Vascular Endothelial σ1-Receptor Stimulation With SA4503 Rescues Aortic Relaxation via Akt/eNOS Signaling in Ovariectomized Rats With Aortic Banding

Hideaki Tagashira; Takayuki Matsumoto, PhD; Kumiko Taguchi, PhD; Chen Zhang, PhD; Feng Han, PhD; Keiko Ishida; Shingo Nemoto; Tsuneo Kobayashi, PhD; Kohji Fukunaga, PhD

**Background:** We previously reported that σ1-receptor (σ1R) expression in the thoracic aorta decreased after pressure overload (PO) induced by abdominal aortic banding in ovariectomized (OVX) rats. Here, we asked whether stimulation of σ1R with the selective agonist SA4503 elicits functional recovery of aortic vasodilation and constriction following vascular injury in OVX rats with PO.

**Methods and Results:** SA4503 (0.3–1.0 mg/kg) and NE-100 (a σ1R antagonist, 1.0 mg/kg) were administered orally for 4 weeks (once daily) to OVX-PO rats. Vascular functions of isolated descending aorta were measured following phenylephrine (PE)- or endothelin-1 (ET-1)-induced vasoconstriction and acetylcholine (ACh)- or clonidine-induced vasodilation. SA4503 administration rescued PO-induced σ1R decreases in aortic smooth muscle and endothelial cells. SA4503 treatment also rescued PO-induced impairments in ACh- and clonidine-induced vasodilation without affecting PE- and ET-1-induced vasoconstriction. Ameliorated ACh- and clonidine-induced vasodilation was closely associated with increased Akt activity and in turn endothelial nitric oxide synthase (eNOS) phosphorylation. The SA4503-mediated improvement of vasodilation was blocked by NE-100 treatment.

**Conclusions:** σ1R is downregulated following PO-induced endothelial injury in OVX rats. The selective σ1R agonist SA4503 rescues impaired endothelium-dependent vasodilation in the aorta from OVX-PO rats through σ1R stimulation, enhancing eNOS-cGMP signaling in vascular endothelial cells. These observations encourage development of novel therapeutics targeting σ1R to prevent vascular endothelial injury in vascular diseases. *(Circ J 2013; 77: 2831–2840)*

**Key Words:** Endothelial nitric oxide synthase; σ1-receptor; Vascular disease

Over the past 20 years, observational, retrospective, interventional and meta-analytic studies have supported the idea that ovarian steroid hormones such as estrogen exert protective effects in women against cardiovascular disease.1 Estrogen receptors (ERs) have multiple functions, including regulation of gene transcription and cell signaling. In the genomic pathway, for example, ERs suppress tumor necrosis factor (TNF)-α-induced activated IkBα/ NFκB signaling and protect vascular smooth muscle cells (VSMCs) from vascular injury.2 In the non-genomic pathway, ER stimulation increases nitric oxide (NO) generation in endothelial cells (ECs), including those of humans, and plays an important role in vasoprotection.3,4 Regulation of endothelial NO synthase (eNOS) activity in ECs is complex. NO is a major regulator of vascular tone and has anti-atherogenic activity, including anti-inflammatory and antioxidant effects.6,7 Several cofactors are required for endothelial eNOS function, including Akt, Ca2+, calmodulin and tetrahydrobiopterin.8,9 Of these, Akt is the likely downstream target for estrogenic action on vascular ECs because 17β-estradiol (10 nmol/L) treatment of human ECs induces Akt activation and in turn eNOS phosphorylation and activation within 5 min.10 We have also found that Akt-eNOS signaling is markedly impaired in the thoracic aorta after aortic banding in ovariectomized (OVX) rats.11

The σ1-receptor (σ1R) has 2 transmembrane segments and is widely expressed in rat peripheral tissues.12 We also confirmed...
that the σR is expressed in rat thoracic aorta. Most recently, Amer et al reported that σR is expressed in human saphenous vein ECs (SVECs). However, the pathological function of aortic σR, particularly in the endothelium following cardiovascular disease, remains unclear.

We have reported decreased σR expression levels in the thoracic aorta as cardiac hypertrophy progresses in OVX rats and that treatment with the σR agonist, dehydroepiandrosterone (DHEA), antagonizes pressure overload (PO)-induced decreases in σR expression in the thoracic aorta. These observations suggest that downregulation of σR expressed in vascular ECs may alter NO-mediated endothelial function. However, DHEA has other steroid-like activities in addition to σR stimulation, further studies using specific σR agonists are required to identify specific σR function in endothelial activity.

SA4503 (1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine dihydrochloride) is a selective and potent σR agonist. Moreover, SA4503 has no affinity for other receptors, ion channels and kinases. There is evidence that σR has a function in central nervous system (CNS) diseases, such as depression, dementia and ischemia; SA4503 treatment reportedly rescues some of these CNS diseases. However, thus far, there is no evidence that SA4503 binds to σR in the vascular endothelium and regulates its functions.

In this study, to define the molecular mechanisms underlying σR activation-mediated vasculoprotection and evaluate the pathophysiological relevance of σR to vascular endothelial dysfunction following OVX and PO, we examined endothelium-dependent relaxation in the descending aorta after chronic SA4503 treatment in OVX rats with abdominal aortic banding. We found that σR in the endothelial cells is downregulated following PO-induced endothelial injury in OVX rats and the selective σR agonist SA4503 rescues impaired endothelium-dependent vasodilation in the aorta from OVX-PO rats.

**Methods**

**Materials**

Reagents and antibodies were obtained from the following sources: anti-σR antibody (Abcam, Cambridge, UK); antiplatelet endothelial cell adhesion molecule-1 (PECAM-1) and anti-eNOS antibody (BD Biosciences, San Jose, CA, USA); antiphospho-Akt antibody (Ser473), total-Akt antibody, antiphospho-Akt antibody (Thr308) and antiphospho-eNOS antibody (Cell Signaling Technology, Beverly, MA, USA); anti-β-tubulin antibody (Sigma, St. Louis, MO, USA). The σR agonist SA4503 was synthesized in the Laboratory of Medicinal Chemistry, Zhejiang University (Hangzhou, China), according to the method of Fujimura et al. The specific σR antagonist, NE-100, was obtained from Santa Cruz (Santa Cruz, CA, USA). Other reagents were of the highest quality available (Wako Pure Chemicals, Osaka, Japan).

**Animals**

All animal handling procedures complied with the Guide for Care and Use of Laboratory Animals and were approved by the Animal Experimentation Committee of Tohoku University Graduate School of Pharmaceutical Sciences. Female 6-week-old Wistar rats weighing 150–200 g were obtained from Japan SLC and housed under climate-controlled conditions with a 12 h light/dark cycle.

**Surgical Procedures**

Bilateral OVX was performed under sodium pentobarbital anesthesia (50 mg/kg, i.p; Tokyo Kasei Kogyo, Tokyo, Japan) as previously described. Adequate depth of anesthesia was confirmed by a negative toe pinch reflex. If anesthesia was insufficient, a top-up dose of 20% of the initial dose was given.

**Experimental Design**

Female Wistar rats were randomly assigned to 7 groups: (1) sham operation (Sham: 7 rats); (2) sham plus SA4503 (Sham+SA: 6 rats); (3) OVX: 5 rats; (4) OVX plus PO plus vehicle (OVX-PO+Vehicle: 6 rats); (5) OVX plus PO plus SA4503 (0.3 mg/kg) (OVX-PO+SA0.3: 5 rats); (6) OVX plus PO plus SA4503 (1 mg/kg) (OVX-PO+SA1.0: 5 rats); and (7) OVX plus PO plus SA4503 (1 mg/kg) plus NE-100 (1 mg/kg) (OVX-PO+SA+NE: 6 rats).

**Immunohistochemistry**

Immunohistochemistry was carried out using a previously described method. Masson’s trichrome stain was performed using a kit (Sigma, St. Louis, MO, USA) according to the manufacturer’s protocol. Evaluation of vascular lesions and vascular remodeling was performed as previously described.

**Western Blot Analysis and cGMP Measurement**

Each frozen sample was homogenized as described. Western blot analysis was also performed as previously described. Briefly, the blots were subjected to ECL immunoblotting detection (Amersham Biosciences, Amersham, Buckinghamshire, UK). The emitted light was captured by a Luminescent Image Analyzer (LAS-4000 mini, Fuji Film, Tokyo, Japan) attached to a CCD camera. Densitometric quantification of Western blot signals was performed with Image Gauge Ver3.0 (Science Lab, Fuji Film, Tokyo, Japan). The relative intensities of Western blot signals were expressed as percentage of the values for sham-operated mice. cGMP measurement was performed using a cGMP enzyme immunoassay Biotrak System (Amersham Biosciences, UK), according to the manufacturer’s protocol as described.

**Results**

σR Expression in Vascular Endothelial Cells and Effect of SA4503 Treatment on σR Expression in the Descending Aorta Under Basal Conditions

We first used immunoblotting to test the effects of SA4503 on
Figure 1. Endogenous vascular sigma-1 receptor (σ1R) expression in the rat aorta and the effect of SA4503 treatment on its expression. (A) Western blot analysis of σ1R, PECAM-1 and β-tubulin expression in the aorta. (B) Densitometry quantification of σ1R and PECAM-1 expressions shown in (A). Data are expressed as percentages of the value of sham-operated rats. Each column represents the mean ± SEM. *P<0.05 and **P<0.01 vs. sham group; *P<0.05 and **P<0.01 vs. OVX group; ***P<0.01 vs. the OVX-PO-vehicle treated group; †P<0.05 vs. the OVX-PO plus SA4503 (1 mg/kg)-treated group. N.S., not significant. Each group consists of 4 rats. (C,D) Immunohistochemical analysis of endogenous vascular σ1R localization in the aorta. Righthand columns in (D) show high magnification images of insets in the Merge column.
the expression of \( \sigma R \) and the vascular endothelial cell marker PECAM-1 in the descending aorta following 4 weeks of PO after aortic banding in OVX rats. OVX alone had no effect on the expression of \( \sigma R \) and PECAM-1 (Figures 1A, B), suggesting that OVX alone does not affect endothelial morphology or function. Reduced PECAM-1 expression suggested vascular injury to aortic ECs. Interestingly, chronic oral administration of SA4503 (0.3 and 1.0 mg/kg) dose-dependently restored both \( \sigma R \) and PECAM-1 expression to levels comparable with those of the sham and OVX rats (Figures 1A, B). Co-administration of NE-100, a selective \( \sigma R \) antagonist, with SA4503 eliminated its ameliorating effect on PECAM-1 levels, but did not alter its effect on \( \sigma R \) expression (Figures 1A, B). In immunoblotting with \( \sigma R \) antibody, \( \sigma R \) short receptor of 12 kDa, a deletion \( \sigma R \) mutant, that we previously identified.

To confirm the expression of \( \sigma R \) in aortic ECs, we performed immunohistochemical analysis of \( \sigma R \) and PECAM-1 in tissue sections from the descending aorta. Because the aorta autofluoresced, background signals in the absence of antibodies are shown in Figure 1C. \( \sigma R \) was expressed in both VSMCs and ECs in OVX and sham rats (Figure 1D). At 6 weeks after OVX-PO treatment, \( \sigma R \) immunoreactivity had largely decreased in both VSMCs and ECs (Figure 1D). PECAM-1 immunoreactivity was also markedly reduced in OVX-PO rats. SA4503 treatment (1.0 mg/kg) for 4 weeks rescued the decreased immunoreactivity in both the smooth muscle and endothelium. Importantly, co-administration of NE-100 did not alter SA4503-induced \( \sigma R \) recovery in smooth muscle, whereas the restoration of PECAM-1 immunoreactivity seen following SA4503 treatment was abolished. These findings suggest that \( \sigma R \) expression in both aortic smooth muscle and endothelium is downregulated under OVX-PO conditions.

To address the effects of SA4503 on vascular lesion formation and vascular remodeling in the descending aorta following 4 weeks of PO after aortic banding in OVX rats, we used Masson’s trichrome staining (Figure 2). Although OVX alone treatment did not cause vascular lesion formation, aortic banding in OVX rats increased vascular remodeling without severe vascular lesion. Notably, chronic oral administration of SA4503 (1.0 mg/kg) restored the vascular remodeling observed in OVX-PO rats. Co-administration of NE-100 with SA4503 eliminated its ameliorating effect on vascular remodeling.

**Effect of Chronic SA4503 Treatment on OVX-PO-induced Impaired Vasodilation Mediated by NO**

Next, we assessed the effects of SA4503 treatment on endothelium-dependent vasodilation following OVX-PO-induced vascular injury. We first investigated acetylcholine (ACh)-induced relaxation using aortic rings isolated from OVX-PO rats. ACh-induced relaxation was dramatically impaired in rings from OVX-PO compared with OVX or sham-operated rats (P<0.05 vs. sham group; P<0.05 vs. OVX group) (Figures 3A, B). Interestingly, chronic SA4503 administration at 1 mg/kg dose-dependently restored ACh-induced relaxation and completely rescued the impaired relaxation (P<0.05 vs. OVX-PO + vehicle group) (Figures 3A, B). Pretreatment of sham-operated rats with indomethacin (at 10^{-5} mol/L) plus L-NNA (at 10^{-4} mol/L) for 20 min completely inhibited ACh-induced relaxation, indicating that the effect is endothelium-dependent and that NO production accounts for the observed relaxation.

Next, we investigated clonidine-induced relaxation using isolated aorta. Clonidine-induced relaxation was significantly impaired in the aorta from OVX-PO compared with OVX or sham-operated rats (P<0.01 vs. Sham group; P<0.05 vs. OVX group) (Figures 3C, D). As with ACh-induced relaxation, chronic SA4503 administration rescued impaired clonidine-induced vasodilation (P<0.05 vs. OVX-PO + vehicle group) (Figures 3C, D). Co-administration of NE-100 abolished SA4503-mediated effects in the aorta (P<0.05 vs. OVX-PO + SA1.0 group) (Figures 3C, D). Unlike ACh-induced relaxation, clonidine-induced relaxation was only partially mediated by NO, based on our observation that indomethacin plus L-NNA pretreatment only partially blocked the effect. Moreover, relaxation induced by sodium nitroprusside (10^{-10}–10^{-5} mol/L) did not differ between the OVX and OVX-PO groups (data not shown). Overall, the data suggested that SA4503-mediated endothelial \( \sigma R \) activation rescues PO-induced impaired endothelium-
1-Receptor Stimulation Rescues Aortic Dysfunction

Effect of Chronic SA4503 Treatment on Vasoconstriction
We next investigated the effects of chronic SA4503 treatment on aortic vasoconstriction in OVX-PO rats. Notably, PE- or ET-1-induced vasoconstriction was unchanged in OVX-PO rats compared with sham-operated rats (Figures 4A,B). Moreover, SA4503 treatment (1.0 mg/kg) also had no effect on PE- or ET-1-induced vasoconstriction with or without NE-100.

Effect of Acute SA4503 Treatment on Vascular Endothelial Function
Because acute treatment with a high affinity $\sigma_1$R agonist reportedly causes systolic constriction in isolated cardiomyocytes, we tested the acute effects (10$^{-6}$ mol/L for 1 h) of either SA4503 or NE-100 on aortic relaxation and constriction using normal female rats. Although SA4503 treatment of isolated aorta slightly enhanced ACh-induced relaxation at ACh concentrations of 10$^{-7}$ mol/L, at ACh or clonidine doses required for maximal relaxation, SA4503 treatment had little effect on relaxation (Figures 5A, B). NE-100 treatment, however, significantly reduced clonidine-induced relaxation but did not affect ACh-induced relaxation (Figures 5C, D).

Next, we investigated effects of acute (10$^{-6}$ mol/L for 1 h) treatment with SA4503 or NE-100 on PE- or ET-1-induced constriction in the aorta from female rats (Figures 5C, D). Neither SA4503 nor NE-100 treatment had any effect on PE- or ET-1-induced aortic constriction (Figures 5C, D). Moreover, we confirmed that there were no significant effects of chronic SA4503 treatment on ACh- or clonidine-induced vasodilation, or PE- or ET-1-induced vasoconstriction in normal female rats (data not shown). Taken together, the observation that SA4503 had no acute effects on vascular tone in isolated aorta suggests that SA4503 would have no effect on vascular tone in healthy humans.
Effect of Chronic SA4503 Treatment on Akt/eNOS/cGMP Signaling

We next investigated the mechanisms underlying the improvement of ACh- and clonidine-induced relaxation in OVX-PO rats. Because SA4503 improved endothelium-dependent relaxation, we asked whether eNOS was modified posttranslationally after SA4503 treatment by measuring eNOS phosphorylation at Ser^1177. As expected, SA4503 treatment increased PO-induced decreased eNOS phosphorylation (P<0.01 vs. OVX-PO+Vehicle group) (Figures 6A,B). Co-administration of NE-100 with SA4503 inhibited SA4503-induced increases in eNOS phosphorylation (P<0.01 vs. OVX-PO+SA1.0 group), but total eNOS expression was unchanged among groups (Figures 6A,B).

To further confirm eNOS activation by SA4503, we investigated the levels of cGMP, a messenger downstream of NO. Consistent with perturbed eNOS phosphorylation, PO-induced...
σ1-Receptor Stimulation Rescues Aortic Dysfunction

production and relaxation in aorta. We also confirmed that SA4503-induced Akt activation accounts for increased eNOS phosphorylation, as we reported previously. 11 SA4503 treatment significantly restored Akt phosphorylation of Ser473 (P<0.05 vs. OVX-PO+Vehicle group) and Thr308 (P<0.05 vs. OVX-PO+Vehicle group). Co-administration of NE-100 inhibited SA4503-induced increases in Akt phosphorylation at Ser473 (P<0.05 vs. OVX-PO+SA1.0 group) and Thr308 (P<0.05 vs. OVX-PO+SA1.0 group), suggesting that SA4503-mediated increases in eNOS phosphorylation are critical for ACh-induced NO production and relaxation in aorta. We also confirmed that SA4503-induced Akt activation accounts for increased eNOS phosphorylation, as we reported previously. 11 SA4503 treatment significantly restored Akt phosphorylation of Ser473 (P<0.05 vs. OVX-PO+Vehicle group) and Thr308 (P<0.05 vs. OVX-PO+Vehicle group). Co-administration of NE-100 inhibited SA4503-induced increases in Akt phosphorylation at Ser473 (P<0.05 vs. OVX-PO+SA1.0 group) and Thr308 (P<0.05 vs.

Figure 6. Effect of SA4503 (SA: 0.3 or 1 mg/kg) on Akt/eNOS/cGMP signaling. (A) Western blot analysis of eNOS total protein, phospho-eNOS (Ser1177), phospho-Akt (Ser473), phospho-Akt (Thr308) and total Akt protein in the aorta. (B) Densitometric quantification of immunoreactive bands shown in (A) (p-eNOS/total eNOS protein). (C) ELISA analysis of basal cGMP content in the aorta. Data are expressed as percentages of the value of sham rats. (D) Densitometric quantification of immunoreactive bands shown in (A) (phospho-Akt (Ser473)). (E) Densitometric quantification of immunoreactive bands shown in (A) (phospho-Akt (Thr308)). Data are expressed as percentages of the value of sham rats. Each column represents the mean±SEM. ¶P<0.05 and ¶¶P<0.01 vs. sham group; *P<0.05 and **P<0.01 vs. OVX group; #P<0.05 and ###P<0.01 vs. the OVX-PO vehicle treated group; †P<0.05 and ††P<0.01 vs. the OVX-PO plus SA4503 (1 mg/kg)-treated group. Each group consists of 4 rats. eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; NE-100 (specific σ1R antagonist); OVX, ovariectomy; PE, phenylephrine; PO, pressure overload.
Reduced Akt phosphorylation was also detected in endothelium-denuded aorta (Figures 7A, B). We also examined potential changes in σR expression and Akt phosphorylation in endothelium-denuded aorta isolated from variously treated rats (Figure 7C). The reductions in both σR expression and Akt phosphorylation in endothelium-denuded aorta following OVX-PO were not as significant as those observed in naive aorta (Figures 6A, 7C (n=3)). Indeed, the reduction in σR expression in endothelium-denuded aorta was relatively small compared with aorta with endothelium (67±8.7% of sham vs. 45±10% of sham, respectively). The reduction in Akt phosphorylation at Ser\(^{473}\) was also less significant in endothelium-denuded aorta (80±15% of sham) than aorta with endothelium (60±6.5% of sham), as was Akt phosphorylation at Thr\(^{308}\) (73±18% of sham vs. 43±10.3% of sham in aorta with

**Effect of Chronic SA4503 Treatment on Akt Phosphorylation in Both the Vascular Endothelium and Smooth Muscle**

To define whether σR stimulation in aortic ECs improves vasodilation, we investigated σR, Akt, and eNOS expression in the aorta with and without endothelium. To do this, we prepared endothelium-denuded strips from OVX-PO rats and subjected them to Western blotting. After removal of the endothelium, we observed a significant reduction in σR (by ≈40%), Akt (by ≈40%) and eNOS (by ≈95%) expressions in the aorta (Figures 7A,B). Reduced Akt phosphorylation was also detected in endothelium-denuded aorta (Figures 7A,B). We also examined potential changes in σR expression and Akt phosphorylation in endothelium-denuded aorta isolated from variously treated rats (Figure 7C). The reductions in both σR expression and Akt phosphorylation in endothelium-denuded aorta following OVX-PO were not as significant as those observed in naive aorta (Figures 6A, 7C (n=3)). Indeed, the reduction in σR expression in endothelium-denuded aorta was relatively small compared with aorta with endothelium (67±8.7% of sham vs. 45±10% of sham, respectively). The reduction in Akt phosphorylation at Ser\(^{473}\) was also less significant in endothelium-denuded aorta (80±15% of sham) than aorta with endothelium (60±6.5% of sham), as was Akt phosphorylation at Thr\(^{308}\) (73±18% of sham vs. 43±10.3% of sham in aorta with

**Figure 7.** Effects of chronic treatment with SA4503 (SA: 1 mg/kg) on endothelial-denuded aorta. (A) Western blot analysis of sigma-1 receptor (σ-R), phospho-Akt (Ser\(^{473}\)), phospho-Akt (Thr\(^{308}\)), total eNOS protein and β-tubulin expression in the intact aorta (Aorta) and in aorta denuded of endothelial cells (–EC). Each group consists of 4 rats. (B) Densitometric quantification of results shown in (A). Each column represents the mean±SEM. (C) Western blot analysis of σ-R, phospho-Akt (Ser\(^{473}\)), phospho-Akt (Thr\(^{308}\)) and β-tubulin expression in endothelium-denuded aorta isolated from variously treated rats. Each group consists of 3 rats. *P<0.05 and **P<0.01 vs. control group. eNOS, endothelial nitric oxide synthase; NE-100 (specific σ-R antagonist); OVX, ovariectomy; PO, pressure overload.
endothelium) (Figure 7C). Taken together, the findings suggested impaired endothelial αR expression and Akt phosphorylation were more significant in the endothelium when compared between the endothelium and smooth muscle.

Discussion

Although acute exposure to the αR agonist (+)-3-hydroxyphenyl-N-(1propyl)piperidine ((+)-3-PPP) reportedly causes marked constriction in isolated cardiomyocytes, up to now there has been no evidence for αR expression in the vascular endothelium nor has it been described in vascular constriction and vasodilation. Our results are the first report showing chronic and acute effects of treatment with αR agonist in the vascular system. We also provide evidence that αR expression markedly decreases in both the smooth muscle and endothelium of the descending rat aorta following OVX-PO and that αR down-regulation in the endothelium is closely associated with impairment of ACh- and clonidine-induced vasodilation. Although αR is also downregulated in smooth muscle cells following OVX-PO, its reduction in smooth muscle seems to have less pathological relevance than the impaired vascular functions observed in OVX-PO rats. To support this idea, αR stimulation had no significant effects on PE- or ET-1-induced vascular constriction in aorta from not only normal female rats but also OVX-PO rats.

Because sodium nitroprusside (SNP)-induced vasodilation was unchanged between sham-operated and OVX-PO rats (data not shown), the impaired Akt and eNOS signaling seen following OVX-PO and that αR down-regulation in the endothelium is closely associated with impairment of ACh- and clonidine-induced vasodilation. Although αR is also downregulated in smooth muscle cells following OVX-PO, its reduction in smooth muscle seems to have less pathological relevance than the impaired vascular functions observed in OVX-PO rats. To support this idea, αR stimulation had no significant effects on PE- or ET-1-induced vascular constriction in aorta from not only normal female rats but also OVX-PO rats.

Although acute exposure to the αR agonist (+)-3-hydroxyphenyl-N-(1propyl)piperidine ((+)-3-PPP) reportedly causes marked constriction in isolated cardiomyocytes, up to now there has been no evidence for αR expression in the vascular endothelium nor has it been described in vascular constriction and vasodilation. Our results are the first report showing chronic and acute effects of treatment with αR agonist in the vascular system. We also provide evidence that αR expression markedly decreases in both the smooth muscle and endothelium of the descending rat aorta following OVX-PO and that αR down-regulation in the endothelium is closely associated with impairment of ACh- and clonidine-induced vasodilation. Although αR is also downregulated in smooth muscle cells following OVX-PO, its reduction in smooth muscle seems to have less pathological relevance than the impaired vascular functions observed in OVX-PO rats. To support this idea, αR stimulation had no significant effects on PE- or ET-1-induced vascular constriction in aorta from not only normal female rats but also OVX-PO rats.

Because sodium nitroprusside (SNP)-induced vasodilation was unchanged between sham-operated and OVX-PO rats (data not shown), the impaired Akt and eNOS signaling seen following OVX-PO and that αR down-regulation in the endothelium is closely associated with impairment of ACh- and clonidine-induced vasodilation. Although αR is also downregulated in smooth muscle cells following OVX-PO, its reduction in smooth muscle seems to have less pathological relevance than the impaired vascular functions observed in OVX-PO rats. To support this idea, αR stimulation had no significant effects on PE- or ET-1-induced vascular constriction in aorta from not only normal female rats but also OVX-PO rats.

In conclusion we provide, for the first time, evidence supporting a potential role of αR expression in the ECs of the aorta.
in attenuating PO-induced vascular endothelial dysfunction. In our model, SA4503 treatment protected against PO-induced vascular injury by upregulating σR expression and stimulating σR-mediated Akt-eNOS signaling in the aorta. These results contribute to our understanding of the mechanisms underlying σR-mediated protective effects in vascular ECs and are relevant to clinical strategies to prevent vascular disorders, including atherosclerosis.

Acknowledgments
This work was supported in part by grants-in-aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (Kakenhi 23390109 and 24659024 to K.F.) and by a research fellowship from the Japan Society for the Promotion of Science (Kakenhi 2444360 to H.T.).

Disclosures
None.

References
23. Kobayashi T, Taguchi K, Yashtiro T, Matsumoto T, Kamata K. Impairment of PI3-K/Akt pathway underlies attenuated endothelial func-
24. Bhuivani MS, Tagashira H, Fukunaga K. Sigma-1 receptor stimulation with fluvoxamine activates Akt-eNOS signaling in the thoracic aorta of ovariectomized rats with abdominal aortic banding. *Eur J Pharmaco-
12011; 650: 621 – 628.
27. Shioda N, Ishikawa K, Tagashira H, Ishizuka T, Yawo H, Fukunaga K. Expression of a truncated form of the endoplasmic reticulum chaperone protein, sigma-1 receptor, promotes mitochondrial energy deple-
29. Molin JC, Bendhack LM. Clonidine induces rat aorta relaxation by nitric oxide-dependent and -independent mechanisms. *Vasc Phar-