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ORIGINAL ARTICLE

A pyridoindole antioxidant SMe1EC2 regulates contractility, relaxation ability, cation channel activity, and protein-carbonyl modifications in the aorta of young and old rats with or without diabetes mellitus

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Abstract We studied the effects of treatment with SMe1EC, a hexahydropyridoindole antioxidant, on vascular reactivity, endothelial function, and oxidonitrosative stress level of thoracic aorta in young and old rats with or without diabetes mellitus. The rats were grouped as young control (YC 3 months old), old control (OC 15 months old), young diabetic (YD), old diabetic (OD), young control treated (YCT), old control treated (OCT), young diabetic treated (YDT), and old diabetic treated (ODT). Diabetes was induced by streptozotocin injection and subsequently SMe1EC2 (10 mg/kg/day, p.o.) was administered to YCT, OCT, YDT, and ODT rats for 5 months. In young and old rats, diabetes resulted in hypertension, weight loss, hyperglycemia, and hypertriglyceridemia, which were partially prevented by SMe1EC2. SMe1EC2 also inhibited the diabetes-induced increase in aorta levels of AGEs (advanced glycosylation end-protein adducts), 4-HNE (4-hydroxy-nonenal-histidine), 3-NT (3-nitrotyrosine), and RAGEs (receptors for AGEs). The contractions of the aorta rings to phenylephrine (Phe) and KCL did not significantly change, but acetylcholine (ACh) and salbutamol relaxations were reduced in OC compared to YC rats. Diabetes induction increased Phe contractions in YC and OC rats, KCL contractions in YC rats, and did not cause further inhibition in already inhibited ACh and salbutamol relaxations in OC rats. We have achieved the lowest levels of ACh relaxation in YD rats compared to other groups. SMe1EC2 did not change the response of aorta to ACh, salbutamol and Phe in YC rats, and ameliorated ACh relaxations in OC and YD but not in OD rats. In YDT and ODT rats, increased Phe and KCL contractions, high blood pressure, and impaired salbutamol relaxations were amended by SMe1EC2. Phe contractions observed in YD and OD rats as well as KCl contractions observed in OC rats were the lowest levels when the rats were treated with SMe1EC2. When the bath solution was shifted to cyclopiazonic acid (CYP) or CYP plus Ca^{2+}-free medium, the contraction induced by a single dose of Phe (3×10^{-6} M) was more inhibited in YD and OD than in YC but not in OC rats. In SMe1EC2-treated rats, neither the presence of CFM nor CFM plus CYP exhibited a significant change in response of aorta to a single dose of Phe. These findings suggest that α1-adrenergic receptor signaling is activated in both age groups of diabetic rats, diabetes activates K^+-depolarization and calcium mobilization via Ca_V especially in the aorta of young rats, and sensitizes the aorta of old rats to the

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regulating effect of SMe1EC2. ACh relaxations were inhibited in YC rats, increased in OC rats and unchanged in YD and OD rats when aortic rings pretreated with TEA, an inhibitor of calcium-activated K^+ channels (K_{Ca}), or 4-aminopyridine (4-AP), an inhibitor of voltage-sensitive K^+ channels (K_V). ACh relaxations were inhibited in YCT, OCT, and YDT rats in the presence of 4-AP or TEA. In ODT rats, 4-AP did not change ACh relaxation but TEA inhibited. These findings suggest that the contribution of K_v and K_{Ca} to ACh relaxation is likely upregulated by SMe1EC2 when the relaxations were inhibited by aging or diabetes. We conclude that SMe1EC2 might be a promising agent for aging and diabetes related vascular disorders.

Keywords Aging · Diabetes · Antioxidant · Aorta · Pyridoindole · Rat · Protein carbonylation · Oxidonitrosative stress · Contractility · Endothelium

Introduction

The enhanced production and accumulation of advanced lipid peroxidation end products (ALEs) and advanced glycoxidation end products (AGEs) have been linked to increased risk for macrovascular and microvascular complications associated with diabetes mellitus (DM) (Karasu et al. 1997a; Karasu 2000; Koçak et al. 2000; Yülek et al. 2007; Ma et al. 2008; Karasu 2010; Ceylan-Isik et al. 2011; Baumann, 2012). Chemical modification of proteins, nucleic acids, and aminophospholipids by reactive carbonyl compounds (RCCs) also accumulate with aging, leading to cytotoxicity and pathological disorders (Negre-Salvayre et al. 2008; Karasu 2010; Lamoke et al. 2015). It has been suggested that the persistent and sustained generation of AGEs and ALEs is a causal factor for the induction and progression of age-related diseases and their complications via impairment of intracellular redox signaling (Karasu 2010; Ergin et al. 2013a, b).

AGEs and ALEs exert deleterious effects by acting directly to induce cross-linking of long-lived proteins to promote vascular stiffness, altering vascular structure and function and interacting with receptor for AGE (RAGE), to induce intracellular signaling leading to enhanced oxidonitrosative stress and generation of key proinflammatory and prosclerotic cytokines (Horváth et al. 2009; Fleming et al. 2011; Ray et al. 2012).

The fact is that the pathogenic consequences of elevated interaction of RCCs and its receptors (RAGE), including active atherosclerotic plaque formation, endothelial dysfunction, and hypertension, increase with aging and DM (Horváth et al. 2009; Fleming et al. 2011; Barlovic et al. 2011; Ray et al. 2012; Yamagishi et al. 2012; Gu et al. 2014; Lamoke et al. 2015).

In this respect, AGEs/ALEs-RAGE signaling pathway presents a promising target for novel therapies, and blocking the vicious cycle of AGE/ALEs-RAGE axis or prevention of excess formation of RCCs is relevant in order to modify the natural history of vascular disease in aging and diabetes (Karasu et al. 1997a; Koçak et al. 2000; Ceylan-Isik et al. 2011). The novel anti-AGEs/ALEs strategies preventing oxidative protein degradation involve the use of free radical scavengers, antioxidants, and cellular redox regulators that may have an essential role in controlling of vascular complications in aging and DM (Karasu 2010; Drummond et al. 2011). In connection therewith, since last decade, we have been evaluating the effects of new pyridoindole compounds on DM-induced metabolic and functional abnormalities and their mechanisms of action. For instance, we have found that a pyridoindole antioxidant stobadine is a beneficial efficacy in the prevention or restoration of cardiovascular complications observed in experimental diabetes through regulation of arterial blood pressure (Karasu 2010; Juranek et al. 2010; Ceylan-Isik et al. 2011). Stobadine manages vascular reactivity by maintaining endothelial ability to produce nitric oxide (NO) at an adequate level and inhibiting vascular smooth muscle Ca^{2+} entry (Ceylan-Isik et al. 2011).

As a newer congener of stobadine, SMe1EC2 (2-ethoxycarbonyl-8-methoxy-2,3,4,4a,5,9b-hexahydro-1H-pyrido[4,3-b] indolinium dichloride) has a remarkable antioxidant efficacy in protecting lipids, enzymes, and some tissue functions against the oxidative attacks (Juranek et al. 2010). In a previous experiment, SMe1EC2 has been shown to improve vascular endothelial function damaged by hyperglycemic PSS in vitro (Zúrová-Nedelcevová et al. 2006). It has been shown that SMe1EC has no effect on the basal tone of the aorta (Broskova et al. 2013), but increases NO-dependent vascular relaxation in short-term diabetic rats (Sotníková et al. 2011). Therefore, it is reasonable to test the effects of SMe1EC2 on the prevention of oxidative stress-sensitive vascular abnormalities in aging and diabetes animal models. In this study, we aimed to investigate the effects of SMe1EC2 on protein-based

nitrotyrosine, HNE-, AGE-adducts, and RAGE aorta levels, as well as endothelial function and reactivity of aorta in diabetic or non-diabetic young and old rats. In addition, it was also examined whether the changes in calcium and potassium channel activities contribute to the vascular effects of SMe1EC2.

Materials and methods

Animals and the treatment protocols

Male Wistar rats (Ankara University, Faculty of Pharmacy, Animal House, Ankara), weighing 250–280 g and 12 weeks of age were housed in an air-conditioned colony room at 22 ± 2 °C and supplied with standard pellet diet and tap water ad libitum. Procedures involving animals and their care were conducted in accordance with the NIH guidelines for the care and use of laboratory animals. The rats were divided into eight groups: (1) young control (YC), (2) young control treated with SMe1EC2 (YCT), (3) old control (OC), (4) old control treated with SMe1EC2 (OCT), (5) young diabetic (YD), (6) young diabetic treated with SMe1EC2 (YDT), (7) old diabetic (OD), and (8) old diabetic treated with SMe1EC2 (ODT). Diabetes mellitus (DM) was induced in some of the young (3 months old) and old (15 months old) rats by a twice intravenous injection with an interval of 2 days of 2 × 20 mg/kg, i.p. mg/kg Streptozotocin (STZ) in a 0.05 mol/l citrate buffer solution (Zúrová-Nedelcevová et al. 2006). Ten days after STZ injection, tail vein blood glucose samples were measured with (Accu-check go®, Roche Diagnostic) to ensure induction of DM. The animals that blood glucose level ≥ 250 mg/dl were accepted to be diabetic. Some of YC, OC, YD, and OD animals were treated once a day with 10 mg/kg SMe1EC2 (Juranek et al. 2010) for 5 months. The animals received vehicle (0.15 M saline) or SMe1EC2 (2-ethoxycarbonyl-8-methoxy-2,3,4,4a,5,9b-hexahydro-1H-pyrido[4,3-b] indolinium dichloride) orally via a gastric tube (gavage) in a maximum volume of 0.5 ml. Adequate measures were taken to minimize pain or discomfort. An initial 10-days period without treatment was introduced to avoid β-cell regeneration and alleviation of hyperglycemia, which is known to occur when antioxidants are administered together with STZ or shortly after induction of diabetes mellitus (Koçak et al. 2000). Before the sacrifice, the old animals were 20 months aged. Standard laboratory scale was used to measure the body weights.

Blood pressure measurement

Blood pressure (BP) was measured indirectly in a conscious and slightly restrained rat by the tail cuff method at the end of the study and 12 h after the last SMe1EC2 or vehicle administration (Koçak et al. 2000). For these measurements, the rats were conditioned to the restraint and the warming chamber for 10–20 min/day for at least 3 days before measurements. BP measurements were performed from 10:00 to 12:00 AM by the same investigator. After 5–10 min of stabilization in a warming chamber (35 °C), a typical run involved ten repetitions of the automated inflation–deflation cycle. The mean of the six readings within a 5–10 mmHg range was taken as the blood pressure.

Vascular function studies

Rats were sacrificed by cervical dislocation. Blood samples (3–5 ml) were collected in heparinized collecting tubes by the intracardiac route. Descending thoracic aorta was dissected and carefully cleaned to remove fat and connective tissues. Aorta rings (3 mm in length) were mounted onto tissue organ baths filled with physiological salt solution (PSS) containing (in mM): NaCl 118, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5, and glucose 11.2. The solution was maintained at pH 7.4 and gassed with 95% O_2 and 5% CO_2 at 37 °C. Rings from control and diabetic animals were equilibrated for 60 min under an optimal resting tension of 2.0 g (determined to be optimum in preliminary experiments). During this period, the PSS in the tissue bath was replaced every 20 min. After equilibration, each aortic ring was continuously stimulated with 10^{-6} mol/l phenylephrine (Phe EC_{50}) until reproducible contractile responses were obtained. Isometric tensions were recorded by a force transducer in a tissue bath system (PowerLab Data Interface Module) connected to a PC running Chart software (v4.2, ADI Instruments, Chalgrove, Oxon, UK). All experiments were performed on endothelium intact aortic rings.

Contractile responses

For studying the vasoconstrictor responsiveness of aorta, the cumulative dose of phenylephrine (Phe 10^{-9} to

10^{-5} M) or KCL (10–60 mM) was added to the organ bath and the developed responses were recorded. We also test the contractile response of aorta to a sub-maximal dose of Phe (3×10^{-6} M) in a Ca^{2+}-free medium (CFM) containing (in mM): NaCl 118, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25, EGTA 2, and glucose 11. A sub-maximal dose of Phe-induced (3×10^{-6} M) transient contraction in CFM was also evaluated in the presence of the endoplasmic reticulm Ca^{2+}-ATPase (SERCA) re-uptake inhibitor cyclopiazonic acid (10^{-6} M). In particular, after the Phe-induced vasoconstriction reached a plateau, the medium in organ baths was switched to CFM (depletion period). Two times fifteen minutes following the depletion period, CFM was added and Phe-induced transient contractions were evaluated approximately 1 min later. Cyclopiazonic acid (CYP) was added to the baths at the time of medium switch. To see if the treatment had affected Ca^{2+} channels, L-type Ca^{2+} agonist KCL-induced contractile responses (10–60 mM) in PSS were also examined.

Relaxation responses

For studying the relaxation response of aorta, the rings were first pre-contracted with a submaximal concentration of Phe (3×10^{-6} M). The cumulative concentrations of acetylcholine ACh (10^{-9} to 10^{-5} M), salbutamol (3×10^{-7} to 3×10^{-5} M), or sodium nitroprusside (SNP 10^{-11} to 10^{-6} M) were then added to the organ bath and the responses were recorded (Karasu 2000; Koçak and Karasu 2002). In another set of experiments, after repeated washing and stabilization of basal tone, the responses to ACh (10^{-9} to 10^{-5} M) in Phe-precontracted rings were compared to the responses obtained after the following treatments: (1) incubation (30 min) with tetraethylammonium (TEA 10^{-4} M), an inhibitor of calcium-activated K^+ channels (K_{Ca}), to explore the participation of these channel subtypes in the arterial response to ACh; and (2) incubation (30 min) with 4-aminopyridine (4-AP 10^{-4} M), an inhibitor of voltage-sensitive K^+ channels (K_V), to explore the participation of these channel subtypes in the arterial response to ACh (Ye et al. 2004).

Protein oxidation/nitrosative stress and RAGE analysis

The descending thoracic aorta dissected out in ice immediately after the rats were sacrificed and stored at − 80 °C for the analyses. The samples were homogenized in phosphate-buffered saline (PBS pH 7.4) and centrifuged (700×g, 5 min at 4 °C) to remove cellular debris. Supernatants were used to all biochemical assays described here. All the results were normalized by the protein content using bovine albumin as standard (Cumaoglu et al. 2010).

In this study, we used the Cell Biolabs, OxiSelect™ Advanced Glycation End Product ELISA Kit for the detection and quantitation of AGE-protein adducts (AGEs). The quantity of AGEs in protein samples is determined by comparing its absorbance with that of a known AGE-BSA standard curve. The levels of the receptor for advanced glycation end-products (RAGE), HNE-histidin adducts (4-HNE), and 3-nitrotyrosine (3-NT) levels in aorta homogenates were also measured by OxiSelect ELISA kits (Cell Biolabs, San Diego, CA) according to the kit's procedures.

Reagents

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA) except with SMe1EC2 (was obtained from the Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences) and were dissolved in distilled water with the exception of cyclopiazonic acid (dissolved in DMSO with a final DMSO concentration of $< 0.01\%$). Preliminary study showed that this concentration of DMSO had no significant effect on any of the experimental protocols tested.

Statistical analysis

Four aortic segments (endothelium intact) were collected per rat. For phenylephrine (Phe), which elicits contraction of aortic rings, results are expressed as the change in isometric tension induced by Phe, normalized by the dry weight of the vascular rings. For Acetylcholine (ACh) or salbutamol, which elicits relaxation of Phe-preconstricted aortic rings, the responses are expressed as percent reduction of tension in the preconstricted state. For each experimental series, data are expressed as the mean ± SEM with "*n*" being the number of rats used. The agonist maximum response (E_{max}) was calculated from concentration–response curve by non-linear regression analysis of the curve using computer-based fitting program and used for comparison (Prism 4, Graphpad, CA, USA). Comparisons

of dose–response curves were made for across all groups by two-way analysis of variance (ANOVA) followed by the Bonferroni post-test. Student's *t* test was used for the comparisons of the contractile response of aorta to Phe (3×10^{-6} M) before and after Ca^{2+}-free medium (CFM) or CFM plus cyclopiazonic acid (CYP). $p < 0.05$ was considered significant.

Results

Body weight, blood glucose, triglyceride, and blood pressure

The general characteristics of the rats have been shown in Table 1. OC rats were significantly heavy relative to YC group ($p < 0.01$). DM caused a reduction in body weights of YC as well as OC animals. SMe1EC2 treatment did not significantly change the weights of YC ($p > 0.05$) but markedly prevented weight loss in YD and OD animals ($0 < 0.05$).

In comparison with YC or YCT group, the difference between the mean final blood glucose levels of OC and OCT rats was not statistically significant ($p > 0.05$). DM led to a persistent hyperglycemia in both young and old rats ($p < 0.001$). However, SMe1EC2 treatment gave a rise to significant ameliorations in blood glucose levels of both groups of diabetic animals ($p < 0.001$).

Plasma triglyceride levels did not significantly change in SMe1EC2 treated control groups compared to untreated corresponding rats ($p > 0.05$). DM increased plasma triglyceride levels in both young and old rats ($p < 0.01$) that were significantly restored by SMe1EC2 treatment ($p < 0.05$).

Aging of the rats resulted in an increase in systolic blood pressure ($p < 0.05$), which was not significantly change by SMe1EC2 treatment. Systolic blood pressure was aggravated by DM in both young ($p < 0.01$) and old rats ($p < 0.001$) but was significantly prevented by SMe1EC2 treatment ($p < 0.05$).

Protein oxidation/nitrosative stress markers and RAGE levels

In OC rats, AGEs levels of aorta were found to be significantly increased and DM caused further augmentation in AGEs levels of old rats (Fig. 1a). DM also significantly aggravated AGEs levels in young rats (Fig. 1a). OD rats showed the highest value of AGEs levels when compared to the AGEs levels of YD or OC rats ($p < 0.05$). AGEs levels was unchanged by SMe1EC2 treatment in YC rats but significantly improved in OCT, YDT ($p < 0.01$), and ODT ($p < 0.001$) animals compared to their corresponding controls (Fig. 1a).

In OD rats, the increased level of AGEs associated with a significant decline in aorta RAGE level (Fig. 1b). In comparison with YC rats, DM led to augmentation in aorta RAGE level more significantly in YD than OD rats ($p < 0.001$). SMe1EC2 treatment did not produce a significant alteration in RAGE level of YC rats ($p > 0.05$) but protected the rats against DM-induced alterations in aorta RAGE level ($p < 0.01$) (Fig. 1b).

In the aorta of OC rats, 4-HNE (Fig. 1c) and 3-NT (Fig. 1d) levels were increased statistically less significant compared with the aorta of YC animals ($p < 0.05$); however 4-HNE and 3-NT levels severely exacerbated in the presence of DM and significantly prevented by SMe1EC2 treatment ($p < 0.001$) (Fig. 1c, d). The increase in aorta 3-NT levels was the most in YD rats compared to other groups of rats (Fig. 1d).

Constriction and relaxation responses

Cumulative addition of phenylephrine (Phe 10^{-9} to 10^{-5} M) or potassium chloride (KCl 10–60 mM) to the organ bath resulted in concentration-dependent contractions of aortic rings in all the group of animals (Fig. 2). Aging itself did not significantly affect the vasoconstrictive response to Phe (Fig. 2a). DM caused a significant increase in the vasoconstrictor effect of Phe in both young and old rats that was reflected by a significant increase in apparent E_{max} ($p < 0.01$). The vasoconstrictive response of aorta to Phe was not statistically different in YCT and OCT rats compared to YC or OC rats ($E_{max}p > 0.05$). SMe1EC2 treatment led to a significant downward shift in Phe-induced vasoconstrictions of YD and OD animals ($E_{max}p < 0.001$). An important feature of Phe-induced constriction was the lowest level in YDT and ODT rats compared to other groups of rats (Fig. 2a).

The responsiveness of aorta to KCL did not significantly change in OC rats compared to YC rats ($E_{max}p > 0.05$) (Fig. 2b). The treatment of rats with SMe1EC2 caused a significant inhibition in response of the aorta to KCL in old but not in young rats compared to their untreated controls. DM led to a significant increase in KCL-induced vasoconstriction only in young rats ($E_{max}p < 0.001$) but not in old rats when compared with non-diabetic corresponding control animals. SMe1EC2

Table 1 The final measurements of body weight, blood glucose, triglyceride, and systolic blood pressure in experimental rats

	Weight (g)	Glucose (mg/dl)	Triglyceride (mg/dl)	Systolic BP (mmHg)
Young control (YC)	$269 \pm 21^{\dagger\dagger fff}$	$105 \pm 8^{fff\#\#\#}$	$82 \pm 4^{ff\#}$	$123 \pm 2^{\dagger\dagger fff\#\#\#}$
Young control + SMe1EC2 (YCT)	$285 \pm 16^{\dagger ff}$	$110 \pm 9^{fff\#\#\#}$	85 ± 8^{ff}	$128 \pm 2^{\dagger ff\#\#\#}$
Old control (OC)	$383 \pm 37^{**fff\#}$	$117 \pm 34^{fff\#\#\#}$	$81 \pm 3^{ff\#}$	$140 \pm 4^{*f\#\#}$
Old control + SMe1EC2 (OCT)	$398 \pm 24^{***fff\#}$	$107 \pm 26^{fff\#\#\#}$	88 ± 7^{ff}	$141 \pm 3^{*f\#\#}$
Young diabetic (YD)	$224 \pm 19^{**\dagger\dagger\dagger\#\#}$	$382 \pm 53^{***\dagger\dagger\dagger}$	$126 \pm 21^{**\dagger\dagger\#\#}$	$159 \pm 5^{**\dagger}$
Young diabetic + SMe1EC2 (YDT)	$252 \pm 22^{*\dagger\dagger\#}$	$288 \pm 47^{***\dagger\dagger\dagger f}$	89 ± 10^{f}	$147 \pm 6^{*\#}$
Old diabetic (OD)	$304 \pm 30^{\dagger f}$	$329 \pm 51^{***\dagger\dagger\dagger}$	$113 \pm 8^{*\dagger}$	$169 \pm 4^{***\dagger\dagger}$
Old diabetic + SMe1EC2 (ODT)	$311 \pm 38^{\dagger f}$	$292 \pm 43^{***\dagger\dagger\dagger f}$	$93 \pm 12^{f\#}$	$134 \pm 5^{*\dagger f\#\#}$

Mean ± SEM, $n = 8$–10 rats per group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs young control; $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ vs old control; $^{f}p < 0.05$, $^{ff}p < 0.01$, $^{fff}p < 0.001$ vs young diabetic; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.01$ vs old diabetic

treatment of both groups of diabetic animals results in significantly downward shift in the vasoconstrictor response to KCL (10–60 mM) ($E_{max}p < 0.05$) (Fig. 2b). We observed the lowest contractile response to KCL in OCT animals compared to other group of animals ($E_{max}p < 0.001$), and the aorta of YD rats displayed the most contractions to KCL than other group of rats ($E_{max}p < 0.05$).

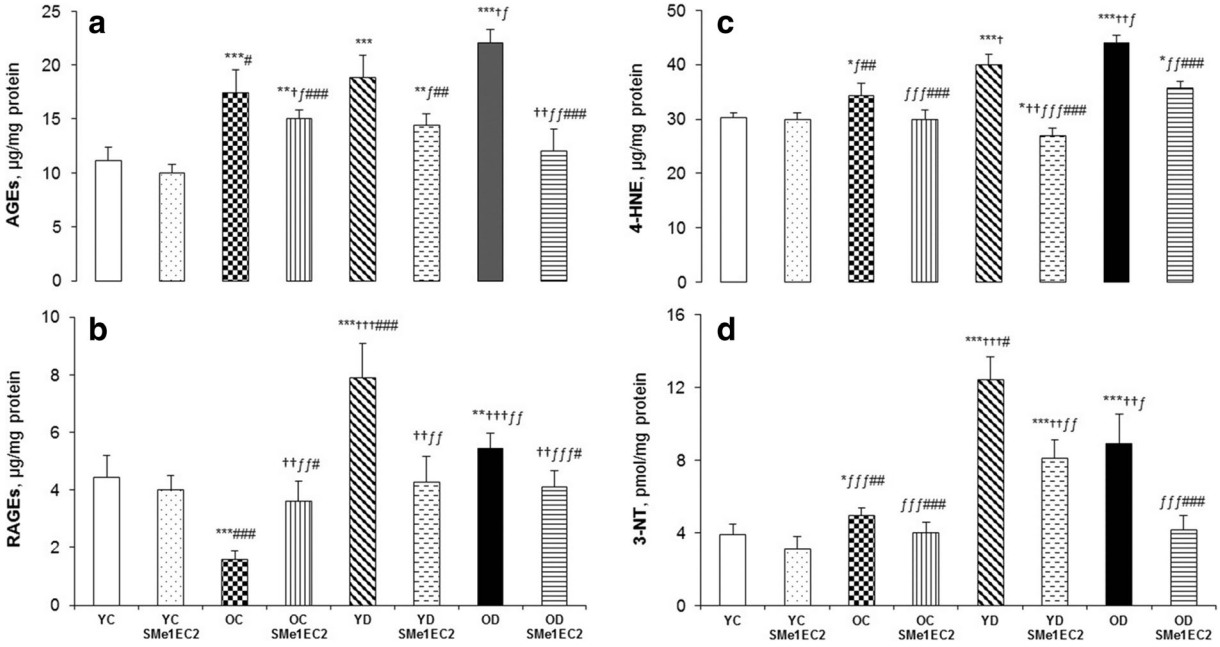

Fig. 1 The results of measurements of AGE-protein adduct levels (**a**), RAGE levels (**b**), 4-HNE-protein adduct levels (**c**), and 3-NT levels in aorta homogenates of rats grouped as young control (YC), young control treated with SMe1EC2 (YCT), old control (OC), old control treated with SMe1EC2 (OCT), young diabetic (YD), young diabetic treated with SMe1EC2 (YDT), old diabetic (OD), and old diabetic treated with SMe1EC2 (ODT). Mean ± SEM, $n = 8$–10 rats per group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs YC; $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ vs OC; $^{f}p < 0.05$, $^{ff}p < 0.01$, $^{fff}p < 0.001$ vs YD; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs OD

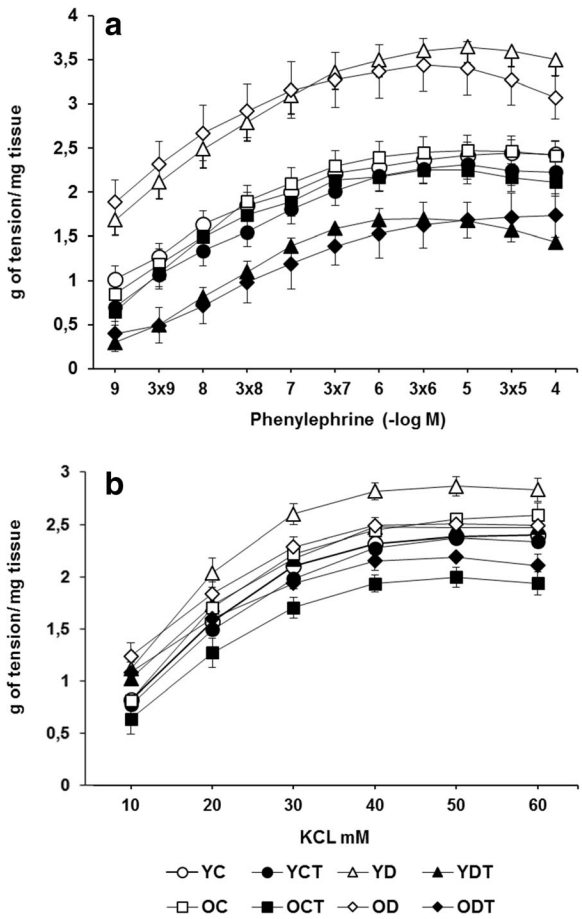

Fig. 2 Concentration-dependent vasoconstrictions to phenylephrine (Phe, 10^{-9}–10^{-4} M) (**a**) and KCL (10–60 mM) (**b**) in aorta rings isolated from rats grouped as young control (YC), young control treated with SMe1EC2 (YCT), old control (OC), old control treated with SMe1EC2 (OCT), young diabetic (YD), young diabetic treated with SMe1EC2 (YDT), old diabetic (OD), and old diabetic treated with SMe1EC2 (ODT). Mean ± SEM, n = 8–10 rats per group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs YC; $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ vs OC; $^{f}p < 0.05$, $^{ff}p < 0.01$, $^{fff}p < 0.001$ vs YD; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs OD

Cumulative addition of ACh (10^{-9} to 10^{-5} M) to the organ bath resulted in concentration-dependent decreases in the tension of aorta pre-contracted with Phe (Fig. 3a). The responsiveness of aorta to ACh significantly decreased by aging ($p < 0.01$), and DM did not produce a further attenuation in OD rats compared to OC rats ($p > 0.05$) (Fig. 3a). DM led to a maximum decrease in ACh-induced relaxation in YD rats compared to other group of rats ($p < 0.01$). The cumulative dose–response curves of ACh were similar in YC and YCT rats, but SMe1EC2 protected aorta against aging-induced ACh hypo-responsiveness, reflected by a significant alteration in apparent E_{max} in OCT rats compared with OC rats ($p < 0.01$) (Fig. 3a). SMe1EC2 treatment also significantly prevented the inhibitory effect of DM on ACh relaxation in YDT rats (p 0.001) (Fig. 3a).

The relaxation response to salbutamol was found to be decreased in OC rats compared to YC animals ($E_{max}p < 0.001$) (Fig. 3b). DM inhibited the relaxation response of aorta to salbutamol significantly ($p < 0.001$), and the E_{max} from the cumulative dose–response curves of salbutamol was similar in YD and OD rats (Fig. 3B). SMe1EC2 treatment did not significantly change the dose–response curve of salbutamol in YCT or OCT rats when compared with untreated control rats ($p > 0.05$). Salbutamol relaxation was inhibited by DM in YD rats in similar degree that we observed in OC rats and was found to be not different in OC, OD, and YD rats. SMe1EC2 treatment was able to improve salbutamol relaxation in YDT and ODT rats ($E_{max}p < 0.001$).

On the other hand, neither aging nor DM or SMe1EC2 treatment affected the responsiveness of aorta to SNP (data not shown). The E_{max} values for the responses of aorta to Phe, KCL, ACh, and salbutamol are demonstrated in Table 2. Either aging, diabetes, or SMe1EC2 treatment had no significant effect upon the pD_2 pattern of contractions or relaxations in all group, indicating that there has not been any significant change in the sensitivity of aortic rings in different experimental groups.

Calcium channel regulation of aorta constriction

To elucidate the mechanism of action of aging, DM or SMe1EC2 treatment on calcium mobilization, the transient vasoconstriction to a single dose of Phe was re-examined in the presence of Ca^{2+} free medium (Krebs solution without Ca^{2+}, CFM) as well as in the presence of cyclopiazonic acid, a SERCA inhibitor, plus Ca^{2+} free medium (Ceylan-Isik et al. 2011). Figure 4 shows the response of aorta to Phe (3×10^{-6} M) before and after CFM (a) and CFM plus cyclopiazonic acid (CYP) (b). Phe constrictions were significantly inhibited in YC but not in OC rats when bath medium was changed by CFM and was further inhibited in the presence of CFM plus CYP ($p < 0.01$). CFM alone or combined with CYP resulted in a significant decrease in Phe constrictions in YD and OD rats ($p < 0.001$). In SMe1EC2 treated rats, Phe constrictions did not significantly change in the rings exposed to CFM or CFM plus CYP compared to before exposures ($p > 0.05$) (Fig. 4b).

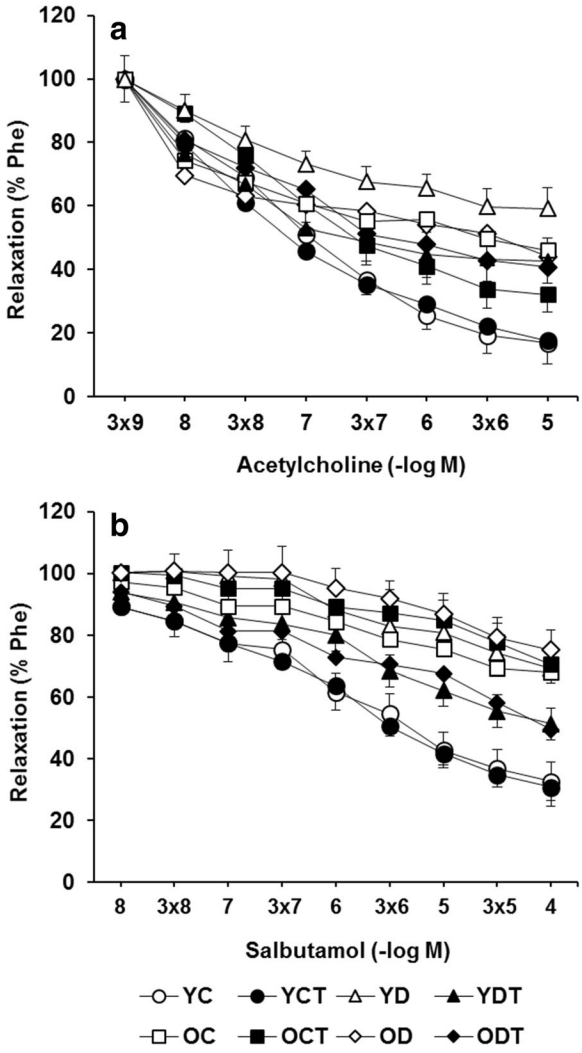

Fig. 3 Concentration-dependent vasorelaxations in response to acetylcholine (ACh, 10^{-9}–10^{-5} M) (**a**) and salbutamol (10^{-9}–10^{-4} M) (**b**) in the phenylephrine-preconstricted (Phe, 10^{-6}–3×0^{-6} M) aortic rings isolated from rats grouped as young control (YC), young control treated with SMe1EC2 (YCT), old control (OC), old control treated with SMe1EC2 (OCT), young diabetic (YD), young diabetic treated with SMe1EC2 (YDT), old diabetic (OD), and old diabetic treated with SMe1EC2 (ODT). Mean ± SEM, $n = 8$–10 rats per group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs YC; $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ vs OC; $^{f}p < 0.05$, $^{ff}p < 0.01$, $^{fff}p < 0.001$ vs YD; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs OD

Potassium channel regulation of aorta relaxation

Figure 5 shows the effects of TEA and 4-AP on ACh-induced relaxations. In YC rats, preincubation of aortic rings with TEA or 4-AP inhibited the relaxant effect of ACh in the same degree ($p < 0.001$) (Fig. 5A1). SMe1EC2 treatment of YC rats resulted in a significant increase in the inhibitory effect of TEA or 4-AP on ACh-induced relaxation compared to SMe1EC2 untreated YC rats ($p < 0.001$) (Fig. 5A2). ACh-induced relaxations have ~40% decrease in OC relative to YC rats, displayed completely normalization by TEA or 4-AP (Fig. 5B1). TEA or 4-AP inhibited ACh-induced relaxation in OCT rats, which showed a significant improvement with SMe1EC2 treatment ($p < 0.01$) (Fig. 5B2). We did not achieve further inhibition with TEA or 4-AP in YD or OD rats characterized by ~50% reduction in ACh-induced relaxations (Fig. 5C1, D1). SMe1EC2-induced amelioration in ACh-induced relaxation by SMe1EC2 treatment was significantly inhibited with TEA or 4-AP in YD rats ($p < 0.001$) (Fig. 5C2). The influence of KCa blockade and Kv inhibition on ACh dose–response curves was considerably different in ODT rats, which showed a better relaxation response to ACh compared to untreated OC rats. Namely, ACh-induced relaxations were markedly inhibited by TEA ($p < 0.001$), it did not change significantly by 4-AP in ODT rats ($p > 0.05$) (Fig. 5D2).

Discussion

This study provides strong evidence of a vaso-protective action of SMe1EC2 in aging and diabetes. The results have shown that SMe1EC2 in old and diabetic rats can serve to maintain physiological blood pressure by maintaining ACh relaxation through mainly smooth muscle hyperpolarization and protection of NO against destructive effects of free radicals. Not only through these mechanisms, but SMe1EC2 also contributes to the regulation of blood pressure by protecting β-adrenergic receptor-mediated relaxation and by controlling α1-adrenergic receptor signaling for contraction. We observed that in the old and diabetic vasculature, SMe1EC2 treatment: (1) prevents impairment of endothelial function, (2) reduces systemic oxidant generation, and (3) suppresses smooth muscle depolarization and calcium mobilization.

It is well known that, the vascular contraction induced by Phe, a selective α1-receptor agonist, is balanced with ACh-stimulated and NO-mediated endothelial relaxation through the mechanism by which Ca^{2+} influx is reduced (Bolotina et al. 1994; Peng et al. 1996). When this regulatory system is inadequate, the aortic contractions to Phe or other adrenergic agents increase

Table 2 The maximum contractions to Phe and KCL and maximum relaxations to ACh and salbutamol in experimental groups

	E_{max} (g tension/mg tissue)		% max. relaxation	
Groups	Phe	KCL	ACh	Salbutamol
Young control (YC)	2.37 ± 0.4	2.42 ± 0.4	84 ± 5	68 ± 3
SMe1EC2 treated young control (YCT)	2.22 ± 0.3	2.39 ± 0.5	82 ± 6	71 ± 4
Old control (OC)	2.27 ± 0.4	2.57 ± 0.8	57 ± 8***	28 ± 6***
SMe1EC2 treated old control (OCT)	2.12 ± 0.6	1.96 ± 0.7***†††	63 ± 8***	27 ± 9***††
Young diabetic (YD)	3.63 ± 0.3***	2.86 ± 0.6**	41 ± 7***	24 ± 4***
SMe1EC2 treated young diabetic (YDT)	1.43 ± 0.4***^{fff}	2.43 ± 0.4^{ff}	65 ± 6***^{fff}	47 ± 4***^{f}
Old diabetic (OD)	3.48 ± 0.8***	2.48 ± 0.4	57 ± 10***	25 ± 3***
SMe1EC2 treated old diabetic (ODT)	1.53 ± 0.7***$^{\#\#\#}$	2.22 ± 0.8*$^{\#}$	58 ± 6***$^{\#\#\#}$	48 ± 7***$^{\#\#\#}$

Mean ± SEM, $n = 8$–10 rats per group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs young control; $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ vs old control; $^{f}p < 0.05$, $^{ff}p < 0.01$, $^{fff}p < 0.001$ vs young diabetic; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.01$ vs old diabetic

(Koçak et al. 2000; Bauer and Sotníková 2010; Karasu 2010), which leads to rise in blood pressure in aging (Faconti et al. 2015) and diabetes (Cohen and Tong 2010). This relationship seems to be not working in the aorta of old rats, because OC rats showed no significant increase in Phe contractions despite inhibition of ACh and salbutamol relaxations. In that case, the reason for the increase in Phe or KCl contractions observed in diabetic rats cannot be explained by the reduced regulatory effect of endothelium-dependent and/or endothelium-independent relaxation alone since diabetes did not produce a further decline in ACh or salbutamol relaxations in YD and OD compared to OC rats. In old age, Phe signaling seems to be not sensitive

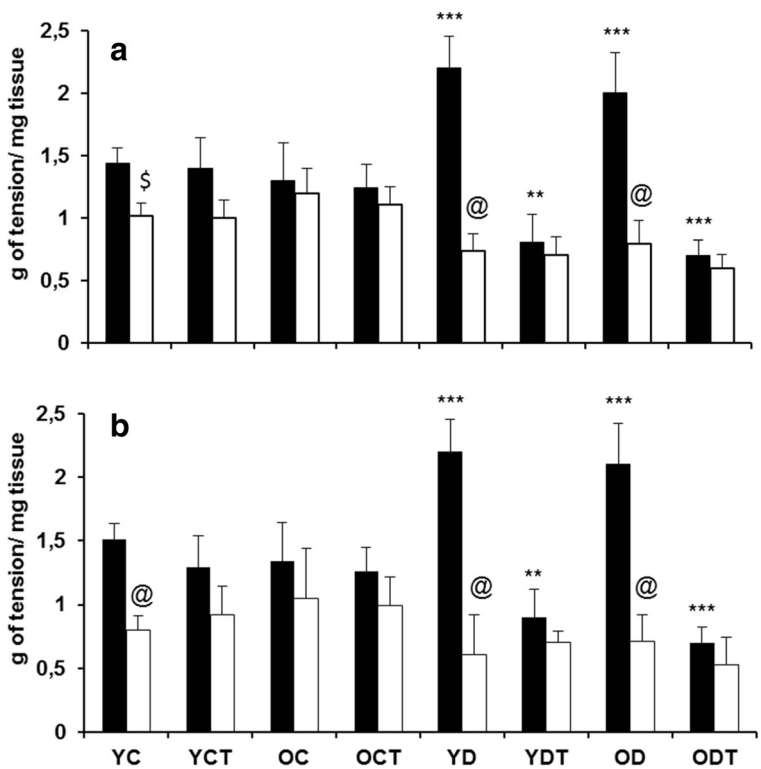

Fig. 4 Vasoconstrictions to a single dose of phenylephrine (Phe, 3×10^{-6} M) in the absence (black bar) or in the presence of Ca^{+2}-free medium (white bar) (**a**) or Ca^{+2}-free medium + cyclopiazonic acid (10^{-6} M) (white bar) (**b**) in aorta rings isolated from rats grouped as young control (YC), young control treated with SMe1EC2 (YCT), old control (OC), old control treated with SMe1EC2 (OCT), young diabetic (YD), young diabetic treated with SMe1EC2 (YDT), old diabetic (OD), and old diabetic treated with SMe1EC2 (ODT). Mean ± SEM, $n = 8$–10 rats per group. **$p < 0.01$, ***$p < 0.001$ vs YC in the presence of Ca^{+2}; \$$p < 0.01$, @$p < 0.001$ vs. without Ca^{+2}-free medium or Ca^{+2}-free medium + CYP

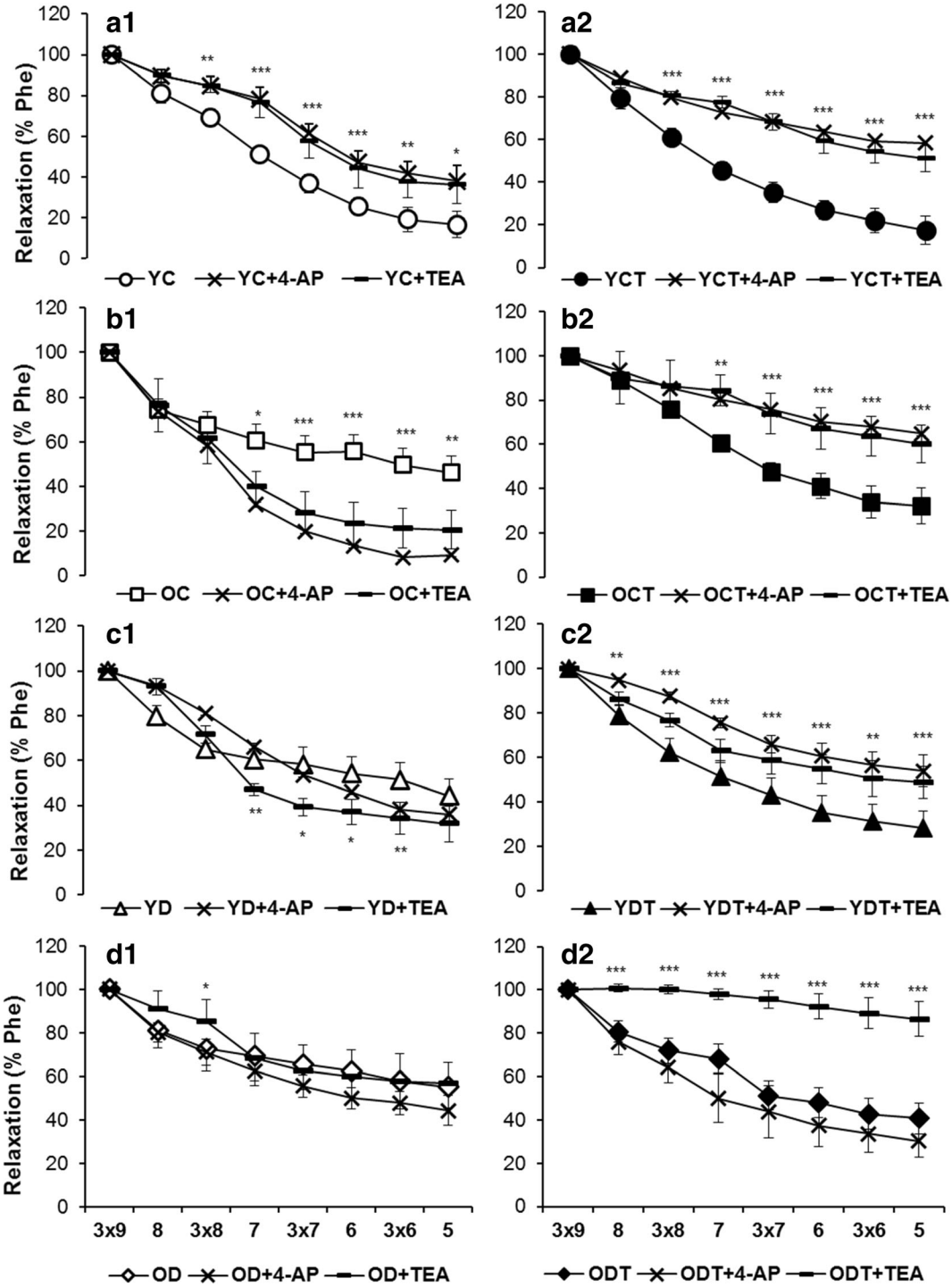

Fig. 5 Vasorelaxation response to cumulatively increased concentration of acetylcholine (ACh, 10^{-9}–10^{-5} M) in the preconstricted (Phe, 10^{-6}–3×0^{-6} M) aortic rings before and after incubation with TEA (10^{-4} M) or 4-AP (10^{-4} M). The rats grouped as young control (YC), young control treated with SMe1EC2 (YCT), old control (OC), old control treated with SMe1EC2 (OCT), young diabetic (YD), young diabetic treated with SMe1EC2 (YDT), old diabetic (OD), and old diabetic treated with SMe1EC2 (ODT). Mean ± SEM, $n = 8$–10 rats per group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs before incubation with TEA or 4-AP

to the reduced endothelial relaxation capacity of vasculature, but an activated vasoconstrictor signaling appears to play an excessive role in YD and OD rats. Contrary to this, we and other investigators previously demonstrated in the rat model of aging or diabetes mellitus that endothelial control of vascular tonus is shifted in favor of vasoconstriction, and insufficient NO and also some arachidonoic acid metabolites mediate to increase Phe contractions (Karasu et al. 1997a; Koçak et al. 2000; Peredo et al. 2006; Reyes-Toso et al. 2007; Sotníková et al. 2011; Ceylan-Isik et al. 2011). The reason for why aging per se did not have an effect on the vasoconstrictor response of aorta to Phe may be related to aging time; in this current study, we used rats that were 20 months old, others usually used 10-month-old rats. In that case, the comparison of aorta relaxations and contractions in the presence of NOS inhibitor L-NAME would be more determinative (Novella et al. 2013).

The Phe contractions in aorta are mediated by the release of Ca^{2+} from the intracellular stores and Ca^{2+} influx through voltage-gated calcium channels (Ca_V) and receptor-operated channels (ROCs) (Horowitz et al. 1996; Hill-Eubanks et al. 2011). KCl also promotes vasoconstriction via smooth muscle membrane depolarization and then Ca^{2+} influx through Ca_V (Hill-Eubanks et al. 2011). Our findings indicate that the mechanisms involving regulation of Ca_V to mediating Phe contractions remain as physiologically active level during aging course, since not only Phe contractions but also KCL contractions stayed as unchanged in OC compared to YC rats. However, when diabetes mellitus developed in young stages of rats, the increased activity of Ca_V is most likely to be a primary contributing factor leading to an increase in Phe contraction and hypertension, because KCL contractions only elevated in YD but not in OD compared to YC, OC, and OD rats. Accordingly, the expressions or activity of contractile proteins such as PKC, which leads to Ca^{2+} flow through Ca_V, has been shown to increase in diabetic vessels due to oxidative and carbonyl stress (Webb 2003; Wang et al. 2012a; Karasu 2010; Chettimada et al. 2014). Because altered Ca^{2+} mobilization and impaired calcium signaling play a crucial role in the development of vascular reactivity abnormalities in aging or diabetes mellitus (Zhu et al. 2001; Karasu 2010; Ceylan-Isik et al. 2011; Ma et al. 2008; Wang et al. 2012a); in details, the function of sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) was assessed by treating the aorta rings in Ca^{2+}-free conditions with or without cyclopiazonic acid (CYP). We found that CFM or CFM plus CYP inhibited Phe contractions in YC and both groups of diabetic but not OC rats, suggesting that exaggerated vasoconstriction seen in diabetic aorta is also related to SERCA and IP3R activation and sensitive to the inhibition with CFM or CFM plus CYP (Table 3).

Phe contractions did not change in YCT and OCT rats but dramatically inhibited in YDT and ODT rats. SMe1EC2 treatment also prevented exaggeration of KCl contractions in YD rats. These are associated with its blood pressure lowering and salbutamol relaxation normalizing effects in YDT and ODT rats. ACh relaxation improving effect of SMe1EC2 was only seen in OCT and YDT animals. In addition, SMe1EC2 treatment kept blood pressure at normal levels in ODT rats without any improvement in endothelial relaxation. Interestingly, Phe contractions in YDT and ODT rats as well as KCl contractions in OCT rats were obtained at the lowest levels compared to other group of rats. These results suggest that exacerbated α1-adrenergic receptor signaling is inhibited by SMe1EC2, and SME1EC2 affects the aorta when aging makes the aorta more sensitive to calcium mobilization via Ca_V. In addition, SMe1EC2 treatment seems to be unable to affect Ca^{2+} release from IP3-sensitive intracellular stores since neither CFM nor CFM plus CYP exhibited a significant inhibition in the contractions of aorta to a single dose of Phe in YCT, OCT, YDT, and ODT rats. This is in agreement with the reported endothelial function protecting effect of SMe1EC2 and its mother substance stobadine in hyperglycemic rats (Zúrová-Nedelcevová et al. 2006; Sotníková et al. 2011). Conversely, there is also an in vitro study showing that SMe1EC2 does not affect the responses of aortic rings to Phe, KCL, and ACh (Broskova et al. 2013).

The increased K^+ efflux causes membrane potential hyperpolarization, leading to vasodilatation that may play a critical compensatory vasodilator role in disease states in which NO-mediated dilation is impaired (Nelson and Quayle 1995). In fact, the vasorelaxation induced by ACh can be attributed in part to the opening of voltage-sensitive K^+ channels (K_v) and large-conductance calcium-activated K^+ channels (K_{Ca}) (Malakul et al. 2008). Kv and K_{Ca} channels are abundantly expressed in vascular smooth muscle cells in aorta and play a crucial role in counteracting of vasoconstriction and high blood pressure (Lísková et al. 2010). Thus, we examined the contribution of K^+ channels to ACh relaxation using a non-selective inhibitor of

Table 3 The table summarizes how the effects of pharmacological agents used in the study change in experimental groups and also in experimental conditions

Agonist	Medium	Response of aorta rings	Alterations in response of aorta rings in experimental groups						
		YC	YCT	OC	OCT	YD	YDT	OD	ODT
Phenylephrine (Phe) (in cumulatively increased concentrations)	Ca^{2+} presence	Constriction	Unchanged*	Unchanged*	Unchanged*	Increased*	Decreased*	Increased*	Decreased*
Phenylephrine single dose (3×10^{-6} M)	Ca^{2+} presence	Constriction	Unchanged*	Unchanged*	Unchanged*	Increased*	Decreased*	Increased*	Decreased*
Phenylephrine single dose (3×10^{-6} M)	Ca^{2+} free	Decreased constriction#	Decreased#	Unchanged#	Unchanged#	Decreased#	Unchanged#	Decreased#	Unchanged#
Phenylephrine single dose (3×10^{-6} M)	Ca^{2+} free + CYP	Decreased constriction#	Decreased#	Unchanged#	Unchanged#	Decreased#	Unchanged#	Decreased#	Unchanged#
Potassium chloride (KCL) (in cumulatively increased concentrations)	Ca^{2+} presence	Constriction	Unchanged*	Unchanged*	Decreased*‡ƒ&	Increased*‡ƒ&	Unchanged*	Unchanged*	Decreased*‡ƒ&
Acetylcholine (ACh) (in cumulatively increased concentrations)	Ca^{2+} presence in phe precontracted aorta	Relaxation	Unchanged*	Decreased*	Decreased* increased‡	Decreased*‡&	Increasedƒ decreased*	Unchanged‡ increasedƒ decreased*	Unchanged &‡ increasedƒ decreased*
Acetylcholine (in cumulatively increased concentrations)	Ca^{2+} presence + tetraethylamonium (TEA) in Phe precontracted aorta	Decreased relaxation§	Increased§	Increased§	Decreased§	Unchanged§	Decreased§	Unchanged§	Decreased§
Acetylcholine (in cumulatively increased concentrations)	Ca^{2+} presence + 4-aminopyridine (4-AP) in Phe precontracted aorta	Decreased relaxationΦ	IncreasedΦ	IncreasedΦ	DecreasedΦ	UnchangedΦ	DecreasedΦ	UnchangedΦ	UnchangedΦ
Salbutamol (in cumulatively increased concentrations)	Ca^{2+} presence in Phe precontracted aorta	Relaxation	Unchanged*	Decreased*	Unchanged‡ decreased*	Unchanged‡ decreased*	Increasedƒ‡ decreased*	Unchangedƒ‡ decreased*	Increasedƒ‡ decreased*

* Comparison with response of aortic rings obtained from the YC rats

‡ Comparison with the response of aortic rings obtained from the OC rats

ƒ Comparison with response of aortic rings obtained from the YD rats

& Comparison with the response of aorta rings obtained from the OD rats

Comparison of responses within the study group in the presence of Ca^{2+} in the medium

§ Comparison of responses within the study group in the absence of TEA in the medium

Φ Comparison of responses within the study group in the absence of 4-AP in the medium

K_{Ca}, tetraethylamonium (TEA), and 4-aminopyridine (4-AP), a selective inhibitor of K_V. ACh relaxations were blunted by pharmacological blockade of K^+ channels with TEA and 4-AP in YC rats, suggesting that K^+ channels opening contributes to vasodilatation evoked by ACh. When YC rats treated with SMe1EC2, ACh relaxation was inhibited more significantly by TEA or 4-AP than those of SMe1EC2 untreated YC rats, suggesting that ACh relaxation is realized independently from endothelial NO release in the presence of SMe1EC2 and the hyperpolarization-mediated relaxation is likely playing a major regulatory role in controlling of vasoconstrictor tonus by SMe1EC2. Interestingly, in OC rats, the presence of TEA or 4-AP led to an increase in ACh-induced relaxation. This response that seems to be counter to the expectation, may occur in the presence of aging-dependent regulation of the membrane potential through changes in K^+ channel activity, which changes the activity of voltage-dependent Ca^{2+} channels. Admittedly, additional experiments that go beyond the scope of the present study will be required to further identify the specific subtype of K^+ channels involved in the vasorelaxations produced by ACh in old rats. On the other hand, pretreatment of the aorta with 4-AP or TEA did not alter the depressed ACh-induced relaxation seen in YD and OD rats. This finding supports the hypothesis that the involvement of K^+ channels in ACh relaxation is inhibited by diabetes. Our results are consistent with those obtained previously in aorta where diabetes was found to inhibit the vasodilation mediated by K_{Ca} or Kv channels (Malakul et al. 2008; Kavak et al. 2009; Wang et al. 2012a). Furthermore, since diabetes-induced decrease in β-adrenoceptor-mediated vascular dilatation has been shown to be primarily associated with endothelium-independent mechanisms, our findings, which show a decrease in salbutamol relaxation in YD rats, can be explained by the dysfunction of KCa or Kv (Karasu et al. 1997b; Ferro et al. 2004; Chai et al. 2005; Ko et al. 2008). Current study also showed that aging alone is an important triggering factor to induce a decrease in salbutamol-induced relaxation, which shows no further inhibition in OD rats. This supports the reduction of the NO-mediated component of salbutamol relaxations due to aging and also overlaps with the finding that diabetes does not cause an additional inhibition in the already inhibited ACh relaxation in old rats. Nonetheless, we do not totally discard a possible decrease of density of β_2-adrenergic receptors by aging (Deisher et al. 1989; Schutzer et al. 2006). In fact, the blunted regulatory role of K_{Ca} as well as NO on Phe-induced contractions in DM has been well documented (Chai et al. 2005; Majithiya and Balaraman 2006). Previous studies also indicated a reduced $K_{ATP,}$ Kv activity in the cholinergic relaxation of aortic rings of STZ-diabetic rats (Chai et al. 2005; Porto et al. 2010). In the base of our findings, it is possible to say that the regulating role of K_{Ca} and Kv on Phe contractions is emerging during aging course. This is consistent with a previously published report, which suggests that the regulating potency of K_{Ca} on NA-induced contractions is similar to that elicited by NO but is more evident in arteries from hypertensive rats than in those from control rats (Lísková et al. 2010). Another remarkable finding of this study is that TEA or 4-AP produces a significant inhibition ACh relaxation in YDT rats; suggesting that the contribution of K_{Ca} and Kv to the relaxant effect of ACh is enhanced in diabetic state by SMe1EC2. Moreover, when old diabetic rats treated with SMe1EC2, the relaxation to ACh was not changed by 4-AP, but was largely inhibited by TEA, indicates the involvement of the activation by Ca^{2+} of K^+ channels in the endothelial-mediated control of vascular tone in old diabetic rats. It has been reported that the opening of K_{Ca} channels in aorta is increased by diabetes (Ye et al. 2004).

In fact, this is the first study indicating the regulatory role of systemic administration of SMe1EC2 on ion channel dynamics, relaxation, and contraction capacity of aorta and blood pressure in YD and OD rats. We inserted a table demonstrating the effects of the test substance on the contractile and dilatative properties of the aorta from each experimental group (Table 2). Present findings are in agreement with the previous works showing that SMe1EC2 has an anti-dysrhythmic effect through regulation of cardiac K_{Ca} (Félétou 2009; Broskova and Knezl 2011). Accordingly, mother substance stobadine has been shown to decrease aortic stiffness and arterial blood pressure in diabetic rats (Broskova et al. 2013). In addition, we cannot exclude the possibility of inhibition of other vasoconstrictors as a potential mechanism of action of SMe1EC2 to preserve aortic relaxation and to protect the increased vasoconstrictive response to phenylephrine. The fact that, SMe1EC2 allows the regulation of active vessel wall components by different pathways, which may also involve the inhibition of other vasoconstrictors like prostanoids or arachidonic acid metabolites which are known to be elevated in diabetes (Peredo et al. 2006).

In the current study, diabetes led to an increase in AGEs, RAGE, 4-HNE, and 3-NT, but SMe1EC2 treatment improved all alterations observed in the stress markers in diabetic rats. This is in accordance with the reported inhibition of endothelial function by carbonyl compounds and oxidonitrosative intermediates in diabetic vessels (Zobali et al. 2001; Negre-Salvayre et al. 2008; Karasu 2010; Wang et al. 2012b; Sell and Monnier 2012). AGEs exert their cellular effects mainly through interaction with cell-surface RAGE (Barlovic et al. 2011; Yamagishi et al. 2012). We found that when the AGE level is increased the RAGE level is decreased in aged rats, implying a receptor down regulation to compensate the destructive effects of excessive production of AGEs and/or possibly other RAGE ligands or an aging-dependent decrease in the synthesis of RAGE protein. In concert of all the aforementioned results, this study revealed that SMe1EC2 is a promising pharmacological agent to be used in the treatment of vascular disorders including hypertension in case of aging and diabetes.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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