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Prolonged treatment with angiotensin1–7 improves endothelial function in diet-induced obesity

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Abstract

Objective—The renin–angiotensin system peptides are critically involved in the regulation of endothelial function with important pathological implications. Angiotensin (Ang) 1–7 has many beneficial effects in the vasculature that modulate the cardiovascular risk. Here, we tested the hypothesis that Ang 1–7 has a protective role against the endothelial defects associated with diet-induced obesity (DIO) in mice.

Methods—Ang 1–7 (with or without Ang II) was delivered subcutaneously for 4 weeks using osmotic minipumps. Vascular studies were performed using aortic rings. Arterial pressure and heart rate were measured in separate cohorts of mice by telemetry.

Results—First, we examined whether chronic administration of Ang 1–7 improves the vascular dysfunctions caused by Ang II. Subcutaneous coinfusion of Ang 1–7 significantly attenuates Ang II-induced endothelial dysfunctions. In addition, DIO mice have significant impairment in the endothelium-dependent relaxation. The contractile responses induced by various stimuli, including serotonin and endothelin-1, were differentially altered in DIO mice. Notably, DIO mice treated with Ang 1–7 for 4 weeks displayed significant improvement in the endothelial function as indicated by the increased acetylcholine-induced relaxation. Consistent with this, chronic treatment with Ang 1–7 reversed the increased aortic expression of NAD(P)H oxidase subunits (p22^{phox} and p47^{phox}) and plasma TBARS associated with DIO mice. In contrast, treatment with Ang 1–7 did not normalize the altered contractions associated with DIO mice.

Conclusion—Our data demonstrate a novel role for Ang 1–7 in improving obesity-associated endothelial dysfunction.

Keywords

endothelial dysfunction; obesity; renin–angiotensin system

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Conflicts of interest

There are no conflicts of interest.

INTRODUCTION

Vascular abnormalities including impaired endothelial function are commonly associated with obesity [1–3]. Clinical and animal studies indicate that obesity is associated with several alterations that may contribute to endothelial dysfunction including the increased activity of the renin–angiotensin system (RAS) [4–6].

There are several mechanisms by which increased RAS activity may compromise vascular function. For instance, in the vasculature, excess of angiotensin (Ang) II is known to interfere with insulin receptor signalling to inhibit glucose uptake and nitric oxide synthase, promote the secretion of proinflammatory cytokines, stimulate the production of reactive oxygen species and the release of endothelin-1 by the endothelium [7,8].

The identification of the biological activity of Ang 1–7 and the subsequent cloning of angiotensin-converting enzyme 2 (ACE2, a key enzyme for the generation of Ang 1–7) and the Mas receptor has established a new regulatory axis in the RAS [9–11]. It is now well established that Ang 1–7 represents an important factor exerting protective influences on various vascular beds involved in cardiovascular regulation [11]. For instance, Ang 1–7 confers protection from hypertension-induced cardiac hypertrophy and fibrosis [12,13], changes in kidney function and structure [14], and alteration in autonomic and baroreflex functions [15].

In the vasculature, Ang 1–7/ACE2 axis has many beneficial effects on cardiovascular regulation [11]. For instance, overexpression of ACE2 improves endothelial function in spontaneously hypertensive rats [16]. In addition, chronic treatment with Ang 1–7 reverses the endothelial dysfunction caused by various conditions, including high salt intake in rats [17] and apolipoprotein E deficiency in mice [18]. The cardiovascular protective effects of Ang 1–7 may be due, at least in part, to negative modulation by this peptide of Ang II actions. Indeed, increasing Ang 1–7 levels or overexpression of ACE2 protect against the deleterious effects of Ang II in several tissues that are relevant for cardiovascular homeostasis [11].

This study was designed to investigate the potential beneficial vascular effects of Ang 1–7 in obesity. First, we tested whether chronic treatment with Ang 1–7 prevents Ang II-induced endothelial dysfunction. Next, we examined whether chronic treatment with Ang 1–7 protects against the endothelial dysfunction associated with diet-induced obesity (DIO) in mice.

MATERIALS AND METHODS

Drugs and reagents

Acetylcholine (ACh), sodium nitroprusside (SNP), KCl, 5-hydroxytryptamine (5-HT) and Ang II were obtained from Sigma-Aldrich Biochemical and dissolved in physiological saline solution. Prostaglandin F_{2α} (Pgf_{2α}) (Lutylase; Pfizer Pharmaceutical, New York, USA) was supplied by the University of Iowa pharmacy. Endothelin-1 (ET-1) and Ang 1–7 were

purchased from Peninsula Laboratories Inc. and dissolved in physiological saline. All concentrations are stated as the final concentration in the organ chamber.

Animals

Male C57BL/6J mice were obtained from the Jackson Laboratories (Bar Harbor, Maine, USA). Mice were housed in a room with constant temperature (23°C) and a 12-h light/dark cycle (lights turned off at 1800 h) with free access to food and tap water. To induce obesity, 5–6 week old C57BL/6J mice were randomly assigned to standard chow [controls, composed of proteins (18.62%), fat (6.25%), fibres (4.53%) and nitrogen-free extracts (53%)] or high fat diet [45% kcal as fat, D12451 from Research Diets, consisted of a special diet composed of proteins (24%), fat (24%) and carbohydrate (41%)] for 38 weeks. Care of the mice used in the experiments met the standards set forth by the National Institutes of Health in their guidelines for the care and use of experimental animals. All procedures were approved by the University Animal Care and Use Committee at the University of Iowa.

Chronic infusions

Osmotic minipumps (Alzet) were implanted subcutaneously to deliver Ang II, Ang 1–7 or vehicle (physiological saline) for 4 weeks before performing the vascular studies. Ang II was administered at a commonly used dose (1 µg/kg per min) that causes endothelial dysfunction in mice [19]. The concentration of Ang 1–7 (0.4 µg/kg per min) was selected on the basis of previous studies showing a lack of effect on body weight and arterial pressure at this dosage [20,21].

To implant the osmotic minipumps, mice were anaesthetized by intraperitoneal injection of Ketamine (91 mg/kg) and Xylazine (9.1 mg/kg) (vascular studies) or isoflurane inhalation (haemodynamic study). The osmotic minipump was inserted in a subcutaneous pocket on the dorsal surface created using blunt dissection. The incision was closed with sutures, and mice were kept warm until full recovery from anaesthesia.

Haemodynamic measurements

We measured arterial pressure and heart rate in mice by telemetry. Mice were anaesthetized with intraperitoneal injection of Ketamine (91 mg/kg) and Xylazine (9.1 mg/kg) and the catheter of a PA-C10 (Data Sciences International, St. Paul, Minnesota, USA) inserted into the left common carotid artery. The transmitter was placed under the skin along the right flank. Mice were allowed to recover for at least 10 days. Baseline arterial pressure and heart rate were recorded for 7 days. Each mouse was then anaesthetized, for few minutes, with isoflurane to implant osmotic mini-pumps delivering Ang II (1 µg/kg per min) with or without Ang 1–7 (0.4 µg/kg per min) for 4 weeks. Arterial pressure and heart rate recordings resumed shortly after the surgery and continued for 4 weeks. At the end of the study, mice were sacrificed with CO₂ asphyxiation (followed by cervical dislocation).

Aortic ring preparation

Separate cohorts of mice were used for vascular studies. These mice were sacrificed with a lethal dose of pentobarbital (50–100 mg/mouse, intraperitoneally). The thoracic aorta was quickly removed and placed in Krebs' buffer with the following ionic composition (mmol/l):

NaCl 118.3, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25, glucose 11. Connective tissue and remaining fat in the adventitia were removed, and the vessel was cut into four equal straight pieces of 4–5 mm in length. Vascular rings were suspended in a myograph chamber (Multi Wire Myograph System – Model 610 mol/l, Danish Myo Technology, Ann Arbor, Michigan, USA) containing 5 ml oxygenated Krebs' solution maintained at 37°C. The rings were connected to a force transducer via two steel pins to measure isometric tension (contraction and relaxation). Resting tension was increased stepwise to reach the final tension of 0.5 g, and the rings were allowed to equilibrate for 45 min. This amount of resting tension is optimal for contraction in these arteries as demonstrated previously [22]. After an initial precontraction (submaximally, 40–50% of maximum) with Pgf_{2α} (10–50 μmol/l) and relaxation with ACh (100 μmol/l) and SNP (100 μmol/l) to establish the integrity and functionality of the vessel, dose–response curves were performed. Vessels were contracted submaximally with Pgf_{2α} as described above. After a stable contraction plateau was reached, dose–response curves were obtained for ACh and SNP. Contractile response in the aorta to ET-1 (0.1 nmol/l to 0.3 μmol/l), KCl (4–160 mmol/l) and 5-HT (10 nmol/l to 0.3 μmol/l) in a dose-responsive manner was examined. A maximum of five dose–response curves were performed per segment.

RNA isolation and quantitative reverse transcriptase-PCR

Total RNA from aortas was extracted using the Trizol method and reverse transcribed with the Super-Script preamplification system (Invitrogen, Carlsbad, California, USA). RNA levels were measured by quantitative RT-PCR using iQ SYBR Green supermix kit (Bio-Rad Laboratories, Hercules, California, USA) and β-actin used as an internal control in a iQ5 Multicolor Real Time PCR Detection System (Bio- Rad Laboratories). The PCR conditions for all genes were as follows: denaturation for 5 min at 95°C, then 40 cycles for 30 s at 95°C and 30 s at 60°C. At the end of amplification, a thermal melt curve was generated, and the samples were analysed by gel electrophoresis to confirm amplification of a single distinct product. Relative amount was obtained using the delta-delta CT method, normalized to β-actin and expressed as fold change relative to controls. The following primers were used: p22^{phox}-sense: TCATC TGTCTGCTGGAGTAT, p22^{phox}-antisense: ACCGACAACA GGAAGTGGG; p47^{phox}-sense: GTCATTTCATAAGCTCCTG GA, p47^{phox}-antisense: CTCTGTGCGTTGCGGATGGT and β-actin-sense: CATCCTCTTCCTCCCTGGAGAAGA and β-actin-antisense: ACAGGATTCCATACCCAAGAAGGAAGG.

Thiobarbituric acid-reactive substances assay

Blood was collected in presence of Na EDTA (1 mmol/l) from the thoracic aorta at time of euthanasia. Plasma was obtained by centrifuging the blood for 20 min at 4°C at 2040g. Samples were snap frozen and stored at –80°C for later analysis. Thiobarbituric acid-reactive substances (TBARS) levels in the plasma were measured using commercial available TBARS kit (Caymen Chemical, Ann Arbor, Michigan, USA) following the manufacturer's protocol.

Statistical analysis

Data are expressed as mean ± SEM. Statistical analysis was performed using unpaired Student's *t*-test (two groups), one or two-way analysis of variance (ANOVA) (multiple

groups) with or without repeated measures followed by a Tukey post hoc test. A *P* value of less than 0.05 was considered significant.

RESULTS

Angiotensin 1–7 attenuates angiotensin II-induced endothelial dysfunction

To test the efficacy of chronic treatment with Ang 1–7, we examined the effect of coadministration of Ang 1–7 on Ang II-induced changes in vascular function. As reported previously [19], subcutaneous infusion of Ang II (1 µg/kg per min) for 4 weeks caused a marked impairment in the endothelial-dependent vasodilatation induced by ACh (Fig. 1a), whereas the endothelial-independent vasodilatation induced by SNP was not significantly altered after Ang II treatment as compared with vehicle (Fig. 1b). Critically, coinfusion of Ang 1–7 substantially attenuated Ang II-induced impairment in endothelial-dependent vasodilatation as indicated by the improved relaxation in response to ACh (Fig. 1a) without affecting the SNP-dependent relaxation (Fig. 1b).

As shown in Fig. 2, aortic rings from mice treated with Ang II for 4 weeks exhibited altered contractile responses as demonstrated by the substantial enhancement in the vasoconstriction induced by serotonin (Fig. 2a). Arteries of Ang II-treated mice also had a slight, but significant, increase in the vasoconstriction triggered by ET-1 (Fig. 2b). Vascular reactivity to KCl tended to be higher in mice treated with Ang II, but this was not significant (Fig. 2c). Coadministration of Ang 1–7 did not improve significantly the contractile defects caused by Ang II (Fig. 2).

Next, we examined the possibility that the improved relaxation in response to ACh following Ang 1–7 administration may be due to the pro-hypotensive properties of this peptide, limiting Ang II-induced increase in blood pressure. In contrast to our hypothesis, infusion of Ang II for 4 weeks caused a comparable and significant increase in systolic, diastolic and mean arterial pressure, measured by radiotelemetry, in presence or absence of Ang 1–7 (Fig. 3a–c). A significant and comparable decrease in heart rate occurred in both groups of mice (Fig. 3d). Assessment of the haemodynamic circadian pattern by analyzing the diurnal changes in arterial pressure and heart rate revealed no significant differences between mice treated with Ang II and Ang II along with Ang 1–7 (data not shown).

Altered vascular reactivity in diet-induced obese mice

Body weight was significantly greater ($P < 0.01$) in the mice fed high-fat diet for 38 weeks (referred to as DIO mice) as compared with lean control mice on normal chow (Fig. 4).

Notably, aortic rings of DIO mice exhibited a significant reduction in the vasodilatory response to ACh (Fig. 5a). This impaired relaxation in DIO mice appears specific to the endothelium-dependent vasodilatation, as the response to SNP was comparable between DIO mice and lean controls (Fig. 5b). These results suggest that the altered response to acetylcholine was a result of endothelial and not smooth muscle dysfunction.

Aortic rings of DIO mice showed contrasting changes in the contractile responses induced by different vasoconstrictors. Indeed, the response to serotonin was slightly but significantly

attenuated in DIO mice as compared with lean controls (Fig. 6a). Conversely, the contractile response to ET-1 was increased in the DIO mice relative to controls (Fig. 6b). On the contrary, the contraction induced by KCl was not significantly different in DIO mice vs. lean controls (Fig. 6c).

Angiotensin 1–7 improves endothelial function in diet-induced obese mice

Treatment with Ang 1–7 for 4 weeks had no significant effect on body weight in DIO mice ($P = 0.097$, Ang 1–7 vs. vehicle, Fig. 4). In contrast, DIO mice treated with Ang 1–7 showed significant improvement in the endothelial function as indicated by the increased ACh-induced relaxation (Fig. 5c). Of note, the response to ACh was comparable ($P > 0.05$) between the DIO mice treated with Ang 1–7 and lean controls. The vasodilatation induced by SNP was similar in the DIO mice treated with Ang 1–7 and vehicle (Fig. 5d).

Acute treatment with Ang 1–7 by its addition directly into the organ bath of vessels did not lead to significant beneficial effects on the relaxations or contractile responses of the aortic rings obtained from DIO mice (data not shown), indicating that prolonged treatment with Ang 1–7 is necessary to improve endothelial function in obesity.

We also tested whether treatment with Ang 1–7 normalizes the altered contractile responses associated with DIO mice. However, vascular contractions to serotonin, ET-1 and KCl were comparable between the DIO mice treated with vehicle and Ang 1–7 (Fig. 6d–f), indicating that the beneficial vascular effects of Ang 1–7 are specific to the endothelial function.

Finally, to gain insight into the mechanisms underlying the beneficial endothelial effect of Ang 1–7 in DIO mice, we measured vascular expression of NAD(P)H oxidase subunits p22^{phox} and p47^{phox}, major sources of reactive oxygen species in vascular cells, in lean and DIO mice. Consistent with previous findings [23], relative to lean controls, DIO mice exhibited significantly higher expression levels of both p22^{phox} and p47^{phox} in the aorta (Fig. 7a, b). Moreover, DIO mice had a significantly elevated circulating levels of TBARS compared with lean controls, suggesting an accumulation of aldehydes from lipid oxidation (Fig. 7c). Importantly, chronic treatment with Ang 1–7 reversed the increased aortic expression of p22^{phox} and p47^{phox} associated with DIO mice (Fig. 7a, b). Ang 1–7 treatment also abolished the elevated plasma concentration of TBARS associated with DIO mice (Fig. 7c).

DISCUSSION

The major goal of the present study was to determine whether Ang 1–7 has a beneficial vascular effect in dietary obesity. We found that DIO mice exhibit the impairment in the endothelium-dependent dilation that is commonly associated with obesity. Notably, DIO mice treated with Ang 1–7 for 4 weeks displayed significant improvement in the endothelial function as indicated by the normalization of acetylcholine-induced relaxation of the aortic rings. Critically, treatment with Ang 1–7 abolished the elevated expression of NAD(P)H oxidase subunits and plasma TBARS levels observed in the aorta of DIO mice. In contrast, treatment with Ang 1–7 did not improve the altered contractile responses associated with

DIO. These data demonstrate a novel beneficial action of Ang 1–7 to improve obesity-associated endothelial dysfunction.

Our observation that chronic treatment with Ang 1–7 prevents the endothelial dysfunctions caused by Ang II infusion are consistent with previous reports indicating that Ang 1–7 acts as an anti-inflammatory factor in part via the inhibition of oxidative stress and the generation of radical oxygen species and to oppose the proatherogenic effects of Ang II [9,11,24]. Of note, the ability of Ang 1–7 to blunt the endothelial dysfunction caused by Ang II occurs despite the presence of hypertension. Chronic treatment with Ang 1–7 was also found effective to prevent the pro-fibrotic and hypertrophic effects of Ang II in the heart without significant alteration in the hypertension induced by Ang II [25]. Together, these findings suggest that the organ-protective effects of Ang 1–7 are due to negative modulation of Ang II action directly in cardiovascular tissues. Supporting such concept are the findings that in aortic endothelial cells, Ang 1–7 opposes Ang II-mediated signalling events, including the activation of NAD(P)H oxidase, which mediates the production of reactive oxygen species [24]. These findings are relevant given the importance of these signalling pathways for the endothelial dysfunctions caused by Ang II [7,8].

Similar to previous reports in obese patients [26] and animal models of obesity [27–29], we found that the endothelial-independent relaxation in response to sodium nitroprusside was not altered in DIO mice, suggesting that the impairment in the response to acetylcholine was a result of endothelial and not smooth muscle dysfunction. In patients [2] and high-fat fed rats [27], obesity was identified as the main contributor to the endothelial dysfunction. The causal role for obesity in the pathogenesis of endothelial dysfunction is further supported by the favourable effects of weight loss on endothelial function in obese patients [30,31]. Obesity-associated endothelial dysfunction has been attributed to reduction in nitric oxide bioavailability, which may be due to decreased biosynthesis or to increased degradation caused by excessive reactive oxygen species [7,32]. Consistent with such possibility, our data show elevated aortic expression of p22^{phox} and p47^{phox} subunits of NAD(P)H oxidase and TBARS in DIO mice.

The ability of chronic Ang 1–7 treatment to improve the impaired endothelium-dependent vasodilation caused by both obesity and Ang II suggest that the elevated Ang II levels may contribute to the obesity-related endothelial dysfunction. Although RAS activity in DIO mice was not assessed in the present study, it is now well established that obesity, including dietary obesity in rodents, is associated with activation of systemic and tissue RAS leading to elevated plasma Ang II levels [33,34] and enhanced vascular reactivity to Ang II [35]. In addition, Ang type 1 receptor antagonism has been shown to improve acetylcholine-induced vascular relaxation in the obese db/db mice [36]. Moreover, in clinical trials, agents that are RAS blockers such as Ang type 1 receptor antagonists or ACE inhibitors were found to have favourable effects on the cardiovascular risk factors associated with obesity [37–39]. However, whether improvement in endothelial function accounts or contributes to the beneficial cardiovascular effects of RAS blockade in obesity remains to be determined.

The contrasting change in the contractile responses induced by serotonin and endothelin-1 in the DIO mice is intriguing. The reduced vascular reactivity to serotonin in DIO mice

contrasts with previous findings demonstrating enhanced serotonin-induced vasoconstriction of arteries obtained from obese women [40], DIO rats [41] and other rat models of obesity [42,43]. Whether the attenuated vascular reactivity to serotonin is specific to the DIO mice remains to be determined. With regard to the enhanced endothelin-1-induced contraction, a similar observation was previously documented in the carotid artery of DIO mice [44]. Overweight and obese individuals also displayed greater endothelin-1-mediated vasoconstriction [45]. Moreover, the elevated contractile effects of endothelin-1 were suggested to contribute to the obesity-associated impairment in endothelium-dependent vasodilation [45]. However, in the present study, we found that in DIO mice, Ang 1–7 treatment had a beneficial effect on endothelial function, but did not eliminate the enhanced contractile response induced by endothelin-1. In contrast to our findings in DIO mice, Benter *et al.* [46] demonstrated that in streptozocin-induced diabetic rats, chronic Ang 1–7 treatment restored the altered vascular reactivity of the carotid and renal artery ring segments and mesenteric bed to both vasodilators and vasoconstrictors. The reasons for the discrepancy in the effect of Ang 1–7 on the contractile responses between our current study and the previous report [46] are not clear, but could be due to several differences including the vascular beds and the animal models analysed in each study.

The continuous increase in the prevalence of obesity is threatening to reverse the progress achieved in the last decade in improving cardiovascular risk management. Impaired endothelial function that is common in obesity not only increases vascular resistance, which promotes hypertension, but also interferes with insulin actions leading to insulin resistance and diabetes [7]. This underscores the importance of understanding the underlying mechanisms to design effective strategies to reverse obesity-associated endothelial dysfunction. Our data indicate that vascular action of Ang 1–7 has an important protective role against the endothelial dysfunction that is associated with obesity. These beneficial vascular actions of Ang 1–7 can be exploited therapeutically.

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Abbreviations

5-HT	serotonin
ACE	angiotensin-converting enzyme
ACh	acetylcholine
Ang	angiotensin
ET-1	endothelin
DIO	diet-induced obesity
Pgf_{2α}	prostaglandin F _{2α}

RAS	renin–angiotensin system
SNP	sodium nitroprusside
TBARS	thiobarbituric acid-reactive substances

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FIGURE 1.

Ang 1–7 improves Ang II-induced endothelial dysfunction. Comparison of the vasodilation induced by the endothelial-dependent dilator acetylcholine (ACh, a) and endothelial-independent NO donor sodium nitroprusside (SNP, b) in aortic rings from mice treated with vehicle, Ang II or Ang II along with Ang 1–7 for 4 weeks before the study. Precontraction of the aortic rings was induced using $\text{Pgf}_{2\alpha}$ and relaxation was expressed as a percentage of the precontraction. * $P < 0.05$ vs. vehicle, † $P < 0.05$ vs. both Ang II and vehicle ($n = 6$ per group).

FIGURE 2.

Ang II-induced alteration in vascular contraction was not affected by Ang 1–7 treatment. Contractile responses induced by receptor-dependent agonists, serotonin (5-HT, a) and endothelin-1 (ET-1, b), or receptor-independent constrictor, potassium chloride (KCl, c) in aortic rings from mice treated with vehicle, Ang II or Ang II along with Ang 1–7 for 4 weeks before the study. * $P < 0.05$ vs. vehicle ($n = 6$ per group).

FIGURE 3.

Ang II-induced hypertension was not affected by Ang 1–7 treatment. Radiotelemetry arterial pressure [AP: diastolic (a), mean (b) and systolic (c)] and heart rate (HR, d) at baseline and 4 weeks after infusion of Ang II with or without Ang 1–7 in mice. * $P < 0.05$ vs. baseline ($n = 5–7$ per group).

FIGURE 4.

Comparison of body weight between diet-induced obese mice and lean controls with or without Ang 1–7 treatment. Body weight was greater in DIO mice relative to lean controls. Ang 1–7 treatment for 4 weeks had no significant effect on body weight in DIO mice. * $P < 0.05$ vs. lean control mice ($n = 8$ per group).

FIGURE 5.

Ang 1–7 improves endothelial function in diet-induced obese mice. (a, b) Comparison of the vasodilation induced by the endothelial-dependent acetylcholine (ACh, a) and endothelial-independent sodium nitroprusside (SNP, b) in aortic rings obtained from lean and DIO mice. (c, d) Effect of 4-week treatment with Ang 1–7 on the aortic rings relaxation induced by ACh (c) and SNP (d) in DIO mice. Precontraction of the aortic rings was induced using $\text{Pgf}_{2\alpha}$ and relaxation was expressed as a percentage of the precontraction. $*$ $P < 0.05$ vs. lean mice, $^{\dagger}P < 0.05$ vs. DIO-vehicle group ($n = 6$ per group).

FIGURE 6.

Ang 1–7 has no effect on the altered contractile responses in diet-induced obese mice. (a–c) Comparison of the contractile responses induced by receptor-dependent agonists, serotonin (5-HT, a) and endothelin-1 (ET-1, b), or receptor-independent constrictor, potassium chloride (KCl, c) in aortic rings obtained from lean and DIO mice. (d–f) Effect of 4-week treatment with Ang 1–7 on the aortic rings contraction induced by receptor-dependent agonists, serotonin (5-HT, d) and endothelin-1 (ET-1, e), or receptor-independent constrictor, potassium chloride (KCl, f) in DIO mice. * $P < 0.05$ vs. lean mice ($n = 6$ per group).

FIGURE 7.

Ang 1–7 abolishes the upregulation of NAD(p)H oxidase in the aorta and circulating TBARS in diet-induced obese mice. (a, b) Elevated RNA expression levels of p22^{phox} (a) and p47^{phox} (b) subunits of NAD(p)H oxidase in the aorta of DIO mice, which can be normalized by chronic Ang 1–7 treatment. (c) DIO mice exhibited elevated plasma concentration of TBARS relative to lean controls and this was normalized by chronic Ang 1–7 treatment. * $P < 0.05$ vs. lean mice and DIO along with Ang 1–7 ($n = 4–15$ per group).