\pm2\%$, $n=5$ for each, $P<0.01$). Depressor effects were not significantly different between LOS-OP and VEH-OP (Fig. 5b; ΔMAP/basal MAP -12 ± 1 vs. $-12\pm2\%$, $n=5$ for each).

DISCUSSION

The major novel findings of the present study are as follows. First, orally administered telmisartan decreased SNS activity by reducing oxidative stress due to blockade of AT_1R-NAD (P) H oxidase in the RVLM of obesity-induced hypertensive rats. Second, the sympathoinhibitory effects of orally administered telmisartan through the reduction of oxidative stress in the RVLM of obesity-induced hypertensive rats is not a class-effect of ARBs. These results indicate that orally administered telmisartan inhibits SNS activity by blocking AT_1R-NAD (P) H oxidase in the RVLM as well as metabolic profiles in obesity-induced hypertension.

In the present study, orally administered telmisartan, but not losartan, induced sympathoinhibition, despite their similar depressor effects. Together with the results regarding oxidative stress, NAD (P) H oxidase activity in the RVLM, and the pressor responses elicited by microinjection of angiotensin II into the RVLM, our findings suggest that AT_1R-NAD (P) H oxidase–oxidative stress in the RVLM is blocked by orally administered telmisartan, but not losartan. A previous study demonstrated that due to its high lipophilic properties, peripherally administered telmisartan penetrates the blood–brain barrier in a dose-dependent and time-dependent manner and inhibits the centrally mediated effects of angiotensin II [24]. We previously demonstrated that orally administered telmisartan inhibits SNS activity by blocking AT_1R in the brain of hypertensive rats [14]. Taken together, we consider that orally administered telmisartan (5 mg/kg per day) could penetrate the blood–brain barrier and inhibit AT_1R in the RVLM to a greater extent than orally administered losartan (30 mg/kg per day), despite the similar depressor effects. Although losartan also could penetrate the blood–brain barrier [25,35], the depressor effects caused by orally administered losartan are mainly due to direct blockade of AT_1R in the vasculature. Furthermore, previous studies

TABLE 2. Basal mean arterial pressure levels in anesthetized experiments

	OR	OP	OP		
			TLM	LOS	VEH
n	5	5	5	5	5
MAP (mmHg)	88 ± 2	107 ± 3*	96 ± 4+	98 ± 4+	114 ± 5

LOS, losartan; MAP, mean arterial pressure; OP, obesity-prone rats; OR, obesity-resistance rats; TLM, telmisartan; VEH, vehicle.
*$P < 0.05$ vs. OR in OP
+$P < 0.05$ vs. VEH in TLM or LOS

demonstrated differences between telmisartan and losartan [33,36–40]. Compared to losartan, telmisartan ameliorates vascular endothelial dysfunction to a greater degree via normalization of tumor necrosis factor-α activation [36]. Telmisartan shows insurmountable behavior, whereas losartan shows surmountable behavior [37]. In terms of inverse agonist activity, neither telmisartan nor losartan stabilize AT_1R in an inactive state in the absence of angiotensin II [38,39]. In terms of agonist activity of peroxisome proliferator-activated receptor (PPAR)-γ, a previous study suggested that orally administered rosiglitazone, a PPAR-γ agonist, promotes a central antihypertensive effect via upregulation of PPAR-γ and alleviation of oxidative stress in the RVLM of spontaneously hypertensive rats [40]. Although both telmisartan and losartan have a function as partial PPAR-γ agonists, only telmisartan achieves this effect in therapeutic doses [33,41]. Therefore, there is a possibility that the beneficial effects on the reduction of oxidative stress may differ among ARBs, and the differences between telmisartan and losartan on sympathoinhibition in obesity-induced hypertension might be related to differences in the antioxidant effects between the two compounds.

In the present study, AT_1R-NAD (P) H oxidase–oxidative stress in the RVLM contributed to sympathoexcitation in obesity-induced hypertensive rats, consistent with our previous study [20]. In the RVLM, oxidative stress is mainly produced by activation of AT_1R [13]. The central renin–angiotensin system mediates SNS activity via oxidative stress [13,32,42,43]. In the present study, we did not measure angiotensin II levels or AT_1R density in the RVLM. The depressor effect caused by microinjection of losartan, apocynin, or tempol into the RVLM, however, was significantly greater in obesity-prone than in obesity-resistant, and pressor effects of microinjection of angiotensin II into the RVLM were significantly greater in obesity-prone than in obesity-resistant. Based on the present and previous results, we consider that the mechanism by which obesity increases oxidative stress in the RVLM involves the activation of AT_1R-NAD (P) H oxidase–oxidative stress in the RVLM. Moreover, adipose tissue is reported to secrete angiotensinogen [44], and a PPAR-γ agonist, which ameliorates insulin resistance, prevents hypertension and oxidative stress in rats with dietary-induced obesity [45]. It is possible that leptin, a polypeptide hormone mediator produced by adipocytes, stimulates oxidative stress generation in the brain [46]. In a previous study, we demonstrated that caloric restriction induces sympathoinhibition via reduction of oxidative stress in the RVLM of obesity-prone rats [20]. Although we did not determine the effects of insulin, leptin, or other adipocytokines on the AT_1R in the RVLM in the present study, we consider that peripheral adipose tissue might induce the activation of AT_1R-NAD (P) H oxidase–oxidative stress in the RVLM of obesity-induced hypertensive rats.

The obesity-induced hypertensive rats used in the present study have central obesity, impaired fasting glucose, low plasma adiponectin levels, hypertension, and sympathetic hyperactivity. These characteristics are consistent with those in previous studies using the same model [11,20], and with those observed in patients with MetS [1–4,6–8]. Furthermore, we demonstrated that either orally administered telmisartan or losartan has beneficial effects on the metabolic profile in obesity-induced hypertensive rats and that telmisartan has sympathoinhibitory effects. The findings of the present study suggest that ARBs are preferable for the treatment of hypertension in patients with MetS, consistent with previous studies [30,31]. Moreover, it is possible that orally administered telmisartan, but not losartan, inhibits SNS activity in patients with MetS.

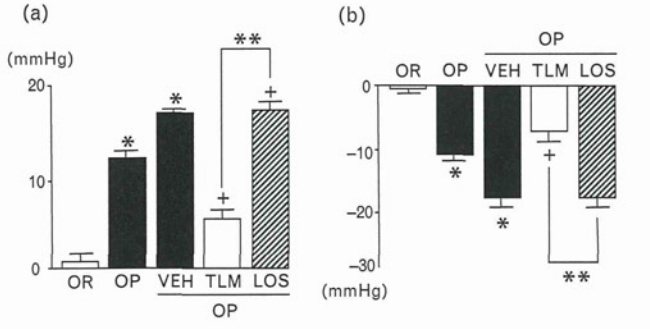

FIGURE 4 (a) Changes in mean arterial pressure caused by the microinjection of angiotensin II or (b) losartan into the bilateral rostral ventrolateral medulla of obesity-prone (OP) rats, obesity-resistant (OR) rats, telmisartan (TLM)-treated OP rats, losartan (LOS)-treated OP rats, and vehicle (VEH)-treated OP rats ($n = 5$ per group). *, $P < 0.05$ vs. OR in OP or VEH; +, $P < 0.05$ vs. VEH in TLM or LOS; **, $P < 0.05$ vs. TLM in LOS.

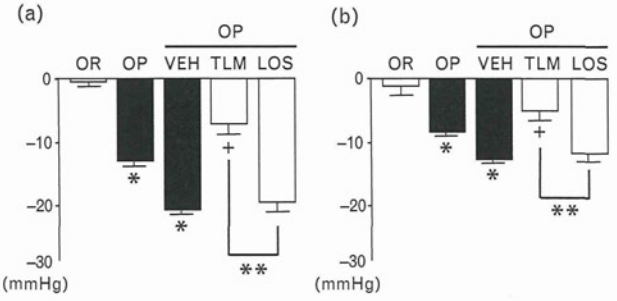

FIGURE 5 Changes in mean arterial pressure caused by the (a) microinjection of tempol or (b) apocynin into the bilateral rostral ventrolateral medulla of obesity-prone (OP) rats, obesity-resistant (OR) rats, telmisartan (TLM)-treated OP rats, losartan (LOS)-treated OP rats, and vehicle (VEH)-treated OP rats ($n = 5$ per group). *, $P < 0.05$ vs. OR in OP or VEH; +, $P < 0.05$ vs. VEH in TLM or LOS; **, $P < 0.05$ vs. TLM in LOS.

Further clinical studies are necessary to determine whether there are differences in the sympathoinhibitory effects among ARBs acting on the brain, particularly in the RVLM and hypothalamus.

In the present study, both telmisartan and losartan attenuated body weight gain during the course of treatment, whereas the daily calorie intake was not reduced. The results are consistent with previous studies in which ARBs improved energy expenditure and metabolic profiles in MetS [47–50]. It is possible that in MetS the ARB-induced attenuation of body weight gain and improvement of energy expenditure cause sympathoinhibition via a reduction of oxidative stress in the RVLM because we previously demonstrated that caloric restriction causes sympathoinhibition via a reduction of oxidative stress in the RVLM of obesity-prone rats [20]. The sympathoinhibition, however, was apparent in only the telmisartan-treated group and not in the losartan-treated group, despite their similar effects on body weight gain. Therefore, in the present study it is unlikely that the ARB-induced attenuation of body weight gain and the improvement of energy expenditure in obesity-prone rats were the main cause of the sympathoinhibition.

There are several limitations to the present study. First, we only examined oxidative stress in the RVLM. Several important nuclei are involved in cardiovascular control, such as the nucleus tractus solitarii and the hypothalamus. Reduction of SNS activity is also achieved by reducing AT_1R activity in the subfornical organ [51]. The increase in oxidative stress in obesity-induced hypertension and the reduction of oxidative stress by orally administered telmisartan may not be unique to the RVLM. In the regulation of SNS activity, however, the RVLM is the most important structure [9,10]. Furthermore, in the RVLM, oxidative stress is the most powerful and important sympathoexciting factor [12–14]. For these reasons, we focused on oxidative stress in the RVLM of obesity-induced hypertensive rats. Second, we cannot exclude the possibility that the benefits of telmisartan in the present study were due to its effects on the kidneys or vasculature. Third, in the present study, we measured blood pressure using the tail-cuff method and oxidative stress in the RVLM using the TBARS method and performed pharmacological examinations by acute microinjection of drugs into the RVLM in anesthetized rats after surgical preparations. Although it is preferable to measure blood pressure and heart rate using radiotelemetry, as in our previous studies [12–14,32], the 20-week observation period in the present study was too long for currently available radiotelemetry systems. Several methods are available for measuring oxidative stress in brain tissue, such as the dihydroethidium or lucigenin fluorescence methods, the electron spin resonance method, and the TBARS method. We previously demonstrated that the TBARS method is both reliable for measuring oxidative stress in the brain tissues and consistent with other methods [12], and we consider that the results obtained by the TBARS method provide a reliable parameter of oxidative stress in RVLM tissue. To inhibit NAD (P) H oxidase or superoxide production, we performed acute microinjection of tempol or apocynin into the RVLM in anesthetized rats. Moreover, we did not evaluate NAD (P) H oxidase isoforms in the present study, and, therefore, the precise mechanisms of NAD (P) H oxidase activation remain unclear. Chronic and awake procedures available by using gene transfer methods targeting AT_1R-NAD (P) H oxidase–oxidative stress in the RVLM locally would strengthen the present findings. Further studies are needed to investigate these issues.

In conclusion, orally administered telmisartan decreases SNS activity by inhibiting oxidative stress due to blockade of AT_1R-NAD (P) H oxidase in the RVLM of obesity-induced hypertensive rats. These results suggest that sympathoexcitation in obesity-induced hypertension is due to AT_1R-NAD (P) H oxidase–oxidative stress in the RVLM, and that orally administered telmisartan inhibits brain oxidative stress-induced sympathoexcitation in MetS through the blockade of AT_1R in the RVLM.

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Conflicts of interest

There is no conflict of interest.

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