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ORIGINAL ARTICLE

Sympathoinhibition caused by orally administered telmisartan through inhibition of the AT_1 receptor in the rostral ventrolateral medulla of hypertensive rats

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In patients and animals with hypertension, sympathetic nervous system (SNS) activation is present. We have demonstrated that angiotensin II type 1 receptor (AT_1R)-induced oxidative stress in the rostral ventrolateral medulla (RVLM), a vasomotor center in the brainstem, causes SNS activation in hypertensive rats. The aim of the present study was to determine whether orally administered AT_1R blockers (ARBs) inhibit SNS activation through an anti-oxidant effect via inhibition of AT_1R in the RVLM of hypertensive rats and, if so, whether the benefits are class effects of ARBs. Stroke-prone spontaneously hypertensive rats (SHRSPs), a hypertensive model with sympathoexcitation, were divided into four groups: SHRSPs treated with telmisartan (TLM), candesartan (CAN), or hydralazine (HYD) and a vehicle group (VEH). Although systolic blood pressure was reduced in the TLM, CAN and HYD groups to the same level, heart rate, SNS activation and oxidative stress in the RVLM were significantly lower in the TLM group only. The pressor effect caused by the microinjection of angiotensin II into the RVLM and the depressor effect caused by the microinjection of tempol, a superoxide dismutase mimetic, into the RVLM were both significantly smaller in TLM, but not in CAN or HYD. These results suggest that orally administered TLM inhibits SNS activation through an anti-oxidant effect via inhibition of AT_1R in the RVLM of SHRSPs; these results are also independent of depressor effects and are not class effects of ARBs.
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Keywords: angiotensin II; brain; oxidative stress; sympathetic nervous system

INTRODUCTION

Sympathetic nervous system (SNS) activation is a main cause of the development and progression of hypertension.[1–4] SNS activation is mainly regulated by the brain,[5–7] and we have demonstrated in rat models with hypertension or heart failure that direct interventions to the brain have beneficial effects because of sympathoinhibition.[8–14] Particularly in the brain, SNS activation is mainly regulated by the rostral ventrolateral medulla (RVLM) in the brainstem, and the functional integrity of the RVLM is essential for the maintenance of basal vasomotor tone.[5,6] We have demonstrated that oxidative stress in the RVLM produced by the angiotensin II type 1 receptor (AT_1R) causes SNS activation.[11,14–17] Upregulation of the central AT_1R is important in the pathophysiology of hypertension.[6,7] Microinjection of AT_1R blockers (ARBs) into the RVLM or intracerebroventricular infusion of ARBs inhibits SNS activation in hypertensive rats.[15,18–20] However, AT_1R or oxidative stress in the RVLM have not been targets for the treatment of hypertensive patients because we do not have suitable oral agents to inhibit AT_1R or oxidative stress in the RVLM of hypertensive patients.

Interestingly, previous animal studies have suggested that peripherally administered ARBs inhibit the central actions of angiotensin II in the brain.[16,21–28] We demonstrated that orally administered ARBs reduced oxidative stress in the brains of hypertensive rats[16,27] and that orally administered telmisartan (TLM) inhibits SNS activation in hypertensive rats.[16] These results suggest that orally administered ARBs have the potential to inhibit SNS activation through reduction of oxidative stress via inhibition of AT_1R in the RVLM. In a previous clinical study, TLM, an ARB, is effective in reducing short-term ambulatory blood pressure variability and SNS activation in hypertensive patients with diabetic nephropathy.[29] However, in other clinical studies, ARBs do not have the same beneficial effects on the autonomic nervous system.[30,31] Moreover, it has not been determined whether the sympathoinhibition caused by orally administered ARBs is a class effect of ARBs.[32] Gohlke *et al.*[26] demonstrated that, following peripheral administration, TLM is able to penetrate the blood-brain barrier in a dose- and time-dependent manner to inhibit centrally mediated effects of angiotensin II and that

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the effects of ARBs might differ depending on the pharmacokinetics and properties of each drug. We hypothesized that orally administered TLM penetrates the blood-brain barrier to a greater extent than any other ARB.

In hypertensive patients, ARBs are preferable for hypertensive patients.[33] New mechanistic insight into antihypertensive treatment could be provided if systemic treatment with ARBs was shown to inhibit SNS activation through inhibition of AT_1R in the brain of hypertensive patients. The aim of the present study is to investigate whether orally administered TLM inhibits SNS activation through the reduction of oxidative stress via inhibition of AT_1R in the RVLM of hypertensive rats. If so, we also aim to determine whether the results are independent of its depressor effects and if these effects are class effects of ARBs. To this end, we divided stroke-prone spontaneously hypertensive rats (SHRSPs) with severe sympathetic hyperactivity, used as the hypertensive model into TLM-, CAN-, hydralazine (HYD)- or vehicle (VEH)-treatment groups. TLM and CAN are widely used ARBs, and both ARBs have powerful blood pressure-lowering effects.[32] We determined SNS activation by 24-h urinary norepinephrine excretion and determined the oxidative stress in the RVLM using the thiobarbituric acid-reactive substance (TBARS) method. We also determined the activity of nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase, which is a key AT_1R-activated component in the creation of oxidative stress moieties in the RVLM. Furthermore, we also performed microinjections of angiotensin II, 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 ethics for Animal Experiments, Kyushu University Graduate School of Medical Sciences, and conducted according to the Guidelines for Animal Experiments by the Kyushu University. Male SHRSPs and age-matched Wistar–Kyoto (WKY) rats (12 to 14 weeks old) weighing 350 to 425 g were fed standard feed during this protocol (SLC Japan, Hamamatsu, Japan). They were housed individually in a temperature-controlled room (22 to 23 °C) with a 12-h/12-h light-dark cycle (lights on at 0700 hours). We divided SHRSPs and the WKY rats into four groups: a TLM-treatment group (TLM rats), a CAN-treatment group (CAN rats), a HYD-treatment group (HYD rats) and a VEH-treatment group (VEH rats).

Oral administration of TLM, CAN or HYD

SHRSPs and WKY rats were treated for 4 weeks. TLM rats received TLM ($2\,mg\,kg^{-1}$ per day, dissolved in 0.5% methylcellulose) and were given this oral gavage once daily (Sigma-Aldrich, St Louis, MO, USA). CAN rats received CAN ($2\,mg\,kg^{-1}$ per day, dissolved in 0.5% methylcellulose) and were given this oral gavage once daily (Sigma-Aldrich). HYD rats received HYD and were given this oral gavage once daily ($5\,mg\,kg^{-1}$ per day, dissolved in drinking water) (Sigma-Aldrich). VEH rats received 0.5% methylcellulose by oral gavage once daily.

Measurement of blood pressure, heart rate and SNS activation

Systolic blood pressure and heart rate were measured once weekly using the tail-cuff method (BP-98 A; Softron, Tokyo, Japan). At 4 weeks, we calculated urinary norepinephrine excretion for 24 h as an indicator of SNS activation as previously described.[9–11]

Microinjection of angiotensin II, tempol or apocynin into the RVLM

At the end of the study, we microinjected angiotensin II bilaterally into the RVLM of all rats ($n = 5$ per group). To inhibit the local oxidative stress in the RVLM, we microinjected tempol (100 pmol) bilaterally into the RVLM of all rats ($n = 5$ rats per group). To inhibit the NAD(P)H oxidase locally in the RVLM, we microinjected apocynin (1 nmol) bilaterally into the RVLM of all groups ($n = 5$ per group). The doses of tempol or apocynin and the procedures of the microinjection are reported in our previous studies.[11,15]

Measurement of TBARS in the RVLM

To obtain RVLM tissue, the rats were deeply anesthetized with sodium pentobarbital ($100\,mg\,kg^{-1}$ IP) and transcardially perfused with PBS ($150\,mol\,l^{-1}$ NaCl, $3\,mmol\,l^{-1}$ KCl and $5\,nmol\,l^{-1}$ phosphate; pH 7.4, 4 °C). The brains were quickly removed, and 1-mm thick sections were obtained with a cryostat at -7 ± 1 °C. The RVLM was defined according to a rat brain atlas as described previously[9,11] and obtained using a punch-out technique. The RVLM tissues were homogenized in 1.15% KCl (pH 7.4), 0.4% sodium dodecyl sulfate and 7.5% acetic acid adjusted; the pH was adjusted to 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.[11,15]

Measurement of NAD(P)H oxidase activity

At the end of the study, NAD(P)H-dependent superoxide production in the RVLM was measured using a lucigenin luminescence assay as described previously.[14,15] Quantification of NAD(P)H oxidase activity was expressed relative to that in WKY rats treated with VEH; this level was assigned a value of 1.

Statistical analysis

All values are expressed as the means ± s.e.m. 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 all groups. Differences were considered to be statistically significant at a P value of <0.05.

RESULTS

Blood pressure, heart rate and urinary norepinephrine excretion

Systolic blood pressure of SHRSPs was significantly lower in each TLM, CAN and HYD rats when compared with VEH rats after 4 weeks of treatment (Figure 1a) and there was no difference among TLM, CAN and HYD rats (Figure 1a). However, heart rate in SHRSPs was significantly lower in TLM rats compared with CAN and HYD rats (Figure 1b). In WKY rats, systolic blood pressure and heart rate were the same among the groups (Figures 1c and d). Urinary norepinephrine excretion was significantly lower in TLM rats versus CAN, HYD and VEH rats in SHRSPs. However, urinary norepinephrine excretion was the same in CAN and VEH rats (Figure 2a). In WKY rats, urinary norepinephrine excretion was the same among all groups (Figure 2b).

TBARS levels and NAD (P) H oxidase activity in the RVLM

In SHRSPs, TBARS levels (Figure 3a) and NAD(P)H oxidase activity (Figure 3b) in the RVLM rats were significantly lower in TLM rats versus CAN, HYD and VEH rats, but there was no difference between CAN and VEH rats. In WKY rats, TBARS levels (Figure 3c) and NAD(P)H oxidase activity (Figure 3d) in the RVLM were the same between all groups.

Effects of microinjection of angiotensin II into the RVLM

In SHRSPs, the pressor effects caused by the microinjection of angiotensin II into the RVLM were significantly smaller in TLM rats than in CAN, HYD or VEH rats (Figure 4a), and they were the same in CAN and VEH rats (Figure 4a). In WKY rats, the pressor effects

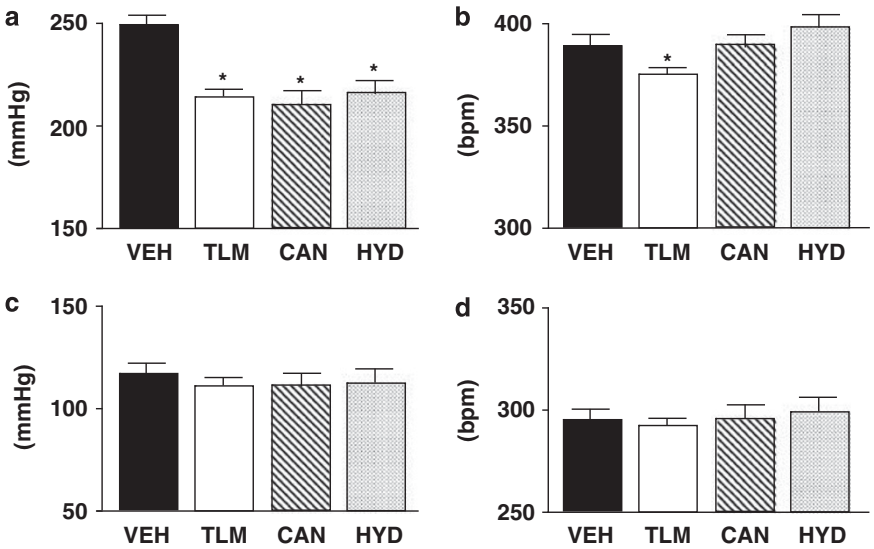

Figure 1 (**a**) Systolic blood pressure of each group of stroke-prone spontaneously hypertensive rats (SHRSPs) ($n=5$ each), (**b**) heart rate of each group ($n=5$ each) of SHRSPs, (**c**) systolic blood pressure of each group of Wistar–Kyoto (WKY) rats ($n=5$ each) and (**d**) heart rate of each group of WKY rats ($n=5$ each). $^*P<0.05$ *vs.* VEH in each strain. CAN, candesartan; HYD, hydralazine; TLM, telmisartan; VEH, vehicle.

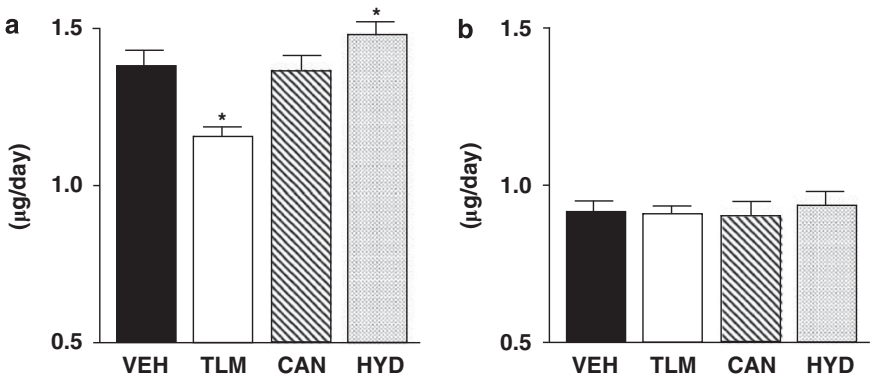

Figure 2. (**a**) 24-h urinary norepinephrine excretion of each group of stroke-prone spontaneously hypertensive rats (SHRSPs) ($n=5$ each) and (**b**) 24-h urinary norepinephrine excretion of each group ($n=5$ each) of Wistar–Kyoto (WKY) rats. $^*P<0.05$ *vs.* VEH in each strain. CAN, candesartan; HYD, hydralazine; TLM, telmisartan; VEH, vehicle.

caused by the microinjection of angiotensin II into the RVLM were the same among groups (Figure 4b).

Effects of microinjection of tempol or apocynin into the RVLM

In SHRSPs, the depressor effects caused by the microinjection of tempol (Figure 5a) or apocynin (Figure 5b) into the RVLM were significantly smaller in TLM rats than in CAN, HYD or VEH rats, and there was no difference between CAN and VEH rats. In WKY rats, the depressor effects caused by the microinjection of tempol (Figure 5c) or apocynin (Figure 5d) into the RVLM were the same in all groups.

DISCUSSION

In the present study, we demonstrated two major findings. First, orally administered TLM inhibits SNS activation through the reduction of oxidative stress via inhibition of AT_1R in the RVLM of SHRSPs. Second, the sympathoinhibition caused by orally administered TLM in SHRSPs is independent of its depressor effect and is not a class effect of ARBs. These results suggest that orally administered TLM might have the potential to be a novel treatment for hypertension via sympathoinhibition.

SNS activation is determined mainly by neural activity in the RVLM in hypertensive patients.[5,6] We demonstrated that oxidative stress in the brain causes hypertension through sympathoexcitation.[11,15–17,34,35] We also demonstrated that AT_1R-inducced oxidative stress in the RVLM causes SNS activation in hypertensive rats.[11,14,15] Direct inhibition of the AT_1R in the RVLM inhibits SNS activation in hypertensive rats.[15,18–20] Peripherally administered ARBs also inhibit the central actions of angiotensin II in the brain.[21–28] Furthermore, we demonstrated that orally administered TLM inhibits SNS activation by reducing oxidative stress in the brains of hypertensive rats.[16] However, it has not been determined whether the sympathoinhibition caused by orally administered ARBs is a class effect of ARBs.[32] The effects of ARBs might differ depending on the pharmacokinetics and properties of each drug.[26] In the present study, orally administered TLM inhibits SNS activation and the angiotensin II-AT_1R-NAD(P)H oxidase-oxidative stress pathway in the RVLM of SHRSPs. However, orally administered CAN or HYD does not have

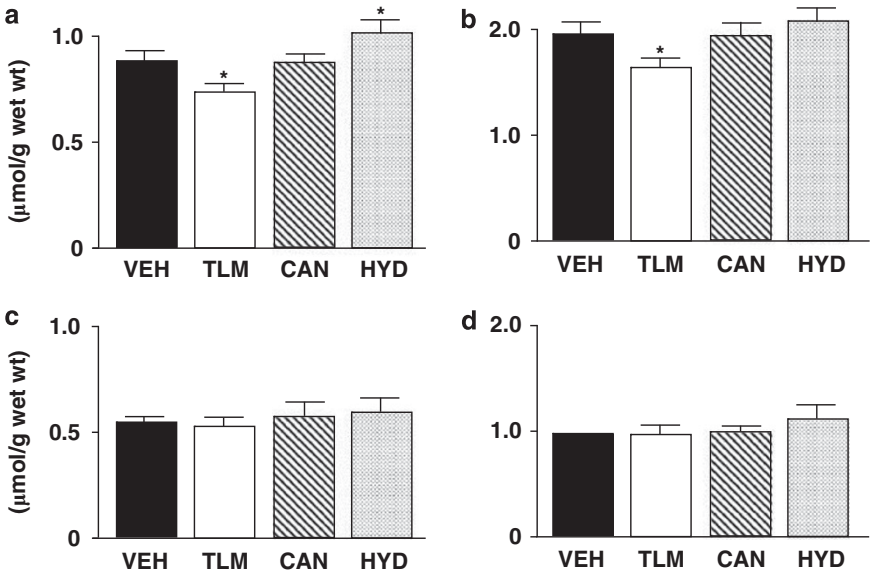

Figure 3 (**a**) Thiobarbituric acid-reactive substance (TBARS) levels in the rostral ventrolateral medulla (RVLM) of each group of stroke-prone spontaneously hypertensive rats (SHRSPs) ($n=5$ each), (**b**) nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase activity in the RVLM of each group ($n=5$ each) of SHRSPs, (**c**) TBARS levels in the RVLM of each group of Wistar–Kyoto (WKY) rats ($n=5$ each) and (**d**) NAD(P)H oxidase activity in the RVLM of each group of WKY rats ($n=5$ each). $^*P<0.05$ *vs.* VEH in each strain. CAN, candesartan; HYD, hydralazine; TLM, telmisartan; VEH, vehicle.

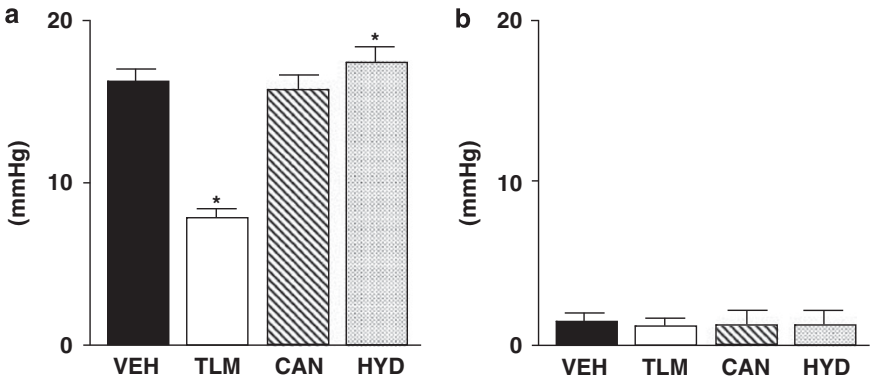

Figure 4 (**a**) Changes in mean arterial pressure caused by the microinjection of angiotensin II into the rostral ventrolateral medulla (RVLM) of each group of stroke-prone spontaneously hypertensive rats (SHRSPs) ($n=5$ each) and (**b**) changes in mean arterial pressure caused by the microinjection of angiotensin II into the RVLM of each group in Wistar–Kyoto (WKY) rats ($n=5$ each). $^*P<0.05$ *vs.* VEH in each strain. CAN, candesartan; HYD, hydralazine; TLM, telmisartan; VEH, vehicle.

these results in SHRSPs despite having similar depressor effects. These results indicate that orally administered TLM might inhibit the AT_1R in the RVLM. These effects are independent of its depressor effects and are not class effects of ARBs.

The mechanisms by which sympathoinhibition, through the reduction of oxidative stress via inhibition of AT_1R in the RVLM, is different between orally administered TLM and CAN should be discussed. In the present study, oxidative stress and NAD(P)H oxidase activity in the RVLM are reduced in the TLM-treated SHRSPs but not in the CAN-treated SHRSPs. The pressor effect caused by the microinjection of angiotensin II into the RVLM and the depressor effect caused by the microinjection of tempol or apocynin into the RVLM are significantly smaller in TLM-treated than in CAN-treated SHRSPs. These results suggest that the pathway of AT_1R-NAD(P)H oxidase-oxidative stress in the RVLM is blocked by orally administered TLM but not by CAN. A previous study demonstrated that, following peripheral administration, TLM penetrates the blood-brain barrier in a dose- and time-dependent manner to inhibit centrally mediated effects of angiotensin II because of the high lipophilicity of TLM.[26] Previously, we also demonstrated that orally administered TLM inhibits SNS activation through the inhibition of AT_1R in the brain of hypertensive rats.[16] We posit that orally administered TLM ($2\,mg\,kg^{-1}$ per day) can penetrate the blood-brain barrier and reach the RVLM of SHRSPs, whereas CAN ($2\,mg\,kg^{-1}$ per day) cannot. Furthermore, previous studies have demonstrated differences between TLM and CAN.[36–39] Both TLM and CAN show clear efficacies.[36] However, the efficacy of CAN is linked to the presence of a carboxyl group at its imidazole-derived moiety, whereas TLM is efficacious despite the absence of a carboxyl group.[36] Moreover, in terms of inverse agonist activity, previous studies have demonstrated that CAN can stabilize AT_1R in an inactive state, therefore acting as an 'inverse agonist,' in the absence of angiotensin II, whereas TLM does not have

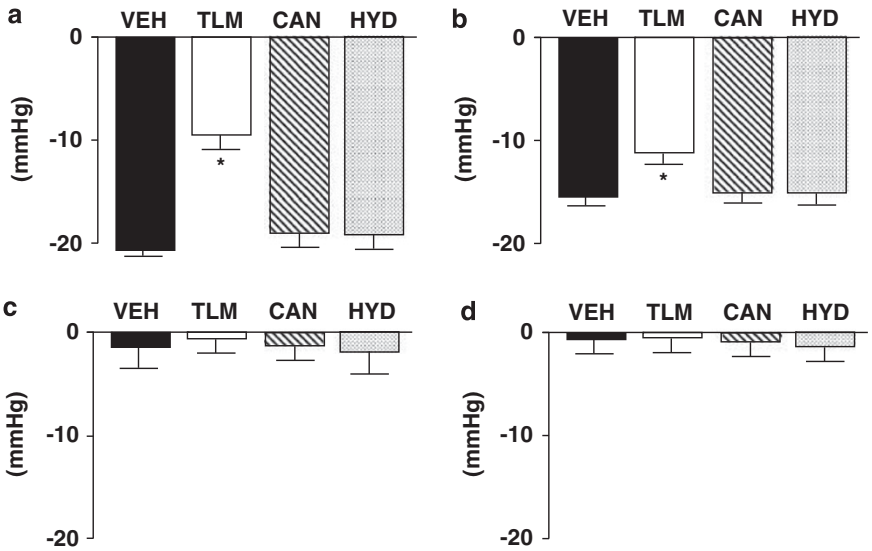

Figure 5 (**a**) Changes in mean arterial pressure caused by the microinjection of tempol into the rostral ventrolateral medulla (RVLM) of each group of stroke-prone spontaneously hypertensive rats (SHRSPs) ($n=5$ each), (**b**) changes in mean arterial pressure caused by the microinjection of apocynin into the RVLM of each group of SHRSPs ($n=5$ each), (**c**) changes in mean arterial pressure caused by the microinjection of tempol into the RVLM of each group in Wistar–Kyoto (WKY) rats ($n=5$ each) and (**d**) changes in mean arterial pressure caused by the microinjection of apocynin into the RVLM of each group in WKY rats ($n=5$ each). $^*P<0.05$ *vs.* VEH in each strain. CAN, candesartan; HYD, hydralazine; TLM, telmisartan; VEH, vehicle.

such an effect.[37–39] In terms of the 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 SHR.[40] Although both TLM and CAN function as partial agonists of PPAR-γ, only TLM achieves this effect at therapeutic doses.[41] Further studies are necessary to investigate the differences in central effects elicited by the various ARBs in terms of efficacy, inverse agonist activity and PPAR-γ agonist activity.

Several studies have suggested that orally administered CAN causes sympathoinhibition and attenuates the central effects of angiotensin II in the brain.[22,24,25,28] Direct inhibition of AT_1R in the RVLM or other areas of the brain inhibits SNS activation,[15,18–20] and superfusion with CAN decreases the electrophysiological activity of RVLM neurons examined using the patch-clamp technique.[42] In previous studies demonstrating sympathoinhibition caused by orally administered CAN in hypertensive rats, the doses of CAN were greater (4,[(ref. 22)] 5,[(ref. 24)] or 10,[(ref. 25)] mg kg^{-1} per day) than those in the present study (2 mg kg^{-1} per day). However, Sakata *et al.*[28] demonstrated that 1 mg kg^{-1} per day of CAN for 2 weeks causes sympathoinhibition in SHR. Although blood pressure of SHRs in their study is lower than that of the SHRSPs in the present study, and the heart rate of the SHRs were similar to that of the WKY rats in their study, these previous studies suggested that the difference in sympathoinhibition between TLM and CAN in the present study may not be due to the dose of CAN. We hypothesize that orally administered CAN (2 mg kg^{-1} per day) was not sufficient to penetrate the blood-brain barrier of SHRSPs.

Interestingly, in the present study, sympathoinhibition through reduction of oxidative stress via inhibition of AT_1R in the RVLM was not obtained in WKY rats. These results are compatible with our previous studies.[11,15] In those studies, overexpression of superoxide dismutase in the RVLM or direct infusion of ARBs into the brain did not reduce oxidative stress and did not inhibit SNS activation in WKY rats.[11,15] From these results, two explanations are possible. First, orally administered TLM (2 mg kg^{-1} per day) could not penetrate the blood-brain barrier, which is not as damaged in WKY rats. In hypertensive rats, the blood-brain barrier is damaged;[43,44] thus, orally administered TLM can easily penetrate the blood-brain barrier of SHRSPs. This possibility would also support our results that orally administered ARB-induced sympathoinhibition through the reduction of oxidative stress via inhibition of the AT_1R in the RVLM is dependent on the penetration of the blood-brain barrier. Second, the role of AT_1R-induced oxidative stress on the regulation of SNS activation may differ between SHRSPs and WKY rats.

The increase in the number of hypertensive patients is a health problem because hypertension is considered to be a risk factor for cardiovascular diseases.[33] ARBs are widely used in hypertensive patients because of their powerful blood pressure-lowering effects and organ-protective effects.[33] However, one of the important treatment targets for hypertension is inadequate SNS activation, and it has not been determined whether ARBs have beneficial effects on SNS activation in hypertension. In the present study, we demonstrated that orally administered TLM, but not CAN, inhibits SNS activation through the reduction of oxidative stress via inhibition of AT_1R in the RVLM of SHRSPs. These results are compatible with a previous clinical study, which indicated that TLM (40 mg per day) causes sympathoinhibition to a greater extent than losartan (50 mg per day) in hypertensive patients with diabetic nephropathy.[29] We also previously demonstrated that orally administered atorvastatin, azelnidipine or amlodipine also causes sympathoinhibition through the reduction of oxidative stress in the RVLM of SHRSPs.[45–47] However, the results of the present study could not directly elucidate the clinical benefits of TLM in hypertensive patients because the dose of TLM in the present study is not a clinical dose, and there are no clinical trials demonstrating the same reduction in heart rate obtained in the present study. Furthermore, damage to the blood-brain barrier may be much more significant in SHRSPs than in hypertensive human patients. To determine whether the benefits of

TLM observed in the present animal study could be obtained in humans with hypertension, further clinical studies are necessary to examine brain oxidative stress, concentrations of ARBs in the brain and the permeability of the blood-brain barrier in hypertensive patients treated with clinical doses of ARBs.

There are several limitations to the present study. First, we examined the AT_1R-NAD(P)H oxidase-oxidative stress pathway only in the RVLM. In addition to the RVLM, some important foci are involved in cardiovascular regulation, such as the nucleus tractus solitarii and the hypothalamus.[6] AT_1R is rich in the specific brain nuclei that regulate SNS activation, such as the anteroventral third ventricle, paraventricular nucleus of the hypothalamus, nucleus tractus solitarii and RVLM.[48–50] Moreover, a high density of AT_1R is present in the brain regions involved in the regulation of SNS activation, such as the circumventricular organs outside of the blood-brain barrier, where peripherally administered ARBs are able to effect change without consideration of the blood-brain barrier, as well as inside of the blood-brain barrier.[50] The reduction of oxidative stress via inhibition of AT_1R caused by orally administered TLM may not be a phenomenon unique to the RVLM. However, in the regulation of the SNS activation, the RVLM is the most important site.[5,6] Furthermore, in the RVLM, oxidative stress is considered to be the most important sympathoexciting factor.[11,15–17] For these reasons, the RVLM is the focus of the present study. Second, we did not examine the dose-dependency of sympathoinhibition caused by orally administered TLM or CAN and the long-term effect of orally administered TLM or CAN in the present study. Our previous study and the present study suggest that the degrees of sympathoinhibition and the depressor effects caused by orally administered TLM are significantly smaller in the present study ($2\,mg\,kg^{-1}$ per day) than in our previous study (5 or $10\,mg\,kg^{-1}$per day).[16] Third, we could not directly demonstrate that TLM penetrates the blood-brain barrier to reach the RVLM; in future studies we will measure the concentration of TLM in the brain tissue.

In conclusion, in the present study, orally administered TLM inhibits SNS activation through the reduction of oxidative stress via inhibition of AT_1R in the RVLM of SHRSPs, and the effects are independent of its depressor effect and are not class effects of ARBs. These results suggest that orally administered TLM might have the potential to be a novel treatment for hypertension resulting from sympathoinhibition through the reduction of oxidative stress via inhibition of AT_1R in the RVLM.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Grassi G, Bertoli S, Seravalle G. Sympathetic nervous system: role in hypertension and in chronic kidney disease. *Curr Opin Nephrol Hypertens* 2012; **21**: 46–51.
2 Grassi G, Seravalle G, Dell'oro R, Mancia G. Sympathetic mechanisms, organ damage, and antihypertensive treatment. *Curr Hypertens Rep* 2011; **13**: 303–308.
3 Grassi G. Sympathetic neural activity in hypertension and related diseases. *Am J Hypertens* 2010; **23**: 1052–1060.
4 Grassi G. Role of the sympathetic nervous system in human hypertension. *J Hypertens* 1998; **16**: 1979–1987.
5 Dampney RA. Functional organization of central pathways regulating the cardiovascular system. *Physiol Rev* 1994; **74**: 323–364.
6 Guyenet PG. The sympathetic control of blood pressure. *Nat Rev Neurosci* 2006; **7**: 335–346.
7 Dupont AG, Brouwers S. Brain angiotensin peptides regulate sympathetic tone and blood pressure. *J Hypertens* 2010; **28**: 1599–1610.
8 Hirooka Y, Sakai K, Kishi T, Takeshita A. Adenovirus-mediated gene transfer into the NTS in conscious rats. A new approach to examining the central control of cardiovascular regulation. *Ann NY Acad Sci* 2001; **940**: 197–205.
9 Kishi T, Hirooka Y, Sakai K, Shigematsu H, Shimokawa H, Takeshita A. Overexpression of eNOS in the RVLM causes hypotension and bradycardia via GABA release. *Hypertension* 2001; **38**: 896–901.
10 Hirooka Y, Kishi T, Sakai K, Shimokawa H, Takeshita A. Effect of overproduction of nitric oxide in the brain stem on the cardiovascular response in conscious rats. *J Cardiovasc Pharmacol* 2003; **41**: S119–S126.
11 Kishi T, Hirooka Y, Kimura Y, Ito K, Shimokawa H, Takeshita A. Increased reactive oxygen species in rostral ventrolateral medulla contribute to neural mechanisms of hypertension in stroke-prone spontaneously hypertensive rats. *Circulation* 2004; **109**: 3257–3262.
12 Hirooka Y, Shigematsu H, Kishi T, Kimura Y, Ueta Y, Takeshita A. Reduced nitric oxide synthase in the brainstem contributes to enhanced sympathetic drive in rats with heart failure. *J Cardiovasc Pharmacol* 2003; **42**: S111–S115.
13 Sakai K, Hirooka Y, Shigematsu H, Kishi T, Ito K, Shimokawa H, Takeshita A, Sunagawa K. Overexpression of eNOS in brain stem reduces enhanced sympathetic drive in mice with myocardial infarction. *Am J Physiol* 2005; **289**: H2159–H2166.
14 Nozoe M, Hirooka Y, Koga Y, Araki S, Konno S, Kishi T, Ide T, Sunagawa K. Mitochondria-derived reactive oxygen species mediate sympathoexcitation induced by angiotensin II in the rostral ventrolateral medulla. *J Hypertens* 2008; **26**: 2176–2184.
15 Kishi T, Hirooka Y, Konno S, Ogawa K, Sunagawa K. Angiotensin II type 1 receptor-activated caspase-3 through ras/mitogen-activated protein kinase/extracellular signal-regulated kinase in the rostral ventrolateral medulla is involved in sympathoexcitation in stroke-prone spontaneously hypertensive rats. *Hypertension* 2010; **55**: 291–297.
16 Hirooka Y, Sagara Y, Kishi T, Sunagawa K. Oxidative stress and central cardiovascular regulation. -Pathogenesis of hypertension and therapeutic aspects-. *Circ J* 2010; **74**: 827–835.
17 Hirooka Y. Oxidative stress in the cardiovascular center has a pivotal role in the sympathetic activation in hypertension. *Hypertens Res* 2011; **34**: 407–412.
18 Koga Y, Hirooka Y, Araki S, Nozoe M, Kishi T, Sunagawa K. High salt intake enhances blood pressure increase during development of hypertension via oxidative stress in rostral ventrolateral medulla of spontaneously hypertensive rats. *Hypertens Res* 2008; **31**: 2075–2083.
19 Gao XY, Zhang F, Han Y, Wang HJ, Zhang Y, Guo R, Zhu GQ. AT1 receptor in rostral ventrolateral medulla mediating blunted baroreceptor reflex in spontaneously hypertensive rats. *Acta Pharmacol Sin* 2004; **25**: 1433–1438.
20 Ito S, Komatsu K, Tsukamoto K, Kanmatsuse K, Sved AF. Ventrolateral medulla AT1 receptor support blood pressure in hypertensive rats. *Hypertension* 2002; **40**: 552–559.
21 Wang LM, Tan J, Leenen FHH. Central nervous system blockade by peripheral administration of AT1 receptor blockers. *J Cardiovasc Pharmacol* 2003; **41**: 539–599.
22 Lin Y, Tsuchihashi T, Kagiyama S, Matsumura K, Abe I. The influence of chronic antihypertensive treatment on the central pressor response in SHR. *Hypertens Res* 2001; **24**: 173–178.
23 Lin Y, Matsumura K, Kagiyama S, Fukuhara M, Fujii K, Iida M. Chronic administration of olmesartan attenuates the exaggerated pressor response to glutamate in the rostral ventrolateral medulla of SHR. *Brain Res* 2005; **1058**: 161–166.
24 Tsuchihashi T, Kagiyama S, Matsumura K, Abe I, Fujishima M. Effects of chronic oral treatment with imidapril and TCV-116 on the responsiveness to angiotensin II in ventrolateral medulla of SHR. *J Hypertens* 1999; **17**: 917–922.
25 Pelosch N, Hosomi N, Ueno M, Masugata H, Murao K, Hitomi H, Nakao D, Kobori H, Nishiyama A, Kohno M. Systemic candesartan reduces brain angiotensin II via downregulation of brain renin-angiotensin system. *Hypertens Res* 2010; **33**: 161–164.
26 Gohlke P, Weiss S, Jansen A, Wienen W, Stangier J, Rascher W, Culman J, Unger T. AT1 receptor antagonist telmisartan administered peripherally inhibits central responses to angiotensin II in conscious rats. *J Pharmacol Exp Ther* 2001; **298**: 62–70.
27 Araki S, Hirooka Y, Kishi T, Yasukawa K, Utsumi H, Sunagawa K. Olmesartan reduces oxidative stress in the brain of stroke-prone spontaneously hypertensive rats assessed by an *in vivo* ESR method. *Hypertens Res* 2009; **32**: 1091–1096.
28 Sakata K, Kumagai H, Osaka M, Onami T, Matsuura T, Imai M, Saruta T. Potentiated sympathetic nervous and renin-angiotensin systems reduce nonlinear correlation between sympathetic activity and blood pressure in conscious spontaneously hypertensive rats. *Circulation* 2002; **106**: 620–625.
29 Masuda S, Tamura K, Wakui H, Kanaoka T, Ohsawa M, Maeda A, Dejima T, Yanagi M, Azuma K, Umemura S. Effects of angiotensin II type 1 receptor blocker on ambulatory blood pressure variability in hypertensive patients with overt diabetic nephropathy. *Hypertens Res* 2009; **32**: 950–955.
30 Krum H, Lambert E, Windebank E, Campbell DJ, Esler M. Effect of angiotensin II receptor blockade on autonomic nervous system function in patients with essential hypertension. *Am J Physiol* 2006; **290**: H1706–H1712.
31 McGowan CL, Notarius CF, McReynolds A, Morris BL, Kimmerly DS, Picton PE, Floras JS. Effect of angiotensin AT1 receptor blockade on sympathetic responses to handgrip in healthy men. *Am J Hypertens* 2011; **24**: 537–543.
32 Mogi M, Horiuchi M. Remote control of brain angiotensin II levels by angiotensin receptor blockers. *Hypertens Res* 2010; **33**: 116–117.

33 Ogihara T, Kikuchi K, Matsuoka H, Fujita T, Higaki J, Horiuchi M, Imai Y, Imaizumi T, Ito S, Iwao H, Kario K, Kawano Y, Kim-Mitsuyama S, Kimura G, Matsubara H, Matsuura H, Naruse M, Saito I, Shimada K, Shimamoto K, Suzuki H, Takishita S, Tanahashi N, Tsuchihashi T, Uchiyama M, Ueda S, Ueshima H, Umemura S, Ishimitsu T, Rakugi H; Japanese Society of Hypertension Committee. The Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2009). *Hypertens Res* 2009; **32**: 3–107.
34 Zimmerman MC, Lazartigues E, Lang JA, Sinnayah P, Ahmad IM, Spitz DR, Davisson RL. Superoxide mediates the actions of angiotensin II in the central nervous system. *Circ Res* 2002; **91**: 1038–1045.
35 Zimmerman MC, Lazartigues E, Sharma RV, Davisson RL. Hypertension caused by angiotensin II infusion involves increased superoxide production in the central nervous system. *Circ Res* 2004; **95**: 210–216.
36 Van Liefde I, Vauquelin G. Sartan-AT1 receptor interactions: *in vitro* evidence for insurmountable antagonism and inverse agonism. *Mol Cell Endocrinol* 2009; **302**: 237–243.
37 Yasuda N, Miura S, Akazawa H, Tanaka T, Qin Y, Kiya I, Imaizumi S, Fujino M, Ito K, Zou Y, Fukuhara S, Kunimoto S, Fukuzaki K, Sato T, Ge J, Mochizuki N, Nakaya H, Saku K, Komuro I. Conformational switch of angiotensin II type 1 receptor underlying mechanical stress-induced activation. *EMBO Rep* 2008; **9**: 179–186.
38 Miura S, Karnik SS, Saku K. Review: angiotensin II type 1 receptor blockers: class effects versus molecular effects. *J Renin Angiotensin Aldosterone Syst* 2011; **12**: 1–7.
39 Cernes R, Mashavi M, Zimlichman R. Differential clinical profile of candesartan compared to other angiotensin receptor blockers. *Vasc Health Risk Manag* 2011; **7**: 749–759.
40 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.
41 Benson SC, Pershadsingh HA, Ho CI, Chittiboyina A, Desai P, Pravenec M, Qi N, Wang J, Avery MA, Kurtz TW. Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPARgamma-modulating activity. *Hypertension* 2004; **43**: 993–1002.
42 Matsuura T, Kumagai H, Kawai A, Onimaru H, Imai M, Oshima N, Sakata K, Saruta T. Rostral ventrolateral medulla neurons of neonatal Wister-Kyoto and spontaneously hypertensive rats. *Hypertension* 2002; **40**: 560–565.
43 Ueno M, Sakamoto H, Liao YJ, Onodera M, Huang CL, Miyanaka H, Nakagawa T. Blood-brain barrier disruption in the hypothalamus of young adult spontaneously hypertensive rats. *Histochem Cell Biol* 2004; **122**: 131–137.
44 Lippoldt A, Kniesel U, Liebner S, Kalbacher H, Kirsch T, Wolburg H, Haller H. Structural alterations of tight junctions are associated with loss of polarity in stroke-prone spontaneously hypertensive rat blood-brain barrier endothelial cells. *Brain Res* 2000; **885**: 251–261.
45 Kishi T, Hirooka Y, Shimokawa H, Takeshita A, Sunagawa K. Atorvastatin reduces oxidative stress in the rostral ventrolateral medulla in stroke-prone spontaneously hypertensive rats. *Clin Exp Hypertens* 2008; **30**: 1–9.
46 Konno S, Hirooka Y, Araki S, Koga Y, Kishi T, Sunagawa K. Azelnidiine decreases sympathetic nerve activity via antioxidant effect in the rostral ventrolateral medulla of stroke-prone spontaneously hypertensive rats. *J Cardiovasc Pharmacol* 2008; **52**: 555–560.
47 Hirooka Y, Kimura Y, Nozoe M, Sagara Y, Ito K, Sunagawa K. Amlodipine-induced reduction of oxidative stress in the brain is associated with sympatho-inhibitory effects in stroke-prone spontaneously hypertensive rats. *Hypertens Res* 2006; **29**: 49–56.
48 Reja V, Goodchild AK, Phillips JK, Pilowsky PM. Upregulation of angiotensin AT1 receptor and intracellular kinase gene expression in hypertensive rats. *Clin Exp Pharmacol Physiol* 2006; **33**: 690–695.
49 Hu L, Zhu DN, Yu Z, Wang JQ, Sun ZJ, Yao T. Expression of angiotensin type 1 (AT1) receptor in the rostral ventrolateral medulla in rats. *J Appl Physiol* 2002; **92**: 2153–2161.
50 McKinley MJ, Albiston AL, Allen AM, Mathai ML, May CN, McAllen RM, Oldfield BJ, Mendelsohn FAQ, Chai SY. The brain renin-angiotensin system: location and physiological roles. *Int J Biochem Cell Biol* 2003; **35**: 901–918.