Activation of receptor for advanced glycation end products contributes to aortic remodeling and endothelial dysfunction in sinoaortic denervated rats

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Objective: The aim of present study was to test the hypothesis that activation of receptor for advanced glycation end products (RAGE) pathway contributes to aortic remodeling and endothelial dysfunction in sinoaortic denervated (SAD) rats.

Methods and results: Experiment 1: 8 weeks after sinoaortic denervation, aortas were removed for measurement of AGE/RAGE pathway. Sinoaortic denervation in rats resulted in enhanced activity of aldose reductase, reduced activity of glyoxalase 1, accumulation of methylglyoxal and AGE, and upregulated expression of RAGE in aortas.

Experiment 2: 5 weeks after sinoaortic denervation, the rats received intraperitoneal injections of 500 μg soluble RAGE (sRAGE) daily for 3 weeks. Treatment of SAD rats with sRAGE attenuated aortic remodeling, marked by reduction in AW/length, wall thickness, proliferation of SMC, and collagen deposition, and improvement of endothelial function. Treatment of SAD rats with sRAGE abated aortic oxidative stress, marked by reduction in formation of malondialdehyde, reactive oxygen species, superoxide, peroxynitrite and 3-nitrotyrosine, and enhancement of ratio of GSH/GSSG. Treatment of SAD rats with sRAGE attenuated aortic mitochondrial dysfunction. Treatment of SAD rats with sRAGE suppressed aortic NFκB nuclear translocation and inflammation. Treatment of SAD rats with sRAGE restored aortic NO formation through upregulating eNOS and dimethylarginine dimethylaminohydrolase-2 and downregulating protein arginine methyltransferase-1.

Conclusion: Activated RAGE contributed to aortic remodeling and endothelial dysfunction in SAD rats, possibly via induction of oxidative stress and inflammation, impairment of mitochondrial function, and reduction in NO bioavailability.

1. Introduction

Arterial baroreflex (ABR) dysfunction predicts mortality and occurs with increasing age and is involved in many cardiovascular diseases including diabetes, hypertension, and hypercholesterolemia [1–3]. Sinoaortic denervation is a classic model of ABR dysfunction, which has been recognized as an independent determinant of end organ damages including cardiac hypertrophy, arteriosclerosis and renal lesion [4]. Lacolley et al. first reported that sinoaortic denervation induced aortic distensibility and structure changes [5]. Morphometric analysis revealed that aortic wall of SAD rats exhibited proliferation of muscle smooth cells (SMC), deposition of collagen, and degradation and apoptosis of endothelial cells [6,7]. Furthermore, Cai et al. demonstrated that ABR dysfunction induced by sinoaortic denervation promoted high-fat-diet induced atherosclerosis in rats [3]. In addition, improvement of ABR dysfunction attenuated high-fat-diet induced atherosclerosis in animals [8]. However, the underlying molecular mechanism by which ABR dysfunction induced vascular injuries was still little understood.

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AGE as well as its receptor (RAGE) has been implicated in several vascular diseases including atherosclerosis [9]. AGE is known to form and accumulate in the vessel wall, where they may act with RAGE, and subsequently elicit oxidative stress and induce proinflammatory or procoagulant cellular responses [10], finally leading to abnormalities of vessel wall including aortic remodeling and endothelial dysfunction.

In current study, we found AGE/RAGE pathway was activated in the aortas of SAD rats. The present study was therefore designed to testify the hypothesis that activation of RAGE contributes to aortic remodeling and endothelial dysfunction in SAD rats.

2. Methods

2.1. Animals and protocol

Male Sprague–Dawley (SD) rats (8 weeks old, 200–250 g) were purchased from Vital-River Animal Ltd. (Beijing, China) and housed in a room under controlled temperature (23–25 °C), humidity (40–60%) and lighting (12-h light/dark cycle) with food and water provided ad libitum. All the animals used in this work received humane care in compliance with institutional animal care guidelines, and were approved by the Local Institutional Committee. All the surgical and experimental procedures were in accordance with institutional animal care guidelines. All other reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA) unless otherwise noted.

Experiment 1: rats were randomly divided into 2 groups of 12 animals each: (1) Sham-operated group (Sham); (2) sinoaortic-denervated group (SAD). 8 weeks after sinoaortic denervation, aortas were removed for measurement of activities of aldose reductase (AR) and glyoxalase 1 (Glo-1), contents of methylglyoxal (MG) and advanced glycation end products (AGE), and protein expression of RAGE.

Experiment 2: rats were randomly divided into 4 groups of 48 animals each: (1) Sham; (2) Sham treated with sRAGE; (3) SAD; (4) SAD treated with sRAGE. 5 weeks after sinoaortic denervation, the rats received intraperitoneal injections of 500 μg sRAGE daily for 3 weeks. Albumin was used as a control at equimolar concentrations. Twenty-four hours after the final injection of sRAGE, measurements of hemodynamic parameters and aortic parameters were performed.

2.2. Animal model

Sinoaortic denervation (SAD) was performed according to the previously described method [3]. (See Supplementary data).

2.3. Measurement of hemodynamic parameters

Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart period (HP) were recorded continuously as previously described [8]. The mean values of these parameters during 24 h were calculated and served as SBP, DBP and HP for study. The mean standard deviation over the mean was calculated and defined as the quantitative parameter of blood pressure variability (BPV) and heart period variability (HPV). Under above-mentioned BP recording conditions, baroreflex sensitivity (BRS) was measured in conscious rat as previously described [8]. The mean of the two measurements with a proper dose served as the final result.

2.4. Glo-1 assay

Glo-1 activity was assayed by spectrophotometry according to the method of McLellan and Thornalley, monitoring the increase in absorbance at 240 nm due to the formation of S-α-lactoylglutathione for 10 min at 25 °C.

2.5. AGE assay

Aortas were homogenized in sterile phosphate-buffered saline containing protease inhibitors and centrifuged at 12,000 × g for 10 min at 4 °C. The level of AGE was quantified using specific ELISA kit for rats according to the manufacturers’ instructions (ELAab Life Science Inc., Wuhan, China). The final result was normalized to protein concentration.

2.6. Methylglyoxal (MG) assay

MG, a precursor in the formation of AGE, was measured in the neutralized perchloric acid extracts of aortas by HPLC methods according to previously published procedures [11].

2.7. Aldose reductase (AR) assay

AR activities were measured in aortic homogenates using spectrophotometric techniques as described previously [11].

2.8. Histomorphometric analysis and immunohistochemistry (IHC) staining

After hemodynamic monitoring, the animals were sacrificed by rapid decapitation. The aortas were immediately excised and rinsed in cold physiological saline. The aortas were cleaned of adhering fat and connective tissue. Just below the branch of the left subclavicular artery a 22-mm long segment of the thoracic aorta was harvested, blotted, and weighed. The aortic weight to the length of aorta (AW/length) was calculated.

The sections were stained with hematoxylin and eosin. Wall thickness (WT) was measured as the distance between endothelium and adventitia. The image was captured and displayed on a computer monitor using the image analysis software (LEICA QUIPS, LEICA Imaging Systems, England).

Masson staining was performed for tissue slices in each group. The blue color represents collagen. Images were quantified with a computerized image analysis system. Collagen deposition was expressed as a percentage of positively stained area to medial area.

For immunohistochemistry, sections were rehydrated, and incubated with proliferating cell nuclear antigen (PCNA, the proliferation index of smooth muscle cell of arteries) antibody (Santa Cruz) overnight at 4 °C followed by incubation with HRP-conjugated secondary antibody. The sections were counterstained with hematoxylin. The number of PCNA positive cell was calculated in each view.

For each vascular cross-section, four views were measured and for each rat, four cross-sections of each vessel were measured for calculating mean values. The operator was blinded to the experimental group during the analysis.

2.9. Quantitative real-time PCR analysis (qRT-PCR)

Total aortic RNA was isolated according to the manufacturer’s protocol. RT-PCR analysis was performed with a QuantiTect™ SYBR® Green PCR (Tiangen, Shanghai, China) according to the manufacturer’s instructions. The RT-PCR data was based on SYBR green amplification. The sequences of primers are listed in Table 15 (supplementary data). The highly specific measurement of mRNA was carried out for IL-6, intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), IL-1β, TNFα, macrophage chemoattractant protein 1 (MCP-1) and β-actin using the LightCycler system (Bio-Rad, Carlsbad, USA). Each sample was run and analyzed in duplicate. Target mRNA levels were adjusted as the values relative to β-actin, which was used as the endogenous
control to ensure equal starting amounts of cDNA. The fold-change relative to control (Sham-operated group) values was obtained and used to express the experimental change in gene expression.

2.10. Western blotting

Nuclear protein was separated by using a Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime Institute of Biotechnology, Shanghai, China). Nuclear purity as determined by Western blotting for the cytosolic marker protein-IkBα (Fig. 5S, supplementary data).

The protein concentration was determined with bovine serum albumin as a standard by a Bradford assay. Equal amount of protein preparations were run on SDS-polyacrylamide gels, electrotransferred to polyvinylidene difluoride membranes, and blotted with a primary antibody against NFκB p65, Lamin B1, RAGE, DDAH-2, PRMT-1, eNOS (Santa Cruz Biotechnology, Inc., CA) overnight at 4°C using slow rocking. Then, they were blotted with HRP-conjugated secondary antibody and HRP-conjugated monoclonal antibody against β-actin. Immunoreactive bands were detected by a chemiluminescent reaction (ECL kit, Amersham Pharmacia).

2.11. Aortic relaxation in response to acetylcholine (ACh) and sodium nitroprusside

Thoracic aortas isolated from 4 groups were cut into rings 2 mm in length and placed in Krebs buffer. Experiment was performed on rings beginning at their optimum resting tone, previously determined to be 3 g for rat aorta. This resting tone was reached by stretching rings in 500 mg increments separated by 10-min intervals. Data were collected using a MacLab system and analyzed using Dose Response Software (AD Instruments, Colorado Springs, CO, USA). Vessel rings were preconstricted with phenylephrine (1 μmol/l), and their vasorelaxant dose responses to acetylcholine (1 nmol/l–10 nmol/l) and sodium nitroprusside (1 mmol/l–10 μmol/l) were recorded.

Thoracic aortas isolated from sham-operated and SAD groups were cut into rings 2 mm in length and placed in Krebs buffer. After preconstriction with phenylephrine (1 μmol/l), vasorelaxant dose responses to ACh (1 nmol/l–10 μmol/l) with and without coadministration of the superoxide dismutase (SOD) mimic, 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl (Tempol, 1 mmol/l, 30 min incubation), were determined.

2.12. Measurement of malondialdehyde (MDA)

MDA concentration is a presumptive marker of oxidant-mediated lipid peroxidation. Aortic homogenates were used for the determination of MDA using a kit (Cayman, Ann Arbor, USA).

2.13. Aortic total ROS, superoxide (O2−), and peroxynitrite (OONO−) production

Aortic total ROS, O2−, and OONO− production were detected as the method described by Elks [13]. (See Supplementary data).

2.14. Measurement of mitochondrial function

Mitochondria were isolated by differential centrifugation of thoracic aortic homogenates. Mitochondrial protein concentration was determined using a DC Protein Assay Kit (Bio-Rad, Hercules, CA, USA). Rates of ATP formation were quantified using a commercially available kit (BioVision, Mountain View, CA, USA). ROS produced by mitochondria was measured using lucigenin-enhanced chemiluminescence in modified Hepes buffer. To

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**Fig. 1.** Aortic AGE–RAGE pathway in SAD rats. 8 weeks after sinoaortic denervation, aortas were removed for measurement of activities of AR (A) and Glo-1 (B), contents of MG (C) and AGE (D), and protein expression of RAGE (E). AR, aldose reductase; Glo-1, glyoxalase 1; MG, methylglyoxal; AGE, advanced glycation end products; RAGE, receptor for advanced glycation end products; SAD, sinoaortic denervated; values are means ± SD. n = 12 in each group; * P < 0.05 versus sham-operated rats.
minimize $O_2^-$ production by redox cycling, 5 $\mu$mol/l lucigenin was employed. The results were corrected for protein content. Measurement of mitochondrial swelling was done by the method of Mariappan [14]. The absorbance was measured at 540 nm.

2.15. Measurement of GSH/GSSG ratios

GSH/GSSG ratios were measured in aortic extracts using a commercially available Bioxytech GSH/GSSG-412 assay kit (Oxis Research, Portland, OR), which utilized the Tietze recycling method.

2.16. Enzyme linked immunosorbent assay (ELISA)

Aortas were homogenized in sterile phosphate-buffered saline containing protease inhibitors and centrifuged at 12,000 × g for 10 min at 4 °C to remove the insoluble pellet. The levels of inflammatory mediators and 3-nitrotyrosine were quantified using specific ELISA kits for rats according to the manufacturers’ instructions (TNF-α, IL-1β, IL-6, and ICAM-1 from R&D Systems, Minneapolis, MN, USA; VCAM-1 from Uscn Life Science Inc., Wuhan, China; MCP-1 from Invitrogen, Camarillo, CA, USA; 3-nitrotyrosine from Northwest Life Science Specialties, Vancouver, WA, USA). The final result was normalized to protein concentration.

2.17. Measurement of NOx

Aortas were homogenized in sterile phosphate-buffered saline containing protease inhibitors and centrifuged at 12,000 × g for 10 min at 4 °C. The levels of NOx, the stable end products of NO, in aortas were measured using a Total Nitrite/Nitrate Assay kit (Biyuntian, Nanjing, China).

2.18. Measurement of NO production

For NO production, aortas were dissected and incubated for 30 min in Krebs–Hepes buffer containing: BSA (20.5 g/l), CaCl$_2$

![Graph](image.png)

*Fig. 2. Effect of treatment with sRAGE on aortic remodeling and endothelial function in SAD rats. Graphs showed AW-to-length ratio (A), WT (B), HE staining, PCNA staining and Masson staining (C), and concentration–response curves to ACh (D) and sodium nitroprusside (E) in isolated thoracic aortas from rats. AW, aortic weight; WT, wall thickness; PCNA, proliferating cell nuclear antigen; ACh, acetylcholine; sRAGE, soluble receptor for advanced glycation end products; SAD, sinoaortic denervated; values are means ± SD. n = 12 in each group; *P < 0.05 versus sham-operated rats; #P < 0.05 versus SAD rats.
(3 mM) and l-Arginine (0.8 mM). NaDETC (1.5 mM) and FeSO$_4$·7H$_2$O (1.5 mM) were separately dissolved under argon gas bubbling in 10 ml volumes of ice-cold Krebs–Hepes buffer. These were rapidly mixed to obtain Fe(DETC)$_2$ solution (0.4 mM), which was added to the aortas and incubated for 45 min at 37 °C. Aortas were immediately frozen in plastic tubes using liquid N$_2$. NO measurement was performed on a table-top x-band spectrometer Miniscope (Magnettech, MS200, Berlin, Germany). The final result was normalized to dry weight of sample.

2.19. Determination of asymmetric dimethylarginine (ADMA)

For the determination of aortic ADMA, we adopted the high-performance liquid chromatography (HPLC)-mass spectrometry method. Briefly, 100 µl of aortic homogenate was mixed with 350 µl of borate buffer (pH 9.0). The sample was placed on an unconditioned Waters Oasis MCX column and then washed with 1 ml of borate buffer, three times with 1 ml of H$_2$O, and then with 1 ml of methanol. The sample was eluted with 1 ml of NH$_4$OH–H$_2$O–methanol (10:40:50), dried under nitrogen gas, and then reconstituted with 50 µl of methanol. A flow rate of 1.2 ml/min was used, and intensity was measured using a series 200 fluorescent detector with excitation at 250 nm and emission at 395 nm (PerkinElmer Life and Analytical Sciences, Shelton, CT).

2.20. Statistical analysis

All the data are presented as mean ± standard deviations. In experiment 1, comparison between two groups was analyzed using paired Student’s t-test. In experiment 2, comparison among groups was analyzed using a two-way analysis of variance followed by Bonferroni t-test. P < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS 11.0.0 software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Aortic AGE–RAGE pathway in SAD rats

When compared to sham-operated rats, enhanced activity of AR (Fig. 1A), reduced activity of Glo-1 (Fig. 1B), increased content of MG (Fig. 1C) and AGE (Fig. 1D), and upregulated expression of RAGE (Fig. 1E) were found in aortas of SAD rats, indicating that AGE–RAGE pathway was activated in aortas of SAD rats.

3.2. Effect of treatment with sRAGE on hemodynamic parameters in SAD rats

As shown in Table 2S, when compared to sham-operated rats, increased SBPV and DBPV, and reduced BRS, similar SBP, DBP, HP, and HPV were found in SAD rats. Treatment with sRAGE in SAD rats or sham-operated rats had no significant effect on these hemodynamic parameters.

3.3. Effect of treatment with sRAGE on aortic remodeling and endothelial function in SAD rats

When compared to sham-operated rats, increased AW/length (Fig. 2A), WT (Fig. 2B), proliferation of SMC (evidenced by increased expression of PCNA, Fig. 2C), and collagen deposition (Fig. 2C) was found in aortas of SAD rats. Treatment with sRAGE in SAD rats

![Graphs showing MDA content (A), formation of ROS (B), O$_2^-$ (C), and ONOO$^-$ (D), 3-nitrotyrosine (E), GSH/GSSG ratio (F), mitochondrial ROS formation (G), mitochondrial ATP formation (H), and optical densities for mitochondrial swelling assay (I) in isolated thoracic aortas from rats. MDA, malondialdehyde; ROS, reactive oxygen species; O$_2^-$, superoxide; ONOO$^-$, peroxynitrite; GSH, glutathione; GSSG, glutathione disulfide; ATP, adenosine triphosphate; sRAGE, soluble receptor for advanced glycation end products; SAD, sinoaortic denervated; values are means ± SD. n = 12 in each group; *P < 0.05 versus sham-operated rats; #P < 0.05 versus SAD rats.](https://example.com/fig3)
reduced AW/length, WT, proliferation of SMC, and collagen deposition in aortas, indicating that treatment with sRAGE inhibited aortic remodeling in SAD rats.

Endothelium-mediated vascular relaxation of aorta in response to ACh (Fig. 2D) was markedly impaired in SAD rats when compared to sham-operated rats. Treatment of SAD rats with sRAGE significantly improved endothelium-mediated vascular relaxation of aorta in response to ACh, indicating that sRAGE improved endothelial function in SAD rats. The endothelium-independent relaxations to sodium nitroprusside were similar among groups (Fig. 2E).

In addition, it was found that incubation with Tempol, a superoxide dismutase (SOD) mimic, restored endothelium-mediated vascular relaxation in response to ACh in denervated rat aortas (Fig. 2S, supplementary data).

### 3.4. Effect of treatment with sRAGE on aortic oxidative stress in SAD rats

When compared to sham-operated rats, increased production of MDA (Fig. 3A) and formation of ROS (Fig. 3B), O$_2^-$ (Fig. 3C), OONO$^-$ (Fig. 3D) and 3-nitrotyrosine (Fig. 3E) and reduced ratio of GSH/
GSSG (Fig. 3F) was found in aortas of SAD rats. Treatment with sRAGE in SAD rats reduced MDA content, suppressed formation of ROS, $O_2^-$, and nitric oxide, and improved ratio of GSH/GSSG in aortas, indicating that treatment with sRAGE abated oxidative stress in aortas of SAD rats. In addition, staining with oxidative fluorescent dye DHE also revealed that $O_2^-$ production in aortas of SAD rats was increased, which was suppressed by treatment with sRAGE (Fig. 3S, supplementary data).

3.5. Effect of treatment with sRAGE on mitochondrial function in SAD rats

When compared to sham-operated rats, enhanced formation of mitochondrial ROS (Fig. 3G), reduced ATP formation (Fig. 3H), and increased mitochondrial swelling (Fig. 3I) were found in aortas of SAD rats. Treatment with sRAGE in SAD rats suppressed mitochondrial ROS formation and increased mitochondrial ATP formation, and reduced mitochondrial swelling in aortas, indicating that sRAGE preserved mitochondrial function in aortas of SAD rats.

3.6. Effect of treatment with sRAGE on inflammation in SAD rats

When compared to sham-operated rats, nuclear NFκB p65 expression was higher (Fig. 4A) in aortas of SAD rats. Treatment with sRAGE in SAD rats decreased nuclear NFκB p65 expression in aortas, indicating that sRAGE suppressed translocation of NFκB into nucleus in aortas of SAD rats.

When compared to sham-operated rats, mRNA levels and protein content of ICAM-1 (Fig. 4B, H), IL-6 (Fig. 4C, I), MCP-1 (Fig. 4D, J), VCAM-1 (Fig. 4E, K), TNFα (Fig. 4F, L), and IL-1β (Fig. 4G, M) were increased in aortas of SAD rats. Treatment with sRAGE in SAD rats reduced mRNA levels and protein expression of ICAM-1, IL-6, MCP-1, VCAM-1, and TNFα, indicating that sRAGE suppressed inflammation in aortas of SAD rats.

3.7. Effect of treatment with sRAGE on NO formation in SAD rats

When compared to sham-operated rats, aortic NO formation (Fig. 5A, B) was reduced and ADMA formation was increased (Fig. 5C). In addition, eNOS and DDAH-2 were downregulated and PRMT-1 was upregulated in aortas of SAD rats (Fig. 5D).

Treatment with sRAGE in SAD rats enhanced protein expression of eNOS and DDAH-2 and reduced protein expression of PRMT-1, and reduced ADMA content and increased NO formation in aortas.

4. Discussion

When the reflex arc is interrupted by sinoaortic denervation, arterial baroreflex (ABR) function is markedly impaired [15], marked by increased BPV and reduced BRS. Whether ABR was involved in the regulation on aortic AGE/RAGE pathway remained unknown. Meerwaldt et al. first reported the association between BRS and AGE in diabetic patients [16], indicating that AGE/RAGE pathway might be involved in the regulation of vascular pathology when ABR function was impaired. In this study, it was first demonstrated that when ABR function was impaired by sinoaortic denervation in rats, aortic AGE/RAGE pathway was activated.

AR is the first enzyme of the polyol pathway. A critical consequence of flux via the AR pathway is the generation of a precursor of AGE–MG [17], which could be detoxified by Glo-1 [18]. In this study, when ABR dysfunction was induced by sinoaortic denervation, aortas exhibited significant activation of the whole AR–MG–AGE–RAGE axis.

To investigate the role of AGE/RAGE pathway in the progression of aortic remodeling and endothelial dysfunction in SAD rats, sRAGE, a soluble portion of the extracellular domain of RAGE, was used as an inhibitor of interaction of AGEs and RAGE. It was found that treatment of SAD rats with sRAGE attenuated aortic remodeling and endothelial dysfunction, but it had no significant effect on BPV and BRS, which indicated that the beneficial effect of sRAGE on vasculature was independent from arterial baroreflex function.

The focus of the current investigation was to probe potential links between the AGE/RAGE pathway and sinoaortic denervation-related vascular dysfunction. Increased oxidative stress and reduced bioavailability of NO contributed to aortic dysfunction in SAD rats [6]. The bioavailability of NO, the major effector of endothelium-dependent relaxation and regulator of SMC...
proliferation [19], can be scavenged by oxidative stress. AGE/RAGE activation might generate oxidative stress by several means: (1) AGE/RAGE activation depleted NADPH [20], which was a necessary cofactor for glutathione reductase, thereby leading to diminished cellular antioxidant capacity; in this study, we found treatment of SAD rats with sRAGE restored reduced ratio of GSH-to-GSSG, which indicated the important effect of AGE/RAGE activation on antioxidant capacity. (2) AGE/RAGE activation impaired mitochondrial function and enhanced mitochondrial ROS formation [20,22]. In this study, aortic superoxide production, measured directly with electron paramagnetic resonance, was markedly elevated in aortas of SAD rats. Peroxynitrite (ONOO−) production was also found increased in aortas of SAD rats. ONOO− formation is known to be catalyzed by nitric oxide (NO) and superoxide (O2−) through the mechanism of the formation of peroxynitrite. This study indicates that activation of the AGE/RAGE pathway contributed to the oxidative stress in aortas of SAD rats.

The reduction of bioavailability of NO in aortas of SAD rats was also due to reduced activity of eNOS, the major enzyme responsible for NO formation in endothelium. In addition, we found that aortic content of ADMA, an endogenous inhibitor of eNOS, was enhanced in aortas of SAD rats. ADMA was mainly generated by PRMT-1, and content of ADMA, an endogenous inhibitor of eNOS, was enhanced for NO formation in endothelium. In addition, we found that aortic content of ADMA, an endogenous inhibitor of eNOS, was enhanced for NO formation in endothelium. In this study, we found that aortic content of ADMA, an endogenous inhibitor of eNOS, was enhanced for NO formation in endothelium. In this study, we found that aortic content of ADMA, an endogenous inhibitor of eNOS, was enhanced for NO formation in endothelium. In this study, we found that aortic content of ADMA, an endogenous inhibitor of eNOS, was enhanced for NO formation in endothelium. In this study, we found that aortic content of ADMA, an endogenous inhibitor of eNOS, was enhanced for NO formation in endothelium. In this study, we found that aortic content of ADMA, an endogenous inhibitor of eNOS, was enhanced in aortas of SAD rats. PRMT-1 expression, and increased ADMA formation. In this work, treatment with sRAGE ameliorated aortic activation of NF-κB, indicating that activation of AGE/RAGE pathway contributed to the oxidative stress in aortas of SAD rats.

Apart from oxidative stress and reduced bioavailability of NO, inflammation played an important role of progression of vascular dysfunction in SAD rats [27]. AGE/RAGE pathway mediated prolonged vascular inflammation, and enhanced the expression of (VCAM)-1 and proinflammatory cytokines, leading to an exacerbation of the inflammatory state [28,29]. In this work, treatment with sRAGE ameliorated aortic activation of NF-κB and reduced the expression of VCAM-1, ICAM-1, TNFα, IL-6, IL-1β and MCP-1, indicating that activation of AGE/RAGE pathway contributed to the inflammation in aortas of SAD rats.

In conclusion, activation of AGE/RAGE contributed to aortic remodeling and endothelial dysfunction in SAD rats, possibly through induction of oxidative stress and inflammation, impairment of mitochondrial function, and reduction in NO bioavailability.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2013.04.033.

References


