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Endothelial Nitric Oxide and 15-Lipoxygenase-1 Metabolites Independently Mediate Relaxation of the Rabbit Aorta

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Abstract

Endothelial 15-lipoxygenase-1 (15-LO-1) metabolites of arachidonic acid (AA), 11,12,15-trihydroxyeicosatrienoic acid (THETA) and 15-hydroxy-11,12-epoxyeicosatrienoic acid (HEETA) and nitric oxide (NO) mediate relaxations to acetylcholine (ACH). However, interactions between NO and the 15-LO-1 pathway have not been explored. Therefore, the effect of physiological and pharmacological concentrations of NO on 15-LO activity and relaxation was studied in rabbit aorta. In indomethacin-treated aortic rings, maximal ACH relaxations of $91.3 \pm 4.0\%$, decreased to $54.5 \pm 3.0\%$ by the NO synthase inhibitor, nitro-L-arginine (LNA), to $49.8 \pm 3\%$ by the guanylate cyclase (GC) inhibitor, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one, to $63.7 \pm 4.9\%$ by the lipoxygenase (LO) inhibitor, nordihydroguaiaretic acid (NDGA) and were completely inhibited by the combination of LNA and NDGA. AA relaxations were not affected by GC inhibition but were reduced by LO inhibition. The NO donor, dipropyleneetriamine-NONOate (DPTA) caused concentration-related relaxations ($EC_{50} = 4.7 \times 10^{-6}$ M). Aortic metabolism of ^{14}C -AA to THETA and HEETA was not altered by EC_{50} concentrations of DPTA but were reduced 10-fold by 10^{-3} M DPTA. In LNA-treated aorta, DPTA (3×10^{-6} M) caused relaxations of $38.25 \pm 4\%$. Maximum relaxations to ACH did not differ in presence and absence 3×10^{-6} M DPTA ($49.5 \pm 5\%$ and $44.2 \pm 4\%$, respectively). These results indicate that NO and 15-LO-1 act in parallel to mediate ACH relaxations and NO does not alter 15-LO-1 activity.

Keywords

Arachidonic acid; vascular relaxation; nitric oxide; 15-lipoxygenase

1. Introduction

Nitric oxide (NO), prostaglandin (PG) I_2 and endothelium-derived hyperpolarizing factors (EDHFs) are synthesized in the endothelium and act in a paracrine manner on smooth muscle cells (SMCs) to dilate arteries and maintain blood pressure (Chawengsub et al., 2009; Cohen and Vanhoutte, 1995; Feletou and Vanhoutte, 1996). These endothelial factors are released by agonists such as bradykinin and acetylcholine (ACH) and shear stress.

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Disclosures

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Decreases or increases in the activity of one dilator is partially compensated by another to maintain arterial tone (Kohler and Hoyer, 2007). Several EDHFs have been identified and include AA metabolites of cytochrome P450 (CYP450) and 15-lipoxygenase (15-LO-1), hydrogen peroxide, C-type natriuretic peptide, and potassium (K) ions (Busse et al., 2002; Campbell and Falck, 2007; Campbell et al., 1996; Chauhan et al., 2003; Chawengsub et al., 2009; Cohen and Vanhoutte, 1995; Edwards et al., 1998). In the presence of nitric oxide synthase (NOS) and cyclooxygenase (COX) inhibition to inhibit the synthesis of NO and PGs, the relaxations are attributed to EDHFs. In rabbit arteries, NO- and PG-independent relaxations to ACH are due to 15-LO-1-metabolites of AA (Campbell et al., 2003; Chawengsub et al., 2008).

15-LO-1 metabolizes unsaturated fatty acids such as AA or linoleic acid (Kuhn and Thiele, 1995). Endothelial 15-LO-1 metabolites of AA, 11,12,15-trihydroxyeicosatrienoic acid (THETA) and 15-hydroxy-11,12-epoxyeicosatrienoic acids (HEETA), are EDHFs (Campbell et al., 2003; Chawengsub et al., 2008; Chawengsub et al., 2009). In rabbit arteries, THETA and HEETA are synthesized in the endothelium and activate small conductance calcium-dependent potassium channels (SK_{Ca}) channels to hyperpolarize vascular SMCs and relax the arteries (Gauthier et al., 2004; Zhang et al., 2007). Inhibition of 15-LO-1 by pharmacological inhibitors or reducing 15-LO-1 expression with an antisense oligonucleotide reduced the synthesis of THETA and HEETA and decreases the relaxations to ACH (Aggarwal et al., 2008a; Desai et al., 2006; Tang et al., 2006). In contrast, increasing the endothelial expression of 15-LO-1 with an adenovirus increased the synthesis of THETA and HEETA and increases the relaxations to ACH (Aggarwal et al., 2008a; Aggarwal et al., 2007). Thus, 15-LO-1 is the rate limiting step in this pathway and changes in its expression are sufficient to alter the synthesis of THETA and HEETA and the dilation of arteries (Aggarwal et al., 2008a; Aggarwal et al., 2007; Tang et al., 2006).

NO influences the expression and activity of several proteins in the endothelium by S-nitrosylation or tyrosine nitration (Handy and Loscalzo, 2006). Additionally, NO binds to prosthetic iron groups such as heme or iron-sulfur clusters leading to either activation or inhibition of the enzymes (Handy and Loscalzo, 2006). With regards to enzymes that participate in endothelium-dependent dilations, NO alters the activity of guanylyl cyclase (GC), COX, CYP450 and arginase (Feletou et al., 2008; Santhanam et al., 2007). For example, NO increases the synthesis of PGI_2 in rat endothelium (Tang et al., 2008b) and decreases the synthesis of CYP450-derived EDHFs (Bauersachs et al., 1996) that cause increased or decreased relaxations, respectively. The effect of NO on 15-LO-1 has not been investigated in detail.

Since both NO and 15-LO-1 are activated simultaneously by the same agonist, there is the possibility of crosstalk between the two pathways. Indeed, there are suggestions of this possible interaction. For example, exogenous 15-hydroxyeicosatetraenoic acid (15-HETE) decreased NO activity in rabbit pulmonary arteries (Ye et al., 2005), over-expression of 15-LO-1 decreases NO-mediated vasodilation in the rabbit capillaries (Viita et al., 2008) and hypercholesterolemia-induces an increase in endothelial 15-LO-1 expression and activity and reduces NO bioavailability and GC activity (Hiltunen et al., 1995; Stokes et al., 2002). Moreover, Holzhutter et al. demonstrated that short-term incubation with high concentrations of NO inactivated purified 15-LO-1 by forming a NO-15-LO-1 complex whereas with longer incubations, NO oxidized the ferrous form of the non-heme iron in the active site of 15-LO-1 resulting in activation of the enzyme (Holzhutter et al., 1997). These contradicting studies indicate that 15-LO-1-derived mediators and NO interact under specific experimental conditions; however, a direct physiological effect of NO on 15-LO-1 activity or function of 15-LO metabolites has not been studied. The goal of our study was to determine if physiological concentrations of NO affect 15-LO activity in vascular cells and

thus we investigated the effect of NO on 15-LO-1 mediated AA metabolism and vascular relaxations. In particular, we wondered if the NO and 15-LO pathways act in parallel or if the 15-LO pathway is only fully active when NO is inhibited.

2. Methods

2.1. Rabbits aortas

Animal protocols were approved by the institutional animal care and use committee of the Medical College of Wisconsin, and procedures were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (1996). Four-week old New Zealand White rabbits (Kuiper Rabbit Ranch, IN) were euthanized with pentobarbital overdose. From the euthanized rabbits, aorta were removed and maintained at 4°C in N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer (mM): 10 HEPES, 150 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 6 glucose, pH 7.4 (Campbell et al., 2003).

2.2. Isometric tension in aortic rings

Thoracic aorta was cut in to 2–3 mm rings. Aortic rings were suspended in a 6 ml tissue bath with Krebs bicarbonate buffer of composition (in mM): 119 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.17 MgSO₄, 25 NaHCO₃, 1.18 KH₂PO₄, 0.027 EDTA, 5.5 glucose, at 37°C and bubbled with 95% O₂ and 5% CO₂ (Campbell et al., 2003). Isometric tension was measured with force-displacement transducers and recorded with a Macintosh computer and MacLab software. The vessels were gradually adjusted to 2 gm resting tension and allowed to equilibrate for 30 min. The vessels were then tested for the maximum response with KCl (30 mM, 1.9±0.3 g) as described previously (Campbell et al., 2003; Pfister and Campbell, 1992). The vessels were contracted by phenylephrine (Phe; approx. 10⁻⁷ M, 1.1±0.2 g), to 50–60% of the maximal KCl contraction. KCl and Phe responses were consistent across all experiments. Cumulative concentrations of ACH (10⁻⁹-10⁻⁶ M) or AA (10⁻⁷ to 10⁻⁴ M) were added to the bath and changes in isometric tension were measured. In some experiments, the rings were treated with indomethacin (Indo; 10⁻⁵ M) with or without L-nitro-arginine (LNA) (3 × 10⁻⁵ M), the GC inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ; 10⁻⁵ M) or the LO inhibitor nordihydroguaiaretic acid (NDGA; 3 × 10⁻⁵ M) or their combination for 10 min, contracted by Phe and relaxations to ACH determined. This concentration of LNA inhibits ACH relaxations of rabbit aorta to a similar extent as 3 × 10⁻⁴ M LNA (Aggarwal et al., 2008c). The concentration of NDGA eliminates methacholine relaxations in rabbit aorta (Singer and Peach, 1983) and inhibited the aortic metabolism of AA to HETEs (Pfister et al., 1998). The concentration of ODQ completely blocked the rise in cGMP stimulated by either NO or SNP in rabbit aortic smooth muscle cells (Weisbrod et al., 1998). Similarly, rings were pretreated with LNA, contracted by Phe and relaxations to cumulative concentration of the NO donor dipropyleneetriamine-NONOate (DPTA) (10⁻⁹ - 10⁻⁴ M) were determined. To measure the ACH relaxations in presence of NO, aortic rings were incubated with Indo and LNA and precontracted with Phe. DPTA (3 × 10⁻⁶ M) was added to these aortic rings and were allowed to relax. The tension in aortas was allowed to stabilize, and this value was considered as a new baseline. Cumulative concentrations of ACH were added to these preparations to measure non-prostanoid and non-NO mediated relaxations. Vasorelaxation is expressed as percentage of maximum precontraction.

2.3. Metabolism of ¹⁴C-AA

Aortas were dissected, cleaned, cut into 2–3 mm rings, weighed and incubated at 37°C with Indo (10⁻⁵ mol/L) (Sigma, MO) in 5 ml HEPES for 10 min. Vehicle or DPTA (10⁻⁶ – 10⁻³ M) and [¹⁴C]-AA (0.5 µCi, 10⁻⁷ M) was added, incubation was continued for 5 min, and then A23187 (10⁻⁵ M) (Sigma, MO) was added. After 15 min, the reaction was stopped

with ethanol (15% final concentration). The tissue was removed and saved while media was extracted using Bond Elute octadecylsilyl columns (Pfister et al., 1998).

The extracts from media or tissue were analyzed by reverse phase high-pressure liquid chromatography (HPLC) using a Nucleosil C-18 (5 μ , 4.6 $\times$ 250 mm) column (Pfister et al., 1998). The solvent system consisted of a 40 min linear gradient (flow rate=1 ml/min) from 50% solvent B (acetonitrile with 0.1% glacial acetic acid) in solvent A (deionized water) to 100% solvent B. Column effluent was collected in 0.2 ml fractions and the radioactivity was determined. Total radioactivity from a sample was normalized to the weight of the tissue used.

2.4. Statistical analysis

The experimental data were expressed as means $\pm$ S.E.M. Radioactivity from the ^{14}C -AA metabolism studies was analyzed by student *t* test. A repeated measure two-way ANOVA followed by Bonferroni post-test was performed to analyze relaxations to each DPTA, ACH or AA concentration and the effect of inhibitors to these relaxations. Values were considered significant at $p < 0.05$ or smaller.

3. Results

3.1. NO and 15-LO-1-mediated relaxations to ACH

ACH relaxations were determined in the aortas in the presence of Indo (Figure 1). NO relaxes arteries by activating GC in the SMCs (Ignarro et al., 1987). Thus, NO mediated relaxations were determined by inhibiting NO synthesis with LNA (Figure 1A) or inhibiting GC with ODQ (Figure 1B) (Olson et al., 1997). Incubation of aortas with LNA or ODQ did not change the basal tension (2 g) of the aortas. LNA reduced the maximum ACH relaxations in Indo treated aortas from $91.3 \pm 4.0\%$ to $54.5 \pm 3.0\%$ indicating that approximately 37% of the relaxation response was due to NO (Figure 1A). Inhibition of 15-LO with NDGA also reduced the maximal relaxations to ACH to $63.7 \pm 4.9\%$. Incubation with LNA in combination with NDGA further inhibited the relaxations to a maximum $18.0 \pm 4.0\%$. Thus, the Indo- and LNA-resistant relaxations were due to LO metabolites of AA. Inhibition with other LO inhibitors such as BW755C and ebselen caused similar inhibition of the LNA- and Indo-resistant ACH-relaxations (data not shown). In the presence of Indo, ODQ also reduced the ACH relaxations from a maximum of $85.3 \pm 3.3\%$ to $49.8 \pm 3.2\%$ (Figure 1B). As with LNA, these results indicate that about a third of the relaxation response was due to NO. When arteries were incubated with the combination of ODQ and NDGA, the relaxations were inhibited further to a maximum $8.4 \pm 1.3\%$. This again indicates that the Indo and ODQ resistant relaxations were due to LO metabolites.

AA also induced relaxations in the Indo-treated aortic rings to maximum $55.6 \pm 7.3\%$ (Figure 1C). These relaxations were not significantly altered by ODQ. The Indo- and ODQ-resistant AA relaxations were almost completely inhibited by NDGA (max $3.3 \pm 2.3\%$). Therefore, in presence of Indo- and ODQ, relaxations to AA are mediated by LO metabolites.

3.2. NO-induced relaxations

Physiological concentrations of NO that cause relaxation were estimated by determining concentration-related relaxations to the NO donor DPTA (Figure 2). DPTA caused concentration-dependent relaxations in rabbit aorta with a maximum relaxation of $103.6 \pm 1.4\%$ at 10^{-4} M (Figure 2A). Concentrations higher than 10^{-4} M DPTA did not cause any further relaxation. The EC_{50} of DPTA relaxations was 4.7×10^{-6} M. The DPTA concentration range of 10^{-7} M to 10^{-4} M mimicked physiologically active concentrations of NO that cause relaxation. Maximal DPTA-induced relaxations of $103.6 \pm 1.4\%$ at 10^{-3} M

were inhibited by ODQ ($34.5 \pm 3.6\%$) (Figure 2B). However, DPTA relaxations at 10^{-4} M, which represents a more physiological concentration of NO, were reduced to ~8% by ODQ. This confirms that exogenously added physiological concentrations of NO relaxes the aortas through the GC-cGMP pathway. Incubation of aortas with NDGA did not alter the DPTA relaxations (Figure 2C) indicating that LO does not affect the NO-mediated relaxations.

3.3. Effect of NO on AA metabolism by 15-LO-1

The effect of NO on the metabolism of ^{14}C -AA in the aortas was determined in presence or absence of DPTA (Figure 3 and 4). All the incubations were performed in presence of LNA to inhibit the endogenous synthesis of NO. Concentrations of DPTA bracketing the EC_{50} 's for relaxation were tested i.e. below the EC_{50} (10^{-6} M), near the EC_{50} (3×10^{-6} M), above the EC_{50} (10^{-5} M) and the supraphysiological concentration of 10^{-3} M. Figure 3 shows representative chromatograms of ^{14}C -AA metabolism in presence of vehicle, LNA, or LNA with 3×10^{-6} M and 10^{-3} M DPTA. Rabbit aorta metabolized ^{14}C -AA to metabolites that comigrated with THETA, HEETA, 15-HETE and 12-HETE (Figure 3A). Quantitative estimates of THETA, HEETA and HETE synthesis are shown in Figure 4. Incubation with LNA alone did not change the synthesis of THETA (Figure 4A), HEETA (Figure 4B) or HETEs (Figure 4C) compared with the vehicle only incubation, respectively. Lower concentrations of DPTA (10^{-6} , 3×10^{-6} and 10^{-5} M) did not change the synthesis of THETA, HEETA or HETEs. However, incubation with 10^{-3} M DPTA significantly reduced the synthesis of THETA, HEETA and HETEs compared with the LNA only incubations. This indicates that high, but not low, physiological, concentrations of NO inhibit aortic 15-LO-1. In all incubations, total added radioactivity was recovered from the incubation media. Thus, neither the metabolites nor AA were trapped in the cellular lipid fractions in the presence or absence of NO.

Various ratios of metabolites were analyzed from the vehicle, LNA or DPTA incubations (Table 1). The ratio THETA:HEETA, THETA:HETEs and HEETA:HETEs did not change in any incubation compared with the vehicle. These comparisons indicate that enzymes downstream of 15-LO-1 such as hydroperoxide isomerase, soluble epoxide hydrolase or glutathione peroxidase that are involved in the pathway of THETA, HEETA and HETE synthesis were also not affected. However, the ratio THETA:AA, HEETA:AA and HETEs:AA was significantly reduced in the incubations with 10^{-3} M DPTA compared with other incubations. The ratio of 12-HETE to 15-HETE also did not change in any incubation compared with the vehicle (data not shown). These results indicate that only a high, supraphysiological concentration of DPTA (10^{-3} M) inhibits 15-LO-1 activity.

3.4. Effect of NO on 15-LO-1-induced relaxations

To determine the affect of NO on 15-LO-1-mediated relaxations, Indo- and LNA-resistant relaxations to ACH were measured in presence or absence of DPTA (Figure 5). LNA inhibited the synthesis of endogenous NO and DPTA (3×10^{-6} M) was used to replace the NO exogenously. Indo- and LNA-treated aortas were first precontracted with Phe and then incubated with either vehicle or DPTA (3×10^{-6} M), a concentration that approximates the EC_{50} . In Indo-, LNA- and vehicle-treated aortas, ACH caused maximum relaxations of $44.2 \pm 4.2\%$ (Figure 5). DPTA alone caused $38.2 \pm 4\%$ relaxation. Addition of cumulative concentrations of ACH to DPTA-treated arteries caused maximum of $75.3 \pm 2.2\%$ relaxations (Figure 5). After subtraction of the baseline relaxation by DPTA, the maximum ACH-relaxations in presence of DPTA were $49.5 \pm 5\%$ (Figure 5B). These relaxations were not different from the relaxations in absence of DPTA. These data indicate that 15-LO-1-mediated relaxations to ACH occur in the presence of physiological concentrations of exogenous NO.

4. Discussion

The goal of our investigation was to determine if NO interacts with the 15-LO-1 pathway during agonist stimulated relaxation. We addressed this goal by determining if endogenous as well as exogenous NO alters LO mediated relaxations of the rabbit aorta. We first investigated if the endogenous NO-mediated GC pathway affected 15-LO-1-mediated relaxations. Endogenous NO synthesis was inhibited by LNA or GC activity was inhibited by ODQ. Both ODQ and LNA cause partial reductions in the relaxations to ACH, and these ODQ-resistant and LNA-resistant ACH relaxations were blocked by LO inhibition. In agreement with these results, LNA-resistant relaxations were inhibited completely by a number of LO inhibitors including BW755C, ebselen and baicalein (Aggarwal et al., 2008c; Oliver et al., 2003). ODQ and LNA caused similar reductions in the relaxations to ACH suggesting that NO's effects are mediated by GC and cGMP pathway exclusively. Moreover, in the presence of Indo, AA relaxations of rabbit aorta were not affected by ODQ but were inhibited by NDGA. This also indicates that the relaxations to AA do not involve GC but were due to 15-LO-1 metabolites. This is consistent with our previous findings that AA decreased the cyclic GMP content of rabbit aorta (Pfister and Campbell, 1992). Therefore, these results indicate that the LNA- or ODQ-resistant relaxations were due to the 15-LO-1 pathway and does not involve GC or its downstream effectors, and that the basal or ACH-stimulated NO does not affect the 15-LO-1-mediated relaxation.

By performing concentration-dependent relaxations using DPTA, we estimated the in vitro physiologically active concentrations of exogenous NO and then determined the effect of these concentrations on aortic 15-LO-1 activity. The NO-donor DPTA (half life of 6 h) caused concentration-related relaxations with 10^{-4} M DPTA causing 100% relaxation. These relaxations were inhibited by ODQ indicating a role of the GC-cGMP pathway. However, with 10^{-3} M DPTA, approximately one-third of the relaxation response remained after inhibition with the sCG inhibitor, ODQ. Similarly, in the rabbit carotid arteries, ODQ-resistant relaxations to NO have been observed (Plane et al., 1998). Because this concentration of DPTA represents a supra-physiological concentration of NO, we did not attempt to identify the mechanisms mediating the ODQ-resistant relaxation. However, because NO concentrations can greatly increase during inflammation (Korhonen et al., 2005), the relaxation responses to 10^{-3} M DPTA could reflect inflammatory NO levels. NDGA did not alter the DPTA-induced relaxations suggesting that 15-LO-1, endogenous 15-LO-1 metabolites and NDGA do not affect the mechanism of action of NO.

NO is a reactive free radical that causes S-nitrosylation or nitration of proteins (Mannick and Schonhoff, 2002). These modifications may inactivate enzymes by degradation, modification, and/or altered localization. To determine the effect of endogenous and exogenous NO on 15-LO-1 enzymatic activity, aortas were incubated with ^{14}C -AA in presence or absence of LNA and/or DPTA. Aortic rings metabolized ^{14}C -AA into THETA, HEETAs and HETEs. The metabolism of AA was not altered by either LNA or LNA and DPTA (10^{-6} to 10^{-5} M). However, a supraphysiological concentration (10^{-3} M) of DPTA significantly decreased the synthesis of THETA, HEETA and 15-HETE. The reason for the inhibition by the high concentration of DPTA was not determined but may be related to the formation of a NO-15-LO-1 complex as described previously (Holzhutter et al., 1997). These experiments showed that physiological concentrations of NO do not alter 15-LO-1 activity and thus THETA and HEETA and NO may act independently of each other.

15-LO-1 synthesizes 15-hydroperoxyeicosatetraenoic acid (15-HPETE) from AA (Chawengsub et al., 2008; Chawengsub et al., 2009). 15-HPETE is either reduced to 15-HETE by glutathione peroxidase or rearranged to HEETA by a hydroperoxide isomerase (Pfister et al., 2003; Schnurr et al., 1996). HEETAs are then reduced to THETAs by soluble

epoxide hydrolase (Chawengsub et al., 2008). The proportion of THETA to HEETA, THETA to HETEs or proportion of HEETA to HETEs did not change with vehicle, LNA or DPTA (10^{-6} - 10^{-5} M) and LNA. Therefore, NO did not alter the enzymatic activity of 15-LO, soluble epoxide hydrolase, glutathione peroxidase, or hydroperoxide isomerase. However, the proportion of the THETA, HEETA or HETEs to AA was significantly reduced with 10^{-3} M DPTA suggesting that high concentrations of NO reduced the activity of 15-LO-1. HPETEs and HETEs are synthesized in the cytosol but are incorporated into membrane phospholipids (Moore et al., 1988). THETAs have not been detected in membrane lipids (Tang et al., 2008a). However, in our studies, the total added radioactivity was recovered in the incubation media indicating that the AA metabolites were not esterified to membrane lipids even in the presence of 10^{-3} M DPTA.

ACH stimulates the release of NO, PGs, THETA and HEETA (Campbell et al., 2003; Chawengsub et al., 2008); however, in the presence of LNA and Indo, relaxations to ACH are only due to THETA and HEETA. Since physiologically active concentrations of NO do not affect the synthesis of THETA and HEETA, the physiological consequences of these findings were determined by measuring ACH relaxations in the presence of DPTA. LNA was used to inhibit endogenous NO synthesis, and a concentration of DPTA approximating the EC_{50} for relaxation (3×10^{-6} M) was added to restore the NO-mediated relaxation component. The relaxations to ACH were not different in the presence or absence of DPTA confirming that indeed, relaxations to THETA and HEETA are not affected by the physiologically active concentrations of NO.

Vascular NO activity decreases with hypercholesterolemia, aging, and diabetes (Collins and Tzima, 2011; Feron et al., 1999; Nakagawa, 2011). We have previously shown that in hypercholesterolemia, 15-LO-1 activity increases while in aging the activity decreases (Aggarwal et al., 2008b; Aggarwal et al., 2008c). These results with hypercholesterolemia and aging indicate that the activity of NO and 15-LO-1 are not related. However, the results also indicate that in disease conditions as hypercholesterolemia, 15-LO-1 activity may increase as a protective mechanism. Based on the literature and the results in this study, we suggest that at physiological concentrations of NO, 15-LO-1 and its AA metabolites act independently of the effects of NO. However, in the disease conditions when NO increases to pathological concentrations, the activity of 15-LO-1 may be altered. As an opposite approach, in a rabbit model of increased 15-LO-1 by adenoviral transduction, VEGF-A165-induced eNOS expression and activity in skeletal muscle capillaries was reduced by 15-LO-1 overexpression (Viita et al., 2008), suggesting an inhibitory effect of 15-LO-1 on eNOS.

In non-vasculature tissues, the effects of NO on LO activity and vice versa, have been described. For example, in immortalized mouse hippocampal cells, LO inhibitors protect against cell-death evoked by 0.5 mM SNP (Czubowicz et al., 2010). It should be noted that this concentration of SNP (and thus NO) represents a supra-physiological concentration. In human neutrophils, the NOS-inhibitor L-NAME decreased 5-LO activity in a guanylyl cyclase- and protein kinase G-dependent manner to decrease the synthesis of proinflammatory leukotrienes (Zagryazhskaya et al., 2010). The increased activity of 5-LO was blocked by inhibiting the 5-LO-activating protein. This indicates that physiological concentrations of NO increase the activity of 5-LO. In another report, the oxidized form of NO, ONOO-, did not cause necrosis of astrocytes in the presence of AA; however, in the presence of NDGA, a LO inhibitor, the ONOO and AA combination was lethal (Palomba et al., 2010). Thus, it was assumed that the AA-metabolites of LO protected against the lethal effects of ONOO. Thus, NO may interact with LO's other than 15-LO in non-vascular tissues.

5. Conclusions

In summary, ACH stimulates NO, HEETA and THETA synthesis which mediate the endothelium-dependent relaxation of rabbit aorta. NO at physiologically active concentrations does not affect the activity of 15-LO-1 or the synthesis of THETA or HEETA. However, at supraphysiological concentrations, NO reduces the enzymatic activity of 15-LO-1. The 15-LO pathway of relaxation is independent of the effect of NO, and the 15-LO-1 and NO pathways act in parallel to cause vasorelaxation.

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Abbreviations

AA	arachidonic acid
15-LO-1	15-lipoxygenase-1
THETA	11,12,15-trihydroxyeicosatrienoic acid
HEETA	15-hydroxy-11,12-epoxyeicosatrienoic acid
NO	nitric oxide
ACH	acetylcholine
LNA	nitro-L-arginine
LO	lipoxygenase
GC	guanylate cyclase
NDGA	nordihydroguaiaretic acid
DPTA	dipropylenetriamine-NONOate
PG	prostaglandin
EDHFs	endothelium-derived hyperpolarizing factors
SMCs	smooth muscle cells
CYP450	cytochrome P450
K	potassium
NOS	nitric oxide synthase
COX	cyclooxygenase
SK_{Ca}	small conductance calcium-dependent potassium channels
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
Phe	phenylephrine
Indo	indomethacin
ODQ	1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one
HPLC	high-pressure liquid chromatography
15-HPETE	15-hydroperoxyeicosatetraenoic acid

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Figure 1.

Acetylcholine (ACH) or arachidonic acid (AA) induced aortic relaxations: Aortas were pretreated with indomethacin (10^{-5} M), precontracted with phenylephrine (10^{-7} to 10^{-6} M) and relaxations to cumulative concentrations of ACH or AA were determined. ACH-relaxations in the aortic rings treated with vehicle, nitro-L-arginine (LNA; 3×10^{-5} M), NDGA (3×10^{-5} M) or LNA and NDGA (A) and, vehicle, ODQ, or ODQ and NDGA (B). AA relaxations in the aortic rings treated with vehicle, ODQ or ODQ and NDGA (C). $n = 8-16$, $** = p < 0.01$, $*** = p < 0.0001$.

Figure 2.

Relaxations to the NO donor, DPTA NONOate (DPTA) in aortas: Aortas were precontracted with phenylephrine (10^{-7} to 10^{-6} M) and relaxations to cumulative concentrations of DPTA were determined. (A) Relaxations to cumulative concentrations of DPTA alone ($EC_{50}=4.7 \times 10^{-6}$ M). Aortas were treated with either vehicle or ODQ (B) or vehicle or NDGA (C) and relaxations to DPTA determined. $n = 8-16$, $** = p < 0.01$, $*** = p < 0.0001$.

Figure 3.

Effects of LNA and DPTA on 15-LO-1 metabolism of ^{14}C -AA in aortic rings with endothelium: Aortic rings were treated with indomethacin (Indo, 10^{-5} M) and incubated with ^{14}C -AA in the presence of vehicle (A), nitro-L-arginine (LNA, 3×10^{-5} M) (B), LNA and 3×10^{-6} M DPTA (C), and LNA and 10^{-3} M DPTA (D). The media was removed, extracted and the metabolites resolved by HPLC. Migration times of known standards are indicated in each panel.

Figure 4.

Effects of LNA and DPTA on the synthesis of 15-LO-1 metabolites in aortic rings with endothelium (see legend to Figure 3) in the presence of vehicle (A), nitro-L-arginine (LNA, 3×10^{-5} M) (B) or LNA treated rings treated with various concentrations of DPTA (C and D). The media was removed, extracted and metabolites resolved by HPLC. Percentage metabolite CPM synthesized per mg tissue is expressed as mean $\pm$ SEM and compared with values in the LNA only incubations. n = set of 3 experiments, ** = $p < 0.01$, *** = $p < 0.0001$

Figure 5.

Effect of NO on acetylcholine (ACH)-induced relaxations: Aortas were pretreated with indomethacin (10^{-5} M) and LNA (3×10^{-5} M) and precontracted with phenylephrine (10^{-7} to 10^{-6} M). Rings were treated with vehicle or DPTA (3×10^{-6} M) and allowed to equilibrate to a stable baseline. Relaxations to cumulative concentrations of ACH were determined. (A) The percentage of relaxation was plotted against the concentrations of ACH. The addition of DPTA relaxed the aortas (shown by star on Y-axis). (B) The baseline for the two treatments was subtracted and the baseline corrected relaxations were plotted against the concentrations of ACH. $n = 10-12$.

Effect of NO on AA-metabolism by 15-LO-1 in rabbit aorta. Ratios of metabolite CPM per mg of tissue were calculated from a set of three experiments.

Table 1

Treatment	THETA/HEETA	THETA/HETE	HEETA/HETE	THETA/AA	HEETA/AA	HETE/AA
Control	1.15 ± 0.17	0.28 ± 0.02	0.25 ± 0.33	0.11 ± 0.02	0.10 ± 0.03	0.41 ± 0.10
LNA	1.39 ± 0.04	0.24 ± 0.03	0.17 ± 0.00	0.17 ± 0.08	0.12 ± 0.06	0.73 ± 0.35
DPTA 10 ⁻⁶	1.45 ± 0.70	0.43 ± 0.20	0.29 ± 0.00	0.19 ± 0.06	0.15 ± 0.02	0.50 ± 0.09
DPTA 3 × 10 ⁻⁶	1.64 ± 1.01	0.28 ± 0.12	0.19 ± 0.04	0.14 ± 0.02	0.15 ± 0.01	0.70 ± 0.40
DPTA 10 ⁻⁵	1.18 ± 0.64	0.36 ± 0.17	0.31 ± 0.02	0.20 ± 0.01	0.25 ± 0.01	0.77 ± 0.42
DPTA 10 ⁻³	1.65 ± 0.11	0.59 ± 0.02	0.35 ± 0.01	0.02 ± 0.00 *	0.01 ± 0.00 *	0.04 ± 0.04 *

* p<0.05 compared with the LNA only incubations.